SciX 2015 ABSTRACTS

(1) **Pre-adaptation: How Basic Research Helps Oceanographers Meet Global Challenges**; Robert S.C. Munier[1]; [1]Woods Hole Oceanographic Institute

This keynote address will speak broadly about pre-adaptation and how basic research in oceanography and ocean engineering provides the technologies, tools and techniques to address societally relevant problems.

(2) **Forensic Microscopy and the Lost Art of Observation**; Christopher Palenik[1]; [1]Microtrace LL

"DNA analyzes one molecule, trace evidence handles the rest." This unofficial slogan of the forensic trace evidence community is a bold statement, but if one stops to consider it, we are each surrounded by literally hundreds of materials and thousands of chemical compounds. Each of these, whether highly engineered to achieve specific properties or naturally occurring, has a story to tell. From a single mineral grain on a sandy beach to a multilayered pigment particle, virtually any material has the potential to play a significant role in a criminal trial, civil litigation, or an internal quality investigation. Yet the ability to exploit this information requires the foresight to find potential evidence, the technical skills to analyze it, and the scientific creativity to exploit the significance of this information. The magnified field of view afforded by a microscope provides a means to observe and analyze materials at a scale that is otherwise invisible to an investigator. In itself, this is not a novel concept, as microscopy has been used in this capacity as a problem solving tool for well over a century. Yet in a world of protocols and standardized methods, the free-form observation platform of microscopy is often discouraged, dismissed, or explicitly limited. Despite this common attitude, case after case illustrates, often in dramatic fashion, the value and elegance of the microscopical approach to the solution a wide range of real world problems. When used today, in concert with other microanalytical methods, this approach affords higher resolution, greater detection limits and wider scope than ever before possible. By means of examples, covering a range of pragmatic, unusual, thought provoking, and sometimes humorous applications of microscopy, this keynote address will illustrate the scientific elegance and practical benefits that a non-scripted but thoughtful microscopical examination can offer to the solution problems, be they academic, industrial or forensic.

(3) **Photons as Reporters of Fundamental Activity in the ICP-MS: Using Lasers to Answer the Five W's**; Paul Farnsworth[1], Lance Moses[1], Jessica Ramsey[1]; [1]Brigham Young University

The five W's of good journalism: who, what, where, when, and why, can be applied to good diagnostic studies of atom and ion behavior in analytical plasmas in general, and to inductively coupled plasma mass spectrometers in particular. Lasers are excellent reporters. The answers to "who" are provided by the selectivity of narrow-band lasers, not only for a targeted atomic species, but also for states occupied by that species. The answers to "what" come in the response to laser-induced perturbations of the plasma in pump and probe experiments. The excellent spatial resolution achievable with coherent beams gives good answers to "where", and time resolution down to the femtosecond level answers "when". The combined answers provided by lasers to "who", "what", "where", and "when" have helped us develop new insights into why inductively coupled plasma mass spectrometers behave as they do and have pointed to possible ways of changing and improving that behavior.

In this presentation I will offer brief examples of the use of lasers to answer different types of questions about ICP-MS behavior, and will then focus on our most recent efforts to understand the effects of helium on laser-ablation sample introductions and to study the relationship between the vacuum interface design and ion beam formation at the entrance of a quadrupole mass analyzer.

(4) **All-Optical Laser Ablation-based Analytical Techniques: Status, Achievements and Directions**; Vassilia Zorba[1], Jhanis Gonzalez[1], Huaming Hou[1], George Chan[1], Xianglei Mao[1], Richard Russo[1]; [1]Lawrence Berkeley National Laboratory

Laser ablation, the interaction of a high-power laser with a material surface which leads to instant vaporization of mass in the form of plasma, is extensively used today as a sampling tool for chemical analysis. Analysis of the optical emission spectra resulting from the laser-induced plasma provides information about the elemental (LIBS) and isotopic (LAMIS) content of any material. Laser ablation sampling is predominantly carried out using nanosecond and femtosecond laser systems. Among these two technologies, femtosecond lasers offer significant advantages in laser ablation, including enhanced non-linear absorption and suppressed thermal effects, which improve sampling and chemical imaging resolution. Additionally, femtosecond lasers can also generate laser filaments, based on the dynamic balance between non-linear Kerr self-focusing and air ionization, which enable pulsed laser beam propagation over extended distances. In this talk, we provide an overview of some of the most recent developments and new applications of LIBS and LAMIS in energy applications (generation, storage), nuclear non-proliferation, and remote isotope sensing with filaments. We also demonstrate three-dimensional chemical imaging capabilities and strategies for improvements in resolution and absolute limits of detection in far- and near-field configurations.

(5) **Laser SIMS Advancements**; David Willingham[1], Benjamin Naes[1], Mindy Zimmer[1]; [1]Pacific Northwest National Laboratory

The efficacy of current state-of-the-art mass spectrometry methods used for non-proliferation and nuclear forensics analyses is reliant on the ability to detect trace elemental signatures from complex sample matrices. Most mass spectrometry techniques are limited by their ionization efficiencies, simply the number of ions detected, divided by the amount of material analyzed, in the range of 1-5 percent. The authors present a novel approach aimed at increasing these ionization efficiencies to nearly 100 percent by combining high energy, ultraviolet (UV) and vacuum ultraviolet (VUV) light sources with secondary ion mass spectrometry (SIMS). The ability to enhance sensitivity and improve the accuracy and precision of isotope ratio measurements translates into less material needed for analysis, decreased analysis time and the discovery of novel signatures.

Data was obtained by combining UV lasers as well as a VUV lamp with dynamic SIMS. SIMS is a mass spectrometric method that generates secondary ions (positive and negative) by impacting solid surfaces with high energy primary ions. Generally, the secondary ions represent a small fraction of all the material removed during the analysis with the rest remaining neutral and, therefore, undetectable. This research aims to photoionize the remaining material removed during SIMS analysis using high energy focused UV/VUV light sources. Three distinctly different light sources were evaluated, an Excimer laser at 193 nm, an Argon ion laser at 244 nm and a VUV lamp at 150 nm.

Preliminary data was collected using lanthanide and actinide standard reference materials in order to demonstrate proof-of-concept. Significant signal enhancements were observed for bulk neodymium oxides as well as uranium oxide particles. Time resolved experiments were performed to confirm the observation of distinct photoionization signals. Additionally, the accuracy and precision of isotope ratio measurements were used to compare with traditional SIMS measurements. These results show that laser post-ionization can be used to increase the ionization efficiency of dynamic SIMS experiments; however, the exact magnitude of ionization enhancement is heavily dependent on the ionization potential of the element of interest as well as the photon energy and spectral distribution of the employed photo-ionization light source.

(6) **Recent Advances in Quantifying Actinide Isotope Ratios by RIMS**; Brett Isselhardt[1], Michael Savina[1, 2], Andrew Kucher[1]; [1]Lawrence Livermore National Laboratory, [2]Argonne National Laboratory

Resonance Ionization Mass Spectrometry holds the promise of rapid, isobar-free quantification of actinide isotope ratios if laser-induced and other sources of systematic bias in the measured ratios can be overcome. We present recent progress in demonstrating this potential using two Pu test materials to demonstrate precision and accuracy in the measured $^{240}Pu/^{239}Pu$ ratio to about 0.5%, without any chemical purification prior to measurement. The minor isotopes ^{238}Pu and ^{241}Pu are also quantified with statistics limited precision and accuracy. The concentration of trace amounts of uranium present in the test materials has also been estimated. In addition, we present an introduction to the new RIMS facility at LLNL. LLNL-ABS-663575

(7) **Real Time Isotopic Analysis of Atmospheric Greenhouse Gases Using Mid-IR Laser Spectroscopy**; David Nelson[1], Barry McManus[1], Joanne Shorter[1], Tara Yacovitch[1], Scott Herndon[1], Mark Zahniser[1]; [1]Aerodyne Research

In this presentation we discuss recent advances in monitoring the isotopes of carbon dioxide and methane, the two most important greenhouse gases (GHG). Greenhouse gas emissions are the primary drivers of global climate change and hence there is a crucial need to quantify their sources and sinks. A general technique to help constrain source and sink strengths in GHG exchange processes is the analysis of the relative proportions of isotopic variants of GHG's. Very high precision measurements of isotopologue ratios are necessary in order to identify sources and sinks because the characteristic changes are small. The standard method of isotopologue measurement has been mass spectrometry, but this technique typically requires significant sample preparation and relatively high instrument maintenance. Laser spectroscopy has the potential to ease these burdens and also to allow easy separation of interfering isobars such as $^{13}C\text{-}CO_2$ and $^{17}O\text{-}CO_2$.

For carbon dioxide, we present recent results demonstrating ultra-high precision measurements of isotope ratios which have the potential to rival the accuracy of mass spectrometric measurements. These measurements were performed using Tunable Infrared Laser Direct Absorption Spectroscopy (TILDAS). We have obtained isotopic measurement precisions exceeding 10 per meg for both $^{13}C\text{-}CO_2$ and $^{18}O\text{-}CO_2$ while measuring ambient air samples with continuous flow. We have also developed an ultra high precision and completely automated method for analyzing air samples from canisters. We alternately and rapidly trap sample gas and reference gas in the optical cell. One measurement requires only ~200 ml (at STP) of air and 16 minutes of measurement time. The resulting repeatability is 6 per meg for $\delta^{13}C\text{-}CO_2$, 7 per meg for $\delta^{18}O\text{-}CO_2$ and 30 per meg for $\delta^{17}O\text{-}CO_2$.

In addition, we present recent laser spectroscopic measurements of the isotopes of methane. These include measurements of $^{13}CH_4$ and CH_3D at ambient atmospheric mixing ratios with unprecedented precision and measurements of the clumped isotope of methane in concentrated gas samples.

(8) **LA-ICPMS: Embracing Challenges of Space and Time**; Bodo Hattendorf[1], Marcel Burger[1], Luzia Gyr[1], Gunnar Schwarz[1], Alexander Gundlach-Graham[1], HAO Wang[1], Detlef Günther[1]; [1]ETH Zurich, Laboratory for Inorganic Chemistry

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) continues to spread into an increasing range of applications. Even though most applications and research are carried out in a geochemical context, the interest in LA as sampling tool for highly sensitive element and isotope analyses is increasing for example in the field of materials sciences and especially biological or bio-medical studies. In this context especially the elemental imaging capabilities from LA sampling are of high interest to study the distribution of various elements in tissue directly or using elemental tags as proxies to determine the distribution of proteins for example. Along this line, there is a remarkable attention to optimization of laser ablation equipment that provides as low as possible aerosol dispersion in order increase the image size or spatial resolution respectively, without increasing analysis time. Together with recent time-of-flight mass spectrometers that can achieve sub-millisecond data acquisition such ablation cells have been successfully applied to determine multi-element distributions in various materials. Examples highlighted in this presentation is the 2-dimensional imaging of a meteorite sample, where not only metal and silicate phases could be distinguished but also the compositional change within the metal phase can be resolved. Another example is the three-dimensional tomography of element distributions in an Opalinus clay where individual pyrite, clay and carbonate domains were determined using a laser spot size 5 µm and a mean depth ablation rate of 1 µm.

(9) **Advances in High Repetition Rate Femtosecond Laser Ablation**; Fanny Claverie[1], Ariane Donard[1, 2], Amélie Hubert[2], Fabien Pointurier[2], Nagore Grijalba[1,3], Nora Unceta[3],Christophe Pécheyran[1]; [1]LCABIE, IPREM UMR UPPA/CNRS 5254, University of Pau and Pays de l'Adour, Pau, France; [2]CEA-DIF, Bruyères le Châtel, Arpajon; [3]Department of Analytical Chemistry, Faculty of Pharmacy, University of the Basque Country, Vitoria-Gasteiz, Spain

Laser ablation - Inductively coupled Plasma Mass Spectrometry is considered as a powerful analytical technique for solid sample analysis. The development of IR-Vis-UV high repetition rate femtosecond lasers has permitted the expansion of the LA/ICPMS applications through different applications. A panel of those emerging applications will be presented in combination with some fundamental aspects related to particles generation/atomization, isotope ratio of short transient signals and wine counterfeiting.

We have developed an algorithm to detect single particles generated by laser ablation and to determine pulse-to-pulse aerosol distribution. An example of the analysis of the aerosol produced by IR fs ablation of pure copper will be presented and compared to particles distribution/counting given by low-pressure cascade impactor.

Lasers are now intensively coupled to MC-ICPMS in order to get better isotope ratios measurement. However, the short transient signal obtained for micrometric particles of natural uranium and the necessity to use simultaneously two types of detectors (faraday cup for abundant isotopes like 238U and electron multipliers for low abundant isotopes like 234U, 235U, 236U) drastically affects the isotope ratios precision. A new approach based on a time resolved correction was developed to improve the internal precision and accuracy up to a factor 10.

Finally, laser ablation – ICPMS technique was used as a new and unambiguous diagnosis tool to fight against wine counterfeiting. A new ablation cell adjustable to different bottle shapes has been successfully developed and multifactorial trace analysis of the packaging (glass, paper, ink and capsule) was carried out. This provides an accurate determination of counterfeited bottles with no or very limited degradation that would not alter the value of the bottle.

(10) **Novel Forensic Applications Using LA-ICP-MS and LIBS**; Jose Almirall[1], Tricia Hoffman[1]; [1]Florida International University

Laser-based methods are used to conduct quantitative analysis of the minor and trace elements in milk powder in food safety and food authentication applications. The generated elemental profile of each sample is used to aid in the creation of a database that will be developed to determine global provenance. Specifically, the goal is to develop a LIBS method for the analysis of milk powders that can more accessible to workers in developing countries due to the lower cost and complexity of LIBS. The more sensitive LA-ICP-MS and LA-ICP-OES are used as reference methods for the elemental profiles generated by LIBS. The study of the plasma fundamentals of a LIBS generated plasma when analyzing the milk powder matrix including measuring the electron density, plasma temperature and optimize the analytical LIBS conditions is presented. We also describe the development of the LA-ICP-OES, LA-ICP-MS and LIBS methods for milk powder analysis directly and compare the analytical figures of merit to reference values of standard materials (IAEA 153 and IAEA155). An optimized quantitative LIBS analysis method using external calibration curves is compared to data from calibration-free LIBS. A tandem LIBS/ LA-ICP-MS method was also used to determine any complementary information from stand-alone LIBS and LA-ICP-MS. The analysis of different milk powder samples acquired in the U.S. for direct analysis by LA-ICP-MS, LA-ICP-OES, and LIBS is presented and compared to the analytical figures of merit (also analyzing reference materials with each analytical run). Finally, the determination of the necessary elemental suite to be incorporated in the analysis scheme is presented and pair-wise comparisons (using ANOVA+Tukey's HSD) for each analytical method to determine the false inclusion and false exclusion rates for the known samples is also presented.

(11) **High-resolution for Direct Isotopic Analysis**; Martin Resano[1], Esperanza García-Ruiz[1], Maite Aramendía[1], Eduardo Bolea-Fernandez[2], Frank Vanhaecke[2], Flavio Nakadi[2],Marcia Veiga[2]; [1]University of Zaragoza; [2]Department of Analytical Chemistry, Ghent University, Belgium; [3]Universidade de Sao Paulo

One of the aspects hampering the acquisition of isotopic information, which needs to be very accurate and precise to be meaningful, is the occurrence of spectral overlaps. In order to overcome this issue there are different possible strategies, but, in many cases, a time-consuming and cumbersome separation step is carried out.

Alternatively, the use of direct solid sampling techniques can provide much faster results in a more straightforward way. However, in this case, it is necessary to use instrumentation that provides sufficient spectral resolution, or else to use reactions (chemical resolution) to avoid spectral overlap. This work presents two different possibilities based on two different analytical techniques: i) high-resolution continuum source graphite furnace molecular absorption spectrometry, and ii) laser ablation ICP-MS/MS, both of which enable direct solid sampling analysis.

The first one is a novel approach that is based on the monitoring of molecular instead of atomic spectra,1 because the former may exhibit isotopic shifts that are large enough to appreciate sufficiently resolved isotopic peaks for each target isotope. For instance, Cl isotopic analysis can be carried out by monitoring the transitions corresponding to 27Al35Cl and 27Al37Cl.2

The second approach is based on using a double mass selection (MS/MS) mode in order to prevent both spectral overlap from atomic ions at the mass-to-charge ratios of the target product ions, but also any measurable effect (mass bias) from the matrix on the ratios obtained, because otherwise perfectly matrix-matched materials are needed to correct for mass discrimination. Application of this approach to Sr isotopic analysis will be demonstrated.

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2. F. V. Nakadi, M. A. M. S. da Veiga, M. Aramendía, E. García-Ruiz and M. Resano, J. Anal. At. Spectrom., 2015, in press, doi: 10.1039/c5ja00055f.

(12) **Expanding LA-ICP-MS Capabilities with Simultaneous LIBS and LAMIS**; Richard Russo[1,2], Jhanis Gonzalez[1, 2], Xianglei Mao[1,2], George Chan[1], Vasillia Zorba[1], Jong Yoo[2], Alex Bol[2], Derrick Quarles[, 2]; [1]Lawrence Berkeley National Laboratory; [2]Applied Spectra, Inc

Laser ablation instantly converts a small quantity of a sample into photons and an aerosol. By measuring both simultaneously, analytical capabilities for dynamic range, element/isotope coverage, accuracy and precision are improved. The technology for photon measurements is LIBS (Laser Induced Breakdown Spectroscopy) which provides major concentrations of most elements and light elements like H, N, O, Be and others which compliments the aerosol based LA-ICP-MS for trace elemental and isotopic capabilities. Our new technology LAMIS (Laser Ablation Molecular Isotopic Spectroscopy) expands the capabilities of LIBS by providing isotopic analysis for these major and light elements. This new analytical technology is combined into one instrument and is a complete toolbox for elemental and isotopic analysis, every element and isotopic on the periodic chart measured for each laser ablation pulse. The lecture will discuss the physics and chemistry underlying the ablation processes for producing the optical plasma suitable to elemental and isotopic analysis, and the aerosol, including laser and detection parameter space for optimization of each modality. Examples will be provided for several applications whereby major, minor, trace elemental and isotopic data are measured for each laser ablation event mapping in 2D and 3D coordinates every element and isotope in a sample.

(13) **Topological Data Analysis: A New Tool for Big Data Exploration**; Ludovic Duponchel[1]; [1]Lille University

An important feature of analytical chemistry is that data of various kinds is being produced at an unprecedented rate. This is mainly due to the development of new instrumental concepts and experimental methodologies. It is also clear that the nature of the data we are acquiring is significantly different. Indeed in chemometrics, we are given data in the form of always longer vectors, where all but a few of the coordinates turn out to be irrelevant to the questions of interest, and further that we don't necessary know which coordinates are the interesting ones. "Big data" in chemometrics is a future that might be closer than any of us suppose. It is in this sense that new tools have to be developed in order to explore and valorize such datasets. Topological data analysis (TDA) is probably one of these [ ]. It was developed recently considering that a data set is a sample or "point cloud" taken from a manifold in some high-dimensional topological space. The sample data are used to construct simplices, generalizations of intervals, which are, in turn, "glued" together to form a kind of wireframe approximation of the manifold. This manifold represents the "shape" of the data from which you can discover sub-populations of samples that cannot be observed with conventional techniques like Principal Component Analysis or Cluster Analysis in general. For this presentation, a big data set will be explored in order to reveal the potential of this new concept.

(14) **Multi-block Data Analysis: New Extensions and Applications in Chemometrics**; Douglas Rutledge[1], Delphine Jouan-Rimbaud Bouveresse[1]; [1]AgroParisTech

Multi-block procedures are used for the simultaneous analysis of sets of several matrices with different types of variables describing the same samples. Multi-blocks methods (also called multi-tables methods) were initially used in sensometry for the analysis of sensory data. For example, when a series of samples are evaluated by a number of judges, each using several (possibly different) characteristics to describe them, it is interesting to know to what extent the judges consider the samples as similar or whether a particular judge evaluates them differently.

One multi-block technique called "Common Components and Specific Weights Analysis" – CCSWA, whose algorithm has been called ComDim (for Common Dimension) in the SAISIR Matlab Toolbox [SAISIR], was proposed in 2000. The objective of ComDim is to describe p data tables observed for the same n samples (i.e., a set of p data matrices (Xi) each with n rows, but not necessarily the same number columns, mi) taking into account the maximum of the common part of the total variance of each table. The method consists in determining a common space for all p data tables, with each matrix having a specific contribution ("salience") to the definition of each dimension of this common space. The use of multi-block methods can be extended to other types of applications:

- Selection of variables, i.e. ComDim_VarSelec

- Detection of significant Factors in an experimental design, i.e. AComDim

- Quantitative analysis (i) either relating the scores obtained in ComDim to dependent variables, or (ii) adapting a quantitative multivariate method to the multi-block situation, i.e., PLS_ComDim, OPLS_ComDim.

Variable selection is an important topic in multivariate data analysis, as the quality of multivariate predictive models can often be improved by selecting informative variables or by eliminating uninformative variables. Many methods can be found in the literature to perform variable selection or elimination. However, as most analytical techniques generate signals described by many variables, the risk of false positives (incorrectly selecting a variable as being informative when it is not) cannot be neglected. Even a careful validation procedure does not always avoid the possibility of false positives. In the case of discriminant models, ANOVA is often used to test each variable separately to determine whether it varies more between groups than within groups. The problem with this method is that although a multiple selection is performed, each variable is selected one by one in a univariate fashion. This is also the case with stepwise methods: the forward selection selects the variables one by one, while the backward elimination removes them one by one. Truly multivariate selection methods are to be preferred. Among the numerous existing methods, one can cite Uninformative Variable Elimination-PLS, Genetic Algorithm-PLS, rPLS or iPLS.

In the first part of this presentation, ComDim will be used for the selection of interesting blocks of variables: the initial data matrix is split into p ($p > 1$) segments, each segment being then considered as an individual block. A multi-block procedure can then be applied to the set of p segments. Examination of the scores of the samples together with the salience values of each block helps to determine the interesting range(s) of variables. The size of the segments is an important criterion. The ComDim method enables to work with blocks of different sizes, so that segments of different widths can be constructed. For example, with spectral data, one can choose to work with segment containing one peak each; hence, each segment has a size defined by the width of the peak. Or one can choose to work with fixed-sized segments, in which case one can decide to split up the data into down to one variable per segment. In this case, although each variable is in a separate block, as all blocks are analyzed simultaneously, it will constitute a multivariate method.

In the second part of this presentation, ComDim will be used to determine the impact of the different factors on sample measurements or signal intensity variations. The effect of each of the factors and their interactions are compared to other sources of variations, either “noise” or uncontrolled factors of influence. This adaptation of ComDim, AComDim, is based on the same concept as ANOVA-PCA (also called APCA) and ANOVA-Simultaneous Components Analysis (ASCA). The experimental data matrix is decomposed into successive matrices containing the mean at each level for each factor or interaction. The residuals matrix obtained after this successive subtraction of the mean matrices is then added back to each matrix of means. In a second step of APCA, a PCA is applied to each matrix of means plus residuals. Groups formed by samples in the PC1–PC2 plane are examined in order to evaluate the significance level of each factor compared to noise.

With AComDim, the separate PCAs from the second step are replaced by a single simultaneous analysis of all matrices with ComDim, a multi-block analysis method. By examining the saliences obtained, it is possible to associate CCs to factors and to determine the importance of each factor compared to the residuals.

(15) **Classical Least Squares Methods for Target Detection in Hyperspectral Imaging**; Neal Gallagher[1]; [1]Eigenvector Research, Inc.

Principal components analysis (PCA) and inverse least squares methods are ubiquitous in chemometrics but alternative techniques such as classical least squares are experiencing a resurgence because the results are more interpretable and practitioners can take more control over their modeling. Classical least squares forms the foundation for generalized least squares (GLS) and extended least squares (ELS), provides a natural framework for de-cluttering (e.g., via weighting) and also allows for easy implementation of constraints on contributions (concentration) that are the objective of the analysis. A comparison between GLS-based target detection and whitened PCA (WPCA) is shown for target detection in hyperspectral imaging. The synergy between target detection and WPCA (that can be considered an anomaly detection approach) results in a sensitive “targeted anomaly detection” procedure that utilizes target relevant information for the analysis.

(16) **Homeopathic ICA: A Simple Approach to Expand the Use of Independent Component Analysis**; Willem Windig[1], Michael Keenan[2], Barry Wise[1]; [1]Eigenvector Reseach, Inc., [2]Wolcott, NY

Independent component analysis (ICA) is an increasingly popular method to resolve complex data sets, such as chemical image data, into a limited number of images of the constituents and their associated spectra. Unfortunately, the pre-requisite of statistical independence severely limits the application of ICA. In this paper we will show that, for a certain class of data, increasing the sparsity of a data set increases the independence of components, which enables the successful application of ICA. The sparsity can be increased by simply adding zeros to the data set or by applying a Haar-wavelet transform. Although this ICA application relies on data dominated by pure components, a certain amount of mixtures is tolerated. ICA will be explained using simple numerical examples and actual data sets obtained by energy dispersive X-ray

spectrometry (EDS) of a Cu-Ni diffusion couple, a braze interface and several other techniques, such as secondary ion mass spectrometry (SIMS) of a mixture and magnetic resonance imaging (MRI) data of a human brain.

(17) **Comparative Study of Classification Trees for the Authentication of Marijuana**; Peter Harrington[1], Xinyi Wang[2], Steve Baugh[1]; [1]Ohio University, [2]Cannaprint

Recently, various states in the US have legalized medical and recreational use of marijuana, Cannabis sativa. Determining the provenance of marijuana is an important legal problem. Marijuana grown legally may be carefully cultivated while bootleg marijuana may be contaminated or adulterated. Marijuana may have been illegally transported either into or out of state jurisdictions. Furthermore, bootleg marijuana may have been cultivated in raw sewage or treated with pesticides or herbicides. In addition, it is believed that the growing conditions may affect the quality and pharmacological effect of various cultivars. Marijuana from different cultivations may be marketed for different uses and to have specific medical benefits. Therefore, authentication of marijuana is a timely and important problem. Metabolic profiling that may be accomplished rapidly by spectroscopic methods has proven useful for the determination of the provenance and quality of various botanical products. However, chemometric models are required to discern the nuances among the spectral profiles.

For targeted analysis of spectral profiles, multivariate classifiers provide discriminatory models that may separate the spectra profiles into known classes. The advantage of using tree-based classifiers is that a divide and conquer approach is used to split the spectra into know classes at each branch of a tree. The tree structure provides an inductive logic in that general class divisions (i.e., large differences between classes found here) occur at the root of the tree and precise class divisions occur near the leaves of the tree (i.e., similar classes found here). In addition, tree classifiers are robust with respect to outlier samples or spectra.

The support vector machine-tree classifier compared favorably with a partial least squares discriminant analysis (PLS-DA) [1] and a fuzzy rule-building expert system (FuRES) [2] that is also a tree-based classifier. The evaluations using bootstrapped Latin partitions [3] compared the classification accuracies for marijuana extracts profiled by mass spectrometry (MS) and proton and COSY nuclear magnetic resonance (NMR) spectroscopy.

References:

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[3] P.B. Harrington, Trends in Analytical Chemistry, 25:11, 1112, 2006.

(18) **High Pressure Studies of Illicit Materials**; Iain Oswald[1], Oliver Sutcliffe[2], Niamh Nic Daeid[3]; [1]University of Strathclyde; [2]Manchester Metropolitan University; [3]University of Dundee

Polymorphism is the ability for a compound to crystallise in more than one three dimensional arrangement of molecules. It is a significant phenomenon that affects many organic materials from pharmaceuticals, pigments to energetic materials due to the fact that a number of physicochemical properties such as melting point, solubility and bioavailability can be altered. One subset of organic materials that have not been explored significantly is illicit materials. This paper presents our work investigating polymorphism in 'legal highs' and other illicit compounds under a range of thermodynamic regimes. Polymorphism of pharmaceutical products is strictly monitored by companies to ensure product quality and safety; this is not the case in clandestine laboratories. The premise behind this work was to explore whether polymorphism would exist under unregulated environments such as those found in clandestine laboratories potentially giving rise to fluctuations in the bioavailability from batch to batch of street samples. In addition to normal crystallisation methods we have explored the effects of pressure (>1000 atm.) taking into account that many of these compounds are found in tableted form.

(19) **Evaluation of Two Wavelengths, 785 and 1064 nm, for the Identification of New Psychoactive Substances using Handheld Raman Spectroscopy**; Amira Guirguis[1], Sarah Girotto[1], Benedetta Berti[1], Jacqueline Stair[1]; [1]University of Hertfordshire

INTRODUCTION New psychoactive substances (NPS) are emerging analogues to established drugs of abuse, yet they are not internationally controlled and pose public health risks. Fluorescing impurities in NPS add challenges to their identification using conventional Raman 785 nm laser. The aim of this study was to evaluate two laser wavelengths, 785 and 1064 nm for the identification of NPS Internet products using handheld Raman spectroscopy.

MATERIALS AND METHODS Two handheld Raman spectrometers, Xantus-1 and First Guard (Rigaku, USA), with laser wavelengths of 785 and 1064 nm, respectively, were employed for the identification of 60 NPS products. Spectra were collected for the reference standards of 10 NPS, eight adulterants and 12 cutting agents and stored in the on-board library. Standards were run as test samples to test the accuracy and selectivity of each instrument. Spectra of NPS products were obtained, through glass vials and Raman responses were measured using a percentage Hit Quality Index. Gas chromatography – mass spectrometry (GC-MS) was employed to confirm the identity of compounds present in the NPS Internet products.

RESULTS AND DISCUSSION Using the GC-MS confirmatory analysis, the content of 54 (90%) NPS products was identified, whereas, the content of 38 (63%) NPS matched to the product labels. Using the standard 785 nm laser (Xantus-1), only one (2%) NPS (i.e., dextromethorphan) was identified and matched to the label. Using the First Guard (1064 nm), the number of identified NPS products increased to 28 (47%). The First Guard identification of 28 NPS and four adulterants were confirmed with GC-MS, whilst using Xantus-1, the content of most products was not identified due to fluorescing impurities with 785 nm radiation. The present study has demonstrated that a longer laser wavelength (i.e., 1064 nm) reduced fluorescence and hence, identification of NPS street-like samples was improved over the conventional 785 nm wavelength.

CONCLUSIONS Raman spectroscopy employing a laser wavelength of 1064 nm has shown promise as a handheld technique for the identification of unknown new psychoactive substances products in-field. Current work is being undertaken to enhance the identification of these substances by improving spectral classifications.

(20) **Forensic Examinations to Determine Illicit Drugs Commonly Seized in the Philippines: From Evidence to Judgment**; Ronald Jefferson Narceda[1]; [1]Philippine Drug Enforcement Agency

Illicit drug industry is certainly a global concern and demonstrates a tremendous challenge to various drug law enforcement agencies. In recent decades, crime rates associated with drug abuse has been increasing and its adverse social impacts are very alarming. These many kinds of drug of abuse are derived from either natural products or chemical synthesis. The former include mainly opiates, cannabis and cocaine. The latter include various amphetamine type stimulants (ATS) such as methamphetamine and methylenedioxymethamphetamine (MDMA). In the Philippines, methamphetamine hydrochloride or shabu is the most common drug of abuse and accounts the majority of all locally reported drug law violations. Marijuana (Cannabis sativa) remains the second most used drug in the country as alternative drug choice to methamphetamine hydrochloride. Furthermore, a number of local seizures also comprise MDMA, cocaine and selected opiates and benzodiazepines. To mitigate illegal drug concerns in the Philippines, the government through the Philippine Drug Enforcement Agency (PDEA) strictly enforces a comprehensive dangerous drugs law (Republic Act 9165) in partnership with various drug enforcement groups in the country. Accordingly, all seized items are subjected to forensic analysis for drug identification to support arresting officers in their effort to apprehend drug offenders. Given that PDEA being the lead agency for anti-drug operations, PDEA laboratory service is well-equipped to extensively analyze different drug evidence seized by law enforcers. Noteworthily, since the Philippines is archipelagic in nature, PDEA forensic laboratories were decentralized strategically nationwide for fast tracking of results. This review summarizes various methods of drug analysis in the country using both traditional and modern methods, and also employing the use of analytical instruments such as FT-IR, RS, GC-FID and GC-MS. It further highlights the Filipino forensic chemists' vital role in providing valuable drug identification reports required to execute suitable charges for drug criminals. Finally, some problems regarding forensic examination in the Philippines are discussed for reference purposes.

(21) **Improved Identification Algorithms for Detection of Counterfeit Medicines by Raman Spectroscopy**; Latevi Lawson[1], Jason Rodriguez[1]; [1]FDA

Potential infiltration of counterfeit drug products—containing the wrong active pharmaceutical ingredient (API) or no API—into the bona fide drug supply poses a significant threat to consumers worldwide. Raman spectroscopy offers a rapid, non-destructive avenue to perform screening of a high volume of samples. Traditional qualitative Raman identification is typically done with spectral correlation methods that compare the spectrum of a reference sample to an unknown. This is readily done for pure materials but is quite challenging when dealing with drug products that contain different formulations of active and inactive ingredients. Typically, reliable identification of drug products using common spectral correlation algorithms can only be made if the specific product under study is present in the library of reference spectra, greatly limiting the scope of products that can be screened. In this presentation we introduce the concept of the Raman barcode for identification of drug products by comparing the known peaks in the API spectrum to the peaks present in the drug product. This method requires the transformation of the Raman spectrum for both API and drug product into a barcode representation by assigning zero intensity to every spectral frequency except the frequencies that correspond to Raman peaks. By comparing the percentage of nonzero overlap between the expected API barcode and drug product barcode, the presence of active ingredient can be confirmed. We report our results using the barcode method to confirm the presence of APIs in common antibiotic and antiviral drugs purchased domestically and internationally.

(22) **Drug Quality and Dissolution Testing at All Points in the Supply Chain: Integration of Scalable Technology in the Health System**; Muhammad Zaman[1], Nga Ho[1], Darash Desai[1]; [1]Boston University

Poor quality medicines, that account for about 30% of drug sales in developing nations, make up $75B out of a $962B global pharmaceutical market and cause nearly 700,000 preventable deaths every year. In Africa alone, 30M women living in malaria-endemic areas become pregnant each year. Malaria is an aetiologic factor in pregnancy complications and contributes to at least 10K maternal deaths and 200K newborn deaths annually. Pregnant women with malaria have a mortality rate close to 50%.Unfortunately 30-50% of antimalarials and other life saving drugs are substandard in sub-Saharan Africa alone. Thus, there is a real need to perform more rigorous testing with an easy-to-use, affordable, high-throughput approach to improve maternal healthoutcomes. Current tools, unfortunately, are too cumbersome, expensive or qualitative and hence ill-suited for testing drugs in resource limited settings.

We have developed a novel platform, PharmaChk, that is aimed to address problems associated with available technologies. Our system is designed to be affordable, user-friendly, robust, quantitative and is able to perform drug ingredient and dissolution testing. PharmaChk uses rapid luminescent and fluorescent assays to quantify drug quality. Our initial work has focused on anti-malarials and we are now extending our work to oxytocin. Our field trials in Ghana focusing on both tablet and injectable formulations showed excellent agreement (within 5%) with the gold standard method (HPLC) despite orders of magnitude cost difference in the two technologies. PharmaChk also outperformed the existing field standard (MiniLab), as PharmaChk, unlike MiniLab, is both quantitative and able to test injectables. Additionally, PharmaChk was able to test more than a hundred samples in a matter of a couple of days, something outside the scope of current field standards. We, along with various partners are now scaling the technology to test drug quality in both urban and rural areas, in both tablet and injectable forms.

Overall, our results from the field studies show high performance and substantial improvement over field based methods in precision, accuracy and ease of use and demonstrate our ability to substantially

(23) **LIBS Outside the Lab**; Christian Bohling[1], Jens-Uwe Günther[1], Angelika Feierabend[1], Andreas John[1]; [1]Secopta GmbH, Berlin

LIBS is a powerful tool for fast elemental analysis. The minimally destructive analytical technique allows precise contactless analysis of samples with different stoichiometric composition without sample preparation.

Nevertheless, a lot of basic conditions have to be considered in order to realize projects outside the lab. In typical industrial LIBS applications like raw material identification, recycling or industrial process control the ambient conditions are far away from being perfect for laser spectroscopic investigations. In contrast to other optical measurement methods, LIBS needs high intensities to generate plasma. Therefore, even marginal contaminations yield a fatal damage of the measurement system. In this talk examples of integrating LIBS sensors in polluted industrial environments are presented. The industry-suitable element analyzer FiberLIBS is used inline in the production process to identify aluminum and steel alloys. The device measures the alloy components with high precision at the moving material. The determined values are compared to the specifications and the result is reported to a master control system.

The big advantage of LIBS in industrial applications: No sample preparation is needed. Competing techniques, e.g. arc spectroscopy or XRF, need well prepared surfaces. The preparation is time and cost intensive. But: LIBS is a surface sensitive technique too. This means surface contamination always has an influence on the measurement result. However, any LIBS system has a nice feature, which might be used for reducing surface effects: laser ablation. Examples of how laser ablation can be used for fast inline sample preparation, even at high speed recycling applications at 3 m/s, are presented.

The newest modification of SECOPTAs MopaLIBS caused an increase of the penetration depth of laser pulses. Additional a synchronized second laser source is used for pre-ablation in order to increase the process speed.

(24) **Stimulated Emission and Lasing in a Laser-Induced Plasma**; Lev Nagli[1], Michael Gaft[1]; [1]Laser Distance Spectrometry Ltd

New type of laser is demonstrated where the active media is laser-induced plasma (LIP). Under appropriate resonant optical pumping of a pre-formed laser plasma plume, stimulated emission and lasing occur. They manifest themselves as emission of intense, polarized and collimated beams in the forward and backward directions in longitudinally pumping scheme. Stimulated emission (SE) and lasing effects that exist only during a pumping pulse are found in plasma of atoms with transitions probabilities higher then 10^7 s-1. Those are atoms from the 13th and 14th groups of the periodic table and also in Ca, Ti, Zr and Fe atoms. Some atoms such as Al and In in LIP possess a three-level lasing system. In Tl plasma, both three- and four-level laser systems are present. Other atoms also, exhibit three, four or quasi-four-level laser systems. Transverse pumping system is checked and the plasma plume lasing is found. Transverse pumping geometry has many advantages for developing higher power lasers compared to the longitudinal pumping. These lasers may be used as coherent light sources in a variety of optical applications. Intense, collimated backward SE in a LIP plume may strongly enhance the selectivity and sensitivity of inductively coupled plasma(ICP-OES) and laser-induced breakdown spectroscopy (LIBS) used for elemental analyses of different substances. Fig. 1 shows image of Fe plasma pumped at 252.3nm. Bright spots are lasing at 452.9nm.

Fig. 1 Image of Fe plasma pumped at 252.3nm. Bright spots are lasing at 452.9nm.

(25) **The Determination of Bioavailability Concentrations of Nutrients in Soils using Chemometric Analysis of LIBS Data**; Josette El Haddad[1], Aissa Harhira[1], Luc English[2], Gilles Clément[2], Charles Nault[2], Alain Blouin[1],Mohamad Sabsabi[1]; [1]National Research Council Canada - Energy, Mining and Environmentt; [2]LOGIAG Inc.

The analyses of the nutrients bioavailability concentration (phosphorus, calcium, aluminum, etc...), the pH and the percentage of organic matter are very important to evaluate soil and crops nutrient requirements. The current analysis (Mehlich 3) methods require time-consuming and expensive sample manipulations. The Laser Induced Breakdown Spectroscopy (LIBS) technique is a potential solution due to its fast analysis, accurate results and minimal laboratory sample manipulations. The ability to rapidly analyze a large number of samples will enhance the adoption of the technology within field management practices and nutrients distribution. The National Research Council Canada collaborated with LOGIAG to build a specific LIBS system for agricultural soil analysis. LIBS spectral analysis by chemometric methods allowed not only the quantification of nutrient concentrations but also the quantification of the pH value and the percentage of organic matter in soils. The results of different chemometric methods will be presented. It will be shown that LIBS analytical performances provide soil test results comparable to the standard method results. This was an important challenge as it will allow the LIBS analytical soil process to be accredited as a recognized and reliable analytical method.

(26) **LIBS Process Analyzer**; Francois Doucet[1], Lutfu Ozcan[1]; [1]ELEMISSION Inc.

Metallic and metalloid alloys play a key role in a very broad range of applications. While being relatively inexpensive, these materials possess mechanical properties which are very well adapted to several fields involving their use, such as structural engineering. However, even a small change in the concentration of an alloying element can have a large impact on the properties of these alloys. The importance of controlling the chemical composition of metal alloys has increased over the years to ensure the quality of final products (pipes, wires, plates, sheets, etc). LIBS (Laser-Induced Breakdown Spectroscopy) is an emerging analytical technique which allows the detection of most elements of the periodic table, with detection limits around parts per million by weight (ppm w/w). Its simplicity of implementation, speed and versatility make it a very attractive technique with great potential for applications requiring a real-time measurement. In this work, we investigated the real-time measurement on a variety of materials using a LIBS process analyzer. In addition, we will report the performance of such an instrument on real samples.

(27) **Laser Induced Breakdown Spectroscopy for Gold Analysis in Ore Samples**; Kheireddine Rifai[1,2], Marcel Laflamme[1], Marc Constantin[1], Mohamad Sabsabi[2], Alain Blouin[2], François Vidal[3],Paul Bouchard[2], Konstontinos Fytas[1]; [1]Université Laval; [2]National Research Council Canada - Energy, Mining and Environment; [3]IRNS - Energie matreriaux et telecommunication

Quebec has 17 mines producing approximately 1.5 billion dollars of gold annually, in addition to creating more than 4,500 direct jobs. However, the mining industry faces major challenges such as lower grade gold deposits and complexity of deposits with higher impurity levels. These factors increase exploration and production costs. XRF, which has been used successfully for a number of metals such as copper, zinc and nickel is inadequate for the desired gold ore application, which requires a detection limit of 50 to 100 times lower.

Our research activities are to evaluate the possibility of using the laser-induced breakdown spectroscopy (LIBS) technique to measure gold concentration directly on rock samples and in real time. In our preliminary activities we have successfully tested the feasibility of the technique on a variety of synthetic samples simulating different types of mineralogical matrices containing gold (quartz, pyrite, etc.). The standard LIBS technique allowed us to obtain a limit of detection of approximately 0,8 ppm on all types of samples, which is adequate for the desired application (~ 1 ppm). Different strategies have been tested in order to overcome matrix effects. In particular, (1) Normalization of the gold signal by the signal of adjacent continuum or by the sum of signals of Si line and Fe line, (2) preliminary classification of matrices using chemometrics calculations from laser plasma emission spectrum over a wide wavelength range. Finally, LIBS was used to map diamond drill cores in order to define an effective strategy for the analysis of rock samples in the field.

(28) **Organic and Inorganic Techniques and Strategies for Analysing Illegal Generic Medicines**; Neville Broad[1,2]; [1]Authenticate Limited (UK), [2]University of Kent (UK)

Raman and fourier-transform infra-red have been at the forefront of the rapid identification of suspect drugs in recent times. However, the use of NIR has been less used with in forensic circles. In this presentation the Author will discuss each of these techniques, which primarily have specificity for organic components of suspect matrixes, with consideration of their advantages and disadvantages.

It is essential that a second identification methodology is employed for the confirmation of a counterfeit drug. This technique should ideally be orthogonal to the first choice. The miniaturization of X-ray fluorescence (XRF) and laser induced breakdown spectroscopic (LIBS) instruments can possibly prove an ideal inorganic, i.e. orthogonal technique. Both of these technologies will also be discussed for, utilizing an experimental study of illegal generic, erectile dysfunction tablets.

(29) **Combating Adulterated Drug Products**; Connie Ruzicka[1], Kelly Park[1], Katherine Alejo[1]; [1]US Food and Drug Administration

The increased availability and use of dietary supplements among consumers has been accompanied by an increased frequency of adulteration of these products with synthetic pharmaceuticals. The presence of these adulterated products in the marketplace is a worldwide problem and their consumption poses health risks to consumers. In response, the FDA has developed a program to evaluate rapid screening tools to be used in assessing the quality and safety of imported dietary supplements. One of the techniques that the Division of Pharmaceutical Analysis (DPA) has developed for the program is ion mobility spectrometry (IMS), a rapid, reliable screening tool that requires minimal sample preparation and produces results in less than a minute. The FDA is currently exploring the use of IMS instruments at US ports of entry to test products labeled as dietary supplements that are suspected of containing undeclared Active Pharmaceutical Ingredients (APIs). This presentation will describe the development of IMS screening methods for detecting undeclared weight loss and male enhancement APIs in products labeled as dietary supplements as well as discuss the efforts used to deploy the instruments in the field. Lastly, the presentation will highlight the results obtained from assessments conducted at two international mail facilities located in the United States.

(30) **Challenges of Counterfeit Drug Detection in the Field**; Pauline Leary[1], John Reffner[2]; [1]Smiths Detection, [2]John Jay College of Criminal Justice

The accurate identification of counterfeit drugs and determination of the source of these goods is an important capability. These drugs have been shown to cause sickness and death and are a threat to public health and safety. In some cases, threats to health and safety are short term and result in acute poisonings or lack of effective treatment; in others they are long term and challenge the development of future medications and global health. Counterfeiting groups include organized-crime and terrorist organizations. These groups present significant challenges to law enforcement. It is necessary that these criminals are identified and brought to justice.

Lab-based methods for the identification and sourcing of these goods include, but are not limited to, methods based upon microscopy, spectroscopy, chromatography and spectrometry. Depending upon the type of drug product, as well as the provenance of the sample, quick and easy differentiation between a counterfeit and authentic sample may be possible. In some instances, establishment of associative links amongst a set of samples or across sample sets may be made, and sourcing is possible. A challenge to the use of these lab-based instruments is the time required to process samples, send them to the lab for analysis, and receive results. Methods that provide equivalent information but may be applied in the field are, therefore, desirable.

Field analysis of counterfeit drugs where actionable, on-scene information is required is an important emerging field. Many different methods and technologies capable of performing this type of field analyses are available including portable infrared, Raman, and gas-chromatography/mass spectrometry systems. These methods are valuable and provide critical information that can be used to detect, identify, and even authenticate samples on scene quickly and accurately.

A review of data collected from counterfeit-drug samples, the value of these analyses, and challenges experienced in field deployment will be discussed. A workflow for the successful use in the field will be presented.

(31) **The Creation of Paper-based Devices to Detect Select Pharmaceuticals**; Toni Barstis[1]; [1]Saint Mary

The World Health Organization defines a counterfeit medicine as a pharmaceutical whose active ingredient is either under-dosed or completely absent.1 The business of pharmaceutical counterfeiting is a major issue worldwide. Not only is the worldwide counterfeit business lucrative, selling more than 200 billion dollars in counterfeit pharmaceuticals in the last few years,2,3 but also harmful to vulnerable communities, such as those with limited analytical resources. To address this global need, we have developed Paper Analytical Devices (PADs), which involve simple colorimetric chemical tests on a paper-based device, to provide an inexpensive, rapid method of screening suspect pharmaceuticals.

This presentation will focus on the PADs research process, specifically on the (1) creation and optimization of PADs to screen for select pharmaceuticals, (2) construction of a computer-aided analysis program that objectively analyzes the image of the completed PAD, and (3) development of instrumental methods of analysis to verify the results of the PADs. Different colorimetric tests have been adapted to paper: some colorimetric tests detect the active pharmaceutical ingredient (API) found in genuine pills while others detect select ingredients (e.g., substituted APIs, starch, chalk) found in counterfeit pills. Users simply scrape the suspect pill across the PAD, dip the PAD in water, and take a picture of the completed PAD. An automated computer-aided analysis program is being constructed in MATLAB to analyze these images and inform the user whether the pill is genuine or counterfeit. Altogether, each PAD has been specially designed to optimize the colorimetric response and to allow the program to locate each lane and account for select imaging issues, such as poor PAD position. Several PADs have been field-tested to assess the reliability and reproducibility of the chemistry, and progress is being made towards finding instrumental methods using UPLC-UV and UPLC-MS analyses to quantify the API in select pharmaceuticals. These instrumental methods are being used to validate results obtained from field tests.

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(32) **Rapid Identification of Counterfeit Medicines from the World Market using Dual Laser Handheld Raman Spectroscopy**; Sulaf Assi[1]; [1]Bournemouth University

Pharmaceutical counterfeiting represents a global problem which affects 10% of the world market. According to the World Health Organisation, counterfeit medicines could result in treatment ineffectiveness at their easiest and lethal effects at their worst. Counterfeit medicines could be encountered anywhere across the wholesale supply chain including manufacturers, wholesalers, pharmacies, hospitals or even patients' homes. This stimulates the need to develop rapid and non-destructive technique to identify counterfeit medicines wherever they were encountered. Handheld Raman spectroscopy fulfills this advantage thus it saves the time and money from importing the sample to the laboratory.

Therefore, this work aims at identifying counterfeit medicines from the world market using dual laser handheld Raman spectroscopy.

A total of 200 branded and generic medicines were purchased worldwide and comprised diverse pharmacological classes, including: Analgesics/anti-inflammatory, antibiotics, antidiabetics, cardiovascular medicines, medicines used for respiratory diseases, sexual stimulants and weight loss medicines. Medicines were measured 'as received' using the Bruker BRAVO handheld Raman spectrometer equipped with dual laser. Twenty spectra were taken per medicine product. Spectral analysis was made using the instrument inbuilt algorithm (probability-based) and offline using the OPUS software version 7.7 (correlation-based). In both cases, spectra of test medicines were compared to those of authentic reference products.

The instrument in-built algorithm compared the probability of match of the test medicine product to the reference library. A p-value above 0.05 showed that the test spectrum matched the authentic spectrum and thus was authentic. The offline algorithm compared the Hit Quality Index (HQI) which compared the correlation between the test and the reference spectrum. An HQI value above 95% indicated that the test spectrum matched the library spectrum and was authentic. In this respect, the probability showed more accurate identification than the HQI algorithm (less type I and type II errors). However, this depended on the dose of the active pharmaceutical ingredient (API) in a medicine product. Thus, mismatches were encountered using both the probability and correlation algorithms in medicines with low doses of APIs.

In conclusion, handheld Raman spectroscopy offered a simple, rapid and non-destructive technique for the identification of counterfeit medicines from the world market.

(33) **Point-of-Care Tumor Detection with Biomarker-Targeted SERS Nanoparticles Topically Applied on Fresh Tissues**; Jonathan Liu[1]; [1]University of Washington

There is a need for intraoperative technologies to comprehensively image surgically excised tissues and to determine if there is residual tumor at the surgical margins. A comprehensive imaging technique that is sensitive and specific for tumor detection would significantly reduce the costs and inconvenience associated with multiple surgeries and could also improve patient outcomes by reducing the sampling errors associated with conventional pathology and the risks associated with re-excision surgeries. We are developing a wide-area imaging strategy to enable the quantitative molecular phenotyping (QMP) of freshly excised tissues during surgery. This technique utilizes surface-enhanced Raman scattering (SERS) nanoparticles (NPs) that are targeted to various disease biomarkers and which are topically applied on surgical specimens to provide molecular-imaging contrast. SERS NPs are attractive due to their low toxicity, high brightness, and their potential for sensitive and multiplexed biomarker detection with laser illumination at a single wavelength. We demonstrate that the QMP technology is able to visualize the expression of a panel of protein biomarkers at the surfaces of fresh tissues following a rapid topical staining (10-min) and rinse removal (15-s) protocol that is appropriate for intraoperative use. Furthermore, we demonstrate a ratiometric imaging method that quantifies the specific vs. nonspecific binding of targeted SERS NPs and eliminates the ambiguities due to nonspecific accumulation of the NP contrast agents. Experiments with cell lines, tumor xenografts in mice, as well as fresh human tissues have been performed to validate the ability of this QMP imaging method to quantify the expression levels of cell-surface biomarkers in receptor-positive tumors with strong agreement to flow cytometry and immunohistochemistry.

(34) **New Reporters for Biological SERS Measurements**; Colin Campbell[1], Patrick Thomson[1], Kate Fisher[1]; [1]University of Edinburgh

Redox potential is of key importance in the control and regulation of cellular function and lifecycle, and previous approaches to measuring the biological redox potential non-invasively in real time are limited to narrow potential ranges. We have developed an approach to measuring redox potential in live cells that relies on Surface Enhanced Raman Spectroscopy. Our approach uses rationally-designed nanosensors that are responsive to potential changes across a pathophysiological range and allows real-time measurements of cells undergoing hypoxic and oxidative stresses.

(35) **High Throughput Optofluidic Surface Enhanced Raman Spectroscopy (SERS) Interrogation: Proof of Concept via Lectin Detection of Cancerous Cells**; Marjorie Willner[1], Jonathan Simpson[2], Kay McMillan[2], Michele Zagnoni[2], Duncan Graham[2], Peter Vikesland[1]; [1]Virginia Polytechnic Institute and State University; [2]University of Strathclyde

High throughput surface enhanced Raman spectroscopy (SERS) is a much needed advance to better realize the full potential of the detailed fingerprint information available with Raman spectroscopy. At present SERS, as an analytical technique, is often plagued by a lack of quantification and reproducibility due to the length of time required for individual sample analysis. In this effort we discuss the development of an optofluidic device that combines the high throughput capability of microfluidics with SERS detection. A two-phase microfluidic based platform with droplet storage allows for the rapid screening of over one hundred individual prostate cells. Specifically, the N-acetyl neuraminic (sialic) acid residue overexpressed on cancerous prostate cells (PC3), compared to non-cancerous cells (PNT2), is targeted with carbohydrate (lectin) functionalized nanoparticles. In-house algorithms were developed to automatically process SERS maps and rapidly distinguish cancerous PC3 cells from both non-cancerous PNT2A cells and empty droplets. Furthermore, the computer vision methods employed are scalable. Herein, we demonstrate the novel use of labeled nanoparticles for SERS detection in a high throughput segmented flow optofluidic device.

(36) **Ultrasensitive Detection with SERS and SEHRS**; Jon Camden[1]; [1]University of Notre Dame

SERS is a well established method for ultrasensitive detection. In this talk we describe new SERS-based assays for the environmental pollutant hydrazine and nuclear forensic targets. In addition, we explore the advantages and sensitivity of using nonlinear scattering processes, such as hyper-Raman, for surface-enhanced detection.

(37) **Surface-enhanced Raman Scattering (SERS) Spectroscopy for Intracellular Chemical Imaging and Protein Analysis**; Katsumasa Fujita[1]; [1]Osaka University

Surface-enhanced Raman scattering has been attracting attention of researchers on the wide range of the research from the basic study of the enhancement mechanism to the application in chemical analysis. Combined with a metallic nanoparticle or tip, SERS can map molecular distributions in a sample with a high spatial resolution. We tried to apply SERS in detection of intracellular molecules with high temporal and spatial resolution. Gold nanoparticles introduced in a living cell exhibited the strong enhancement of Raman scattering from intracellular molecules and allowed us to obtain a SERS image in 2 sec by using slit-scanning Raman microscopy. With the combination of dark-field imaging of nanoparticles and laser tracking for SERS excitation, we further increased the temporal resolution to 50 msec with the position detection accuracy of 65 nm. The developed system allowed us to trace the motion of a nanoparticle under intracellular transportation and the simultaneous detection of SERS provided information of endogenous molecules on the transportation pathway. We also developed a new technique for the analysis of small molecule binding sites in proteins. By tagging small molecules with alkyne, proteins binding with the tagged molecules can be identified by detecting the Raman peak of alkyne at the silent region. We have combined the alkyne Raman detection with chromatography and successfully extracted small molecule binding peptides from the protein lysates.

(38) **Raman Optical Activity of Amide and Disulfide Groups in Peptides and Model Systems**; Vladimir Baumruk[1], Marketa Pazderkova[1, 2], Vaclav Profant[1], Lucie Bednarova[2], Petr Malon[1]; [1]Charles University in Prague, Faculty of Mathematics and Physics; [2]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences

Our aim is to utilize ROA to get unique information on peptide/protein conformation, which is otherwise difficult or even impossible to obtain. We have focused on investigation of amide and disulfide groups. Utilizing tailor-made model structures (dilactams with two interacting non-planar amide groups), special model peptides and even biologically active molecules (neurohypophyseal hormones and their agonistic and antagonistic analogs, antimicrobial peptides) we have traced the specific spectral manifestation of disulfides and non-planar amides. The experimental ROA results were supplemented by the data obtained by complementary chiroptical methods – electronic (including vacuum UV) and vibrational circular dichroism. When used in a concerted fashion, these techniques provide complex information on peptide/protein secondary structure. Where possible, the experimental chiroptical data were compared to advanced ab initio calculations.

(39) **Vibrational Chiroptical Spectroscopy in Natural Product Chemistry: Have we Achieved Enough?**; Joao Marcos Batista Junior[1]; [1]Federal University of Sao Carlos - UFSCar

Vibrational optical activity (VOA), which consists of vibrational circular dichroism (VCD) and Raman optical activity (ROA), is no longer a curious novelty in the field of molecular spectroscopy. The development of commercial instrumentation and reliable *ab initio* calculations for predicting theoretical spectra has made the use of these chiroptical methods a routine in many laboratories, both in academia and pharmaceutical industry, for stereochemical analysis of chiral small molecules as well as macromolecules. VOA presents some advantages over other methods available to determine three-dimensional structure of chiral molecules. VCD and ROA can be measured virtually for any molecule, directly in solution, without the requirement of single crystals, chiral auxiliaries, derivatizations, UV-Vis chromophores, or simulations of excited-state wavefunctions. In addition, as these two spectroscopic methods represent global molecular properties, all degrees of freedom of a molecule can be investigated, at least in theory. Nevertheless, at the same time, it becomes more difficult to extract stereochemical information from VOA spectra, which increases the dependence on the accuracy of the calculations. VCD and ROA also require larger amounts of sample and longer acquisition times than do optical rotation and electronic circular dichroism (ECD), for example. Since the concept of chirality is ubiquitous in nature and the determination of the absolute configuration and conformations of chiral molecules is of paramount importance for natural product chemistry, the application of VOA to this field of research has faced a tremendous increase over the last fifteen years. However, there is still much room for improvement. In this talk, I will present a critical overview of the evolution in the application of vibrational chiroptical spectroscopy to natural product chemistry over the last decade. The main difficulties involved in acquiring quality VOA spectra of natural products (both experimental and calculated) as well as future perspectives on the use of VCD/ROA will also be provided.

(40) **Insights into the Vibrational Nature of Carbohydrates from Raman Optical Activity**; Shaun Thomas Mutter[1]; [1]University of Manchester / Manchester Institute of Biotechnology

Raman optical activity (ROA) is a powerful analytical technique with high sensitivity to stereochemistry that makes it ideally suited to the study of carbohydrates, an important class of biomolecules with poorly understood structure-function relationships. Despite the potential of ROA for this application, this area is still relatively unexplored and compared to other biomolecules little is known about the vibrational nature underpinning observed spectra.

To explore this vibrational nature a combined molecular dynamics (MD) and quantum chemical approach has been used to calculate the ROA spectra of several monosaccharides. Full MD simulations have been run, using carbohydrate specific force fields, to incorporate hydration effects and allow detailed conformational analysis. These MD runs provide ideal starting points for quantum mechanics / molecular mechanics (QM/MM) ROA calculations that allow the carbohydrate to be modelled at DFT levels of theory and also allow for the inclusion of explicit water molecules at MM levels of theory. Excellent results are reported for this approach, when compared to experimentally obtained spectra, for several monosaccharides, including β-D-xylose, D-glucuronic acid and N-acetyl-D-glucosamine.

Inclusion of conformational data and explicit solvent molecules in the calculation of carbohydrate ROA spectra leads to significant improvements over gas phase and implicitly solvated calculations and can lead to insights into otherwise unobtainable data, including assignment of observed peaks. New Raman and ROA assignments for several peaks of monosaccharide spectra are presented, with a particular emphasis on features recurrent across multiple molecules as well as vibrational modes identified to show particular importance to the solution phase conformationally dynamic nature of carbohydrates.

(41) **Studying the Distribution of Deep Raman Spectroscopy Signals using Liquid Tissue Phantoms with Varying Optical Properties**; Martha Vardaki[1], Benjami Gardner[1], Nicholas Stone[1], Pavel Matousek[2]; [1]School of Physics, University of Exeter; [2]Central Laser Facility, STFC Rutherford Appleton Laboratory

Cancer is nowadays one of the most common causes of death worldwide with more than 8 million victims only in 2012. The aim of current diagnostic methods is the accurate diagnosis of the disease at an early stage. However, these common tests (e.g. biopsy) are invasive and unpleasant for the patient.

Methods based on vibrational spectroscopy (Raman, IR) have the advantage of being non-invasive and chemically specific, while have already been demonstrated as a potential tool for disease diagnosis.

Apart from the conventional Raman spectroscopy, recent advances have established the feasibility of using deep Raman sub-surface techniques to probe the disease specific molecular signals in depths well above a few hundred micrometers (up to 2.7 cm). To date this has been mainly explored in the breast cancer diagnosis in order to identify malignant calcifications and in bone disease diagnosis.

However, in these studies, the measured Raman scattering has only been explored in terms of signal intensity versus tissue thickness. A major unexplored aspect of deep Raman is the spatial distribution of the origin of Raman scattering throughout a sample volume and its dependence on optical properties of the sample.

In this study we employed liquid tissue phantoms, consisting of a scattering agent (Intralipid), an absorption agent (Indian ink) and a synthesized calcification powder (HAP) similar to that found in cancerous tissues, to simulate human tissues in both volume and optical properties. Measuring the tissue phantoms in a transmission deep Raman setup, we studied experimentally the photon migration of the Raman as a function of optical properties of the medium (surrounding tissue) and the location of calcifications within the phantom.

Our results, simulate the potential *in vivo* measurement conditions for human prostate and breast samples and assess the feasibility of such measurements. The goal of the study was to inform the development of future non-invasive cancer screening applications *in vivo* and provide insight into light propagation and Raman scattering distribution in deep Raman measurements, exploring also the effect of the variation of laser and Raman photon relative absorbance in the phantoms.

(42) **Standoff UV Raman Spectroscopy: Spatial Heterodyne Raman Spectrometer for Planetary Applications**; Nirmal Lamsal[1], S. Michael Angel[1], Shiv K. Sharma[2], Tayro Acosta-Maeda[2]; [1]University of South Carolina; [2]University of Hawaii

A goal of our research is to develop improved Raman spectrometers for planetary exploration (e.g. small with high sensitivity). This fits the stated needs of NASA for improved technologies for chemical measurements on future space missions. For planetary and other applications in extreme environments, we developed the spatial heterodyne Raman spectrometer (SHRS). The design of the SHRS allows very high resolution Raman measurements to be made in an exceptionally small package, even in the deep UV. In addition, the SHRS has a very wide field of view allowing large sample areas to be measured. In this paper, we describe for the first time, standoff UV SHRS measurements and we show how the SHRS is uniquely suited to measure photosensitive materials. We also show the first fluorescence-free Raman spectra using a deep-UV SHRS. And, we demonstrate deep-UV SHRS Raman measurements in bright light conditions without vibration isolation.

(43) **Flipping the Analytical Chemistry Classroom**; Christopher Harrison[1]; [1]San Diego State University

With growing class sizes and an ever changing student, the traditional classroom lecture may not be the most effective means of aiding our students in learning complex materials, such as analytical chemistry. One significant drawback of the traditional lecture based approach to education is the almost exclusively one-way flow of information that occurs, particularly as class sizes increase. Consequently, rather than addressing individual student's challenges, capabilities, and potential we often teach to the middle, hoping that the weakest don't fall too far behind and that the brightest aren't too bored.

A remedy for this may be the flipped classroom. This approach takes the lecture out of the classroom and into a digital video. This transformation opens the classroom time up to new possibilities where active learning can be maximized. This talk will delve into Professor Harrison's approach to creating flipped classroom videos, how students are engaged in the classroom, and the general outcomes of these experiences (including some lessons on what not to do) through three semesters of teaching analytical chemistry as a flipped classroom.

(44) **Arduino Powered Instrument Design and Construction in Undergraduate Analytical Chemistry Courses**; Celeste Morris[1]; [1]Northern Kentucky University

Construction of instrumentation based on Arduinos and microprocessors are ideal for analytical courses to enhance student understanding of signal transduction and instrument design for chemical analysis. In analytical courses, students built a spectrophotometer with a motorized diffraction grating and data acquisition controlled by an arduino. Students also constructed arduino-powered instruments in a summer workshop which covered analog and digital circuitry, Arduino and microprocessor technology. Students then applied these technologies for design and construction of basic scientific instrumentation for chemical analysis including pH probes, temperature sensors and the StrØmlingo DIY atomic force microscope. Our experience has shown that instrument construction provides students with a unique opportunity to explore signal transduction and principles of chemical analysis.

(45) **Multiperspective Views in Teaching Laboratory Techniques**; Kevin Davies[1]; [1]Florida Gulf Coast University

In laboratory courses, we strive to teach students high-quality lab technique, and the essentials for the correct use and interpretation of hardware. Unfortunately, while teaching technique, the students are often looking in the wrong place - or are looking in only one place when they need to be aware of what is happening in two or more places. (e.g. during titration, students may potentially need to simultaneously monitor the burette readings, manipulate the stopcock, and swirl the analyte by hand.) This talk will describe the use of multiple simultaneous perspectives (first person direct view, third person over-the-shoulder, etc.) to allow students to watch these action simultaneously, and will detail the design and development of similar videos.

(46) **Social Media in the Blended Classroom**; Kate Hayden[1]; [1]Birmingham-Southern College, Birmingham Al

As scientists and educators, we recognize that not only are the demographics and needs of our classroom rapidly changing, but the market demands of scientists for less traditional career paths are changing as well. Our students now require a skill set that enables them to not only master course content and knowledge but also allows them to become critically thinking problem solvers. In order to address the ever changing needs of all our students, faculty and institutions are looking for innovative ways to decrease cost while increasing access and relevance of classroom experiences to future careers. At Birmingham-Southern College, we have found that utilization of current technology, such as social media, and other blending learning techniques has allowed us to further customize student learning experiences in ways that are relevant and impactful. These techniques also allow us to create a network of mentorship and peer to peer learning that can extend beyond the classroom and campus to more fully maximize student engagement. In my undergraduate Biochemistry course at BSC, I utilize a flipped-classroom model in

which a student's first encounter with course content is outside of the class via video lectures and reading assignments. Through the flipped model I can now use our face to face meetings for group inquiry based learning, case studies and discussions in hopes that I am providing more opportunities for my students to gain critical thinking and problem solving skills while mastering the material at a deeper level. To facilitate the flipped model and to encourage student engagement outside of class I have employed Facebook. Through Facebook I am able to manage my course, provide opportunities for online discussions, and create an online learning community for peer networking and online study groups. Overall, the students reportedly found Facebook to be helpful for organizing and disseminating course content, enjoyed Facebook's ease of use and real time applications for outside of class discussions and reported feeling more engaged with the real life applications of course content because of the online journal club facilitated through Facebook.

(47) **Perspectives from the Flip: The Active-Learning Experience in Analytical Chemistry**; Jared Baker[1]; [1]Elmira College

The inverted, or flipped, classroom is a pedagogical technique that has increasingly been recognized for its efficiency and effectiveness. Consequently, it has begun to be applied in a growing number of primary, secondary and higher education chemistry classrooms. There are a variety tools and manners in which to employ the flipped classroom, however, all emphasize an active-learning experience. One way to ensure that class-time is free for active-learning is to deliver the content via lecture videos that are watched by the student prior to class. With a strong focus on reasoning and quantitative skills, Analytical Chemistry is a course where the inverted model is a natural fit. Not only does the class at Elmira College focus on an active-learning experience, but it also relies heavily on a variety of technological tools in order to enhance the learning experience for the student both in and out of the classroom. This talk will discuss a number of techniques used in the inverted Analytical Chemistry classroom, including quick response codes, interactive writing surfaces, cell-phone polling and video production techniques, and how they serve to enhance the analytical chemistry classroom. Perspectives on the first-time execution and refinement of the course during three iterations will be discussed as well as the assessment of student perception and performance.

(48) **General Remarks**; Teizo Kitagawa[1]; [1]University of Hyogo

General remarks to open the session on Advanced Techniques for Infrared Spectroscopy on Structure-Function Relations of Proteins.

(49) **Coupling Mechanism in the Reaction of Cytochrome C Oxidase Revealed by Newly Developed Time-Resolved IR Measurements**; Satoru Nakashima[1]; [1]Graduate School of Life Science, University of Hyogo

Cytochrome *c* oxidase (CcO) is a terminal enzyme of the respiratory chain and catalyzes the oxygen reduction reaction that is coupled with the proton pumping across the membrane. The coupling mechanism of oxygen reduction reaction and proton pumping are still unrevealed. To observe such proton pumping related protein dynamics, IR spectroscopy can give unique information. Thus time-resolved IR (TRIR) measurement during the reaction is essential to elucidate this coupling mechanism. However, strong water absorption ordinarily obstructs IR measurements of proteins in buffer solution. We have recently succeeded to construct a new TRIR system, which enables to pursue the protein reactions in aqueous solution under physiological conditions. This system consists of femto second laser as a brilliant light source and dispersion type spectrometer combined with intelligent algorism. By using this system and other time-resolved spectroscopic method, CO photolysis of CcO has been studied in detailed. From the observed results, the ligand attached to CuB site just after the photodissociation, and escaped toward bulk solution within 2 μsec. Accompanied with this ligand behavior, the iron-His bond became weak, and α-helix structure along proton pump pathway, changed its structure to close the gate of the water channel. These linkages of protein parts are the 'information relay system' of this enzyme and they facilitate the proton pumping during the reaction. In the next step, we have been developing a new flow system aimed at measuring TRIR spectra on the oxygen reduction reaction of CcO in aqueous solution. Oxygen, which is necessary for the reaction, is supplied by the inserted artificial oxygen lung. Starting this reaction is controlled by CO photolysis of CO-bound CcO. To measure the IR spectra in aqueous solution, the light path of the flow cell must be made extremely thin (50 μm). To evaluate the system, time-resolved visible absorption spectra were observed, and proper functioning of the system was confirmed. Especially, a new intermediate where oxygen binds to CuB was found at the initial stage of the reaction by this flow cell system.

(50) **Infrared Spectroscopic Study on the Structure and Dynamics of Sodium Pump Rhodopsin**; Keiichi Inoue[1,2]; [1]Nagoya Institute of Technology; [2]PRESTO, JST

Microbial rhodopsin is photoreceptive transmembrane protein mainly found in the genomes of microorganisms such as eubacteria, archaea and unicellular eukaryotes. Most ubiquitous microbial rhodopsin is outward light-driven H^+ pumps. They convert light-energy to H^+ gradient, the motive force for ATP-synthesis and various H^+-coupled transports. Inward Cl^- pumps are also found in a part of eubacteria and archaea. In 2013, we discovered a novel class of light-driven pump, outward sodium (Na^+) pump, *Krokinobacter* rhodopsin 2 (KR2), from a marine eubacterium, *Krokinobacter eikastus*. While Li^+ is also transported by KR2, it pumps H^+ in the presence of only larger cations K^+, Rb^+ and Cs^+. The chromophore of KR2 is all-*trans* retinal bound to the protein in the seventh-transmembrane helix (helix-G). Upon light illumination, it isomerizes to 13-*cis* form, and photocyclic reaction including four photo-intermediates (K, L, M and O) is triggered. By time-resolved transient absorption spectroscopy, we have revealed that an H^+-transfer to avoid electric repulsion between Na^+ and protonated retinal Schiff-base and an Na^+-uptake from the cytoplasmic side occurs upon K-to-L/M and L/M-to-O processes, respectively. However, the detailed structural insights about each state were not obtained in these studies. Here, we report our recent results of Fourier-transform infrared spectroscopy (FTIR) for KR2 which provide various structural information about peptide backbone, retinal chromophore, internal water, hydrogen bonding network and so on. By low-temperature FTIR spectroscopy at 77 K, light-induced difference FTIR spectra between the first K-intermediate and dark state were obtained. It showed that the retinal Schiff-base of KR2 is more strongly hydrogen bonded in the dark state compared with a typical H^+ pumping rhodopsin, bacteriorhodopsin (BR). Then, upon *trans*-*cis* isomerization after light activation, the distortion of chromophore mainly occurs in the part near to Schiff-base. Furthermore, the presence a larger number of water molecules in the protein compared to other microbial rhodopsins was observed, and one of them was revealed to be strongly hydrogen-bonded showing a lower O-D stretch at 2333 cm^{-1}. In the presentation, the results about the other intermediates and mutants of KR2 will be explained.

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(51) **Infrared Studies of the Photosynthetic Oxygen Evolving Complex**; Bridgette Barry[1], Udita Brahmachari[1], Zhanjun Gup[1]; [1]Georgia Institute of Technology

While it is known that some proteins employ internal water molecules to catalyze reactions, there are relatively few methods to study the role of water in enzymology. The position and role of water is dynamic and time resolved techniques, such as infrared spectroscopy, are necessary. In

oxygenic photosynthesis, water is the substrate of the enzyme, photosystem II (PSII). PSII converts water to molecular oxygen through four successive, photo-driven oxidation events at a Mn_4CaO_5 cluster. This reaction is essential for the maintenance of aerobic life on earth. A tyrosyl radical, YZ, is essential in photosynthetic water oxidation and transfers oxidizing equivalents from an oxidized, primary chlorophyll donor, to the metal center. From X-ray crystal structures, the metal ions of the catalytic site and YZ are predicted to be extensively hydrogen bonded in an internal water network that also involves amide carbonyl groups and amino acid side chains. This hydrogen-bonded network is predicted to link YZ with the lumenal surface of the thylakoid membrane; the pathway includes an extrinsic subunit known as PsbO. Such an extensive network may be essential in facilitating proton release from substrate water and may play other roles in catalysis. To test the role of the water network, reaction induced FT-IR spectroscopy was employed. PSII was prepared in selected S states by laser flash illumination; these S states correspond to steps along the catalytic cycle to produce oxygen from water. This technique shows that the network is dynamic. In addition, reaction induced FT-IR data are consistent with the conclusion that the water network can be transiently protonated and deprotonated during the catalytic cycle. Taken together, these spectroscopic results support the presence of a catalytically functional, water network, extending from YZ to the lumenal surface of PSII.

(52) **Novel Time-Resolved IR Spectroscopies to Elucidate The Mechanism of Channelrhodopsin**; Joachim Heberle[1]; [1]FU Berlin

The discovery of the light-gated ion channel channelrhodopsin (ChR) set the stage for the novel field of optogenetics where cellular processes are controlled by light (1). Channelrhodopsin has revolutionized neurophysiological experimentation where neurons are controlled with optogenetics for fast, specific excitation or inhibition within systems as complex as freely moving mammals.

Despite the fact that the crystallographic structure of a ChR chimeric construct was solved, the underlying molecular mechanism of light-induced cation permeation remains largely elusive. We have traced the structural changes of ChR2 by time-resolved IR spectroscopy, complemented by electrophysiological measurements (3-6). The vibrational changes were resolved across the entire chemical time range (10^{-14}-10^{1} s) including the open states of the channel. Analysis of the amide I vibrations suggests a transient increase in hydration of transmembrane α-helices which tally the onset of cation permeation.

To address structural changes of the channel, ChR2 was subjected to pulsed electron double resonance (pELDOR) spectroscopy (7). Comparison of spin-spin distances in the dark state and after illumination reflect conformational changes in the conductive state involving helices B and F.

Structural changes in proteins are related to proton transfer reactions. Transient protonation and deprotonation of specific amino acid side chains were monitored. It is demonstrated that reprotonation of aspartic acid 156 is rate-limiting to the closure of the cation channel.

Based on the opportunity to trigger ion gating by light, we believe that ChR will become a model system for detailed biophysical studies on the mechanism of ion channels with utmost temporal resolution and spatial sensitivity.

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(53) **Finding Needles in Haystacks: Scanning Tunneling Microscopy Reveals the Highly Site-Specific Reactivity of TiO2 surfaces**; Melissa Hines[1]; [1]Cornell University

Nanocatalytic titanium dioxide can be produced in a wide variety of faceted shapes and polymorphs, each with different reactivity. This faceting is a macroscopic manifestation of highly site-specific surface reactions. We show that these site-specific reactions literally write a record of their chemical reactivity in the morphology of the surface — a record that can be quantified with scanning tunneling microscopy (STM). Seemingly paradoxically, the sites targeted by these highly site-specific reactions are extremely rare. The most interesting site — the site where the reaction occurs most rapidly and most selectively — is the hardest one to find. This highly reactive site, the key to the reaction, is the needle in the haystack, often occurring in densities below 1% of a monolayer and thus invisible to surface spectroscopies. Nevertheless, these rare reactions enable an exquisite degree of atomic-scale control if only we can learn to harness them.

The peroxo ligand is one of the most promising structure-directing agents in TiO_2 synthesis; however, its role in growth and polymorph control is not well understood. Using a combination of experiments and kinetic Monte Carlo simulations, we show that base-catalyzed peroxide reactions target very particular sites on the rutile (110) surface — sites that are present in concentrations of less than 1% of a monolayer — producing highly homogeneous surfaces characterized by atomically flat terraces and straight atomic-height steps. This reaction produces surface steps with a different orientation, a different structure, and a different reactivity from those prepared using standard surface science techniques, which emphasizes the importance of studying reactivity in relevant environments. The observed reactivity is explained by a simple, site-specific model that is based on metal oxo coordination chemistry.

(54) **Atomic Scale Spectrometry and Structure - Correlating Atom Probe Tomography and Transmission Electron Microscopy**; David Diercks[1], Brian Gorman[1]; [1]Colorado School of Mines

Individually, atom probe tomography (APT) and transmission electron microscopy (TEM) have been used to analyze materials down to the atomic scale. These have provided insights into materials' atomic structures and compositions and the resulting materials' properties. Therefore, analysis by both of these techniques on the same specimen may appear to be largely redundant. However, the information each produces is actually quite complementary.

TEM is fundamentally a visual technique through which size and morphology can be readily determined. In addition, diffraction and high resolution imaging allow for orientation determination, phase identification, and defect characterization. While spectroscopic methods in the

TEM generate compositional information, they have several limitations such as: sensitivities down only to ~ 1at%, being 1-D or 2-D averages over a specimen thickness and interaction volume, and having detection biases towards lighter (electron energy loss spectroscopy) or heavier (energy dispersive x-ray spectroscopy) elements. The latter issue requires the use of standards for true quantitative analysis.

APT has excellent compositional sensitivity to the parts-per-million level through time-of-flight mass spectrometry. This also allows for easily resolving particular isotopes with equal sensitivity. Additionally, the resulting data inherently contain the 3-D information of the specimen. However, without additional information, the accuracy of the 3-D reconstruction can be quite uncertain, with several reconstruction shapes and volumes being equally valid. Depending on the information desired, this can result in different answers. Furthermore, APT detects changes in local composition, therefore if a feature of interest, such as a dislocation or grain boundary, is not chemically different from the surrounding material, APT alone may not be sufficient for recognizing that the analyzed region contained the feature of interest.

Correlation of APT with TEM imaging on specimens both before and after APT analysis allows for the generation of more accurate reconstructions in addition to the quantitative local characterization of features to a degree that might not be possible with the application of the techniques individually. Examples of the use of these correlative techniques for interfaces and grain boundaries in photovoltaic materials, proton conductors, lithium-ion conductors, and oxygen conductors will be presented.

(55) **Single Atom Alloys as a Strategy for Selective Heterogeneous Hydrogenations**; Charles Sykes[1]; [1]Tufts University

Hydrogenation reactions are central to the petrochemical, fine chemical, pharmaceutical, and food industries and are of increasing interest in energy production and storage technologies. Typical heterogeneous catalysts often involve noble metals and alloys based on platinum, palladium, rhodium and ruthenium. While these metals are active at modest temperature and pressure, they are not always completely selective and are expensive. We have demonstrated that single palladium atoms can convert the otherwise catalytically inert surface of an inexpensive metal into an ultraselective catalyst. We used high resolution imaging to characterize the active sites and temperature programmed reaction spectroscopy to probe the chemistry. The mechanism involves facile dissociation of molecular hydrogen at individual palladium atoms followed by spillover onto the copper surface, where ultraselective catalysis occurs by virtue of weak binding. The reaction selectivity is in fact much higher than that measured on palladium alone, illustrating the unique synergy of the system.

(56) **Epitaxial Graphene: Not So Plane and Simple**; Phillip First[1]; [1]Georgia Institute of Technology

Many interesting properties are related to graphene's "Dirac cone" electronic structure, where electrons (and holes) travel with a velocity independent of their energy, similar to massless photons. Relative to other materials, the mobility of electrons and holes in graphene is extraordinary, placing it among potential partners to silicon for electronic devices. I will discuss our atomic-scale measurements of the electronic structure of epitaxial graphene grown epitaxially on silicon carbide. The simple Dirac cone is dramatically altered when the graphene plane is stressed through bending and shear, which can be controlled by patterning the silicon carbide sibstrate prior to graphene growth.

(57) **Photopatterned Electroless Gold Deposition: Optimizing Film Patterning and Nanoscale Structure for Applications**; Y M Nuwan Bandara[1], Buddini Karawdeniya[1], Julie Whelan[1], Brian Velleco[1], Jason Dwyer[1]; [1]University of Rhode Island

Patterned metal structures on insulators have importance as conductive elements for electronics, and, with appropriate structural features on the nanoscale, as key functional elements for optical sensing. Electroless plating—a solution-based process with low equipment overhead—is a compelling route to creating such films. We developed and optimized electroless gold plating chemistry for silicon nitride-an important nanofabrication material. This chemistry produces films with favorable nanoscale structure, and is amenable to straightforward patterning on the microscale. With this focus of creating conductive features on silicon nitride, we photochemically coupled organic molecular overlayers to the surface to inhibit the interaction of the plating bath species with the silicon nitride surface. Since the chosen metal ion-silicon nitride interaction is crucial for our electroless deposition chemistry, gold deposits with a greater propensity on free silicon nitride than on these molecular overlayers. The same photocontrolled surface-linking chemistry on silicon nitride was used as a base for surface polymerization reactions. Rather than using the organic layers as gold plating suppressors, we used them to promote gold deposition on, and likely along, the polymer chains. Microscopic inspection of electrolessly gold plated polymeric surfaces revealed clear differences in the gold grain shapes and distribution compared to the gold on a polymer-free surface. All of our electrolessly gold-plated substrates were effective for performing SERS experiments; the gold-coated polymer-functionalized silicon nitride substrates, however, were several times more effective at amplifying the signal than unmodified silicon nitride.

(58) **Correlation Analysis between Aging Grade & Crystallite Size and Spectral Characteristics of the Laser-Induced Plasma**; Jidong Lu[1], Meirong Dong[1], Jun Li[1], Xuan Dong[1]; [1]South China University of Technology

Heat-resistant steel is an important part and factor affecting the safety of the boiler in the power plant. The traditional analysis method is time-consuming and requires additional treatment for the sample. Laser-induced breakdown spectroscopy (LIBS) is a powerful analytical technique that can realize in situ, rapid detection and analysis for both natural and man-made materials. In our work, LIBS was employed to analyze the failure prediction of heat transfer surface in thermal power plants due to the matrix effect. The aging grade and crystallite size are two important features indicated the failure of the transfer surface. Therefore, the correlations between the aging grade & crystallite size and the spectral characteristics were studied. The results showed that there is a good agreement between the intensity ratio of CrI/FeI, MoI/FeI and the aging grade of T91 samples. Good unique value correlations between the intensity ratio of the ionic to atomic line intensities (Fe, Cr, Mn, Ni) and the crystallite size of TP347H are found. It indicates that LIBS is a promising spectrochemical analytical technique for the failure prediction of heat transfer surface in thermal power plants.

(59) **Detection of Molecular Emission Bands by LIBS: Application to the Quantitative Analysis of Nitrogen in Solid Materials**; Meirong Dong[1], Jidong Lu[1], Jianhua Yu[1], Bo Zhang[1], Yue Pan[1]; [1]South China University of Technology

Laser-induced breakdown spectroscopy (LIBS) has been demonstrated as a powerful analytical technique for various types of samples. However, the extension of this technique to the analysis of non-metal elements still remains a challenging task. Nitrogen is a very important non-metal element in solid materials. It can be carried out by measuring nitrogen emission intensity from the LIBS plasma. The existence of nitrogen in air would increase the complexity of nitrogen measurement. To overcome this limitation, this research was performed under different atmospheres (air and argon) to investigate the useful emission for the quantitative analysis of nitrogen in solid materials. The strong nitrogen atomic emission in air and low intensity in argon indicated that the nitrogen is very difficult to detect in the solid materials. But either in air or in argon, there is

strong molecular band CN related with N. The results showed that there is good correlation between CN emission and nitrogen concentration, while there is little correlation between nitrogen atomic emissions. The correlation coefficient (R) is related with the laser energy. In air environment, there is good correlation under low laser energy while as the laser energy increase, the correlation coefficient is decreased. In argon environment, the correlation coefficient is higher than 0.8 and it increased as the laser energy increased. It indicated that as the laser energy increase, the chemical reaction (C+N2→CN+N) has more contribution for the formation of CN, especially for the high carbon concentration sample, which caused the poor correlation between the molecular emission band and nitrogen concentration.

(60) **Excited State Decay of N2+ at Stratospheric Pressures**; Kumarasiri Konthasinghe[2], Andreas Muller[2], Adam Hopkins[1]; [1]Alakai Defense Systems; [2]University of South Florida

Long-range detection of radioisotopes is important for both environmental monitoring and treaty Sucompliance verification. Highly radioactive alpha emitters are known to ionize atmospheric nitrogen, leading to a concentration gradient of ions near the source. We report here on a novel approach to detection of these alpha particle emitters by laser-induced fluorescence LIDAR of N_2^+ emissions. Our initial tests have been conducted in a corona discharge ionization over a wide range of pressures, stratospheric to one atmosphere, and indicate short lifetimes and complex relaxation/charge transfer pathways.

(61) **Cold Atmospheric Plasma: An Inside Look through Optical Diagnostics**; Liesl Krause[1,2], Prasoon Diwakar[1], Ahmed Hassanein[1]; [1]Center for Materials Under Extreme Environment, School of Nuclear Engineering, Purdue University, [2]Department of Electrical and Computer Engineering, College of Engineering, Villanova University

A new technology used in emerging medical applications is cold atmospheric plasma (CAP). This is generated using a “pen” that emits plasma at room temperature, using various gasses to generate and carry the plasma effluents and species. Recently, success has been shown when cold atmospheric plasma is applied to oncology treatments (including apoptosis), accelerated wound healing, pathogen disinfection, and various material-changing surface effects. However, the mechanisms behind these effects are still speculative and not well understood. The goal of this study is to use multiple diagnostic techniques including fast photography, two color emission spectroscopy and optical emission spectroscopy to better characterize the plasma properties and eventually correlate this information to further test the plasma’s interaction with biological samples. The plume dynamics are observed using fast photography methods with an ICCD. This allows determining visible intensity, plume length, and peak intensity, especially as gas flow rates are varied as well as the type of gas mixture used. A two color emission spectroscopy approach is used for determination of plume temperature. Plume images are recorded using narrow band optical filters at 480 nm and 510 nm. A ratio of intensities is determined and used to predict the temperature. A novel image processing code is used to automate the process of estimating the ratio of intensities from filtered images on a pixel-by-pixel basis. Additionally, optical emission spectroscopy is used to determine the chemical species, electron density, and rotational temperature at various spatial locations in the plasma plume. Detailed characterization of plasma along with the role of various gas mixtures on plasma properties provides new insight into why the plasma interaction on biological samples causes positive health effects. Further mechanisms on the plasma interaction with cancer cells as well as human pathogens will be discussed.

(62) **Exploring the Effect of Sample Properties on Spark-Induced Breakdown Spectroscopy**; Michael Marino[1], Payson Dieffenbach[1], Liesl Krause[2], Prasoon Diwakar[1], Ahmed Hassanein[1]; [1]Center for Materials Under Extreme Environment, School of Nuclear Engineering, Purdue University; [2]Department of Electrical and Computer Engineering, College of Engineering, Villanova University

Optical emission spectroscopy techniques such as laser-induced breakdown spectroscopy (LIBS) and spark-induced breakdown spectroscopy (SIBS) provide portable and robust methods for elemental detection in real-time. Both techniques involve the ablation of a target sample, followed by excitation of analyte in the plasma, and by optical emission at element-specific wavelengths as the excited atoms and ions relax back to their ground state. These emissions are then utilized for quantitative and qualitative analysis of the sample. For both techniques, the main obstacles have always been signal intensity, accuracy, and sensitivity of detection. The main advantage of the SIBS method is more safe operation, while still maintaining the portability of the technique. With the availability of more advanced high powered pulse generators, SIBS provides an attractive alternative for multi-elemental detection in real time. In this study, detailed characterization of spark induced plasma, analyte emission intensity, plasma temperature, electron density, and plasma persistence has been studied for various metallic samples with varying physical properties.Target samples, including Mg, Al, Cu, Ta, Sn, Fe, Co, W, and Mo were chosen based on their diverse set of properties, including: melting point, boiling point, first ionization potential, and conductivity. The role of sample properties on temporal evolution of SIBS signal and plasma characteristics was studied by varying the spark energy from 30 mJ to 180 mJ. Certain parameters such as the conductivity of the material greatly affect the SIBS signal intensity output. Mechanisms of SIBS plasma evolution are discussed in the context of material properties and optimal signal detection approaches are proposed. Principle component analysis is used to determine the dominant material properties that affect the SIBS signal intensity and plasma properties.

(63) **Elemental Analysis of Medicinal Plants from India used for the Treatment of Cardiovascular Heart Diseases by Atomic Absorption Spectroscopy and Nondestructive Instrumental Neutron Activation Analysis**; Bharati Pardeshi[1]; [1]PDEA

INTRODUCTION: India is a developing country where both incidence and prevalence of heart disease has been increasing gradually and unless concerted efforts are made and national policy of prevention of risk factors are undertaken. Minerals and trace elements are chemical elements required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health and not produce by our body. Medicinal plants are highly beneficial for the prevention of different diseases and are potential sources of minerals and vitamins. Practically all human societies have utilized plants not only as sources of nutrition but also as therapy against diseases and ailments. Plants contain various phytochemicals and these phytochemicals can play an important role in reducing occurrences of many diseases by boosting up various organ functions of the human body by supplying necessary nutrients and minerals. 30 to 40% of today’s conventional drugs used in the medicinal and curative properties of various plants are employed in herbal supplement botanicals, nutraceuticals and drug.

AIM: The authors explored the mineral element content of 10 herbs, because mineral elements may have significant role in the development and treatment of cardiovascular heart diseases, and the presence or absence of mineral elements was noted.

METHODS: The objective of this study was to conduct a elemental analysis of medicinal plants used by the traditional medical practitioners in the India for heart diseases. The practitioners advised consumption of these 10 medicinal plants to prevent or reduce the chances of occurrences of various heart disorders. Medicinal herbals prescribed for heart diseases were purchased from medicinal plant shop and were analyzed by Instrumental Neutron Activation Analysis (INAA) using 252Cf Californium spontaneous fission neutron source(flux * 109 n s-1) and the induced

activities were counted by γ-ray spectrometry and Atomic Absorption Spectroscopy (AAS) techniques (Perkin Elmer 3100 Model) available at Department of Chemistry University of Pune ,INDIA , was used for the measurement of major, minor and trace elements.

RESULTS: 15 elements viz. Al, K, Cl, Na, Mn by INAA and Cu, Co, Pb Ni, Cr, Ca, Fe, Zn, Hg and Cd by AAS were analyzed from different medicinal plants from India. A critical examination of the data shows that the elements Ca , K, Cl, Al, and Fe are found to be present at major levels in most of the samples while the other elements Na, Mn, Cu, Co, Pb, Ni, Cr, Ca, Zn, Hg and Cd are present in minor or trace levels.

CONCLUSION: The beneficial therapeutic effect of the studied herbs may be related to their mineral element content. The elemental concentration in different medicinal plants used in the treatment of degenerative heart diseases is discussed.

KEYWORDS:Atomic Absorption Spectroscopy, Instrumental Neutron Activation Analysis, medicinal plants , Trace Elemental Analysis, mineral contents, cardiovascular disease

(64) **Chlorine Isotope Determination by High-Resolution Continuum Source Graphite Furnace Molecular Absorption Spectrometry**;
Esperanza García-Ruiz[1], Flavio V. Nakadi[1,2], Marcia A.M.S. da Veiga[2], Maite Aramendía[1,3], Martín Resano[1]; [1]University of Zaragoza; [2]Universidade de Sao Paulo; [3]Centro Universitario de la Defensa-Academia General Militar de Zaragoza

Recently, the use of optical techniques for isotopic analysis is getting attention, as they may offer an intriguing alternative to mass spectrometry techniques. This work investigates the possibility of obtaining isotopic information via the monitoring of the absorption spectra of a gaseous diatomic molecule generated in a graphite furnace and using a high-resolution (approx. 1.5 pm per pixel) monochromator (HR CS GFMAS). To test this concept, Cl was chosen as the analyte and AlCl as the target species. The results demonstrate that, unlike what occurs with atomic spectra, under optimum conditions it is possible to acquire isotopic information by HR CS GFMAS in a straightforward way, as it is feasible to observe band heads for each Cl isotope (actually, for $Al^{35}Cl$ and $Al^{37}Cl$) that are separated, i.e., they act like two different molecules absorbing at different wavelengths. The method proposed, based upon the addition of both Pd and Al and the selection of peak height values, enables Cl isotopic analysis with precision values around 2% RSD for solutions with Cl contents at the mg/L level. Accurate values, within this uncertainty, can be directly obtained without requiring any method for mass bias correction. The potential of isotope dilution for calibration is also explored, and it is proven how this approach can help in providing accurate results in situations where the occurrence of chemical interferences, a case frequently encountered for the HR CS GFMAS technique, hampers the use of other calibration approaches, as demonstrated for water analysis.

(65) **Significance of Plasma-Ambient Conditions in Emission Features of Laser Ablation Plasmas**; Patrick Skrodzki[1,2], Niral Shah[1], Jason Becker[1,2], Sivanandan Harilal[1], Mark Phillips[1], Brian Brumfield[1],Nicole LaHaye[1]; [1]Pacific Northwest National Laboratory; [2]Purdue University

The expanding field of nuclear forensics for safeguard endorsement, nuclear fuel prospecting, non-proliferation, etc., requires innovative methods of analysis. Laser ablation (LA) for optical emission spectroscopy (OES) is practical; however, sensitivity of LA-OES in detecting special nuclear materials (SNM) is poor. Heavy SNM such as U produce crowded spectra due to electron abundance. Furthermore, studies imply U signal dramatically varies with ambient conditions, e.g. pressure, nature of ambient gas. Consequently, this study considers mechanisms for U signal enhancement and/or quenching under various environmental conditions. LA-OES incorporates the ns Nd:YAG fundamental harmonic using a glass sample containing ~1.3% (w/w) U in primarily Ca, Si, and Na matrix. Signal features of merit include U I and II transition intensities as well as persistence measured with optical time-of-flight (TOF) investigations. Potential explored mechanisms for signal quenching related to ambient conditions include plasma chemistry (e.g. uranium oxide formation) and confinement effects of N_2 and air compared to inert control gas Ar investigated through shadowgraphy.

(66) **Develpment of High-Density Microplasma Emission Source using 3-D Molding Process Based on Microsterolithography**; Ken Kakegawa[1], Ryoto Harigane[1,2], Mari Aida[1], Hidekazu Miyahara[1], Shoji Maruo[2], Akitoshi Okino[1]; [1]Department of Energy Sciences, Tokyo Institute of Technology; [2]Department of Mechanical Engineering, Yokohama National University

The analysis of trace elements in small amount of body fluid such as saliva or sweat has been expected for new diagnosis method of disease. If portable and high-sensitive analyzer for such samples could be developed, the health management in daily life will be easier since these fluids can be sampled on their own. As one method to realize such system, there is growing interest in Micro Total Analysis Systems (μ-TAS). In the μ-TAS devices, the sample introduction, detection and other parts are hyphenated with microchannel and build on small single chip. In comparison with conventional fluid channel, sample consumption and loss can be dramatically reduced because inner volume of the microchannel is much smaller. However, the sufficient analytical sensitivity has not been obtained in elemental analysis using the conventional μ-TAS. The main reason of this problem is that the detector suitable for μ-TAS has not been developed.

In this study, we developed a new atomic emission source for μ-TAS. As high-sensitive trace element analysis methods, inductively coupled plasma has been used for sample excitation or ionization source. But the plasma source cannot be applied to μ-TAS because the plasma volume is large and the discharge current density is not high. Therefore, we proposed high density barrier discharge plasma for μ-TAS. The microchannel was fabricated using the 3-D molding process based on microsterolithography[1]. In this process, the microstructure can be made of various materials including especially SiO_2 which has excellent light transmittance. A pair of 80 μm-wide 5 μm-long electrodes was placed on the microchannel of 80 μm-wide and 100 μm-depth. The plasma gas was flowed through the microchannel and dielectric barrier discharge was generated by applying AC or pulse voltage.

In order to obtain suitable plasma for elemental analysis, the plasma parameters such as the electron density were investigated by changing the supply voltage, frequency, gas species and plasma gas flow rate. Plasma properties of the developed source and conventional plasma sources will be compared.

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(67) **Thermomechanical Characterization of Resilin by Combining Contact Resonance Atomic Force Microscope and Nano Thermal Analysis**; Ehsan Rezaei[1], Charles Nguyen[1], Anastasia Desyatova Desyatova[1], Deepak Rudrappa[2], Paul Blum[2], Joseph Turner[1]; [1]Mechanical and Materials Engineering, University of Nebraska-Lincoln; [2]School of Biological Sciences, University of Nebraska-Lincoln

Contact resonance atomic force microscope (CR-AFM) methods are relatively new techniques used to quantify the elastic/viscoelastic properties of materials including polymers, elastomers, and biological materials. CR-AFM exploits the resonant frequencies of the AFM probe both in and

out of contact. The interaction between the tip and the sample causes the resonances to shift higher and have an increase in peak width. Analytical models of the probe vibrations can then be used to deconvolve the beam dynamics from the spectra to recover the local material influence. More recently, U-shaped AFM thermalevers have been developed which offer the potential for tip heating by passing a current through the probe. Here, a thermalever is used with Lorenz force excitation to sweep the free and contact frequency spectra. These probes have one distinct advantage over rectangular AFM probes in that specific modes allow in-plane and out-of-plane tip-sample motion to be excited independently at the same location. Combining the CR-AFM and local heating, a new measurement approach is possible for investigating the thermomechanical properties of viscoelastic materials. In this poster, we applied the method to obtain the properties of biological samples. Resilin is one of the most efficient biological elastomeric proteins known today with resilience of over 90%, long fatigue life and high elongation at failure. Recently produced recombinant resilins have demonstrated promising mechanical properties for a wide range of engineering applications. However, their thermomechanical and viscoelastic properties at the nanoscale have not yet been characterized. Recombinant pro-resilin was manufactured based on the first exon of the Drosophila CG15920 gene and crosslinked into a hydrogel using ruthenium-mediated rapid photochemical crosslinking. Thermomechanical properties of dehydrated resilin gel specimens were characterized using nano thermal analysis and glass transition temperatures were obtained at a local spatial scale of tens of nanometers. The fact that resilin hydrogels can be thermally pre-treated allows the manufacturing of resilin polymers with heterogeneous tunable properties through nanoscale level pre-treatment. We also demonstrate the use of this approach for measurement of the resilin damping properties. Both the elastic and shear loss tangents of resilin are reported based on the local CR-AFM measurements.

(68) **The Use of XYZ Sample Manipulator in Quadrupole Glow Discharge Mass Spectrometer**; Maciej Miśnik[1,2], Piotr Konarski[1], Aleksander Zawada[1,3]; [1]Institute of Tele and Radio Technology, Warszawa; [2]Gdańsk University of Technology, Gdańsk; [3]Military University of Technology, Warszawa

Glow discharge mass spectrometry (GD-MS) technique is often used in elemental composition of solids and depth profile analyses. One of major drawbacks of this technique is a background that originates from the components of gas atmosphere in which the glow discharge takes place. Particularly troublesome is the presence of water vapors that enter the glow discharge cell at each change of the sample. Recently this problem was partially corrected by A.A. Ganeev et al., who used a sample revolver in the ion source, which allows to simultaneously load 6 samples [1].

In our work we present a construction, which allows a linear translation of samples using a vacuum manipulator with three degrees of translation (XYZ). Our system also allows to mount several samples at a time and thus reduces the number of necessary ventings of the discharge cell. Our construction allows the sample movement in the range 10 mm x 10 mm relative to the stationary intermediate cathode. The movement of the sample can take place during analysis, i.e. at the real time when voltage is applied to the sample. We present the first results of the use of X-Y-Z sample manipulator in a glow discharge analyzer. The analyzer uses a quadrupole mass spectrometer [2]. The glow discharge is carried out at a pressure of approx. 0.5 mbar Ar with a discharge voltage of 0.8-2.5 kV DC and a discharge current of 0.5-5 mA. We will present preliminary results of the depth profile analysis of several layered systems, recorded during the movement of samples. We use intermediate cathode made of 0.1 mm thick tantalum sheet with several diameter diaphragms (0.5, 0.75, 1 and 1.5mm).

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(69) **The Use of Transition Rate Diagrams to Identify Changes in Discharge Processes when O2 or H2 is Present in a Cu/Ne glow Discharge**; Edward Steers[2], Zdenek Weiss[1], Sohail Mushtaq[2], Volker Hoffmann[4], Viktoria Weinstein[2]; [1]LECO Instrumente Plzeň spol. s r.o.; [2]London Metropolitan University, London; [3]Imperial College London; [4]IFW Dresden

Analytical glow discharge (GD) sources are not in thermal equilibrium and the intensities of spectral lines, particularly of ionic lines, are determined by the relative importance of the various individual excitation processes, particularly Penning Ionization, Penning Excitation and asymmetric charge transfer (ACT). These processes are often affected by the presence of impurities in the plasma gas and impurity gases can also introduce new excitation mechanisms, for example, ACT by hydrogen atoms (H-ACT) [1]. One way to identify these processes is to compare the line intensity distributions occurring with different plasma gases. However, "Transition Rate (TR) diagrams", recently introduced by Weiss[2], are a powerful new way to study the excitation processes occurring in low pressure glow discharges (GD), including cascade excitation, and to interpret the changes in intensities that occur if, for example, small amounts of hydrogen are present in a discharge. We will present the principles of TR diagrams, using the Cu II spectrum as an example and then use them to compare and discuss the various excitation processes taking place in analytical glow discharges in neon when small quantities of oxygen[3], hydrogen or nitrogen are present in the plasma gas. These new results suggest that as well as H-ACT, ACT can occur with hydrogen *molecules*, (H_2-ACT)

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(70) **Does Asymmetric Charge Transfer Play an Important Role as the Ionization Mode in Low Power-Low Pressure GD-MS?**; Edward Steers[1], Sohail Mushtaq[1], Glyn Churchill[2], DeAnn Barnhart[2], Volker Hoffmann[3], Karol Putyera[4],Juliet Pickering; [1]London Metropolitan University; [2]Nu Instruments Ltd.; [3]IFW Dresden; [4]Evans Analytical Group

It has been suggested that in low power (~ 5W) analytical glow discharges (such as VG9000 type sources) running in low pressure noble gases ~1 hPa, electron impact ionization and Penning ionization (PI) are the main ionization pathways[1] , and that although asymmetric charge transfer (ACT) may occur in such sources, there is only minor contribution from this process[2]. On the other hand, it has been shown that departures from a regular pattern of relative sensitivity factors (RSF) can be attributed to ACT[3]. We have used a Nu Instruments Astrum high resolution glow discharge mass spectrometer (~1 kV & ~3 mA) to compare ion signals of the constituent elements in various standard reference materials using

argon or krypton as the plasma gas. Comparison of the ratios of the ion signals of each constituent element to that of the plasma gas shows that for oxygen the ratio in krypton is more than ten times higher than that in argon (oxygen ground state ions are produced by Kr-ACT). For many elements, the ratios are very similar but that for tungsten is significantly higher with krypton, whilst for iron the reverse holds. These effects are thought to be linked to the arrangement of ionic energy levels of the elements concerned and the resulting relative importance of ACT and PI. Detailed GD-OES studies have shown that W II line intensities with krypton are greater than those with argon; i.e. Kr-ACT is much stronger for tungsten than Ar-ACT. Ions produced in an excited state decay to the ionic ground or metastable states and make a significant contribution to the total tungsten ion signal.

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(71) **Forensic STR Profiling Based Smart Barcode, a Highly Efficient and Cost Effective Human Identification System**; Andleeb Zahra[1,2,3], Bilal Hussain[1], Amer Jamil[4]; [1]Government College University Faisalabad Pakistan; [2]COmsats Institute of Technology Islamabad Pakistan; [3]Koc University, Istanbul , Turkey; [4]University of Agriculture Faisalabad Pakistan

Human identification is the ultimate goal in forensics, both in criminal casework and in paternity testing, which can be based on DNA profiling of individuals. For this purpose analysis of short tandem repeat (STRs) markers has become a highly useful tool. We have developed a system based on a novel idea that will be highly beneficial in human identification on the basis of DNA fingerprinting. It is a web based application named as Human Identification Barcode System (**HIBS)**. It provides a unique molecular signature or molecular ID of an individual in form of a barcode. This system is based on isolation of DNA from blood followed by amplification by polymerase chain reaction, detection of bands on agarose electrophoresis, and designing of human barcode using self-designed system. HIBS is a web based application that can be accessed via www.hibs.com.pk. Being web based system, it can be accessed from anywhere and it can be implemented on security checkpoints so that a person can be identified on basis of its DNA rather than conventional methods of identification which can be falsified. The barcode generated for each individual on the basis of DNA fingerprinting will be stored in database, and may also be added in CNIC. This will be extremely beneficial against any culprits including suicide bombers, target killers etc as even a single blood spot, a few skin cells, root of hair etc will be enough to unveil the identity of such persons. On the other hand it will also be helpful to relieve the persons with false accusations. This is a secure and smart system that would be highly useful for human identification in future. Moreover, the system is developed as a low-cost technology for easy implementation without the need of expensive equipment.

(72) **Estimation of the Age of Bloodstains under Different Environmental Conditions with Fourier Transform Infrared Spectroscopy and Multivariate Statistical Analysis**; Zhenyu Lu[1], Brianna Cassidy[1], Stephanie Dejong[1], Katherine Witherspoon[1], Michael Myrick[1], Stephen Morgan[1]; [1]University of South Carolina

Bloodstains, which are among the traces encountered most frequently at crime scenes, are important for potential extraction and amplification of DNA for suspect identification, as well as for spatter pattern analysis to reveal a sequence of events. Estimating the age of blood stains with good accuracy and precision has been an elusive goal for forensic investigations. Estimates of blood stain age can contribute to verify witness' statements, limit the number of suspects, and confirm alibis. As a result, estimate the age of blood stains with good accuracy and precision has been an elusive goal for forensic investigations.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used in forensic detection of blood stains and age estimation because of signature absorbances in the mid-infrared region. We have observed position shifts and intensity changes for these peaks as a result of the denaturation of blood proteins and water desorption with aging. Principal component analysis (PCA) and partial least square regression (PLSR) were used in this work to combine these changes in a multivariate calibration for blood age. Calibration experiments over several months at 30 ^{o}C under a variety of humidity (65%, 75%, and 85%) and substrate conditions (acrylic and cotton) enable prediction of blood stain age under different environmental conditions. A short term aging experiment over a period of 23 days on both cotton and acrylic samples has a coefficient of determination up to 0.97 with an uncertainty of 1-2 days, while another long term aging experiment over a period of 83 days has a coefficient of determination up to 0.92 with an uncertainty of 6-11 days.

The results provide a nondestructive and simple approach using ATR-FTIR paired with multivariate statistics analysis to predict blood age up to several months. Calibration models built under a variety of humidity and substrate conditions will contribute to the understanding of mechanisms of blood aging.

(73) **An Experimental Study of the Forensic Luminol Test for Detection of Bloodstains**; Brianna Cassidy[1], Zhenyu Lu[1], Kathrine Witherspoon[1], Jennifer Martin[1], Stephanie DeJong[1], Raymond Belliveau[1], Michael Myrick[1], Stephen Morgan[1]; [1]University of South Carolina

Luminol has been used for blood-stain detection by forensic investigators for over 60 years. When luminol (3-aminophthalhydrazide) is sprayed onto areas containing blood, luminol reacts with the heme moiety of hemoglobin to give a faint bluish-white chemiluminescence. Studies on the sensitivity of the luminol technique have reported a wide range of detection limits. In 2006, Webb, *et al*. recounted detection of five-million times dilute blood using luminol, whereas the FBI had determined luminol to be nonindicative for bloodstains more than one-hundred times dilute. The reason for the large range in reported detection limits stems from a lack of experimental control. Though evidently important, bloodstains are often prepared in a way which cannot guarantee reproducibility, non-quantified amounts of luminol are sprayed on bloodstains in an uncontrolled fashion, and detection of the chemiluminescent response is qualitatively measured by human observation. Recently, we submitted a preliminary patent on a technique which allows reproducible creation of bloodstain samples. Using this sample preparation technique, we have designed experiments which better control blood and luminol application. Furthermore, we employed a Nikon D80 CCD camera to capture light emitted from the blood-luminol reaction and used pixel intensities from raw images to quantify the response. This technique has allowed quantifiable observation of the effect bloodstain dilution has on the chemiluminescent blood-luminol response. Moreover, this technique allows calculation of the luminol limit of detection for bloodstains on fabric.

(74) **Lost in Translation: Bridging Vibrational Spectroscopy Knowledge from Scientists to End Users**; Luisa T.M. Profeta[1], Alan Ford[1], Alen Tomczak[1], Jack Burton[1], Ken Pohl[1]; [1]Alakai Defense Systems

Vibrational spectroscopy equipment marketed to the military and first responder personnel typically are beta tested to work out mechanical and operational kinks prior to final release. However, one critical aspect that is ever evolving in instrument design is understanding operational constraints of fieldable equipment, training available, data collected and how data is used. Since most end users are not trained spectroscopists, it is critical that the training they receive allows them to obtain data which has value and/or can be used to make critical decisions. This process of training and programming instrumentation to optimize both decision making and data quality can be compared to providing language translation between two parties. This presentation will address how spectroscopists need to approach non-spectroscopists. It will detail critical questions and conversations necessary to develop instrumentation to encompass the needs of the end user while retaining the ability for the instrumentation to provide precise and accurate measurements meaningful for scientific support.

(75) **Novel Concept for Forensic Analysis of Biomarkers**; Jan Halamek[1]; [1]Department of Chemistry, University at Albany, SUNY, Albany, NY

The analysis of biomarkers prominent area of forensic science, specifically the analysis of blood in order to identify the presence of various drugs along with DNA for matching purposes. While forensics has grown rapidly over the years, the methods currently used are still in need of vast improvements. Current techniques require sample collection to be performed at the crime scene followed by transportation to a laboratory facility for the analysis to take place. However, due to the high rate at which crimes are committed, this process is far too slow. In areas with high crime rates, it is also possible that the laboratory analyses are too time consuming and result in large backlogs that hinder investigations for days, weeks, and even months.

The research presented here addresses this situation by introducing the use of bioaffinity-based assays for quick and straightforward on-site analyses of blood and fingerprint samples. These enzyme cascades use substrates that are naturally present in the samples in order to identify various traits of the sample originators such as age, gender, ethnicity, and general health conditions. Because only miniscule amounts of enzymes and substrates are necessary, this method requires only very small amounts of samples. Furthermore, the bio-affinity based cascades are remarkably versatile and can be adjusted for the analysis of a wide range of substrates for the discernment of different physical traits.

This could lead to the revolution of the field of forensic science and result in the acceleration of many criminal investigations.

(76) **New and Practical Methods to Characterize Organic Gunshot Residue**; Sydney Brooks[1], Brittany Yeager[1], Suzanne Bell[1]; [1]West Virginia University

Traditional analysis for firearm discharge residue (FDR) relies on the detection of gunshot residue (GSR) particles formed from metals in the primer. Recently, propellant residues (OGSR) have been proposed as alternative/additional target for FDR detection. To be of practical use, any methods developed for forensic use must focus on existing expertise and equipment. Most forensic science laboratories have gas chromatography mass spectrometers (GC/MS) that are capable of detecting most of the organic compounds of interest. However, few GC/MS methods have proven sufficiently sensitive and reproducible to consider for routine casework application. Here, alternative methods, including direct headspace analyses were evaluated for forensic application; with the only viable approach found to be an exhaustive swab extraction followed by drying/reconstitution and GC/MC analysis. Acceptable figures of merit necessitated selected ion monitoring for detection. This presentation will describe the various sample preparation methods including the best solvent extraction procedures along with associated figures of merit. Results from hand swabs from shooters and non-shooters using various weapons and propellants will be presented with an emphasis on forensically relevant and realistic scenarios (i.e, 1-2 shots).

(77) **The Surprising Effect of Temperature on the Weathering of Gasoline**; Ashley Cochran[1], Heather Birks[1], Tyler Williams[1], Glen Jackson[1]; [1]West Virginia University

Arson investigations often involve the identification and characterization of ignitable liquid residues in fire debris. The most commonly used ignitable liquid is gasoline because it is so readily available and particularly effective. Gasoline often evaporates from its containers as it is stored as well as through exposure to high temperatures such as during a fire. Such evaporation is often termed weathering, and it is widely accepted that ignitable liquid residues are more likely to resemble weathered liquid samples than pristine liquid samples.

Prior research has investigated the weathering characteristics of gasoline under a variety of experimental conditions, but to date this has been limited to basic pattern recognition of the resulting chromatograms. Relatively little work has been done to understand how weathering conditions—such as temperature, pressure or convection—affect the distribution of the various classes of volatile components. For the current study we weathered gasoline samples at atmospheric pressure using a stream of nitrogen gas and under vacuum. At both pressures, samples were weathered to 75%, 90%, and 95% by weight, and at three different temperatures of 30°C, 60°C, and 90°C. After weathering, samples were analyzed using an Agilent Gas Chromatograph-Mass Spectrometer (GC-MS) with conventional conditions on a 30 m DB5 column. The resulting chromatograms showed that the earliest eluting, most volatile compounds tend to remain at significantly ($\alpha<0.05$) higher relative abundance when weathered at higher temperatures. The effect is that samples weathered at higher temperatures appear less weathered (on a percent level) than one would expect. These results are consistent with the known relationships between vapor pressure and temperature for the different compounds, as we demonstrate through weathering of a simplified synthetic mixture of 7 common compounds found in gasoline, and the simulated weathering of the same compounds based on published Antoine constants.

This work may help explain why real fires often cause gasoline residues to appear far less weathered than one would expect based on the expected weathering temperatures, alone.

(78) **Raman and Laser Induced Fluorescence Spectroscopy on Ageing Fingerprints**; Lars Landström[1], Christian Lejon[1], Therese Mikaelsson[1], Göran Kidfelt[2], Milja Kanerva[2], Cecilia Vahlberg[2],Kent Rosengren[2], Per Ola Andersson[1]; [1]CBRN Defence and Security, Swedish Defence Research Agency (FOI); [2]Nationellt Forensiskt Center (NFC)

Raman peak intensities, relative the ~1450 cm^{-1} peak, were monitored as function of time on both male and female fingerprints. The investigated peaks were assigned to originate from carotenoids, squalene, and unsaturated fatty acids, and different time constants were found for the peaks (assuming an exponential decay of the time dependent intensity data). In addition, the relative intensity of laser induced fluorescence spectra,

using two different excitation wavelengths (280 and 360 nm), were also monitored over time on the same fingerprints. The overall decay in relative intensity were similar for male and female fingerprints and the results were compared to the results obtained by Raman spectroscopy. The preliminary results presented here strongly indicate the possibility to estimate the age of fingerprints using non-destructive spectroscopic techniques.

(79) **Detection of Trace Evidence Particles by Mid-Infrared Laser Reflectance Imaging**; Raymond Belliveau[1], Stephanie DeJong[1], Lu Zhenyu[1], Brianna Cassidy[1], Stephen Morgan[1], Michael Myrick[1], Michael Myrick[1]; [1]University of South Carolina, Department of Chemistry and Biochemistry

The characterization of particles for forensic purposes is well established using optical and electron microscopy, and spectroscopic techniques, but identifying trace evidence for further examination often proves to be difficult and time-consuming. The purpose of this work is to investigate the use of mid-infrared laser reflectance imaging to detect the location of forensically relevant micro-scale particles on macro-scale surfaces. Of particular interest are particles on natural fiber surfaces. Cellulosic materials have a low reflectance in the 10 μm wavelength region, so, particles having high reflectance at 10μm show high contrast during imaging. Using an experimental set-up consisting of a CO_2 laser operated at 10.6 μm, and an infrared camera, we apply this technique to the detection of trace evidence particles of different sizes and compositions as an application in the forensic sciences.

(80) **A Raman 'Spectroscopic Clock' for Bloodstain Age Determination: The First Week After Deposition**; Kyle C. Doty[1], Gregory McLaughlin[1], Igor K. Lednev[1]; [1]University at Albany

Knowing the time since deposition (TSD) of an evidentiary bloodstain is highly desired in forensics, yet it can be extremely complicated to accurately determine in practice. Although there have been numerous attempts to solve this problem using a variety of different techniques, currently no established, well-accepted method exists. Here, a Raman spectroscopic approach was developed for determining the age of bloodstains up to one week old. Raman spectroscopy, along with two-dimensional correlation spectroscopy (2D CoS) and statistical modeling, was used to analyze fresh bloodstains at ten time points under ambient conditions. The 2D CoS results indicate a high correlation between several Raman bands and the age of a bloodstain. A regression model was built to provide quantitative predictions of the TSD, with a cross-validated root mean squared error of 0.13 and R2 of 0.97. It was determined that a 'new' (1 hour) bloodstain could be easily distinguished from bloodstains at other ages, which is very important for forensic science in helping to establish the relevant association of multiple bloodstains. Additionally, all bloodstains were identified as blood by comparing the measured spectra to multidimensional body fluid spectroscopic signatures. These results demonstrate that Raman spectroscopy can be used as an analytical tool for discriminating between bloodstains on the scale of hours to days. This approach shows promise for immediate practical use in the field to predict the time since deposition with a high degree of accuracy.

(81) **Self-Absorption Measurements of Resonant Aluminum Lines**; David Surmick[1], Christian Parigger[1]; [1]University of Tennessee Space Institute

Laser-Induced breakdown spectroscopy is applied to determine plasma parameters from atomic line profiles. At relatively early times in the plasma evolution, line profiles become distorted due to line self-absorption effects that are more pronounced for higher electron densities, N_e. Here we present N_e and electron temperature, T_e, measurements of laser-induced plasma that is initiated on the surface of an aluminum sample and evaluate the level of self-absorption. Experimental spectra are collected following laser ablation of an aluminum alloy 6061 target. Breakdown is generated using 12 ns pulsed, Q-switched Nd:YAG radiation. Spatially and temporally resolved spectra are recorded using an intensified, charge-coupled detector. Electron densities are determined from Stark-broadened line widths of the Al 394.4 nm and 396.15 nm resonant lines. The aluminum N_e is inferred using results from past and present aluminum laser ablation experiments in which breakdown was initiated in a hydrogen gas atmosphere. The Al lines are de-convolved from instrument broadening to find the Stark width contribution. Optical breakdown events in air are also investigated. Singly ionized nitrogen, N^+, emissions are used to find N_e. The electron temperature is assessed from Saha-Boltzmann type considerations of both Al and N^+ lines. Self-absorption effects and consequence for electron density determination are addressed through a detailed experimental characterization in which a plane reflecting mirror re-images the plasma onto itself prior to detecting the spectrum.

(82) **Laser-Induced Plasma Diagnostics with the Hydrogen Balmer Beta Line**; Ghaneshwar Gautam[1], Christian Parigger[1]; [1]University of Tennessee Space Institute

Laser-induced micro-plasma is generated in laboratory air by employing Q-switched Nd:YAG radiation at the fundamental wavelength of 1064 nm and at a repetition rate of 10 Hz. The plasma is imaged onto the spectrometer slit with two quartz lenses. Spatially and temporally resolved spectra are collected with and without a doubling mirror by using a Czerny-Turner type spectrometer equipped with an 1800 grooves/mm grating and an intensified, charge-coupled camera. The recorded spectra are wavelength calibrated and detector-sensitivity corrected. Stark broadened spectral line profiles of the hydrogen Balmer beta, H_β, are used to determine the plasma electron density, N_e. A computer program is designed to fit the recorded data using tabulated profiles for the H_β line. The determination of the electron density from values of the Stark width and the H_β peak separation is investigated. The effects of self-absorption of the H_β line are evaluated for various time delays from generation of optical breakdown. Spatially resolved micro-plasma may be analyzed by applying Abel and/or Radon inversion techniques for the purpose of extracting details of the expanding plasma kernel. Standard tabulated data are utilized for the Boltzmann plot method to infer the electron excitation temperature. The use of peak-separation values for finding N_e is further elaborated with respect to its future application as a plasma electron density diagnostic. The electron density is also determined from nearby nitrogen lines. The doubling mirror is used to determine the self-absorption correction factors, K_λ. Subsequently, these factors are implemented to construct the corrected profiles.

(83) **Bio-distribution of Magnetic Gold Nanoparticle in Liver through Changes of Ca Channel Pump Detected by LIBS Technique**; Ola Ahmed, Hisham Imam[, 2], Abdel Rahman Zekri[3]; [1]National Cancer institute, Cairo University, Egypt; [2]National institute of laser enhanced science (NILES), Cairo University, Egypt; [3]National Cancer institute, Cairo University, Egypt

Laser-induced breakdown spectroscopy technique (LIBS) was used in thesis study to address the effect of gold magneto nanoparticles on the liver cancer cells (HepG2) by examining the variation in genotoxicity and cytotoxicity.

HepG2 cell line was cultured at 105 cells/ml and expanded, after which, they were incubated with gold magneto nanoparticles at concentrations 10, 25, 50 μg/ml respectively for 12 hours. The effect of these nanoparticles in cell apoptosis as well as in autophagy was evaluated by acridine

orange staining and Annexin V/propidium iodide double staining as well as apoptosis specific PCR array assay for evaluation of apoptosis in the treated cells.

At a concentration of nanocomposite 10, 25, 50 µg/ml, cells showed increase in the apoptosis figure from 11.25% ± 3.1% to 28% ± 2.04%, 39 % ± 3.6%, 60% ± 4.3% respectively. Apoptosis specific PCR array also, showed very high expression of TNF, BIRC3 and NFKB1 with increasing in the fold regulation ratio of 155.03,158.03 and 149.2- fold respectively.

On the other hand, extra investigation of these treated cells by LIBS shows an increasing in the iron oxide nanoparticles concentration as well as calcium concentration. This increase in the calcium content in the treated cells may play an important role in the program cell apoptosis which was prove in our study by both Flow cytometry as well as by profile apoptosis PCR array.

Finally, our data showed the possibility of the using LIBS as inductor of cells apoptosis by measurement Ca concentration changes in liver cancer cells (HepG2 cells).

(84) **Discrimination of polymers from plasma parameters using laser Induced Breakdown spectroscopy**; M Atif; [1]King Saud University
M. Atif , W. A. Farooq, Walid Tawfik, J. P. Singh
Department of Physics and Astronomy College of Science, University Riyadh Saudi Arabia
Institute for Clean Energy Technology, Mississippi State University
Abstract: Three different types of polymers, High Density Polyethylene (HDPE), Polypropylene (PP) and Allyl Diglycol Carbonate (CR39) were investigated using Laser Induced Breakdown Spectroscopy. The plasma was generated using focused beam from fundamental frequency of Q-Switched Nd:YAG laser on the target. The emitted light from the plasma was collected with Optical fiber and spectra were recorded after passing the light through the spectrometer equipped with CCD camera. LIBS plasma temperature was determined using Boltzmann plot and found to be 6800 K, 7250K and 5250K for HDPE, PP and CR39 respectively. The number density was around 7.8 x1015 cm-3 which was estimated from the stark broadened profile of the spectral line.
This technique is simple and can be used in the polymer industry for online discrimination of polymers.

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(85) **The Role of Gas Dynamics on the Formation of AlO in Laser-Ablation Plumes**; Sivanandan Harilal[1], Brian Brumfield[1], Jeremy Yeak[2], Mark Phillips[1]; [1]Pacific Northwest National Laboratory; [2]PM & AM Research
Laser ablation is used for numerous applications under various ambient conditions. Laser ablation plume expansion into vacuum conditions is less complex compared to their expansion in the presence of a reactive gas like air. In the latter scenario, the plasma chemistry will redefine the plume hydrodynamics and the evolution of the chemical composition of the plume. A laser-produced Al plasma in ambient air is an excellent system to study the role of plasma chemistry on plume hydrodynamics or vice versa. The formation of AlO molecules during Al plasma interaction with air is an intriguing process that involves reaction of Al with atomic and molecular oxygen. These reactions are influenced by complex interactions between the plume and air ambient gas which is moderated by expanding shockwaves. In the present work we investigated the role of gas dynamics on the formation of AlO in a laser produced Al plasma in air at atmospheric pressure levels. For producing plasmas, a high-purity Al target is ablated in air using 6 ns pulses from a Nd:YAG laser. Focused shadowgraphy, spectrally integrated and monochromatic fast gated imaging were used to obtain the gas-dynamic features of Al plume in air, while optical emission spectroscopy is used for evaluating spatio-temporal features of Al ions, neutrals and AlO in the plasma. A comparison between the persistence of AlO in laser ablation plumes generated by ns and fs lasers will also be presented.

(86) **Multiblock Analysis Applied to LIBS and XRF Data of Geological Materials**; Faten Ammari[1], Léna Bassel[1], Catherine Ferrier[3], Delphine Lacanette-Puyo[4], Rémy Chapoulie[1], Bruno Bousquet[2]; [1]Université Bordeaux Montaigne, IRAMAT-CRP2A, UMR 5060 CNRS; [2]Université de Bordeaux, CELIA, UMR 5107 CNRS; [3]Université de Bordeaux,PACEA, UMR 5199 CNRS; [4]Université de Bordeaux,I2M-TREFLE, UMR 5295 CNRS
Laser-induced breakdown spectroscopy (LIBS) and X-Ray Fluorescence (XRF) are analytical methods providing the elemental composition of the samples and allowing on-site measurements. They are subsequently perfectly adapted to geosciences.

The general context of our study is to understand the mechanism of formation of calcareous deposits, known as “white disease”, on the walls of prehistoric caves which is a problem that threatens the conservation of many decorated caves listed as a UNESCO world heritage.

In this work, a laboratory cave presenting two calcitic facies named moonmilk and coralloides was considered. Representative samples of the two facies (moonmilk and coralloides) and their supporting rocks (limestone and clay) were studied.

Despite of the omnipresence of calcium inside all the samples, it was shown that minor elements such as strontium or magnesium were also detected by LIBS and XRF. The results of multivariate analyses on LIBS and XRF data allowed the discrimination between the samples and the most relevant variables were selected for each analytical technique. Finally, a mutiblock approach was used and the two analytical methods were compared.

(87) **Improved Electron Collisional Line Broadening for High Resolution LIBS Modeling in the Plasma Kinetics Code ATOMIC**; Heather Johns[1], David Kilcrease[1], James Colgan[1], Elizabeth Judge[1], James Barefield[1], Samuel Clegg[1]; [1]Los Alamos National Laboratory
The Los Alamos National Laboratory (LANL) spectral modeling code ATOMIC produces emissivity and opacity calculations for plasmas based on atomic structure data from the LANL suite of atomic physics codes, including oscillator strengths, energy levels, and ionization cross sections. The ATOMIC code has been employed to generate theoretical LIBS spectra of complex geologic samples. It uses these atomic data to obtain atomic level populations for both non-equilibrium plasmas and those in local thermodynamic equilibrium for single and multi-element plasmas. The ability to model complex systems with many thousands of lines in a reasonable computational time has required compromises in the

treatment of electron collisional line broadening. However, this mechanism is dependent on local electron density and temperature conditions, which can impact the accuracy with which spectroscopic modeling can determine plasma conditions for a given experimental spectrum. A model overestimating the amount of electron collisional line broadening in a line produced by an ion can underestimate electron density and overestimate temperature, but the situation for neutrals is more complex. Previously ATOMIC relied on a hydrogenic approximation1, which overestimates broadening in low temperature plasmas2. To improve the accuracy of our spectroscopic modeling using ATOMIC, we recently improved our electron collisional line broadening model based on the work of Dimitrijevic and Konjevic, but utilizing our calculated atomic structure information2-4. While spectroscopic modeling of LIBS plasmas from detectors with resolving power ~ 1000, such as ChemCam5,6 has already been accomplished with this model, to treat higher resolution LIBS spectra produced in the laboratory at Los Alamos, with resolving powers in excess of 10,000, will require additional improvements to the electron collisional line broadening model. We will evaluate improvements based on the work of Hey and Breger7 and more modern gaunt factors8,9 against select tabulated experimental electron collisional line broadening widths and demonstrate the use of the resulting treatment for spectroscopic modeling of high resolution LIBS spectra.

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(88) **Temporally and Spatially-Resolved Absorption Spectroscopy of Atomic Oxygen in an Air Spark**; Brian Brumfield[1], Sivanandan Harilal[1], Mark Phillips[1]; [1]Pacific Northwest National Laboratory

Understanding the physical properties and chemistry of laser-induced plasmas (LIPs) remains an active area of research, and experimental measurements of LIPs using optical diagnostics have proven beneficial. While LIPs have been studied extensively using emission spectroscopy, less work has been carried out using absorption spectroscopy. Absorption spectroscopy with narrow linewidth cw lasers complements optical emission measurements during the early time-evolution of LIPs, and also enables measurements over longer timescales and at higher spectral resolution than are typically possible using emission.

In this work, tunable diode laser absorption spectroscopy of atomic oxygen is used to investigate the physical conditions and the dissociation mechanisms of O_2 in an air spark generated from a focused nanosecond laser. Of particular interest is investigating the different dissociation mechanisms of O_2 in regions in and around the air spark kernel, requiring combined high spatial and temporal resolution. An external cavity diode laser was used to probe absorption from the metastable level ($2s^22p^33s$, $^5S_2 \rightarrow 2s^22p^33p$, $^5P_3$) of oxygen at 777.2 nm. The air spark was generated by focusing 60 mJ pulses with a 6 ns duration at 1064 nm obtained from a Q-switched Nd:YAG laser. The experimental configuration provides a spatial resolution of 80 μm and a time resolution of 100 ns. Results show the spatial and temporal variations in the atomic oxygen density, electron density, and kinetic temperature around the air spark, obtained by fitting the measured absorption spectra to Voigt profiles. These results are compared with information obtained using emission spectroscopy, and we will discuss the differences between regions inside the air spark kernel and the surrounding regions affected by shock-wave propagation.

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(89) **Laser-induced Breakdown Spectra of Rocks at Variable Ablation and Collection Angles**; Elly Breves[1], Kate Lepore[1], M. Darby Dyar[1], Steven C. Bender[2], Robert L. Tokar[2]; [1]Mount Holyoke College; [2]Planetary Science Institute

Understanding the effects of rock surface tilt relative to laser-induced breakdown spectroscopy ablation and collection optics may aid in interpretation of standoff data such as those acquired by ChemCam during the ongoing Mars Science Laboratory mission. LIBS spectra of pressed-powder rock samples with compositions ranging from basalt to rhyolite were acquired under a Mars-analog 7-Torr CO2 atmosphere 1) while simultaneously varying ablation and collection angle from 0-60° and 2) while varying only collection angle from 0-60° keeping the ablation angle fixed. Total spectral intensity increased as ablation/collection angle or collection angle only approached normal (0°) to the sample surface. This effect was more pronounced for basalts than for dacites and rhyolites, suggesting that spectra of denser compositions with higher refractive indices have greater variability with tilt. For most samples, however, the ratio of the integrated emission line intensity to the integrated continuum intensity increased as ablation/collection angle or collection angle only increased. This effect may be driven by collection angle only, since it was observed in both geometries. In increasing the collection angle, the strongest continuum emission from the central part of the plume may be increasingly excluded from the collection area. The ratio of Si(II)/Si(I) line intensities (634 and 288 nm, respectively) was monitored as a proxy for plasma temperature variations. For the fixed ablation/variable collection angle dataset, little systematic change in Si(II)/Si(I) was observed for any sample. In the variable ablation/variable collection angle dataset, Si(II)/Si(I) increased as ablation/collection angle decreased. This suggests that plasma temperature and the proportions of species are driven by ablation beam energy density variations with ablation angle. Indeed, a similar change in Si(II)/Si(I) was observed by varying the ablation beam pulse energy directly while keeping geometry fixed. Predictions of unknown rock compositions from standard spectra may be improved by restricting the calibration set to spectra with Si(II)/Si(I) similar to the unknown spectrum. Work is in progress to explore relationships between rock composition and the magnitude of these geometry-dependent variations in LIBS signal.

(90) **Application of the Laser-Induced Breakdown Spectroscopy Technique in Steel and Metal Industry**; Vincenzo Palleschi[1,2], Emanuela Grifoni[1], Stefano Legnaioli[1,2], Stefano Pagnotta[1], Giulia Lorenzetti[1]; [1]Applied and Laser Spectroscopy Laboratory, ICCOM-CNR, Pisa, Italy; [2]National Interuniversity Consortium of Materials Science and Technology (INSTM)

The monitoring of the processes in steel and metal industry calls for techniques that are capable of measuring the composition of the metallic alloys at a distance and on moving conveyor belts. In many cases, such as, for example, in the recycling of automotive scrap, the geometry of the objects to be analyzed can vary, and surface coatings can be present. Independently on the specific application, the problems associated to the on-line analysis are often similar; in particular, the LIBS signal are in general quite low, therefore it is important the use of the most advanced experimental strategies for improving the signal/noise ratio. At the same time, we must use robust and fast methods for the analysis of the thousands of spectra that accumulate in few minutes, in the on-line applications of LIBS.

In this communication, we discuss the application of the Laser-Induced Breakdown Spectroscopy (LIBS) technique to two industrial project, funded by the European Commission, in which our Laboratory is involved, to illustrate how we faced and successfully resolved the above-mentioned problems.

(91) **Determination of Elemental Composition of Shale Rocks by Laser Induced Breakdown Spectroscopy (LIBS)**; Jinesh Jain[1], Alexander Bol'shakov[2], Hervé Sanghapi[1], Christina Lopano[1], Dustin McIntyre[1], Richard Russo[2]; [1]National Energy Technology Laboratory; [2]Applied Spectra, Inc.

Organic rich shale formations are unique in the production of natural gas because those not only serve as a source for the gas but also form the reservoir. In addition, the formations that are depleted of the gas are potential candidates for geologic storage of CO2 accompanied by an enhanced gas recovery (EGR). Both CO2 and natural gas adsorption/desorption seem to have a correlation with mineral composition of the shale rocks. Also the rocks that contain higher amount of organic material have greater ability to generate natural gas and potentially a greater capacity of CO2 storage. In order to ascertain the utility of laser induced breakdown spectroscopy (LIBS) for elemental characterization of shale rocks we analyzed the outcrop and core samples from the Marcellus Shale. It is advantageous to use LIBS because it enables a rapid in situ sample analysis with little or no sample preparation and can perform multi-element analysis including total carbon. In this study, a powdered sample was pressed to form a pellet and used for LIBS analysis. Optimization of laser pulse energy, detection gate width, and gate delay for well resolved spectra with good Signal-to-noise ratio (SNR) and signal-to-background ratio (SBR) was carried out. Careful selections of spectral lines of analytes, which do not suffer from potential self-absorption, were considered. Partial least squares regression (PLS-R) and univariate calibration curves were used for quantification of analytes. The samples were also analyzed using ICP-OES and a comparison between LIBS and ICP-OES results will be presented. Development of a LIBS method would provide rapid analysis of shale samples and would potentially benefit the depleted gas shale carbon sequestration research.

(92) **Signal Enhancement in Double-pulse LIBS of Various Metals in Relation to their Physical and Thermal Properties: A Statistical Analysis**; Patrick Skrodzki[1], Jason Becker[1], Prasoon Diwakar[1], Ahmed Hassanein[1]; [1]Center for Materials Under eXtreme Environment, School of Nuclear Engineering Purdue University

Laser-induced breakdown spectroscopy (LIBS) is a well-known analytical technique for elemental detection entailing spectral analysis of plasma formed through laser ablation. Among numerous advantages, LIBS is practical for robust, in-situ and remote analysis of various single- or multi-element materials, namely metals and dielectrics. Although the standard technique suffers from low sensitivity in comparison to other chemical detection techniques, alternative methods exist which bode signal enhancement over the standard. Double-pulse LIBS (DPLIBS) involves optimization of spatial and temporal coupling for two laser pulses in order to improve spectral emissions from plasma formed via laser ablation. However, signal improvement often varies significantly between targets of varying properties. The objective of this study is to compare signal sensitivity between conventional single-pulse LIBS (SPLIBS) and DPLIBS techniques for pure element samples of varying properties. The study investigates laser-material interaction for Nd:YAG SPLIBS and near-collinear Nd:YAG-CO2 DPLIBS scheme. Analysis involves correlation of such material properties as atomic weight, melting and boiling point, thermal parameters, and ionization potential to observed signals and plasma characteristics. Target samples chosen for varying properties include metals of different atomic number : Al, Mg, Co, Fe, Sn, Cu, Cr, Ta, and W. Signal intensity, signal-to-noise and signal-to-background as well as plasma persistence, excitation temperature and electron density constitute parameters of merit for signal as well as plasma analyses, respectively. Statistical analyses including regression and principal component analysis (PCA) suggest thermal properties parallel intensity variation while signal (describing also background of spectra) varies largely with the atomic mass of the target element between proposed SPLIBS and DPLIBS techniques. Ultimately, mechanisms involved in signal enhancement for DPLIBS are discussed in context of laser-material interaction and material physical properties regarding statistical results.

(93) **The Role of Material Properties on Emission Spectra in Nano- and Femtosecond Laser-Induced Breakdown Spectroscopy**; Jason Becker[1], Patrick Skrodzki[1], Prasoon Diwakar[1], Ahmed Hassanein[1]; [1]Center for Materials Under eXtreme Environment, School of Nuclear Engineering Purdue University

Laser-induced breakdown spectroscopy (LIBS) serves as an efficient, non-invasive method for material analysis through ablation of target materials with single or multiple lasers followed by investigation of the spectral characteristics of materials plasmas. Laser-material interaction plays a critical role in the evolution of LIBS plasma which ultimately affects the LIBS spectral response. This study demonstrates the role of sample material properties on both nanosecond and femtosecond LIBS signal intensity, continuum emission, species persistence, plasma temperature, and plasma electron density. This information is crucial in optimizing the parameters for improvement of LIBS signal for various applications. For nanosecond LIBS, the plasma was generated using a 1064 nm, 9 ns full width half maximum (FWHM) laser pulse from Nd:YAG laser. For femtosecond LIBS, the plasma was generated using an 800 nm, 40 fs FWHM laser pulse from a Ti-Sapphire laser. Nine different metals were selected for this study, ranging from low- to high-Z materials. These metals were selected based on their varying atomic mass, melting point, boiling point, ionization potential, and thermal parameters. Signal intensity, S/N, S/B, species persistence, and continuum emission were determined for all samples and correlated to thermal properties of the metal samples. Principal component analysis and dendogram analysis were performed to identify dominating material properties influencing nanosecond and femtosecond LIBS plasma emission. Detailed mechanisms of laser-material interactions and influence of material properties on LIBS plasma are discussed.

(94) **Protonation Effects of Alumina Surface on the First Electronic Transition of Liquid Water Studied by Far-Ultraviolet Spectroscopy**; Takeyoshi Goto[1], Yukihiro Ozaki[1]; [1]Kwansei Gakuin Unversity

Liquid water shows high absorptivity in the far-ultraviolet region (FUV, 120-200 nm). It is very challenging to measure FUV absorption spectra of liquid water. Our research group has developed an attenuated total reflection (ATR)-FUV spectroscopy with an alumina reflection prism.

However, the penetration depth of evanescent wave of the probe light (dp) is shorter than 50 nm owing to short wavelength of the FUV light; therefore, the measured ATR-FUV spectrum of liquid water depends strongly on the alumina surface. [1]

The first electronic transitions (A←X) of pure water and 1 M sulfuric acid solution on α-alumina prism were studied using the ATR-FUV spectroscopy with variable angle (VA) method. The A←X bands of pure water and the sulfuric acid solution observed around 8.4 eV (147 nm) were blue-shifted, and their absorptivities were increased, as the incident angle of the probe light was larger. Because the increase in the incident angle accompanies with shortening the dp length, these spectral changes were associated with the increase in the interfacial phase contribution to the measured VA spectra. Liner decomposition of the VA spectra based on Lambert's law revealed the A←X bands of the bulk water (distance from the alumina surface > 2 nm) and interfacial water (<2 nm). For both the samples, the A←X bands of the interfacial phases were markedly blue-shifted by ~0.25 eV and red-tailed relative to the bulk phases. These electronic state differences between the bulk and interfacial phases mainly arose from the deformation of hydrogen-bond (H-bond) structure and energy of water in the interfacial phases by the alumina surface. The interfacial spectrum of the sulfuric acid solution shows that the A←X bandwidth was much broader than that of pure water, because the electric double layer formed by the protonated surface aluminol groups increased the diversity of H-bond in the interfacial phase. In conclusion, this study revealed how A←X transition of liquid water was affected by the alumina substrate due to the protonation states of the surface aluminol groups by newly developed VA-ATR-FUV spectroscopy.

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(95) **Electronic Transitions of Hydrated Amino Acids in the Wavelength Region 145−300 nm Studied by Far-Ultraviolet Spectroscopy**; Takeyoshi Goto[1], Yukihiro Ozaki[1]; [1]Kwansei Gakuin Unversity

Many chemical compounds show high absorptivities in the far-ultraviolet region (FUV, 120-200 nm); therefore, it is very challenging to measure FUV absorption spectra of liquid samples. Our research group has developed an attenuated total reflection (ATR) method, which makes it possible to measure easily FUV spectra of liquid samples by employing an alumina reflection prism.

In this study, valence electronic transitions of 20 naturally occurring amino acids in aqueous solution were studied with ATR-FUV spectroscopy in the wavelength region from 145 nm to 300 nm. [1] From the measured ATR spectra of aqueous sample solutions, the intense aqueous solvent absorption was subtracted using a modified Kramers-Kronig transformation method in order to reveal the electronic transitions of the hydrated amino acids. The FUV spectra of the amino acids varied with the protonation states of the backbone and side-chain molecular structures. The primary chromophore of glycine (Gly) is the π-π* transition of the carboxyl group observed in the wavelength region around 160-170 nm. The π-π* bands of Gly depended on the protonation states of the amine and carboxyl groups. The conversion from the protonated form to the zwitterion made the π-π* band split into one blue-shifted by 12 nm and the other red-shifted by 11 nm. The amino acids whose side-chains possess the conjugated π electron groups; histidine, phenylalanine, tryptophan, and tyrosine, showed the peculiarly intense and characteristic spectral patterns. The FUV spectra of cysteine (Cys) showed that the deprotonation of the thiol group caused the absorptivity intensely increased and spread to the longer wavelength region. The n-σ* transition of the thiol group in Cys was much more intense and widely spread than those of hydroxyl groups in serine and threonine. The electronic transitions of the hydrated amino acids in the FUV region were subject to the intra- and inter-molecular electronic interactions of the solute-solute as well as the solute-solvent, and those are essential factors to elucidate FUV photochemical processes of the amino acids in aqueous solution.

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(96)) **Thermal Analysis of Thermally Reversible Gels Made of a Bio-based, Biodegradable Polymer**; Brian Sobieski[1], Liang Gong[1], John Rabolt[1], Isao Noda[1], Bruce Chase[1], Steve Aubuchon[1,2]; [1]University of Delaware; [2]TA Instruments

A combination of thermal gravimetric analysis (TGA) and modulated differential scanning calorimetry (MDSC) was used to analyze the thermal properties of a series of thermal-reversible gels made of a solution of dimethylformamide (DMF) and bio-based, biodegradable poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (Nodax¬TM). Gelation of the polymer solution is due to physical interactions between the solvent and solute and phase instability, not from chemical crosslinking. MDSC heat/cool results indicate that the hydroxyhexanoate (HHx) content of the polymer, not the polymer solution concentration, determine the gelation temperature. The results also show that gelation is a kinetic process, meaning it will take longer to gel based on both the HHx content and solution concentration. Because the lower HHx content polymers are more crystalline, these results would suggest that gelation of these solutions may be due to formation of crystalline domains. This result is further supported by the appearance of an endotherm before the solubility exotherm, possibly signifying a crystallization process before solubility occurs. It is important to note that these peaks do not correspond to any glass transition, crystallization, or melting peaks in the pure polymer. The MDSC results have yielded a starting point for understanding the gelation of this polymer in solution. Further analysis will be conducted using a variety of characterization techniques, such as dynamic light scattering, infrared spectroscopy, and atomistic modeling to develop deeper insight into the interactions that govern thermal-reversibility in this polymer solution.

(97) **Temperature Dependence of FUV Spectra for Aqueous Solutions of Alkali Halide to the Freezing-point of Eutectic**; Yusuke Morisawa[1], Yuka Nishikawa[1], Akifumi Ikehata[2]; [1]Kinki University; [2]NFRI, NARO

We are interested a hydration of ions in aqueous solutions of alkali halide because that can influence the taste and health. For example, sodium chloride is a principal components of common salt, and prime example of sodium halide. Its aqueous solution has to control for the taste of the food and health maintenance of human body. An intense absorption band originated from charge transfer from anion to solvent (CTTS) was found around 175-300 nm for the solutions. Particularly for the CTTS band of Cl- which is overlapped with the first absorption band of water, was difficult to measure by using conventional spectrometers. In recent years, however, it was found that the band can be measure in wide range of the concentration and temperature by using an attenuated total reflectance spectroscopy in far-ultraviolet (ATR-FUV).[1] In this presentation we will report the temperature dependence of FUV spectra of aqueous solutions of NaF, NaCl, NaBr, and NaI from room temperature to below the freezing point of eutectic. We found that the FUV spectra sharply changed between liquid phase and eutectic. We will discuss the effect of

the environmental change for the electronic states of the hydrated anions and alternation between phase transitions. [1] Ozaki, Y.; Morisawa, Y.; Ikehata, A.; Higashi, N., Applied Spectroscopy, 2012, 66, 1-25.

(98) **Computing Molar Extinction Coefficient of Capsaicin Using Absorbance Spectroscopy in the Visible Range**; Abraham Lopez[1], José Javier Báez Rojas[1], Jorge Castro Ramos[1], Karen Esmonde-White[2]; [1]National Institute of Astrophysics Optics and Electronics; [2]University of Michigan, Medical School

All materials has optical properties that depend of their composition and chemical structure, due this materials can absorb or scatter light. Also this materials could be active molecules in natural products and have medicinal, antibacterial or anti-fungal properties. One of this natural active molecules is the capsaicin present in hot peppers or chilies has analgesic properties and is the principal active constituent in pepper spray. The amount of capsaicin in peppers depend of the variety of this and where and how is cultivated. The most used techniques includes the HPLC that is time consuming, or new research propose different models with the same accuracy. A commercial oleoresin for alimentary pure capsaicin was examined as-received. Dilutions were prepared by adding capsaicin in pure ethanol in for final concentrations range of 0.1% to1% [W/V], spanning the estimated amount in chilies. Absorption spectra were collected using USB400 spectrometer from OceanOptics© plugged by a bifurcated fiber probe to cuvette holder and this to a source with bandwidth from 360nm to 1100nm, this experimental setup was used to identify the spectral region in which capsaicin absorbs light. Using the Lambert-Beer law, we calculated in visible range (500-700nm) by simple linear regression analysis was gotten the molar extinction coefficient. Absorption spectra show that dilution has an effect on the optical behavior of capsaicin. Throughout the concentration range, capsaicin spectra had a broad maximum absorption peak between 500nm to 600nm with a small peak at 660.6670nm. Our results suggest that capsaicin has optical absorption properties can be modeled using the Lambert-Beer law. We demonstrated feasibility of optical absorption in the visible spectral region for quantify capsaicin. Capsaicin visible spectra contained strong bands which were used to measure the molar extinction coefficient. Our work complements previous work to measure capsaicin optical properties in the UV spectral region and establishes a frame work for future studies comparing our visible optical method to chromatography. Eventually, a portable spectrophotometer may provide a non-destructive and rapid measurement outside of the laboratory, enabling capsaicin quantification throughout the chillie's life.

(99) **Measuring Thermal Properties of Oilseeds using Unilateral Nuclear Magnetic Resonance sensor**; Maria G. A. Carosio[1], André de S. Carvalho[, 2], Luis F. Cabeça[3], Luiz A. Colnago[1]; [1]EMBRAPA Instrumentation; [2]Institute of Chemistry of São Carlos; [3]Federal Technological University of Parana

Portable, low cost unilateral NMR (UNMR) sensor, composed of a unilateral magnet and a surface coil probe, has been used to remotely measure chemical and physical properties of samples. In this paper we are showing that we can use the UNMR sensor to measure the temperature and thermal diffusivity of intact oilseeds and oilseed in the soil. A TD-NMR method is based on the exponential dependence of transverse relaxation time (T_2) of vegetable oil with temperature. The T_2 measurements were carried out in a homemade UNMR 0.6T (24MHz for ^{1}H) sensor and a Tecmag console. The analyses were performed with CPMG pulse sequence using tau=50 μs, 3000 echoes, π/2 pulse=1.5 μs, π pulse=3 μs, acquisition time of 64μs, last delay of 500ms and 100 scans. With a calibration curve the temperature of intact seeds can be measure in a few seconds using T_2 value. When a seed is immersed in a low temperature bath, its temperature decays exponentially, dependent on thermal diffusivity and sample dimension. Temperature is an important parameter used in the design and optimization of heating and cooling process in food industry. Thermal diffusivity (λ) of the macadamia nuts were calculated by the time evolution of oilseeds temperature, measured by TD-NMR. The λ of the nuts were approximately of 0.55 $x10^{-7}$ m^2 s^{-1}, similar to these values measured by thermocouple method (0.60 $x10^{-7}$ m^2 s^{-1}), Dickerson method (0.80 $x10^{-7}$ m^2s^{-1}) and reported in literature for nuts and oilseeds. We thank FAPESP (2009/09734-1) and EMBRAPA Instrumentation for support.

(100) **Moving Window Two-Dimensional Correlation Spectroscopy of the Early Stage Crystallization of Polyethylene**; Ying Jin, Anthony Kotula[1], Angela Hight Walker[1], Kalman Migler[1], Young Jong Lee[1]; [1]National Institute of Standards and Technology

Two-dimensional correlation spectroscopy and the moving window method were performed to investigate phase transition processes of n-alkane and polyethylene. The well-studied n-alkane was first performed the concept and followed by PE. The mesomorphic phase in PE is determined and the phase transition temperatures are obtained quickly after very simple calculations of the temperature-dependent Raman spectra. The new findings were future supported by 2DCOS results. This work helps understanding the crystallization pathway of polyethylene (PE) which is critical to the processing, properties and ultimate performance of the polymeric materials.

(101) **Spectroscopic Markers for Uranyl Phosphates: A Vibrational Study**; Dale L. Perry[1], Nataliya Kalashnyk[, 2], Eric Faulques[3], Florian Massuyeau[3]; [1]Lawrence Berkeley National Laboratory, University of California, Berkeley; [2]Institut Matériaux Microéléctronique Nanosciences de Provence (IM2NP), Université d' Aix-Marseille, UMR CNRS 7334; [3]Institut des Matériaux Jean Rouxel, Université de Nantes, UMR CNRS 6502

Optical spectroscopic fingerprints of several uranium phosphates relevant for environmental sustainability have been investigated. The studied minerals contain uranium(VI) cation coordination centers linked to phosphate functional groups and water molecules. Easy and fast identification of these minerals in their bulk state is possible by using either Raman, infrared, optical, or photoluminescence spectroscopy. Simple density functional theory vibrational modeling is presented to identify the main vibrational lines. In the solid state, vibrational frequencies of the uranyl ion and room-temperature energies of the absorption and emission vary from one sample to another due to their sensitivity to the uranium coordination sphere. Therefore, direct speciation of uranium in the minerals by photon-induced spectroscopic techniques can be operative without subsequent sample dissolution, having a strong potential to substitute conventional time-demanding chemical analyses employed in mineralogy. The affordable methods of spectroscopy described in this work can be readily employed in optical remote sensing to identify uranyl species in groundwater, soil, or other geologic samples and in biological specimen for the purpose of tracking radionuclide transport, pollution, and soluble uranium remediation by uranyl phosphates precipitation.

(102) **Elemental Characterization of Glass Tesserae via X-Ray Fluorescence Spectrometry**; Andrew Sparks[1], Mary Kate Donais[1]; [1]Saint Anselm College

Abstract: Mosaic tesserae retrieved from an archeology dig in Orvieto, Italy were analyzed via X-ray Fluorescence (XRF) spectrometry. The glass tesserae were categorized visually according to their colors: Dark Green, Light Green, Glass Green, Dark Blue, Teal, Aqua, Light Blue, Red, Black, and Glass Blue. Previous research within our group1 permitted the observation that the Red tesserae were significantly different in

composition than the others and would be an interesting subject for further investigations. Additional analyses of the Red tesserae were performed, and the spectral data was compared to the other tesserae colors in an attempt to better characterize the cause of the significant differences. Further analyses of other tesserae colors were also conducted to better understand their composition.

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(103) **First Electronic Transition of Water Molecules in Binary Solutions Studied by Far-Ultraviolet Spectroscopy**; Kodai Kishibe[1], Takeyoshi Goto[1], Hiroto Tanaka[1], Yukihiro Ozaki[1]; [1]Graduate School of Science and Technology, Kwansei Gakuin University

Liquid water is essential to biological systems, and it is important to reveal its physicochemical features. Far-ultraviolet spectroscopy (120-200 nm) provides electronic state information including σ and π electron transitions. The first electronic transition ($A \leftarrow X$) of liquid water is observed around 150 nm, assigned to n-σ^* transition [1]. Because the absorptivity of liquid water in the FUV region is very intense (~10^5 cm^{-1} at 150 nm), an attenuated total reflection (ATR) method with a sapphire prism has been developed for FUV spectral measurements of liquid water [2]. This study explores the $A \leftarrow X$ transitions of water molecules in hydrophobic media using water/ 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) binary solutions. The FUV absorptivity of HFIP in the measured region is very weak and negligible. The important advantage of using HFIP as a sample is that only a broad band for water and interaction was observed.

The ATR-FUV spectra of water/ HFIP binary solutions (mole fraction (X_{HFIP}): 0-1) were measured at 25.0 ± 1.0 ℃. The molar absorption coefficient (ε) spectrum of each solution was determined with Karmers-Kronig transformation. The $A \leftarrow X$ transition energies of the binary solutions distinctively varied with HFIP concentration. The $A \leftarrow X$ bands were shifted to shorter wavelength at $0 < X_{HFIP} < 0.2$ and to longer wavelength at $0.2 < X_{HFIP} < 0.69$. The $A \leftarrow X$ transition energy profile along X_{HFIP} was similar to molar enthalpy excess of mixing at 298 K [3]. This indicates that the changes of the $A \leftarrow X$ transition energies of water molecules in hydrophobic media were mainly governed by the ground electronic state energy and that the hydrophobic interaction of HFIP enhanced the hydrogen bonding among water molecules.

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(104) **Thermal Behavior and Lamella Structures of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Studied by Low-Frequency Raman, Terahertz Spectroscopy, and Small Angle X-ray Scattering**; Dian Marlina[1], Mengfan Wan[1], Koh Yoshida[1], Hiromichi Hoshina[2], Harumi Sato[3], Yukihiro Ozaki[1]; [1]Graduate School of Science and Technology, Kwansei Gakuin University; [2]RIKEN; [3]Graduate School of Human Development and Environment, Kobe University

Nowadays, the study and development of biodegradable polymer which is more compatible with the environment condition gained more attention. Poly(3-hydroxybutyrate) (PHB) is one of the most famous biodegradable polymers which has been widely used for various applications. In order to reduce the highly crystalline of PHB, it has been copolymerized with poly(3-hydroxyvalerate) (PHV), giving rise to P(HB-*co*-HV) copolymers. The PHB and P(HB-*co*-HV) crystallization behaviors are determined by weak inter-molecular hydrogen bond among polymer chains. Since Low frequency vibrational modes (below 300 cm^{-1}) originate from inter- and intra-molecular interactions, low-frequency Raman and Terahertz spectroscopies can be powerful tool to clarify the development of higher order structure of the polymer. X-ray scattering is the principal method used for obtaining information about lamellae thickness in a polymer crystal structure. In this study, we have investigated thermal behaviors of P(HB-*co*-HV) with several HV contents in the low-frequency vibrational mode by using Raman and Terahertz Spectroscopies. Furthermore, SAXS measurement also was observed to deeply understand about lamella thickness of all samples.

Film samples of P(HB-co-HV) (HV = 0, 9, 15, 21 mol%) were prepared by hot-pressed method. In the 50-150 cm^{-1} vibrational region, there are three Raman peaks, while Terahertz spectra only have two peaks. These Raman and Terahertz peaks correspond to the inter-molecular vibrational mode of C-H···O=C hydrogen bonds and intra-molecular vibrational mode of spring like motion in the helical structure. Generally, Terahertz spectra clearly show the differences among all samples. In the room temperature, the absorbance ratio of Terahertz peaks decrease as increasing HV contens. During heating process, the two peaks absorbance decrease gradually and disappear completely at melting temperature. Moreover, C-H···O=C hydrogen bonds peak position shift to lower wavenumber. These thermal behaviors indicate the PHB crystallinity decrease due to the hydrogen bonds deformation as temperature increases. Furthermore, detailed thermal behavior investigation of HV contents to the PHB crystallinity and X-ray measurement results will be shown in the current day.

(105) **Non-hydrolytic Processing of Transition Metal-Doped TiO2 Nanostructures for Photocatalytic Applications**; Swati Naik[1], Gabriel Caruntu[1]; [1]Central Michigan University

Sol-gel is a versatile and well-established solution-based technique for synthesis of TiO2 nanoparticles, but the major problem in this method is the uncontrolled reaction rate involving hydrolysis and condensation of the titanium alkoxide precursor. Therefore, non-hydrolytic approaches alleviate the spontaneous precipitation of the titanium precursor along with allowing the control over the morphology of the resulting nanoparticles by varying the experimental conditions. Although TiO2 has been known as possessing excellent photocatalytic properties, its relatively wide band gap (Eg = 3.2eV), restricts drastically its performance since the photocatalytic activation of TiO2 occurs only in the UV range, which represents only 5% of the potentially usable solar radiation. To efficiently solve this problem and keep unaltered its other physical properties, the bandgap of TiO2 can be engineered upon chemical doping with various cationic and/or anionic species.

In this project, we studied the effect of chemical doping of nanostructured TiO2 with various transition metal ions, namely Fe, Co, Ni, Cr and Mn. To this end, a modified non-hydrolytic sol-gel method was used. Interestingly, a transition from anatase to rutile phase TiO2 was observed upon varying the annealing conditions. Various techniques, including diffraction (X-ray and electron), vibrational spectroscopy (Raman, UV-vis, FTIR) and surface analysis have been used to characterize structurally, electronically and morphologically the M-doped nanostructured TiO2. The performance of the resulting nanopowders in the photocatalytic degradation of methyl orange on doped TiO2 was also investigated in detail.

(106) **Coupling Single Molecule Spectroscopy and Electrochemistry in Zero-Mode Waveguides**; Lawrence Zaino, Dane Grismer, Donghoon Han, Paul Bohn[1]; [1]University of Notre Dame

Single molecule spectroscopy remains a powerful technique for studying single molecule dynamics. Certain molecules, such as flavin mononucleotide, are fluorescent in the oxidized state but do not emit in the reduced state. This allows for fluorescence to be used as a reporter for the redox state of the molecule. Electron transfer dynamics of single molecules have been studied under diffusive control at single occupancy conditions. The zero-mode waveguide (ZMW) serves as the working electrode for potential control of the molecules. By utilizing a ZMW to confine the optical field and as the working electrode, distinct differences in fluorescence can be seen at $E_{eq} > E_{app}$ and $E_{eq} < E_{app}$. As expected, when the ZMWs are under single occupancy conditions, changes in fluorescence intensity are closely correlated to applied potential. This has been shown in constant potential, potential sweep and potential step experiments. Under potential sweep conditions at low scan rates, the fluorescence intensity indicates intermediate redox states, which may be indicative of a flavosemiquinone species.

(107) **Measurement of Spatially Confined Nanoclusters of Porphyrins Using Conductive-Probe Atomic Force Microscope**; Xianglin Zhai[1], Neepa KuruppuArachchige[1], Pedro Derosa[2], Jayne Garno[1]; [1]Louisiana State University; [2]Louisiana Tech University

Protocols for patterning porphyrin nanodots on Au(111) were developed based on protocols with immersion particle lithography. Porphyrins with and without a central metal ion, 5,10,15,20-tetraphenyl-21H,23H-porphine (TPP) and 5,10,15,20-tetraphenyl-21H,23H-porphine cobalt(II) (TPC) were patterned using steps of solution immersion of a gold substrate masked with silicon mesospheres. Individual nanodots of porphyrins were spatially isolated into well-defined, nanoscale arrangements directed by a template film of a nanopatterned thiol monolayer. A surface mask of silica mesospheres was used to prepare nanopores within an alkanethiol matrix film. Nanopores within a methyl-terminated alkanethiol matrix were backfilled with porphyrins by an immersion step. By controlling the concentration and immersion interval, nanodots of porphyrins were generated. The porphyrin nanostructures exhibited slight differences in dimensions at the nanoscale, to enable size-dependent measurements of conductive properties. The conductivity along the vertical direction of the nanodots was evaluated using conductive probe-atomic force microscopy (CP-AFM). Changes in conductivity were measured through the TPP and TPC nanodots. The TPP nanodots exhibited semi-conductive behavior. The TPC nanorods exhibited behavior typical of a conductive film. These studies evaluate the applicability of particle lithography for preparing surface platforms for the measurement of conductive properties at the nanoscale for porphyrins.

(108) **Turn-On Fluorescence as a Strategy for Monitoring the Catalyzed Reduction of Nitrite by Pd-on-Au Nanoparticles**; Anthony Stender[1], Emilie Ringe[1]; [1]Rice University

Fluorescence detection techniques are highly valued for their high sensitivity as well as for their selectivity. Turn-on fluorophores comprise a unique class of organic molecules that exhibits relatively weak or no fluorescence in its native form. However, upon reacting with a specific target analyte, the fluorophore instantly becomes highly fluorescent. As a result, turn-on fluorophores can be used to detect trace amounts of analyte without serious concerns regarding fluorescence from non-reacting fluorophores. Turn-on probes can also be exploited to time the appearance of an analyte in a chemical reaction.

The majority of current turn-on fluorophores have been designed and tested for the detection of metal ions or organic functional groups in biological systems. Expanding the current catalog of turn-on fluorophores for non-biological reaction chemistry would prove extremely beneficial for monitoring reaction products and short-lived intermediate species, particularly for the field of catalysis.

In this study, we exploited turn-on fluorescence to monitor the liquid-phase catalyzed reduction of nitrite to nitrogen gas and ammonia. Nitrates are a common ingredient in fertilizer products, and they are sometimes found at elevated levels in drinking water, where they slowly convert into nitrite. Because of health concerns related to both nitrates and nitrites, researchers are attempting to find a catalyst that will effectively reduce these anions to nitrogen gas. Palladium-on-gold nanoparticles were previously proven to be an effective catalyst in reducing nitrite to nitrogen gas; however, NH3 and NH4+ are also created as byproducts. Naphthalene-2,3-dicarboxaldehyde (NDA) is a well-known turn-on fluorophore typically used in biological settings for the detection of primary amines. NDA also requires cyanide or thiols to fluoresce, both of which are known catalyst poisons. In our experiments, NDA was utilized to detect the formation of NH3/NH4+ during the reduction of nitrite. Experiments were performed under varied reaction conditions (e.g. pH, concentrations, etc.) to determine the source of NH3/NH4+ production. In order to elucidate important nanoscale factors, we have scaled down from the bulk scale to the single-particle level through the use of fluorescence, dark field, and electron microscopy.

(109) **Studies of Electronic States of CNT/Rubber Nanocomposites by using Attenuated Total Reflectance Spectroscopy in the Ultraviolet Region**; Yusuke Morisawa[1], Kenta Kobashi[1, 2], Ichiro Tanabe[2], Harumi Sato[3], Takeyoshi Goto[2], Yukihiro Ozaki[2]; [1]Department of Chemistry, School of Science and Engineering, Kinki University; [2]Department of Chemistry, School of Science and Technology, KwanseiGakuin University; [3]Graduate School of Human Development and Environment, Kobe University

Carbon/rubber nanocomposites have been attracted much attention as novel materials which have a great property and/or new function. The nanocomposites are made by mixing nano carbon materials such as carbon nanotubes (CNT) or graphene as a filler to a rubber. Interactions between rubbers and fille of those nanocomposites have been investigated by using vibrational spectroscopy, IR and Raman. The electronic states however, have been studied rarely since absorption of rubbers are observed in UV region with the much strong intensity. Recently we have developed an attenuated total reflection spectrometer in the UV region (ATR-UV). By using the ATR-UV, we achieved spectra measurements of several kinds of CNT/rubber composites in the UV region. Pure natural rubber (NR) and CNT/NR nanocomposites were investigated. The amount of CNT was changed from 0.5, to 10 parts by weight per hundred parts of rubber (phr). Absorption band due to p-p* transition of NR shifted to longer wavelength in CNT/NR than pure NR, which means that the transitional energy of NR decreased by interaction between NR and CNT. CNT/SBR nanocomposites have also been studied by ATR-UV, and show the band shift in p-p* transition of SBR too. These results give the information of electronic states of carbon/rubber nanocomposites.

(110) **Investigation of Electronic States of Nano Carbon/Polymer Nanocomposites by Attenuated Total Reflectance-Ultraviolet Spectroscopy**; Kenta Kobashi[1], Ichiro Tanabe[1], Yusuke Morisawa[2], Harumi Sato[3], Takeyoshi Goto[1], Yukihiro Ozaki[1]; [1]Graduate School of Science and Technology, Kwansei Gakuin Univ; [2]Graduate School of Science and Technology, Kinki Univ; [3]Graduate School of Human Development and Environment, Kobe Univ

Nano carbon/polymer nanocomposites have been attracted much attention as new functional materials. The nanocomposites are made by mixing nano carbon materials; carbon nanotubes (CNT) or graphene, for example; as a filler to a polymer. In this study, by using our original far-

ultraviolet (FUV, <200 nm) spectrometer that employs attenuated total reflectance (ATR) system, we achieved spectra measurements of several kinds of polymers in the FUV region. Spectra of carbon nanotubes and graphene in black powder states were also successfully measured as a very broad band in near-ultraviolet (NUV, 200-400 nm) region. In addition, in order to reveal electronic states of carbon/polymer nanocomposites, we measured FUV-NUV spectra of two nano carbon/polymer, CNT/poly(dimethylsiloxane) (PDMS) and CNT/ ethylene propylene diene rubber (EPDM), nanocomposites by ATR spectroscopy. Spectra of pure polymer of PDMS and CNT/PDMS nanocomposites were measured. The amount of CNT was changed from 0.02, to 0.06 phr (phr = parts by weight per hundred parts of rubber). For the CNT/PDMS with 0.02 and 0.04 phr, an absorption around 190 nm degreased. On the other hand, for the CNT/PDMS with 0.06 phr, band intensity around 190 nm also decreased, while band intensities around 175 nm and 150 nm increased. These results indicate that the electronic states of PDMS in nanocomposite changed by effects of the CNT filler. We obtained spectra of pure EPDM and CNT/EPDM nanocomposites, and these spectra also showed that electronic states of EPDM in nanocomposite were changed by CNT filler. In this case, we calculated ratios of absorbance at 190 nm and 268 nm to 150 nm. These two ratios were plotted against the amount of CNT (from 0.5 to 10 phr). Both plots changed suddenly at 1 phr, which means that electronic states of CNT/EPDM changed at 1 phr. These investigations provide new insights about the electronic states of nano carbon/polymer nanocomposites and lead to the developments of high quality polymer nanocomposites.

(111) **Structural and Magnetic Properties of Cobalt Substituted Magnetite/Ferrihydrite Composites**; Dale L. Perry[3], K. I. Camacho[1], N. Pariona[1], Arturo I. Martinez[1], E. Baggio-Saitovitch[2]; [1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Unidad Saltillo; [2]Centro Brasileiro de Pesquisas Físicas, Río de Janeiro; [3]Lawrence Berkeley National Laboratory, University of California, Berkeley

The magnetite/ferrihydrite composites were characterized by X-ray diffraction, Mössbauer spectroscopy, transmission electron microscopy (TEM), magnetometry, and first-order reversal curve (FORC) diagrams. It was found that the concentration of cobalt dications plays an important role on the structural and magnetic properties of the products. For all the samples, magnetic measurements yielded non-saturated wasp-waist hysteresis loops at a maximum field of 12 kOe. Additionally, the FORC diagrams revealed the components of wasp-waist hysteresis loops which consist of mixtures of single domains and superparamagnetic particles. TEM analysis revealed the morphology and structure of the composites which consists of strongly agglomerated, poorly crystallized ferrihydrite nanoparticles attached to cobalt substituted magnetite nanoparticles formed in the boundaries of the agglomerates.

(112) **Evaluation of Different Strategies for Laser-Induced Aerosol Mixing and Filtering in Order to Improve the Capabilities of LA-ICP-MS.**; Jorge Pisonero[1], David Blanco[2], Natalia Beltrán[2], Nerea Bordel[1]; [1]Department of Physics, University of Oviedo; [2]Department of Manufacturing Engineering, University of Oviedo, Campus of Gijón

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) is a powerful technique for microanalysis that can provide 2D-imaging, depth profiling, and elemental and isotopic ratio analysis. In this sense, it has been demonstrated that the use of low laser frequencies enhances the capabilities of this technique for some applications. For instance, elemental fractionation is minimized due to reduce melting processes, and aerosol mixing from consecutive laser shots is avoid increasing the spatial resolution [1]. However, low laser frequencies induce transient ion signals due to the rapid wash-out times of the new ablation cell that might affect the measurement of elemental and isotopic ratios and the interpretation of depth profiles.

In order to solve these problems while operating the laser at low repetition rates, different devices have been developed. For example, the squid smoothing device was developed by Laurin Technic. This device consists of a tube (through which the laser-induced aerosol is transported) that is divided into ten tubes with different lengths to be finally joined again into one single tube. In this way, after recombination of the various portions of the aerosol in a single line, the signal is smoothed. Alternatively, Tunheng and Hirata [2] developed two types of stabilizer (blaffled type and cyclone-type) that mix the pulsed-flow and reduce the laser-induced shock wave, achieving a stable signal. Moreover, Z. Hu et al. [3] proposed a device, consisting of a copper cylinder filled with steel wire.

In this study, different smoothing strategies are preliminary evaluated making use of additional gas flows, expansion of the aerosol and/or splitting of the aerosol, in order to achieve a smooth ion signal that minimally affects the wash out times. Moreover, it is investigated the effect of magnetic field gradients applied to the laser-induced aerosol. In particular, permanent magnets and electromagnets are investigated to filter the laser induced aerosol.

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(113) **Laser Ablation-ICP-Mass Spectrometry : Sensitive, Rapid and User Friendly Analytical Technique of Trace Metals for Both Geochemical and Biochemical Samples**; Takafumi Hirata[1]; [1]Kyoto University

Laser ablation sampling technique combined with ICP-mass spectrometry (LA-ICPMS) has become one of the most sensitive and versatile analytical tool for elemental imaging for minerals, fossils or various biological tissue samples. Laser sampling under the atmospheric pressure conditions can provide high analytical capability to accept large-sized samples ranging from 10 μm to 25 mm with the optimum spatial resolutions. With the 75 μm laser beam, from major elements (e.g., C, Na or Ca) to trace-elements (e.g., Ni, Se or Mo) can be monitored. With newly developed square-shaped laser beam can provide flat sample surface even after the laser ablation. After the survey scan using the square-shaped laser pit, elemental imaging with high-spatial resolution can be achieved by the laser ablation using the 5 – 10 μm pit sizes without any additional sample preparation procedures. With the present analytical protocol, multiple elemental images with different spatial resolution can be obtained. With the finely controlled energy fluence of the laser, only the biochemical tissue samples, placed onto the glass substances, were preferentially ablated whereas no laser ablation was made on the glass substrate (soft ablation). This is because the energy fluence employed for

the laser ablation of the biochemical samples was significantly lower than the energy threshold for the glass materials. With the preferential and total ablation of only biochemical samples, we can manage to obtain the homogeneous depth and volume of the sampling.

To take a full advantage of the quantitative imagings, we have developed new software to obtain the imaging data from the repeated line profiling analysis. With the present software, possible correlation among the analytes can be easily evaluated from only the specific area, or lines. Moreover, possible contamination or secondary moving of the elements can also be tested. Another advantage of the present software is to accept almost all the time-profiling information achieved by various analytical techniques. Analytical features achieved by the combination of the LA-ICPMS technique and the present software will be demonstrated.

(114) **State of the Art in Bio-Imaging by LA-ICP-MS**; Philip Doble[1]; [1]UTS

Bio-imaging by LA-ICP-MS finds numerous applications in diverse areas of investigations such as paleontology, neurodegenerative disorders and basic mechanistic biology; and is now considered a key platform technology for Metallomics research. This presentation will highlight some recent applications of bio-imaging by LA-ICP-MS, as well as recent innovations in antibody metal tagging protocols that have facilitated the development of a universal imaging approach for the simultaneous measurement of elements and molecules.

(115) **Development of a Laser Ablation−Inductively Coupled Plasma−Mass Spectrometry Cell and Deconvolution Approaches for Fast High Resolution 3D Imaging**; Stijn J. M. Van Malderen[1], Johannes T. van Elteren[, 2], Frank Vanhaecke[1]; [1]Department of Analytical Chemistry, Ghent University, Belgium, [2]Analytical Chemistry Laboratory, National Institute of Chemistry, Ljubljana, Slovenia, [3], [4]

Laser Ablation−Inductively Coupled Plasma−Mass Spectrometry (LA-ICP-MS) is a powerful tool to visualize the spatial distribution of nuclides on a μm-level over a solid sample in a wide variety of application fields. Practically all LA-ICP-MS imaging studies focusing on high lateral resolution produce an elemental map by ablating the material under investigation according to a grid of distinct positions, and relating the isolated response of each ablation position to a corresponding pixel. The lateral resolution achievable with this approach is limited by the beam waist dimensions of the focused laser beam, which is either restricted by the diffraction limit in the far field or the sensitivity available for the targeted nuclides. The speed of data acquisition and sensitivity are often limited by the dispersion of the aerosol. This fundamental study pursuits a combined strategy of ablation cell development and (pre- and post-) acquisition methodology to overcome these limitations, thus enhancing the sensitivity, lateral resolution and throughput capabilities of LA-ICP-MS beyond state-of-the-art[1]. The laser ablation cell and transport assembly developed[2] in-house are characterized by a fluid dynamic design favourable to aerosol transport, enabling discrete pulse responses, resolved via signal deconvolution, at laser pulse repetition rates up to 200-300 Hz. Overlapping ablation positions inside a 2D or 3D sampling grid results in an image or volume in which the nuclide distribution is artificially convolved with the point spread function (PSF) of the LA system. Image deconvolution was applied to reconstruct the nuclide distribution such that features below the physical size of the laser beam become resolved. The PSF is derived from atomic force microscopy topography maps of single pulse craters , allowing the approach to account for the laser beam shape and aberrations, and the laser-solid interaction, which in turn enhances the lateral resolution of the reconstructed volume.

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(116) **Femtosecond Laser Ablation-Based Mass Spectrometry: An Ideal Tool for Stoichiometric Analysis of Thin Films on the Nanoscale**; Nicole LaHaye[1,2], Jose Kurian[, 3], Prasoon Diwakar[2], Lambert Alff[3], Sivanandan Harilal[1]; [1]Pacific Northwest National Laboratory; [2]Purdue University; [3]Technische Universitat Darmstadt

An accurate and routinely available method for stoichiometric analysis of thin films is a desideratum of modern materials science where a material's properties depend sensitively on elemental composition. We thoroughly investigated femtosecond laser ablation inductively coupled plasma mass spectrometry (fs-LA-ICP-MS) as an analytical technique for determination of the stoichiometry of thin films down to the nanometer scale. The use of femtosecond laser ablation allows for precise removal of material with high spatial and depth resolution that can be coupled to an ICP-MS to obtain elemental and isotopic information. We used molecular beam epitaxy-grown thin films of $LaPd_{(x)}Sb_2$ and T'-La_2CuO_4 to demonstrate the capacity of fs LA ICP MS for stoichiometric analysis and its spatial and depth resolution. Comparison is made between the stoichiometry obtained using laser ablation versus standard liquid nebulization-based sample introduction. Here we demonstrate that the stoichiometric information of thin films with a thickness of ~10 nm or lower can be determined through the use of fs-LA. Furthermore, our results indicate that fs-LA-ICP-MS provides precise information of the thin film-substrate interface and is able to detect the interdiffusion of cations by performing shot-by-shot analysis of the material removal, also demonstrating the precision of depth analysis made available by this technique. Spatial mapping of elemental concentrations is also performed and indicates that there is a spatial variability of deposition using the molecular beam epitaxy technique for thin film production.

(117) **To be Frank and Bright— What More Could One Hope for in a Scientific Colleague and Friend?**; Gary M. Hieftje[1], Elise A. Dennis[1], Alexander Gundlach-Graham[2], Steven J. Ray[1]; [1]Indiana University; [2]ETH Zürich

After a number of years “in the saddle”, every faculty member can cite a number of former research-group members who really made a difference; people who were unusually productive or insightful, who challenged themselves and others, who set a standard that others tried to meet. Frank V. Bright, recipient of the 2015 Spectrochemical Analysis Award of the ACS Analytical Division, was one such person who, in fact, met all these criteria. When Frank first arrived at Indiana University as a post-doctoral associate, he asked what I wanted him to do. As is my custom, I said he could choose his own area of pursuit, and loaned him a thick file of research ideas to serve as a guide. Over the next couple of years, Frank methodically plowed through the file, project by project, and added many of his own variants and original ideas. His intensity, enthusiasm, work ethic, and joy of life left an indelible imprint on both my research group and Indiana University.

In this presentation, selected anecdotes from the Frank Bright “imprint” will be recounted. However, for scientific justification, recent studies into “Distance-of-Flight Mass Spectrometry” (DOFMS) and zoom-Time-of-flight mass spectrometry (zoom-TOF) will be described. Like Frank Bright’s ongoing research, these new approaches to mass spectrometry offer new capabilities and are a notable departure from current methods.

(118) **Interplay of Chromatography and Spectroscopy with Carbon Nanoparticles**; Luis Colon[1], Zuqin Xue[1], Karina Tirado-González[1], Amaris Borges-Muñoz[1]; [1]University at Buffalo - SUNY

As the proliferation of nanomaterials continues, studies of carbon nanoparticles have gained considerable attention in recent years. Our research group is studying the use of carbon dots (C-dots) and nanodiamonds (ND) nanoparticles as potential adsorbents for chromatography. At the same time, we use chromatographic methods to characterized carbon-based nanoparticles. In one example, we have synthesized C-dots having attractive photoluminescent properties with potential applicability in the biological and medical fields. Using chromatographic methods, we have produced fractions with refined and unique properties, allowing for brighter C-dots to be isolated from an apparent low quantum yield mixture. These more luminescent fractions of C-dots also displayed improved biological compatibility and usefulness as cellular imaging probes. Concurrently, C-dots and/or ND modified silicas are being studied as potential adsorbents for chromatography. Initial chromatographic testing suggests that modified non-hydrogenated ND silica support and C-dots modified silicas promote hydrophilic interactions with solutes. The hydrogenated ND-modified silica supports showed more hydrophobic interaction with solutes than the non-hydrogenated ND. Our findings underscore the importance of isolation/ characterization of C-dots, which is critical to properly assess fundamental properties of nanomaterials before establishing their applicability, being a chromatographic adsorbent or a photoluminescent probe.

(119) **Quantum Dots and Upconverting Nanoparticles: Using Paper Platforms for Multiplexed Optical Sensing by Resonance Energy Transfer**; Ulrich Krull[1], M. Omair Noor[1], Feng Zhou[1], Samer Doughan[1], Yi Han[1], Anna Shahmuradyan[1], Uvaraj Uddayasankar[1]; [1]University of Toronto Mississauga

Multiplexed solid-phase nucleic acid hybridization assays on paper-based platforms would be a useful technology for rapid detection of genetic markers for pathogens and genetically-based disease. Quantum dots (QDs) and upconversion nanoparticles (UCNPs) can serve as energy donors in fluorescence/luminescence resonance energy transfer (FRET/LRET) for transduction of nucleic acid hybridization. The surface of paper was modified with imidazole groups for immobilization of green-emitting QDs (gQDs) and red-emitting QDs (rQDs) as FRET donors and two types of oligonucleotide probe sequences as biorecognition elements. Addition and subsequent hybridization of Cy3-labeled and Alexa Fluor 647-labeled oligonucleotide targets provided the proximity for FRET sensitized emission from the acceptor dyes. Single nucleotide polymorphism discrimination was achieved, and the three dimensional matrix of paper substrates provided for high sensitivities, facilitating the use of low-cost detection platforms such as iPad cameras. An iPad camera provided detection limits at the high femtomole level, and at the zeptomole level for amplicons of 150 bases after isothermal amplification.

To advance the sensitivity of bioassays, a paper-based two-color oligonucleotide detection assay with tunable sensitivity has also been developed using a single type of UCNP. Excitation was at 980 nm, thereby avoiding autofluorescence and suppressing optical scatter. Water soluble UCNPs were designed to emit both green and red bands simultaneously by the upconversion process. Two different single stranded oligonucleotide probes were assembled on the UCNPs. Selective hybridization of two targets, each with a different color dye as label, provided the proximity for LRET-sensitized emission from the dyes. It was possible to tune the detection limit and sensitivity for each target by changing the relative ratio of the probes, and target concentration could be quantitatively determined in undiluted serum.

Exploration of methods to achieve even lower detection levels include compaction of the solid-phase matrix to enhance nanoparticle proximity for cross-talk, and implementation of metal nanoparticles to introduce plasmonic processes. Improvement of multiplexing capacity is being achieved using UCNP/QD LRET pairs. Introduction of intrinsically-labeled fluorescent probe molecules has been adopted to provide for the capability of detection of unlabeled target oligonucleotide.

(120) **Genome-Inspired Aptamers: Affinity Derived From Nature**; Linda McGown[1]; [1]Rensselaer Polytechnic Institute

Aptamers are oligonucleotides that are selected for high affinity binding to specific targets. They offer important advantages over antibodies for analytical and medical applications; however, despite intensive effort and substantial investments of time and money since their introduction some 25 years ago, the number of targets to which aptamers have been discovered is relatively small. This is attributed to a number of factors, most often to the selection process itself, which is laborious, non-standardized and difficult to fully automate. There are other, fundamental concerns related to existing, combinatorial approaches, including quality of the "random" combinatorial oligonucleotide library, under-representation of certain structural motifs, inefficiencies and bias of PCR amplification, need for the target in purified form, and non-specific binding. This talk describes a new, nature-inspired route to aptamer discovery that reverses the selection process in order to overcome practical and fundamental limitations of existing aptamer selection approaches. Rather than selecting an aptamer from a combinatorial library of oligonucleotides for high affinity binding to a specific target, we use naturally occurring, genomic DNA sequences to capture proteins from natural pools such as nuclear protein extracts. This new approach is a paradigm shift in aptamer discovery - by using naturally occurring DNA sequences to capture targets from natural "pools", we take advantage of the eons of biological evolution that produced particular DNA sequences that interact selectively with particular proteins to perform biological functions. Moreover, linking aptamer discovery to nature increases the chances of uncovering protein-DNA affinity binding interactions that have biological significance as well as analytical utility. This talk will focus on genomic G-rich sequences that form G-quadruplex (G4) structures. Although G4 is a highly successful motif for aptamers, it tends to be underrepresented in combinatorial libraries. Those that are present are limited to two-tier structures and prone to inefficient PCR amplification. In contrast, nature provides a large diversity of multi-tiered G4 structures throughout the human genome that are excellent candidates for aptamers, particularly to protein targets. The applicability of the new approach extends beyond genomic DNA and proteins, to any naturally occurring oligonucleotides as potential aptamers to targets of interest.

(121) **Seeing the Light: Studies of the Ocular Surface**; Frank Bright[1]; [1]SUNY-Buffalo

Multi-purpose solutions (MPS) are a single solution that functions to simultaneously rinse, disinfect, clean and store soft contact lenses. Several commercial MPS products contain polyhexamethylene biguanide (PHMB) and/or polyquaternium-1 (PQ-1) as antimicrobial agents. In this presentation the speaker will describe the creation of an in-vitro small unilamellar vessicle (SUV) model of the corneal epithelial surface and he will assesses the interactions of PHMB and PQ-1 with several model biomembranes by using a battery of spectrochemical analysis techniques.

(122) **Raman Spectroscopic Approaches - Possible Solutions for Unmet Medical Needs?**; Juergen Popp[1,2]; [1]Leibniz Institute of Photonic Technology, [2]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University Jena

A comprehensive analysis to determine diagnostic, prognostic and predictive factors in a few steps or ideally in one-step requires the development of new, fast and reliable approaches that support and supplement routine medical diagnostics and therapy. In the past years, medical

photonics has witnessed the development of optical / photonic approaches that are potentially in a position to meet these aforementioned challenges. In this context, spectroscopic approaches like e.g. Raman spectroscopy are especially noteworthy. In this contribution, we will summarize our recent results in implementing various Raman-approaches for sepsis and cancer, as these types of diseases harbor unmet needs regarding diagnosis and therapy. We will start with highlighting the potential of Raman microspectroscopy for an early diagnosis and therapy sepsis. In the field of sepsis, the fast identification of pathogens, their resistances and the specific host is crucial for choosing the appropriate initial antibiotic therapy to save lives in intensive care units. It will be shown that Raman hold great promise to address these challenging tasks. Besides single cells, whole tissue sections like biopsy specimens can be characterized by means of Raman-microspectroscopy enabling an objective evaluation of the tissue samples for an early diagnosis of cancer (= spectral histopathology). Raman spectroscopy as an emerging biomedical tool in recent years is based on steady improvements in instrumentation for excitation and collection, and in particular on the availability of fiber optic probes. The potential to couple the Raman system via optical fibers to the point of measurements has enabled within the last years besides ex-vivo Raman studies on excised tissue also in-vivo Raman studies, i.e. Raman endospectroscopy. Here we briefly review and summarize some of our latest results concerning the development and application of compact on-site Raman sensing concepts for medical diagnosis. In this context we introduce novel linear and nonlinear Raman fiber probes for in-vivo tissue screening to reliably diagnose and screen cancer and other diseases in internal organs like e.g. colon, stomach or aorta.

(123) **Practical Considerations in Using Raman Spectroscopy for Aging of Blood Stains**; Anita Mahadevan-Jansen[1], Kiana Jansen[1], Maggie O'Connor[1], Maurice Aalders, Isaac Pence[1]; [1]Vanderbilt University

Blood is a key component of many crime scenes and can provide valuable information about those involved. A major limitation in forensics is a lack of non-destructive techniques that can provide accurate information to form a timeline. Using optical techniques to evaluate blood has been shown to provide insight about when a crime has occurred. Current research includes the use of diffuse reflectance spectroscopy to determine the age of a bloodstain; however, measurements lose accuracy as a bloodstain ages. We propose Raman spectroscopy for this application. Previous work on Raman spectroscopy for biological fluids in forensics has differentiated between blood and other bodily fluids as well as determined if the blood was from a human or animal. However, no work has been reported about the potential of Raman spectroscopic techniques to non-destructively determine the age of a bloodstain. Here we report our investigation of changes in the Raman spectra of blood as a function of time. Raman spectra were acquired using a commercial Raman microscope and custom sample slide for precise time course measurements. Spectra were then characterized for differences between the signals over time. These findings form the basis of a model that will enable accurate prediction of bloodstain age using Raman spectroscopy. Development of these optical techniques for crime scene analysis has the potential to aid in the formation of a realistic timeline and evaluate alibis without the presence of a victim's body

(124) **Unsupervised and Supervised Multivariate Statistical Analysis of a Large Lung Spectral**; Max Diem[1]; [1]Northeastern University

Spectral histopathology (SHP) is an approach for the classification and diagnosis of tissue sections by spectral methods, in particular, Fourier transform infrared (FTIR) microspectral imaging coupled with multivariate statistical analytical methods. We report an extension and continuation of the approach originally pioneered by Lasch et al, namely to use unsupervised methods to delineate areas of high spectral homogeneity for the training of supervised classifiers to detect tissue abnormalities in SHP.

Datasets comprising hundreds of patients, in tissue micro-array as well as large tissue section format have been collected at several laboratories worldwide for different cancer types and organs. We report here recent progress on a lung cancer study that now contains well over 600 patients, and that has revealed astounding sensitivity for the classification and sub-classification of lung cancers.

(125) **Advanced Statistics of Raman Spectroscopic Data for Disease Diagnostics and Forensic Purposes**; Lenka Halamkova[1], Kyle C. Doty[1], Gregory McLaughlin[1], Elena Ryzhikova[1], Oleksandr Kazakov[1], Igor K. Lednev[1]; [1]University at Albany, SUNY

Raman spectroscopy contributes to the various research areas and has advanced in recent years with increasing practical applications. This is mainly due to technical improvement, the versatility of sampling methods and the availability of advanced statistics that helps in the data analysis. Over the last years, advances in the instrumental design of Raman spectroscopy overcame the problem with undesired fluorescence, low sensitivity and reproducibility. Raman spectroscopic data gives the vibrational characteristics of analyte, which can be understood as "fingerprint" type of information. The impact of chemometrics to the application of Raman spectroscopy for forensic purposes and medical diagnostics will be discussed in this contribution. Specifically, we will demonstrate a great potential of Raman spectroscopy for detection, identification and subsequent analysis of traces of body fluids like blood, saliva, semen etc. found at a crime scene. In addition, Raman spectroscopy is capable to provide information about the changes in biochemical composition and molecular structure of the body fluids caused by a disease. The application of Raman spectroscopy of blood for Alzheimer's Disease diagnostics will be shown.

(126) **Speed Acquisition Improvement in Raman Imaging via Compressive Sensing**; Nicolas Spegazzini[1], Rishikesh Pandey[1], Ishan Barman[2], Ramachandra Rao Dasari[1]; [1]Massachusttes Institute of Technology; [2]Johns Hopkins University

Raman spectroscopy is well-established as a powerful analytical tool in biophotonics. Confocal Raman image, combine Raman spectroscopy with confocal microscopy and can obtain 2D and 3D-dimensional information. Moreover is possible acquire label-free imaging in high-resolution using the fingerprint peaks of biomolecules of interest such as nucleic acid and protein. However, observing a living cell with Raman microscopy is quite challenging due to the intrinsically weak Raman signal intensity of endogenous cellular components. We propose a method in order to improve the speed in Raman imaging by compressive sensing in order to reconstruct an accurate Raman image of a cell while keeping number of samples to a bare minimum (undersampling). We demonstrate the efficacy of the proposed approach compared to the conventional galvano mirror XY scanning. Using cell Raman imaging as an example the approach exhibits a reduction in the speed acquisition over the conventional raster-scanning. This method can offer a new potential alternative direction for Raman imaging in continuous process monitoring and drug delivery in cells.

(127) **Advanced Chemometric Strategies for Food Authentication**; Federico Marini[1]; [1]University of Rome La Sapienza

Quality control and the verification of typicality and authenticity of the products - in a word, authentication - are issues that play a major role within food chemistry. From an analytical standpoint, since several factors may determine the quality of a food, the approaches to the authentication of the products are increasingly directing towards the use of one or, rather, more fingerprinting techniques that allow the rapid, often not destructive and, possibly, holistic characterization of the goods. However, the complexity of food matrices often makes the recorded signals be affected by the contribution of different sources of variability, not always related to information sought.

Accordingly, the chemometric strategies enforced for tackling with the aforementioned authentication problems need to take into account such possible variability in order to produce reliable models. As a direct consequence, the objective of the present communication is to present some innovative chemometric approaches for solving problems of characterization and authentication of foodstuff, which allow on one side to obtain detailed information on the effect of possible sources of variability on the experimental signals and on the other to build reliable mathematical models also in the cases in which the presence of these sources of variability may result in the need to build mathematical-statistical models of high complexity.

Moreover, with the aim of obtaining a characterization as holistic as possible of the food of interest, the advantages of data fusion strategies - that permit the integration of the profiles obtained with different instrumental techniques – will be addressed.

(128) **Instrumental Approaches and Chemometric Analyses for Establishing Authenticity of Botanical Products**; Paula N. Brown[1], Michael Chan[1], Jamie Finley[1], Christina E. Turi[2], Andrew R. Lewis[3]; [1]British Columbia Institute of Technology; [2]University of British Columbia; [3]Simon Fraser University

Current quality control approaches of establishing identity specifications are not only required for compliance with good manufacturing practices (GMP), but for prevention of economically motivated adulteration. Botanical authenticity is classically achieved by examination of diagnostic macroscopic and microscopic features, these techniques remain useful for minimally processed biomass such as dried roots. However, modern ingredient supply chains are often far removed from classically identifiable material and traded as highly pulverized dried plant powders. There is continuing demand for high quality, authentic products in the marketplace, and the volume of this demand significantly outpaces research efforts to produce reliable analytical methods that verify botanical identity. The combination of analytical instrumentation with chemometric analyses shows promise as a tool for qualitative determination of identity and purity with respect to defined adulteration in botanical materials. Technologies for acquisition of plant metabolite profiles have developed rapidly in conjunction with new analytical tools for chemical separations, mass spectrometry, and NMR spectroscopy have emerged. A standard metabolomics data set contains vast amounts of information and can either investigate or generate hypotheses. Key factors in using plant metabolomics data effectively are experimental design, availability of reference materials, sample preparation and selection of statistical analyses performed. There exists a need to evaluate approaches to delineate sources of variance, including pre-processing of datasets. This talk will demonstrate practical experiences in the development of methods using phytochemical profiling for characterization and authentication of botanical materials as a GMP requirement. Botanical materials such as Echinacea, Panax, Hydrastis, and Ligusticum species based on NIR and NMR plant metabolite acquisitions will be used to illustrate the utility of multivariate models to characterize and authenticate botanical materials.

(129) **A UV-Vis-PCA Approach to Botanical Identity Confirmation using DNA Validated Botanical Reference Materials**; Jeremy Stewart[1]; [1]Gaia Herbs, Inc

The quality of data generated by an analytical method is beholden to the quality of the reference material utilized in the analysis. This presentation describes a chemometric technique useful for generating validated botanical reference materials based on DNA analysis and qualifying raw materials used in dietary supplement manufacturing.

(130) **Chemometrics in the United States Pharmacopeia**; Lucy L. Botros[1], Jeffrey C. Moore[1], Alan R. Potts[1]; [1]U.S. Pharmacopeial Convention

The U.S. Pharmacopeial Convention (USP) is a scientific nonprofit organization that sets standards for the identity, quality, and purity of medicines, food ingredients, and dietary supplements by the application of the tests, procedures, and acceptance criteria set forth in relevant compendia. USP has recently applied chemometrics in the development of alternative test procedures for the authentication of naturally-sourced materials with high chemical and physical variability, including food ingredients and excipients. Through this development process and with industry collaboration, USP has recognized the scientific validity of setting critical standards for the application of chemometrics to authenticate and identify such materials. This paper will discuss USP's current thinking on supporting and developing appropriate guidelines for quality control applications of chemometrics as alternative methods to currently published monograph procedures in the USP-NF and FCC. Guidelines include the development of a chemometrics general chapter in the USP-NF, which captures critical aspects of developing, validating and maintaining models as part of an alternative procedure, and provides insight on potential chemometric tools and acceptance criteria. Additional FCC standards providing further guidance on validating procedures that are aimed at adulterant detection in food ingredients are also in development. A specific application of chemometrics to skim milk powder authentication and adulteration detection will be presented.

(131) **Spectral Fingerprinting: Following the Transition from Raw Botanical to Finished Product**; James Harnly[1]; [1]US Department of Agriculture

Conceptually, authentication of raw botanical materials is simple; build a model, set statistical limits, and determine if the test material fits the model. The most crucial aspects of the authentication process, however, are selection of the reference samples and the analytical method with which to build the model. Ideally, the reference samples will reflect anticipated variation arising from genetics, environment, and processing. The method should account for as many of the samples' chemical properties as possible, i.e., be as robust as possible. Spectral fingerprinting, with multivariate analysis, has proven to be a powerful tool for discriminating between genus and species, growing locations and years, cultivation methods (conventional vs organic farming), and post-harvest processing. The transition from a raw material to a finished commercial product presents additional variables that must be considered. Many commercial products are based on an extract which has dried on an excipient. The extraction process will exclude some small molecules and the excipient will introduce others. To complicate matters, the excipients vary widely. Thus, a model based on raw materials will almost certainly exclude commercial products unless the material is simply ground and put into capsules. In addition, the extraction and drying processes usually destroys DNA necessary for barcoding. As a result, the final product is best authenticated using marker compounds and the more, the better.

(132) **Nanomaterial Sensors for Trace Chemical Detection**; Ling Zang[1]; [1]University of Utah

Our lab has developed various molecular and nanostructured sensors that can be employed for realtime detection of chemical agents with sensitivity down to ppt for vapor and pg for particulate sampling.1-4 The sensing mechanism is based on either fluorescence modulation (quenching or turn-on), or chemiresistor mode. The high sensitivity can be achieved by dispersing the sensor molecules into porous media like silica gel, or fabricating the molecules into nanofiber structure via self-assembly in solution. In collaboration with industries, these sensor technologies have now been transitioned into chip scale devices for screening explosives, drugs, and toxic industry chemicals.

The nanofibers fabricated from the sensor molecules (usually pi-conjugated) possess one-dimensional (1D) confined optical and electronic properties along the long-axis, such as the long-range exciton diffusion and expedient charge transport. The former enables amplified fluorescence quenching sensing, while the latter is conducive to chemiresistor sensing. When deposited on a substrate the intertwined nanofibers form a porous super net structure with large surface area that can capture analyte molecules from the air through molecular diffusion and surface adsorption. Combination of these features makes organic nanofibers unique materials for development into optoelectronic sensors with high sensitivity and fast response. The detection selectivity relies on two-fold approach, 1) the bottom-up fabrication method of nanofibers, for which the building block molecules can be modified with different binding groups, and 2) software algorithms that allow for differential sensing through sensor array employing multiple nanofibers.

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(133) **Fluorescence Detection of Explosives: A Study Towards Optimization of an Array of Thin Film Optical Sensors**; William Euler[1], Hui Qi Zhang[1], Mingyu Liu[1], Matthew Mullen[1]; [1]University of Rhode Island

The threat of terrorists using explosive devices in nearly any location has led to a world wide effort to improve detection of all types of explosives. The challenge is that nearly all explosives have room temperature vapor pressures in the parts per billion or parts per trillion range, so any detector must be able to detect picogram (or less) quantities of analyte in a dirty background. Fluorescent detection is exquisitely sensitive but suffers from poor selectivity so false alarms are a serious problem. Selectivity can be improved by chemically synthesizing fluorophores designed to target specific analytes but this leads to highly expensive systems. Our approach has been to use off-the-shelf laser dyes that have high quantum yields and to create arrays to provide selectivity. By using a layered thin film design (mechanical substrate/transparent polymer/fluorophore) the intensity of the emitted light can be amplified by two or three orders of magnitude, which will allow inexpensive optics to be used in a hand held instrument. This talk will focus on the underlying photophysics that allows the sensing to be both sensitive and selective. Submonolayer thin films of the fluorophores are readily detected because of the amplification mechanism. Consequently, the amount of explosive analyte required to perturb the fluorescence in an easily measurable fashion is equally small. The fluorescence emission can also be affected by the substrate onto which the fluorophore is deposited. The polarity and the thickness of the polymer modulate the emissive response and examples of this will be shown. The combination of fluorophore and polymer properties can be used to create arrays that are both sensitive and selective using readily available and inexpensive materials.

(134) **Issues in Explosive Detection: Sampling**; Jimmie Oxley[1]; [1]University of Rhode Island

A number of sensitive explosive trace detectors (ETDs) have been developed over the last few decades--chief among them in the IMS (ion mobility spectrometer). These instruments are capable of detecting most explosives at nanogram levels. However, one of the major problems these and other ETDs face is getting the sample from whatever surface it is on, through the instrument, and into the detector. As researchers we proposed to address the issue of swabbing. The collection and analysis of explosive residue is complicated by the fact that swabbing material which has good sorbative properties cannot also be expected to readily release the residue into the inlet of a detection device. Vendors of various instruments requiring swabs have addressed this problem by using materials which have poor pickup but good release properties, e.g., cloth, Teflon, Nomex, and metal mesh. We wished to develop swabs with enhanced adhesion of residues and the capability of releasing the majority of the residue on demand. In this talk we will address the issues surrounding such as study as well as our approaches to enhanced pickup and release of explosive residue.

(135) **HPIMS in Explosive Detection and Forensic Applications**; Ching Wu[1], Anthony Midey[1], Adam Griachen[1], Mark Osgood[1]; [1]Excellims Corporation

Ion mobility spectrometers (IMS) are common tools for detecting trace amounts of explosives, chemical warfare agents, and illegal drugs in a variety of homeland security and forensic analysis scenarios. The advantages and limitations of the technology under different operating environments have become well known. Compared to other spectrometric chemical analysis technologies, e.g., mass spectrometry, IMS is touted as a low resolution technique. The IMS advantages of very high sensitivity, small size, low power consumption, and ambient pressure operation are in some cases completely offset by or, at a minimum, reduced by the lack of resolution sufficient to prevent unwanted responses to interfering chemicals. In the past ten years, we have made significant efforts in improving IMS resolving power while maintaining its good analytical performance. In addition, we have recently developed an high performance ion mobility spectrometer (HPIMS) that can be interfaced to a variety of ionization source configurations achieving detection capabilities of different classes of chemicals. Beside electrospray ionization, the HPIMS system is designed to accept a corona discharge source, a Ni63 radio ionization source, and others. Test results for detection of explosives, contrabands, and other substances using HPIMS and HPIMS-MS will be reported.

(136) **HMTD Decomposition: A Kinetic Study**; Lucus Steinkamp[1], Lauryn DeGreeff[2], Kevin Johnson[2], Greg Collins[2], Susan Rose-Pehrsson[2]; [1]National Research Council; [2]Nova Research, Inc., U.S. Naval Research Laboratory

Hexamethylenetriperoxidediamine (HMTD) is one member of a class of clandestinely prepared peroxide explosives favored by individuals who desire an easily synthesized primary explosive for initiating larger explosive charges. Although the precise vapor pressure of HMTD is somewhat disputed, the explosive is generally regarded as nonvolatile, unlike its counterparts, triacetone triperoxide (TATP) and diacetone diperoxide (DADP) which have relatively high vapor pressures. The lack of volatility makes characterizing and reliably generating molecular HMTD vapor a significant challenge but does not preclude HMTD from having a perceptible odor due to the ease with which HMTD decomposes into smaller, more volatile compounds. A study is currently underway to probe the kinetics of this decomposition mechanism under ambient conditions and establish how factors, such as storage conditions, affect decomposition and give HMTD its distinct odor. This information will inform efforts to regenerate a characteristic HMTD vapor signature for testing trace explosive detection technologies and how canine training aids may be affected by prolonged storage.

(137) **Quantitative Analysis of Coal Using Laser Induced Breakdown Spectroscopy**; Zhe Wang[1], Zongyu Hou[1, 2], Tingbi Yuan[1,2]; [1]State Key Lab of Power Systems, Department of Thermal Engineering, Tsinghua-BP Clean Energy Center, Tsinghua University; [2]China Guodian Science and Technology Research Institute

Fast or online coal analysis is extremely important for combustion optimization, energy saving and emission control in power plant. Laser-induced breakdown spectroscopy (LIBS) has a great potential in online coal analysis due to its advantages such as fast measurement and multi-elements analysis. Currently, LIBS is under through a critical time before successful commercialization in quantitative analysis, while the main obstacles are the low signal repeatability and prediction accuracy. Averaging the spectra from multi-pulse was a commonly used method to improve the signal repeatability of LIBS. While partial least square (PLS) was a popular multivariate model to improve the accuracy of LIBS analysis.

In this work, it was illustrated that the averaged spectrum can eliminate only the random noise but has no effect for nonrandom noise, and the traditional PLS model will accumulate the variations of different spectral lines so as to reduce the repeatability of the predicted value. Thus, using PLS and averaged intensity will reduce the repeatability of the predicted results. The dominant factor based PLS model with spectral line standardization was proposed to improve the repeatability and accuracy. The spectral line intensities from a single pulse were corrected by the unique property of the plasma so as to get standard line intensities with standard plasma parameters. The standardized line intensities and the lines of major elements were then used to construct the dominant factor because they are more reliable and repeatable. The other lines are used to compensate the residue of the dominant factor. Comparing to traditional PLS model, the relative standard deviation (RSD) of the predicted carbon content was reduced from 2.11% to 0.89%, and the error of the calibration was reduced from 1.87% to 1.26%. The precision and accuracy of the results were very close to the national standard of coal chemical analysis. Besides the carbon content analysis, the hydrogen content analysis and proximate analysis of coal were also demonstrated in laboratory and onsite. The results of LIBS are all very close to the national standard of chemical analysis, indicating the feasibility and prospect of LIBS on quantitative analysis.

(138) **Choices and Improvements in Baseline Removal in LIBS Spectroscopy**; Melinda (Darby) Dyar[1], Thomas Boucher[2], Stephen Giguere[2], CJ Carey[2], Sridhar Magadevan[2]; [1]Mount Holyoke College; [2]University of Massachusetts at Amherst

Laser-induced breakdown spectroscopy (LIBS) uses atomic emission lines to characterize the chemical composition of samples lased into a plasma. Emission lines are superimposed upon a background resulting from Bremsstrahlung continuum and ion-electron recombination processes that are not relevant to the atomic emission signal of interest. This signal must be subtracted from the spectra prior to quantitative analysis. An ideal continuum removal algorithm would result in 100% of the predictive information remaining in the signal and 0% in the removed continuum. This ideal is inadequately attained in common existing implementations. Moreover, there are literally dozens of possible algorithms for baseline removal, but comparisons among them are virtually non-existent.

Here we apply six different baseline removal algorithms to the task of continuum removal from a suite of 93 LIBS spectra from randomly-chosen geological samples. Continuum removal methods tested included asymmetrical least squares (ALS), adaptive iteratively reweighted penalized least squares (airPLS), fully automatic baseline correction (FABC), iterative thresholding (IT), Kajfosz-Kwiatek (K-K), and Hermite polynomials (HP) with 2, 1, 2, 2, 2, and 1 associated adjustable parameters, respectively. We arbitrarily chose nine major elements in wt.% oxide as variables to test prediction accuracy resulting from implementation of the various baseline removal algorithms prior to PLS analysis, and compared the results in terms of root mean square errors (RMSE). We created a pipeline that randomly selected a continuum removal method and adjustable parameter or combination thereof, predicted the major element composition, calculated RMSE, and recorded the results. The lowest RMSE was selected after running the pipeline continuously for 24 hours.

Results show that while many combinations of baseline correction parameters improve model accuracy, the wrong choice of correction method or parameters can dramatically degrade performance. Reductions in RMSE relative to uncorrected data were observed after applying the FABC and IT methods, but applying ALS or airPLS had little effect on model accuracy. Both the K-K and HP methods increased the RMSE. We will report subsequent progress on this research, including comparisons with projection-based methods, such as orthogonal basis methods and Fuzzy Optimal Associative Memory techniques and alternate methodologies for optimizing this process.

(139) **Analysis of Wear Metals in Engine Oil using LIBS**; Markus Gaelli[1], Amy Bauer[1]; [1]TSI Incorporated

During regular use of machines and engines, contamination of lubricating oil is inevitable. The oil itself is degraded by heat, causing it to be less effective as a lubricant. In addition, the components of the device wear, depositing particulate material into the oil. As this wear increases, problems can evolve due to metal particle build-up in the oil and reduced performance of worn elements. Early detection of increased wear via oil analysis can prevent catastrophic failure and greatly reduce maintenance costs.

LIBS has previously been evaluated to measure metals dissolved in oil. Prior work was focused on measurement of metallo-organic compounds dissolved in oil, for which standard are readily available. The current work will focus on small metal particles suspended in oil and deposited on a sacrificial analytical surface, which is more similar to the actual expected measurement scenario than earlier work. The small number of individual particles in the sampling region introduces difficulties; depending on the number density of particles in a solution, many laser shots will interact only with the oil matrix. This raises issues of statistics, and calibration concerns. These issues have been well-explored in the application of LIBS to airborne aerosols, which are also discrete sample particles in a matrix that may not yield otherwise interesting signals. We will be making use of these algorithms to make our analysis quantitative. This presentation will focus on experimental details to overcome the challenges described above, as well as ways towards a practical application.

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(140) **Exploring Matrix Effects on Quantitative Analysis of LIBS Data from Rock Powders Doped with Cr, Ni, Mn, Co, Zn, and S**; Kate Lepore[1], Elly Breves[1], M. Darby Dyar[1]; [1]Mount Holyoke College

LIBS quantitative analysis is complicated by chemical matrix effects related to abundances of neutral and ionized species in the plasma, collisional interactions within plasma, laser-to-sample coupling efficiency, and self-absorption. Atmospheric composition and pressure, incidence angle, and energy distribution also influence plasma intensity and temperature distribution. Matrix effects have been well-documented using emission lines from major elements, but some workers believe that minor and trace species are unaffected. To test this hypothesis, we doped five very different bulk rock compositions ranging from a nearly pure silica sandstone to high-Fe mantle rock with Ni, Cr, Mn, Co, Zn, and S in concentrations ranging from 10-80,000 ppm. LIBS data were acquired in the Mineral Spectroscopy Laboratory at Mount Holyoke at constant distance, laser power, and incidence angle under a 7-Torr CO_2 atmosphere to simulate Mars conditions. Data were processed using software with steps analogous to that used for ChemCam data from the Mars Curiosity rover, including normalization to each of three wavelength regions corresponding to the three spectrometers. Independent X-ray fluorescence analyses quantified the composition of each of 180 powders.

In any given matrix, characteristic emission lines of the doped elements give rise to peaks with intensities increasing as a function of concentration, as expected, but the relationships between peak intensity and concentration are linear only below 1000 ppm. Above that concentration, the doped element is present in sufficient amounts in each sample to contribute to matrix effects and distort the univariate calibration line. Moreover, different matrices doped with identical abundances of the same transition metal (or S) showed widely varying emission line intensities in both normalized and un-normalized data. For example, the intensities of Ni lines between 300 and 210.5 nm arising from identical concentrations are 2-3x greater in the Fe-rich rock matrices than in the high-Si sea sand matrix. These results confirm that calibration curves developed on a single matrix will produce reasonable predictions only when matched to the matrix of the unknown being predicted, and perhaps only over a limited range of concentrations. Multivariate analysis provides more accurate, generalized calibrations for all six elements tested.

(141) **Process Patent Protection: Protecting Intellectual Property via Natural-Abundance Stable Isotopes**; John Jasper[1], Martin Pavane[2], Dean Eyler[3], Ila Sharma[4], Albert Lee[4]; [1]Nature's Fingerprint / MIT LLC; [2]Cozen O; [3]Gray Plant Mooty; [4]Chemir Analytical Services

The ambient distribution of light stable isotopes in biopharmaceutical synthetic pathways permits the identification and differentiation of potentially-infringing pathways. After reviewing three cases of product identification, we examine three cases of process authentication, protecting ~$2 billion in biopharmaceutical products: one of false advertising and two of process patent infringement. The three cases of product authentication demonstrate the dynamic range of the light stable isotopes in differentiating sources of pharmaceutical materials. The false-advertising case was substantially a product case because the green-tea L-Theanine and the client- and competitor L-Theanine were markedly different in carbon- (~15‰) and nitrogen isotopic (~10‰) composition. The competitor L-Theanine process was revealed from court documents and chemical-isotopic insight. The competitor was accused of falsely advertising the source of their L-Theanine. The second case of process patent infringement was a straightforward case of infringement. An infringer used synthetic intermediates that were readily available on the market to produce the infringing product. When confronted with the isotopic evidence of process infringement, an out-of-court, business resolution was reached. The third case of process infringement was a case of wrongful accusation of infringement. The carbon-isotopic records of both the product and process studies show that (i) the products are of different origins and (ii) the defendant had in fact used a different synthetic pathway so that he was not infringing the patent of the plaintiff.

(142) **A Tiered Analytical Approach for Investigating Poor Quality Emergency Contraceptives**; Facundo Fernandez[1], Maria Eugenia Monge[1], Prabha Dwivedi[1], Manshui Zhou[1], David Jenkins[2], Paul Newton[3,4]; [1]Georgia Institute of Technology; [2]Product Quality and Compliance, FHI 360; [3]Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital; [4]WorldWide Antimalarial Resistance Network, Churchill Hospital

Reproductive health has been deleteriously affected by poor quality medicines. Emergency contraceptive pills (ECPs) are an important birth control method that women can use after unprotected coitus for reducing the risk of pregnancy. In response to the detection of poor quality ECPs commercially available in the Peruvian market we developed a tiered multi-platform analytical strategy. In a survey to assess ECP medicine quality in Peru, 7 out of 25 different batches showed inadequate release of levonorgestrel by dissolution testing or improper amounts of active ingredient. One batch was found to contain a wrong active ingredient, with no detectable levonorgestrel. By combining ultrahigh performance liquid chromatography-ion mobility spectrometry-mass spectrometry (UHPLC-IMS-MS) and direct analysis in real time MS (DART-MS) the unknown compound was identified as the antibiotic sulfamethoxazole. Quantitation by UHPLC-triple quadrupole tandem MS (QqQ-MS/MS) indicated that the wrong ingredient was present in the ECP sample at levels which could have significant physiological effects. Further chemical characterization of the poor quality ECP samples included the identification of the excipients by 2D Diffusion-Ordered Nuclear Magnetic Resonance Spectroscopy (DOSY 1H NMR) indicating the presence of lactose and magnesium stearate.

(144) **Meeting Authentication Challenges with Spectroscopic Solutions**; Jeffry Denault[1], Robert Beal[1]; [1]Eli Lilly and Company

The challenges associated with chemical authentication of suspect counterfeit pharmaceutical products are becoming more difficult as (1) counterfeit samples are becoming closer replicates of authentic products and (2) reporting requirements to regulatory agencies are increasing. A range of rapid spectroscopic technologies have become more prevalent with the commercialization of portable/handheld analyzers allowing the opportunity to provide high quality data to address both issues quickly. This talk will address general challenges related to authentication and the implementation of spectroscopic technologies for authentication of pharmaceutical products.

(145) **Raman Spectral Fingerprinting for Biologics Counterfeit Drug Detection**; Ravi Kalyanaraman[1], Anna Luczak[1], Jeremy Peters[1], Varsha Ganesh[1]; [1]Bristol-Myers Squibb

World Health Organization (WHO) estimates that less than 1% of pharmaceutical products sold in developed countries, and 10% to 15% or more in developing countries are believed to be counterfeits. Although most of the counterfeits reported are drugs that contain small molecular entities as active ingredients, recent trends also include biologics based drugs and the battle against counterfeit biologics is growing. In 2009, Pharmaceutical Security Institute (PSI) reported that 3.5% of all counterfeit drugs were biologics, compared to 1.2% in 2006. With the recent FDA biosimilar approval and the Avastin counterfeit example reported in the United States, it is becoming increasingly important that pharmaceutical companies find ways to rapidly screen and detect biological counterfeits. This talk will feature a novel technique of using Drop

Coat Deposition (DCD) for the drug sample combined with con-focal Raman spectroscopy for biologics drug fingerprinting. Specific examples will include monoclonal antibody and fusion protein drugs and the spectral analysis focused on the determination of protein secondary structure.

(146) **Rationally Designed Mixed-Monolayer Glyconanoparticles for the Detection of Cholera Toxin by SERS**; Duncan Graham[1], Jonathan Simpson[1], Derek Craig[1], Karen Faulds[1]; [1]University of Strathclyde

Pathogen establishment in a host relies on its exploitation of multi-functional attachment molecules presented by host cells. One such unit is the GM1-ganglioside, a glycosphingolipid, which is displayed on the surface of intestinal cells. The carbohydrates exhibited by this unit are targeted by vibreo cholerae through the lectin, cholera toxin, to initiate infection. The same interactions can be exploited, using carbohydrate coated metallic nanoparticles, for the detection of this pathogen. These so called glyconanoparticles, coupled with surface enhanced Raman spectroscopy (SERS), allow for the sensitive detection of the species of interest. Rationally designed silver glyconanoparticles have been developed for the sensitive (56 ng/mL), low volume detection of cholera toxin B-subunit (CTB) in 5 minutes. PEGylated galactose and sialic acid are added in a specific ratio to coat the particles in GM1-ganglioside mimics for interaction with CTB and display a synergic effect greater than either glycan alone. This demonstrates the first rational design of a glyconanoparticle which mimics the GM1 ligand, allowing selective interaction with the target protein, CTB.

(147) **Metabolite Identification by Sheath-Flow SERS**; Zachary Schultz[1], Matthew Bailey[1], Kevin Jacobs[1]; [1]University of Notre Dame

The chemical specific nature and high –sensitivity of surface enhanced Raman spectroscopy (SERS) has tremendous promise for chemical diagnostics. Challenges to utilizing SERS in clinical assays include reproducibility and quantitation. To address these challenges, we have demonstrated a sheath-flow interface for SERS detection in flow. This sheath-flow SERS interface uses hydrodynamic focusing to confine analyte molecules eluting/migrating out of a column onto a planar SERS substrate where the molecules are detected by their intrinsic SERS signal. Advantages of the sheath-flow SERS detector include small samples sizes (25 – 100 nL) and reproducible SERS signals. Importantly, the sheath flow environment is resistant to irreversible absorption, which enables high throughput and continuous characterization. Because detection is done in flow, this sheath-flow SERS detector can be readily incorporated for post-separation characterization. In particular, SERS characterization shows promise for the analysis of isomeric structures. Recent work in our laboratory has investigated metabolites and other small molecules present in biological fluids. The online interface suggests a complementary approach to the traditional mass spectrometric characterization, which may enable more complete coverage of the metabolites identified. Our work suggests a new route to identifying these molecules for diagnostic assays. Using SERS to identify and quantify metabolites may provide improved diagnostics.

(148) **Development of SERS for Monitoring Small Molecule Metabolites**; Mark McDermott[1,2], Shereen Elbayomy[1,2], Albert Cao[1,2]; [1]University of Alberta; [2]National Institute for Nanotechnology

The measurement of small molecule metabolites in biological samples is a research area of increasing interest due to the promise of using the information for diagnostic purposes. There are already a number of tests that rely on the detection of a single metabolite. The ability to simultaneously detect and quantitate a number of metabolites (6-30) will provide researchers with a powerful tool to link metabolite profile to disease states and may lead to invaluable point-of-care tests.

We and others have shown that chemically modified spherical gold nanoparticles have been used as extrinsic labels for surface enhanced Raman scattering (SERS) detection of surface based assays. Rod-shaped gold nanoparticles should provide higher sensitivity and potentially stronger binding via multivalent interactions. The challenge in employing gold nanorods for this application is the replacement of the shape directing surfactant layer on the surface. We have developed a modification procedure to replace the cetyltrimethylammonium bromide (CTAB) layer on gold nanorods with a self-assembled monolayer (SAM) terminated with ethylene glycol (EG) and COOH groups. The nanorods are made SERS active by place exchange with 4-nitrobenzenethiol (NBT). We will present results that track the modification procedure. Antibodies are conjugated to the nanorods through the terminal –COOH group. The antibody-conjugated nanorods were used for SERS readout in sandwich immunoassays and provide extremely low detection limits for the small molecule thyroxine. A direct comparison with a commercial ELISA will be presented.

(149) **Stimulated Raman Spectroscopy for SERS of Biological Systems**; Renee Frontiera[1], W. Ruchira Silva[1], Emily L. Keller[1]; [1]University of Minnesota

The development of quantitative SERS methodologies for bioanalysis necessitates accurate quantification of SERS enhancement factors, or the amount by which the plasmonic substrate increases the Raman signal. Accurate characterization of the enhancement factor of SERS biosensors can be challenging due to difficulties in differentiating between surface and resonance enhancement. Typical molecules used as SERS biomarkers are often strongly fluorescent, making it nearly impossible to measure resonance Raman cross sections due to the overwhelming isoenergetic fluorescence signal. We use femtosecond stimulated Raman spectroscopy to measure cross sections of fluorescent SERS molecules, enabling quantitative measurements of enhancement factors on a variety of plasmonic substrates. We use an optical etalon to tailor the temporal profile of our pump pulse, which promotes acquisition of the stimulated Raman signal over fluorescence. Here we discuss our results on quantifying resonance Raman cross sections of a commonly used near-infrared SERS dye, 3, 3'-diethylthiatricarbocyanine (DTTC). We find that the cross sections for most of the vibrational modes observed in the resonance Raman spectrum exceed 10-25 cm2/molecule. These cross sections lead to strong SERS signals even with weakly enhancing SERS substrates. In addition to providing some surface enhancement, the plasmonic substrate also strongly quenches the fluorescence, enabling the intrinsically strong resonance Raman signal to be observed. This work will lead to more accurate determinations of SERS enhancement factors with DTTC. This will enable careful quantification of SERS substrates in the biologically relevant near-infrared region of the spectrum, ultimately leading to a more widespread use of these versatile probes in bioimaging and biosensing.

(150) **The Use of Surface Enhanced Raman Scattering (SERS) as an Alternative High-Throughput Screening Method for Applications in Industrial Biocatalysis**; Chloe Westley[1], Yun Xu[1], Andrew Carnell[2], Nicholas Turner[1], Royston Goodacre[1]; [1]Department of Chemistry, University of Manchester, Manchester Institute of Biotechnology; [2]Department of Chemistry, University of Liverpool

Biocatalysis utilises enzymes to perform chemical transformations, offering significant advantages over traditional chemical processes in terms of enhanced specificity and increased sustainability. A major limitation surrounding biocatalysis is the availability of robust, rapid, high-throughput screening methods to select enzymes with enhanced fidelity for the substrate as well as high product yield. At present, commonly used analytical techniques include fluorometric assays, NMR and LC-MS. However, these have associated disadvantages such as lengthy sample preparation, high equipment cost, long acquisition times, and in some cases insufficient structural information is obtained. Therefore, alternative screening

methods have been investigated, in particular surface enhanced Raman scattering (SERS) – the significant enhancement of Raman signals when molecules are absorbed onto a roughened metal nanoscale surface.

SERS yields molecular specific information, and we show that SERS can be used in a label- free manner to monitor enzymatic biotransformations as the functional group complement of the substrate(s) and product(s) changes quantitatively. Due to its sensitivity, ability to conduct analysis in aqueous environments and its label-free approach, SERS is an ideal analytical technique to be used for high-throughput screening of biocatalysts.

In this study, we directly monitored the xanthine oxidase catalysed conversion of xanthine to uric acid, using an inexpensive, portable Raman instrument. In combination with partial least squares regression (PLS-R), we reduce the acquisition time 30-fold, with results being highly comparable and in excellent agreement with comparative HPLC analysis used for benchmarking. We then extended our work to a three-analyte system (including the precursor hypoxanthine) with similar levels of substrate(s) and product(s) quantification in terms of predictive accuracies as well as a 36-fold reduction in acquisition time, thus rivalling currently established analytical techniques.

In addition, the product, uric acid, is an important biomarker for several diseases including gout, various cardiovascular diseases, preeclampsia and multiple sclerosis. We have successfully explored the use of SERS (in combination with multivariate curve resolution alternative least squares, MCR-ALS) in monitoring the change of reaction kinetics on introduction of varying concentrations of oxypurinol, a xanthine oxidase inhibitor and active metabolite of the commercially available drug, allopurinol, ultimately adding biological significance to the application.

(151) **Structure of Porous PMMA Thin Film Examined with Multifocus Raman Microspectroscopy**; Koichi Iwata[1], Ashok Samuel[2], Soshi Yabumoto[2], Kenichi Kawamura[3]; [1]Gakushuin University; [2]National Chiao Tung University, Taiwan; [3]Tokyo Instruments

Multi-focus micro Raman spectroscopy is a promising method for measuring Raman images faster than conventional single-focus micro Raman spectroscopy. With this method, Raman excitation light is focused into multiple positions under an objective lens of a microscope. The scattered light from these foci is collected separately with optical fibers and forms different images on the entrance slit of a spectrograph. The intensity of the light dispersed by the spectrograph is recorded simultaneously by a two-dimensional CCD detector. We have constructed a multi-focus micro Raman spectrometer that forms 21 x 21 foci under a microscope.

We examine three-dimensional structure of porous thin film of PMMA (polymethyl methacrylate) with the multi-focus micro Raman spectrometer. Raman images recorded for different depths of the film indicate that the structure of the pores changes depending on the depth in the film. There are a large number of pores with a diameter of 6 micrometers in average formed on the film surface. The diameter of the pore decreases, however, to 2 micrometers inside the film, at 2 to 3 micrometers from the surface. Pores with smaller diameters form an interconnected complex network in the inner bulk. It is now a practical task to record layers of Raman images for examining the three-dimensional structure of sample materials with the multi-focus micro Raman spectrometer.

(152) **Linear and Non-Linear Fiber-Based Raman-spectroscopy for Biophotonic Applications**; Juergen Popp[1,2]; [1]Leibniz Institute of Photonic Technology; [2]Institute of Physical Chemistry and Abbe Center of Photonics

Raman spectroscopy has matured to become one of the most powerful analytical methods in biophotonics i.e. for addressing biological / biomedical and life science problems. The advancement of Raman spectroscopy as an emerging biophotonic tool in recent years is based on steady improvements in instrumentation for excitation and collection, and in particular on the availability of fiber optic probes. Here we briefly summarize some of our latest results concerning the development and application of Raman fiber concepts for biophotonics, especially for medical applications and environmental sensing. We will start with introducing novel linear Raman fiber probes for a point-wise intravascular monitoring of the artheriosclerotic plaque in living rabbits. Moreover we will present a coherent anti-Stokes Raman scattering (CARS) imaging fiber probe consisting of several thousand single cores integrated into a compact fiber. Since the chosen fiber technology enables the separation of the scanning and the distal ends, the design of a CARS probe head without moving parts and therefore with very small diameters is possible offering the advantage of easier sterilization and recycling. In the second part we will present concepts for fiber enhanced Raman spectroscopy for drug monitoring by utilizing innovative optical hollow core fibers allowing for a fast, sensitive and selective quantification of drugs in very small volumes. These fibers act as a miniaturized sample cell for analyte flow and Raman excitation light guiding improving the sensitivity required for the detection of low concentrated drugs. Finally we introduce novel miniaturized and highly sensitive Raman gas sensing approaches allowing for multi-gas. Urgent issues for Raman gas sensing include in-situ characterization of variations in microbial gas transformation in the soil. Raman multi-gas sensing helps to monitor the composition and temporal and spatial variation of biogenic gases that result from the complex interdependencies of aboveground and belowground physical, chemical, and biological processes and for the built-up of a database for ecosystem analysis.

(153) **Label-free Raman mapping of Living Mammalian Cells - A Valuable New Tool for Investigating Complex Cellular Systems**; Katherine Hollywood[1], LornaAshton[1, 2], Katherine Lau[3], Saba Khan[1], Nicholas Lockyer[1], Mark Dunne[1],Karen Cosgrove[1], Alan Dickson[1], Royston Goodacre[1]; [1]University of Manchester, UK; [2]Lancaster University, UK; [3]Renishaw PLC, UK

Raman mapping offers a powerful opportunity for the analysis of single cells due to its high spatial resolution (< 1 μm), speed of data acquisition and non destructive nature. Raman mapping is capable of providing vast amounts of spatially resolved biochemical information regarding the composition of cellular and sub-cellular components. In our laboratories we have recently established the capability to undertake 'true' live cell Raman microspectroscopy by coupling a Raman microscope with a near infrared (NIR) laser source and an *in situ* microscope incubator.

We will present our recent work whereby we have tracked the uptake and localization of dithranol, an anti-psoriatic drug, within human keratinocyte cells (HaCaT) in real-time over 24 h and at a clinically relevant concentration. The analysis of Raman maps collected from a number of cells demonstrated a variable uptake mechanism and localization pattern within the cells, which is perhaps not surprising due to the heterogeneous nature of a cell population.

We will also present our current work in which we investigate the glucose stimulated insulin secretion (GSIS) response from a genetically engineered human β cell line (EndoC-βH1) and human Islets of Langerhans. The GSIS response mechanism is of great interest in the search for cellular-based therapies for the treatment of diabetes and the current method for this detection is *via* ELISA. Our hypothesis is that we can use

Raman spectroscopy to monitor the effect of stimulation on the cell and also use Raman spectroscopy to detect the insulin secretion into the culture medium in real-time.

This work documents the exciting applicability of Raman spectroscopy for the analysis of 'truly' live mammalian cells. The analysis of live cells overcomes the inherent issues associated with the analysis of fixed cells that only present a single 'snapshot' of cellular metabolism. Thus, this technique could be applied to the analysis of multiple model cell systems and could be used to address a wide-range of biological questions.

(154) **Rapid, Quantitative Spectroscopic Imaging using Coherent anti-Stokes Raman Scattering**; Marcus Cicerone[1]; [1]National Institute of Standards and Technology

Over the past ten years, coherent Raman imaging (CRI) has evolved from a curiosity to a practical tool for investigating some classes of biological and material questions. An important key to this evolution has been the ability to rapidly obtain information from many spectral peaks. Most vibrational spectroscopic information is found in the fingerprint region where spontaneous Raman can be used to achieve > 3:1 signal to noise ratio for weak fingerprint peaks in biological systems, but typically requires acquisition times of several seconds; too slow for imaging. Coherent Raman methods have previously been unable to acquire high quality fingerprint spectra. We have overcome this limitation by developing a highly efficient signal excitation paradigm and appropriately harnessing the nonresonant background (NRB) signal that accompanies the resonant signal of interest. With these and other innovations, we have developed a CRI approach based on broadband coherent anti-Stokes Raman scattering (BCARS) that provides an unprecedented combination of speed, sensitivity, and chemical selectivity. Using this system we are able to obtain high quality Raman spectra in the fingerprint and CH stretch regions from biological specimens at 3.5 ms, enabling rapid, label-free chemical imaging of even delicate samples. I will briefly put our approach in context with the broader CRI field, describe key technical features of the present imaging system and provide application examples in materials and biology. I will also briefly discuss focus areas for future advances, and speculate on ultimate performance limits for coherent Raman imaging.

(155) **Bioorthogonal Stimulated Raman Imaging for Biomedicine**; Wei Min[1], Lu Wei[1]; [1]Columbia University

Innovations in optical imaging technology have significantly impacted modern biology and medicine. While most of the contemporary bio-imaging modalities harness electronic transition (fluorescence), nuclear spin (magnetic resonance imaging) or radioactivity (positron emission tomography), vibrational spectroscopy has not been widely used yet. Here we will discuss an emerging chemical imaging platform, stimulated Raman scattering (SRS) microscopy, which is capable of generating concentration maps of targeted chemical bonds in living systems with high sensitivity, specificity and resolution. When coupled with stable isotopes (e.g., deuterium and 13C) or bioorthogonal chemical moieties (e.g., alkynes), SRS microscopy is well suited for probing in vivo metabolic dynamics of small bio-molecules which cannot be labeled by bulky fluorophores. Physical principle of the underlying optical spectroscopy and exciting biomedical applications such as imaging lipid metabolism, protein synthesis, DNA replication, protein degradation, RNA synthesis, glucose uptake, drug trafficking and tumor metabolism will be presented.

(156) **Learning Risk-Taking as a Young Female Chemist**; Sarah Maurer[1]; [1]Central Connecticut State University

To be successful in chemistry, a young scientist must find a research project with an advisor to support them, stand up for their data, be active in planning a research timeline, work through failure and maintain enthusiasm for research. This is not easy for any student, but these tasks tend to be more difficult for young women who were taught from an early age to be: seen and not heard, helpful, selfless, helpless, and/or obedient. Certainly not every woman struggles with these issues and men suffer from them as well. I offer advice for taking those first scary steps into selfishness, asking for what you want, and standing up for yourself. I have found that being myself and investing in things I enjoy has brought greater rewards than caution and acquiescence.

(157) **Challenges in Managing a Diverse Workforce**; Fred LaPlant[1]; [1]3M

The percentage of women employed in the physical and life sciences continues to increase, tracking closely with academic enrollment. However, because there is an offset in experience, the percentage of women managers and scientists in senior positions still lags the general trend. Although it is changing, management positions in industry are still predominantly filled by men, who likely have very different life experiences from their direct reports. This talk will discuss some of the challenges that face a manager with a diverse workforce: setting expectations that are both fair and equal; providing accurate and appropriate feedback; and understanding the opportunities and limitations of being a guide and mentor.

(158) **Don't Call Us Dropouts (please)! Choosing Nontraditional Career Paths in the Sciences**; Emily Monosson[1]; [1]Independent

At a recent lecture about maintaining women in the sciences and the influence of labels and bias' a graduate student stood up and said several of her peers had gone part-time, "dropping out" of science for family, asking what could be done to prevent this (the so-called dropping out.) I don't recall the speaker's response, but she missed the irony. My first suggestion would be don't label them as drop-outs! There are many different paths through the sciences, not all choose academia or industry; others prefer part-time – but are no less committed to science, some take indirect paths meandering through one field after another acting more like a pollinator than a specialist. My career path began meandering soon after meeting my husband – also a scientist – and I haven't looked back. I have done research, taught, consulted and now focus on writing. The combined experiences led first to the publication of a book of essays about combining science and family written by women in science to my current interest in research and writing about evolutionary toxicology. I am certain I would not have developed these projects if I had followed the straight and narrow. There are many ways we contribute to science. Rather than labeling those who chose non-traditional career paths as drop-outs – if we support them, they may continue contributing to science in surprising ways.

(159) **Adventures Abroad! Pursuing International (European) Academic Positions**; Ingeborg Iping Petterson[1]; [1]Biomedical Physics, University of Exeter

This talk is aimed at those interested in pursuing a PhD, or who are near to completing one who are exploring 'the next step' in academia. There are a considerable number of PhD and postdoctoral positions available outside of the USA, but your personal life, attitude, and future career goals all weigh in on if going abroad is 'right' for you.

I will give some tips, and discuss pros and cons derived from my own experiences in pursuing both a PhD and a postdoctoral position in Europe. I will also discuss some of the unexpected challenges I faced in this international career path.

How does a PhD position in Europe differ from one in the US? What can strengthen an application for international positions? What are the advantages, disadvantages and other considerations in going abroad for education or work? My presentation will answer all of these questions, and more!

(160) **How a Frozen Banana Shaped my Career Path**; Heather Brooke[1]; [1]CAMO Software Inc.

In this talk, I'll discuss my career path: from my first spark of interest, through graduate school, and becoming a respected scientist in the field of spectroscopy and chemometrics.

(161) **Probing Ion Lipid Interactions by Vibrational Sum Frequency Spectroscopy**; Paul Cremer[1]; [1]Penn State University

Infrared-visible sum frequency spectroscopy, a surface specific nonlinear optical method, allows one to obtain vibrational spectra from aqueous interfaces. In our laboratory, this technique is being used in combination with fluorescence microscopy and microfluidic platforms to probe the behavior of two dimensionally fluid lipid bilayers in the presence of varying salts in aqueous solution. We have found evidence for tight binding of transition metal ions such as copper to phosphatidylserine lipids in a complex containing one metal ion and two lipids. Specifically, copper ions bind to the lone pairs on the amine groups as well as the neighboring carboxyl groups to form a square planar complex in which two protons are released. The formation of this complex has direct implications for the phase behavior of the membrane. Moreover, the release of the protons from the interface means that the surface charge remains unchanged upon the bivalent binding of the metal cation. This is significant because the double layer adjacent to the lipid interface remains essentially unchanged even after a saturation concentration of metal ions has bound to the surface. The nature of the interfacial hydration waters as well as potential biological implications from these findings will be discussed.

(162) **Protein Structures and Folding at Interfaces Probed by Chiral Sum Frequency Generation Vibrational Spectroscopy**; Elsa Yan[1]; [1]Yale University

Characterization of protein secondary structures at interfaces in situ and in real time is important to understand biological processes associated with cell membranes and develop biometerials and biosensors. However, such characterization remains challenging because it requires techniques that are both selective to interfaces and protein secondary structures. Here, we demonstrate that chiral sum frequency generation spectroscopy (cSFG) can provide peptide amide I and N-H stretch vibrational signals that are free of water background and characteristic to parallel beta-sheet, anti-parallel beta-sheet, alpha-helix, 3-10 helix and random coil. This enables cSFG to distinguish protein secondary structures at interfaces, similar to circular dichroism spectroscopy for protein characterization in solution. Using cSFG, we followed the kinetics of misfolding of an amyloid protein, human islet amyloid polypeptide (IAPP) that is associated with type II diabetes. We observed the misfolding of IAPP from disordered structures to alpha-helices and then to beta-sheets upon interactions with a lipid surface. These results demonstrate the selectivity of cSFG to interfaces and secondary structures, implying its potential in addressing a wide range of fundamental and engineering problems related to protein at interfaces.

(163) **Molecular Structure at Solid Surfaces: Understanding the Role of Bulk Effects**; Dennis Hore[1]; [1]University of Victoria

Even-order nonlinear spectroscopies such as second harmonic (SHG) and sum frequency generation (SFG) are valued for their sensitivity to interfacial structure as they are capable of discriminating from adjacent bulk phases based on symmetry. Visible-infrared SFG spectroscopy additionally harnesses the sub-molecular structural probe of a vibrational spectroscopy by tuning the infrared laser over molecular resonances. As a result, over the past two decades, SFG spectroscopy has been successfully applied to a wide variety of solid, liquid, and vapour interfaces, revealing signatures of the molecular organization that provide clues to the surface structure. For some systems - such as a dilute solution of molecules in contact with a solid surface - negligible concomitant changes in the bulk occur, compared to the structure that is evolving during the adsorption. In other situations, such as the drying process of polymers, major structural changes occur in the bulk phase, and these have consequences on the surface structure that is developing. In such cases, complementary bulk characterization is necessary, as the second-order nonlinear techniques will be blind to any changes that do not involve a polar ordering of molecules. For example, a decrease in the nonlinear optical response may the result of reduced orientation, or a decrease in the surface number density of that particular species. This talk will illustrate a few areas in which bulk vibrational measurements provide valuable clues as to the origins of the surface features.

(164) **Direct Small-Molecule Detection in a Primary Antibody Assay using Second > Harmonic Generation**; John Conboy[1]; [1]University of Utah

There are multiple clinical areas where quantification of small-molecules, peptides and low molecular weight proteins is crucial for the purpose of tracking disease, diagnostics, and illicit substance screening. Antibodies have the chemical specificity and high affinity needed to detect and quantify the presence of such species. In fact, the combination of their high affinity and exquisite selectivity has afforded antibodies a unique place in bio-specific detection and quantification. However, utilizing antibodies for the direct label-free detection of low molecular weight substances in a primary antibody assay is problematic. Sandwich assays are not possible due to the lack of multiple antigenic binding sites. Competitive ELISAs have been developed and are used clinically but suffer from a limited dynamic range and the inherent complexities of the methods. We have developing a noninvasive highly sensitive small-molecule immunoassay detection scheme using second-harmonic generation (SHG). The use if SHG for the direct label-free detection of several small-molecule drugs will be discussed, illustrating this highly sensitive and label-free detection scheme in a primary antibody small-molecule immunoassay.

(165) **What Makes Aqueous Foams Stable? A Combined Oscillating Bubble and Vibrational Sum-Frequency Spectroscopy Study**; Patrick Koelsch[1], Matthias J. Hofmann,[2], Robert Weikl[2], Hubert Motschmann[2]; [1]University of Washington, Department of Bioengineering; [2]University of Regensburg, Institute of Physical and Theoretical Chemistry

Aqueous foams play an important role in a variety of chemical processes, particularly the stability of liquid foams is a critical parameter not only in food, cleaning, and cosmetic products, but also for unwanted by-products in industrial processes. The macroscopic phenomenon has been extensively studied, but our understanding of its microscopic origin is still limited. Here we study foam formation of aqueous solutions containing two of the most common surfactants: the anionic sodium dodecyl sulfate (SDS) and the cationic cetyltrimethyl ammonium bromide (CTAB). We are using a combination of different techniques to probe various aspects of the interface: the oscillating bubble technique grants access to frequencies up to 500 Hz of the elastic modulus. Furthermore, foam stability measurements and vibrational sum-frequency generation (SFG) spectroscopy in the CH- and OH-stretching regions have been performed. At surfactant concentrations that show an elastic behavior, only unstable foams can be observed and the spectral signatures indicate that electrostatic forces are governing the orientation and arrangement at the interface. At concentrations of the surfactant solutions leading to a viscoelastic behavior, the spectral signatures in the OH region are dominated

by the exchange process of the surfactant and are not of electrostatic origin. We suggest that in case of viscoelastic surfaces, the corresponding dissipative process is related to the disruption of the water H-bonding network by the continuous exchange of surfactants. These results have implications in the development of foaming and anti-foaming agents for a variety of industrial processes.

(166) **Characterization of the Metalloproteome of Histoplasma capsulatum and its Implications Regarding the Pathogenic Response Under Low Zn Stress**; Anna Donnell[1], Alexey Porollo[2], George Deepe[1], Joseph Caruso[1]; [1]University of Cincinnati, [2]Cincinnati Children

Histoplasma capsulatum (*Hc*) is a dimorphic fungus and causes a respiratory infection known as Histoplasmosis which may develop into a progressive infection, especially for immunocompromised individuals. One of the first lines of defense against *Hc* is macrophage (MØ), yet *Hc* has the ability to avoid immune defenses by replicating within the MØ. One of the body's responses to this infection, using the *Hc* strain G217B, is the simultaneous sequestration of Zn and generation of Reactive Oxygen Species (ROS) through NADPH within MØ as shown by Vignesh et al.

In order to determine how the expression of metalloproteins in Hc changes under low Zn stress, the metalloproteome must first be characterized. The use of Size Exclusion High Performance Liquid Chromatography (SEC-HPLC) coupled to Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) allowed for the separation of proteins based roughly on hydrodynamic radii and nearly simultaneous metal detection. Chromatographic fractions were subjected to tryptic digestion and analyzed by tandem mass spectrometry (LC-MS/MS). Protein identifications were made through use of a custom, strain specific database. A custom annotation server was created with a simple user interface so that rapid selection of metalloproteins from the protein identifications could be achieved. This approach was applied to *Hc* grown in normal and low Zn stress conditions in vitro in an attempt to distinguish proteomic changes. Future work involves protein quantification and studies regarding the pathogenic response to oxidative stress to better understand the host-pathogen relationship.

(167) **The Use of Stable Isotope Labeling in Mass Spectrometry Based Bioanalysis**; Stephan Hann[1,2], Teresa Mairinger[1], Eva Oburger[3], Markus Puschenreiter[3], Gunda Koellensperger[4]; [1]1Division of Analytical Chemistry, Department of Chemistry, BOKU Vienna, Austria; [2]Austrian Center of Industrial Biotechnology (acib); [3]Institute of Soil Research, BOKU Vienna, Vienna, Austria; [4]Institute of Analytical Chemistry, University of Vienna, Austria

Nowadays, enriched stable isotopes and isotopically labeled compounds are frequently used in biological sciences in the context with elemental and molecular mass spectrometry. Most of the applications aim at quantification of selected analytes or at tracer studies, e.g. in systems biology or nutrition sciences. We will demonstrate the benefit of stable isotope labeling with recent examples of the above-described approaches. The first experiment aimed at the investigation of phytosiderophores, a group of root exudates released by grass species (Poaceae) for iron mobilization. We conducted a rhizotest experiment with different soils and wheat, grown under normal and Fe-deficient conditions. A significant increase of root exudation and release of the phytosiderophore 2´-deoxymugineic acid was found for Fe-deficient wheat. These results were obtained by an LC-MS/MS based approach using in-house synthesized $^{13}C_4$ - 2´-deoxymugineic acid. Moreover, metal mobilization by 2´-deoxymugineic acid was investigated in different top soil extracts via quantification of metal-phytosiderophore complexes with LC-ICP-MS employing on-line isotope dilution strategies. The data revealed quantitative complexation of the extracted metal fractions. In recent years, metabolomics has been recognized as an important field supporting research and development in industrial biotechnology. At BOKU Vienna we design mass spectrometry based methods for tracer experiments employing both enriched isotopes and isotopically labeled compounds. The first example discusses a sulfur tracer experiment with the yeast Pichia pastoris, which is widely used for protein production. SEC-ICP-SFMS was utilized to trace 34S labeling with $Na_2{}^{34}SO_4$ during a chemostat cultivation. Accurate determination of $^{34}S/^{32}S$ ratios provided data on intracellular protein formation, intracellular degradation, secretion and dilution by growth in the kinetic model and served as a decisions basis for further strain improvement. Our second example addresses the optimization of such microbial cell factories by introduction of a ^{13}C-tracer feeding ^{13}C-labeled glucose during cultivation. GC-QTOFMS technology was used for determination of isotopologue and tandem mass isotopomer distributions of primary metabolites. Subsequently performed ^{13}C metabolic flux analysis assesses in vivo fluxes, which are ideal descriptors of the dynamics of a metabolic network. The data spot bottlenecks and allow further optimization of the host organism improving product yield and quality.

(168) **Applications of ICPMS and MC-ICPMS at Chemical Metrology, National Research Council Canada**; Lu Yang[1]; [1]National Research Council Canada

ABSTRACT: Among various atomic spectrometry techniques, inductively coupled plasma mass spectrometry (ICPMS) is, undoubtedly, one of the most powerful analytical techniques for trace elements determination and speciation. It offers low LODs, wide linear dynamic range, multi-element detection capability, high sample throughput and the ability of isotopic analysis [1]. Analytical precision can be further enhanced by use of multi-collector instrumentation (MC-ICPMS). Recently, MC-ICPMS has become a powerful tool for achieving the highest accuracy and precision of isotope ratios measurements in various scientific fields [2]. At Chemical Metrology National Research Council Canada, developments of methodologies with highest accuracy and precision for such measurements are required for the production of Certified Reference Materials (CRMs). Recent research applications of ICPMS and MC-ICPMS for the trace metals measurements, element speciation [3], protein quantification[4] and high precision and accuracy isotope ratio measurements[5] in biological samples will be presented and discussed in details in this talk.

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(169) **ICP-MS for Multiplex Analysis of Copy Number Variations In Tumor Cells**; Maria Montes-Bayon[1], Tamara Iglesias[1], Marta Espina[1,2], L. Maria Sierra[1,2], Elisa Blanco-González[1]; [1]University of Oviedo; [2]Oncology University Institute (IUOPA)

Regulatory agencies have introduced the concept of "Genomic Biomarker" (GB) which is defined as a DNA or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes, and/or response to therapeutic or other intervention. Therefore, robust, reproducible

accessible genomic biomarkers can be used clinically to screen for diagnose, to monitor the activity of diseases, and also may be useful to guide molecularly targeted therapy and personalised regimens or to assess therapeutic response. Genomics biomarkers include DNA characteristics such as DNA modifications (e.g. methylation) or gene copy number variations. Genome-wide copy number variations (CNVs) in single cells is of special importance for a variety of applications in basic research and clinical diagnosis of diseases like Alzheimer's disease, autism, schizophrenia, breast cancer or obesity. In addition, recent studies have associated genomic CNVs with chemoresistance (tumoral cells that are intrinsically resistant to chemotherapy or become resistant after an initial partial response). Therefore, the development of suitable technologies for determination of relevant genomic biomarkers is an area of increasing interest.

In the present work we will illustrate the simultaneous determination of gene copy number variations for three different genes related to cisplatin resistance by means of gel electrophoresis coupled on-line with inductively coupled plasma mass spectrometry.

(170) **Are Matrix Effects in ICP-MS Independent of Analyte Ion Mass (With or Without High Negative Voltage Ion Extraction)?**; Shi Jiao[1], John W. Olesik[1]; [1]Ohio State University

Matrix induced changes in sensitivity (decreases in up to an order of magnitude) can degrade ICP-MS accuracy. Matrix effects are generally thought to be more severe for light analyte ions and heavy matrix ions, mainly based on the comprehensive characterization by Tan and Horlick [1] using a first generation ICP-MS. These observations are consistent with space charge repulsion or shielding in the positive ion beam. However, using a more current ICP-MS instrument we found that the while matrix effects are more severe due to high (mM) concentrations of high mass matrix ions than equimolar low mass matrix ions, matrix induced changes in sensitivity were similar for all analyte ions . This has implications on the both the choice of internal standards and fundamental causes of matrix effects in ICP-MS.

The severity of ICP-MS matrix effects can be reduced by increasing the time the sample spends in the hot plasma prior to the sampling orifice (by reducing the nebulizer gas flow rate). There are conflicting reports in the literature whether a highly negative extraction lens decreases the severity of ICP-MS matrix effects.

We have carried out a comprehensive characterization of matrix effects in ICP-MS as a function of matrix mass, analyte mass, lens voltage and nebulizer gas flow rate, on more current instruments (PerkinElmer ELAN 6000, that includes a positive focusing lens, and ThermoFinnigan Element 2, that has a -2000 V extraction lens).

The effect of matrix elements with a wide mass range (Na, Cu, Y, In, Cs, Tb, Lu, and Tl) on analyte sensitivities with a wide mass range (Li, B, Mg, Sc, Co, Ga, As, Sr, Cd, Ba, Eu, Yb, Bi, and U) were investigated. On both instruments, the severity of matrix effects were similar for analyte ions with different masses. The severity of matrix effects was slightly less using the Element 2. However, on both instruments the direction (enhancement or reduction of analyte signals) and severity of matrix effects depended on focusing lens voltage and nebulizer gas flow rate.

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(171) **Fiber Fiber Enhanced Raman Multi-Gas Spectroscopy for Breath Analysis**; Torsten Frosch[1], Timea Boegoezi[1], Stefan Hanf[1], Tobias Jochum[1], Juergen Popp[1,2,3]; [1]Leibniz Institute of Photonic Technology; [2]Friedrich Schiller University, Institute for Physical Chemistry; [3]Friedrich Schiller University, Abbe Centre of Photonics

Fiber enhanced Raman multi-gas spectroscopy is a novel technique for online and real-time monitoring of disease markers in exhaled breath. Human breath consists of major gaseous components (CO2, O2, N2), low concentrated gases, natural isotopes (12/13C16/18O2, 14/15N2) and various volatile organic compounds. Raman spectroscopy provides excellent chemical selectivity for identification of all gaseous components (except noble gases) with almost no cross-sensitivities but has a lack in sensitivity.

Recently, elaborate hollow optical sensor fibers were developed for Raman gas spectroscopy. These photonic fibers tremendously improve the interaction of the guided light and the gaseous and volatile sample components within the hollow core. This new technique, fiber enhanced Raman spectroscopy (FERS), enhances the analytical sensitivity at very low sample demand of some nL. FERS is ideally suited for rapid online monitoring of a suit of gases simultaneously in a complex mixture in a high dynamic range from 100% down to sub-ppm levels. FERS has high potential as miniaturized point-of-care technique in clinical application.

An important application is the hydrogen breath test, where patients with oligosaccharide malabsorption disorder (e.g. lactose intolerance) expire H2 and CH4 in concentrations in the range of several tens of ppm, generated by anaerobic metabolism of oligosaccharides by colon bacteria Escherichia coli and the activity of the large bowel bacterium Methanobrevibacter smithii. Other potential applications are in the diagnosis of: Heliobacter pylori infection, diabetes mellitus, hypercholesterolemia, oxidative stress, and lung cancer.

FERS is an arising technique and will help for better elucidation of disease markers in exhaled breath in combination other established methods.

(172) **IR Imaging: Applications in Wound and Transplant Pathology**; Michael Walsh[1], Bennett Davidson[1], Hari Sreedhar[1], Vishal Varma[1], Peter Nguyen[1], Sanjeev Akkina[1],Aliya Husain[2], Suman Setty[1], Andre Kajdacsy-Balla[1], William Ennis[1]; [1]University of Illinois At Chicago; [2]University of Chicago

Pathology, the study of tissues, is driven by the availability of information that can be derived from staining. This approach, however, is reliant on specific antigens being stained that can fail to take into account the entire biochemistry of cells within a tissue. This can lead to Pathology having limited prognostic value in predicting how a patient will progress and whether intervention would be of value. IR imaging has recently seen huge technical advances that allow for the imaging of the entire biochemistry of a tissue section in a few minutes, providing a novel route to giving additional prognostic information back to the pathologist and thus the patient. In this talk, we will discuss some of the recent advances in using IR imaging for the prediction of disease trajectory of immunosuppressed patients who have chronic wounds or have received organ transplants. In both cases, there is very limited information that can be derived using conventional pathology that can predict outcome. We have demonstrated that there are biochemical markers that can predict success of chronic wound closure and organ transplant outcome using IR imaging earlier than conventional pathology techniques. In addition, we will present recent data using a discrete frequency Quantum Cascade Laser imaging approach to allow for rapid tissue imaging.

(173) **Shining Light Inside Middle Ear: What Raman Spectroscopy Tells Us about Infection?**; Rishikesh Pandey[1], Nicolas Spegazzini[1], Tulio A Valdez[2], Ishan Barman[3], Ramachandra Rao Dasari[1]; [1]MIT; [2]Connecticut Children; [3]Johns Hopkins University

Otoscopic diagnosis of middle ear conditions is primarily dependent on a physician's ability to identify visual patterns in order to provide the adequate interpretation of a disease process. Although this method is of substantive clinical significance, it hides a plethora of pathologic and physiologic information that could have important therapeutic implications. Diagnosis of middle ear infection remains challenging in the clinical setting and suffers from significant observer variability and accuracy of physician diagnosis with currently available methods has been shown to be lacking with accurate diagnosis rates for otitis media ranging between 40 and 80%.

Exploiting the endogenous molecular contrast may provide sufficient information to aid in obtaining objective and reproducible disease diagnoses. We have recently attempted to explore the untapped potential of Raman spectroscopy to obtain biochemical signatures that can reveal latent information on pathological conditions including otitis media. In this talk, we present the first demonstration to diagnose different stages of infections based on changes in the spectral characteristics of mucin using Raman spectroscopy. This exciting result opens up the door for further investigations in actual clinical settings such as in real-time disease diagnosis without perforating the tympanic membrane.

(174) **Multi-centre Raman Spectral Histopathology of Deparaffinised Oesophageal Tissues.**; Jennifer Dorney[1], Martin Isabelle[, 2], Gavin Rhys-Lloyd[2], Catherine Kendall[2], Riana Gaifulina[3], Aaran Lewis[3],Geraint Thomas[3], Katherine Lau[, 4], David Reece[, 4], Nick Stone[1]; [1]University of Exeter, Exeter; [2]Gloucester Hospital, Gloucester, United KIngdom; [3]University College London; [4]Renishaw PLC UK

The potential for Raman spectroscopy to provide early and improved specificity of diagnosis on a wide range of tissue and biopsy samples in situ is well documented. The standard diagnosis methods of histopathological and immunohistochemical analysis provide valuable clinical information but require time-consuming tissue processing and are not routinely available during surgery, nor are they amenable to in vivo use. Vibrational spectroscopy offers a complimentary diagnosis tool containing detailed information relating to molecular structure and composition, but is not yet used to a significant extent in a clinical setting.

In this study, we present the results of identification and discrimination of cancerous and non-cancerous tissue samples from patients presenting with Barrett's Oesophagus, from three different Raman instruments across three different locations.

Fresh frozen tissue sections, 10 intraepithelial metaplasia (IM) tissue samples and 10 adenocarcinoma (AC), taken from 20 patients, were thawed and used for spectroscopic analysis. Consecutive tissue sections were also collected for H&E tissue staining. Each centre received a consecutive frozen tissue section from each sample and the same areas of interest are measured in each centre using a 785nm laser in a Renishaw Raman microspectrometer. A routine histopathology review of H&E stained sections was carried out to provide gold standard training and validation for the spectral classification models.

In this project, three clinical centres and a Raman spectrometer vendor are building on existing technology to provide pathology with an automated, fast and simple to use method. Current histopathological techniques for the detection and classification of cancer often lead to ambiguous diagnoses. We expect this method will help to improve diagnostic rates, specificity of diagnosis and early detection.

(175) **Addressing Variability of Tissue Raman Spectroscopy for Clinical Diagnostics**; Isaac Pence[1], Anita Mahadevan-Jansen[1]; [1]Vanderbilt University

Noninvasive clinical diagnostics of disease states has been a widely investigated topic for the biomedical photonics and vibrational spectroscopy communities. The demonstrated sensitivity of Raman scattering techniques to malignant and benign disease states has implied the potential for quantitative and real-time evaluation. However, numerous sources of variability related to physiology, instrumentation, and experimental protocol have dramatic impacts on clinical data collection, analysis, and interpretation. Using Raman spectroscopy to study both dermatology and gastroenterology applications, we seek to characterize and account for potential sources of variability within healthy tissue and both inflammatory and cancer related diseased states. Specifically, sensitivity to variations as a function of patient measurement, instrument configuration, signal preprocessing, and data analysis. Here, we present a fiber-optic probe-based 785nm Raman spectroscopy study of in vivo measurements addressing practical and experimental issues associated with clinical implementation of Raman spectroscopy for real-time detection of disease. In vivo measurements were made with multiple versions of a single probe design for each organ on interest: studies of both Inflammatory Bowel Disease and healthy colon were measured endoscopically, while skin lesions and adjacent or contralateral normal measurements were collected with a conventional fiber-optic probe. Diagnoses for both diseases groups were performed by attending clinicians through visual inspection of patients prior to data collection and subsequent histopathology. We report an analysis of the spectral variability of healthy and inflamed colon tissues as well as normal skin and common benign lesions made in a clinical setting along with an evaluation of system performance. Furthermore, we present preliminary findings on correcting for variations induced via data collection with multiple systems and probes, even of the same design. System evaluation and characterization and comparison are critical steps in the formation of a spectral databases needed for large scale application of Raman spectroscopic techniques for robust discrimination of disease with quantitative metrics, real-time implementation, and non-invasive detection.

(176) **Pattern Recognition/Machine Learning Classification Strategies for Forensic Evidence**; Stephen L. Morgan[1], Nathan C. Fuenffinger[1]; [1]University of South Carolina, [2]University of South Carolina

We discuss the applicability and utility of approaches that have been applied to forensic data for classification based on measured evidence properties. If forensic scientists are to make better use of advanced statistical methods, the operational basis for technique must be well understood along the correct interpretation of results and the limitations to which each approach is subject. Although principal component analysis (PCA) has arguably been the method most widely applied in forensic science over the last 20 years, PCA is not a classification technique in itself. Linear discriminant analysis (LDA), and its extension to multiple groups (canonical analysis), is widely applied to decision-making with two-group classification; quadratic discriminant analysis (QDA) is less widely used. These methods all take into account correlations among feature measurements to find optimal solutions for class assignments. At the other extreme, naïve Bayes (NB) classifiers are simple and can be effective, despite assuming complete independence of features (ignoring correlation among features) in classification samples. Performance of these and other classification methods will be shown over several difference data sets), and pitfalls and limitations will be discussed. One limitation to wider use of classification techniques is that the new practitioner often faces a steep learning curve in both comprehending and using available software. We have developed some application programs that run in the Microsoft Windows environment and offer a user-friendly

interface and intuitive results panel for interactive exploration of the utility of pattern recognition and demonstrate their applicability to several data sets.

(177) **Development and Evaluation of a Searchable Database for the Characterization and Comparison of Forensic Evidence using Spectrochemical Methods**; Tatiana Trejos[1], Claudia Martinez[1], Ruthmara Corzo[1], Kiran Subedi[1], Rhett Williamson[1], Peter Torrione[2], Jong Yoo[3], Jose Almirall[1]; [1]Florida International University; [2]CoVar Applied Technologies; [3]Applied Spectra, Inc

A searchable database was designed and validated as a tool to improve the chemical information gathered from the analysis of forensic evidence. Printing inks and electrical tapes were selected for this study due to their occurrence in forensic casework and homeland security investigations. The database contains over 300 samples from printing ink sources (toner, intaglio, inkjet and offset) and 94 samples from black electrical tape sources that represent some of the global diversity of these materials. The samples were chemically characterized by three to five different spectrochemical methods (infrared spectroscopy, scanning electron microscopy energy dispersive spectroscopy, pyrolysis gas chromatography mass spectrometry, laser ablation inductively coupled plasma mass spectometry and/or direct analysis in real time coupled to mass spectrometry). An automated search of a database after comparing one or more instrumental characterizations enables the user to significantly narrow down the possible sources of ink and tape samples contained within the database. The statistical methods used in the database are based on partial-least squares discriminant analysis (PLSDA) algorithms, KNN classification, and multi-sensor confidence-level fusion. The current version of the database makes use of a min/max-normalized Euclidean distance metric. The database software uses algorithms for classification, assuming the existence of both a “reference” and an “experiment” (or training and testing) databases. The experiment database was treated as “unknown blind samples” and was built from duplicate control samples analyzed at different time intervals, examined by different analysts and/or different instruments. The developed algorithm generates a similarity “score” of the chemical similarity between samples and reliably associates chemically identical or similar samples for most of the techniques with LA-ICP-MS performing best. The results showed that decisions made using data fusion across multiple techniques are often significantly better than decisions made using any of the techniques in isolation. Parameters of forensic interest such as error rates, discrimination potential, inter and intra-source variations are reported for each matrix and each analytical method. The proposed database will advance the capabilities in the field by comparing, associating or eliminating chemical and morphological profiles of forensic evidence in an automated manner utilizing a variety of statistically sound algorithms and practical visualization tools

(178) **A Bayesian Approach to Interpretion of Multi-element Data**; James Curran[1]; [1]University of Auckland

The 2009 National Academy of Science Report "Strengthening Forensic Science in the United States: A Path Forward" held the treatment of DNA evidence up as a gold standard for the interpretation and presentation of forensic evidence. Whether one agrees that DNA is, in fact, such a gold standard or not, the point remains that the advances in the (statistical) interpretation of evidence have not progressed as far as they have in DNA.

In this talk I will discuss why we need statistical interpretation of forensic evidence, and why the Bayesian, or Likelihood Ratio approach fits most closely with the requirements of the court. I will also talk about the issues that are peculiar to the treatment of evidence arising from multi-element concentration data, and the current challenges to a full adoption of the Bayesian methodology.

(179) **Chemometric Approaches for the Analysis of Chemical Attribute Signatures Generated from Forensically Relevant Samples**; Adam B. Hall[1]; [1]Northeastern Univeristy, [2]Boston University School of Medicine, [3]IonSense, Inc.

Current methods for the extraction and analysis of ignitable liquids, smokeless powders and adulterated drugs of abuse suffer from lengthy chromatographic separations prior to MS analysis. In an effort to decrease case-submission-to-reporting turnaround times, we have evaluated higher-throughput methods for use by the forensic science community. DART ionization prior to mass spectral detection has been utilized for the evaluation of generated chemical attribute signatures from forensically relevant samples. These signatures were then interrogated utilizing chemometric approaches in an effort to further classify and determine whether or not two samples could have originated from a common source.

For several applications we demonstrate the use of statistical analysis models that can discriminate analyzed samples in a fraction of the time required for traditional chromatographic methods. In order to evaluate the robustness of such an approach for use in forensic casework, predictive models were constructed using machine-learning techniques and evaluated for their performance in classifying forensically relevant samples. The models generated were able to accurately predict the presence or absence of targeted analytes with error rates between 0 - 4%. Predictive classification models were developed using a combination of pre-processing steps, principle component analysis and machine-learning techniques.

Predictive modeling has advantages over current mass spectral libraries, which are limited to the identification of pure compounds. The ability to employ chemometric approaches for the analysis of the generated data demonstrates an attractive and viable alternative to conventional techniques for ignitable liquids, smokeless powders and adulterated drugs of abuse in forensic casework.

(180) **Evaluation of Analytical Figures of Merit for the Analysis of Nitrogen, Phosphorous, and Sulfur Using Laser Induced Breakdown Spectroscopy (LIBS)**; C. Derrick Quarles Jr.[1], Charles Sisson[1], Jhanis J. Gonzalez[1,2], Richard E. Russo[1,2]; [1]Applied Spectra, Inc.; [2]Lawrence Berkeley National Laboratory

One of the major benefits for using Laser Induced Breakdown Spectroscopy (LIBS) is its ability to detect elements that are difficult or impossible to analyze by most analytical techniques. This is the case, for example, for non-metals such as H, N, O, and halogens such as F, where these elements are difficult or impossible to analyze by XRF or conventional ICP-MS systems. Applied Spectra, Inc. has developed a commercially available Laser Ablation (LA) – LIBS tandem system (J200) that can be coupled with any ICP-MS instrument. In this study, a J200 LIBS instrument equipped with a 266 nm wavelength laser was used to evaluate the figures of merit during the analysis of nitrogen, phosphorous, and sulfur by LIBS while simultaneously analyzing other elements by LA-ICP-MS. A set of soil, plant, and fertilizer samples were used to determine the best detector option for N, P, and S detection and to establish the analytical figures of merit.

(181) **Applications of a New Single Stage Accelerator Mass Spectrometer to Trace Detection and Nuclear Forensics**; Albert Fahey[1], Kamron Fazel[1], Kenneth Grabowski[1], Evan Groopman[1]; [1]Naval Research Laboratory

A new Single Stage Accelerator Mass Spectrometer (SSAMS) able to accept positive ions as the input species is being tested and developed for a wide range of measurements that will cover many elements in the periodic table. Currently, a secondary ion mass spectrometer (CAMECA ims-4f) is being used as an ion source for the SSAMS. This allows the measurement of small, solid samples that typically are not analyzed by Accelerator Mass Spectrometry (AMS).

First results from the SIMS-SSAMS will be presented along with modeling and characterization of the instrument. Background for additional measurements will be discussed covering isotopic and elemental abundance measurements of minor elements in minerals and other samples. In addition, some discussion of the analysis of cosmogenic radionuclides and other chronometers will be discussed.

Future plans to adapt other sources that are typically used in conventional mass spectrometry will be reviewed, and salient parameters of operation will also be discussed. Applications to Nuclear Forensics will also be addressed and preliminary data shown.

(182) **X-Ray Microscopy of Nuclear Materials**; Jesse Ward[1], Greg Eiden[1], Andrew Duffin[1]; [1]Pacific Northwest National Laboratory

Spectromicroscopic techniques that can non-destructively measure the structure and chemical composition of materials with high resolution are a powerful complement to currently available nuclear forensics techniques. Scanning transmission X-ray microscopy (STXM) beamlines available at advanced synchrotron facilities can produce soft X-ray images with nanoscale spatial resolution and perform absorption scans with sub-eV spectral resolution. Since soft X-ray absorption spectra are highly sensitive to the oxidation state and physical and chemical structure of the analyte, chemical assignments can be made on a fine scale. This talk will demonstrate how STXM and complementary techniques (specifically, electron tomography and electron energy loss spectroscopy) can be used to perform chemical imaging of heterogeneous samples at high spatial resolution. In addition, we will demonstrate how these tools can be used to study the chemical aging and weathering of uranyl fluoride. Chemical imaging can reveal information about the sample not available to techniques which measure the isotopic composition alone.

(183) **Advances in Analysis of Samples for Nuclear Non-Proliferation at CEA/DIF**; Bruno Bernard-Michel[1], Fabien Pointurier[1], Maxime Bridoux[1], Anne-Laure Fauré[1], Amélie Hubert[1], Olivier Marie[1], Anne-Claire Pottin[1]; [1]CEA-DIF, Bruyères le Châtel

The CEA/DIF analytical laboratory is a member of the NWAL (NetWork of the Analytical Laboratories) and carries out bulk and particle analysis on environmental samples on behalf of the safeguards program of the IAEA.

Both qualitative and quantitative methods are implemented at the CEA/DIF in order to fully capture the characteristics of nuclear materials, from trace amounts (particles of a few micrometres) to more significant levels (milligrams or grams). Samples characterization is no longer focused solely on the isotopic composition but rather consists in a combination of methods (determination of chemical impurities, physical characteristics, isotopic composition, and presence of organic compounds). Indeed, one of the challenges in the field of nuclear non-proliferation is the identification of new signatures that can be used to decipher the origin, production history and intended use of the nuclear material under study.

As a result, a broad range of analytical techniques are nowadays developed in our lab to provide relevant information about nuclear materials.

We will review here our latest advances on extraction of process signatures, such as:

1/ the implementation of radio-chronometry methods to determine the purification date of uranium compounds,
2/ the identification of chemical composition of uranium compounds contained in micrometer-size particles by micro-Raman spectrometry,
3/ the analysis, at trace levels, of organic compounds (for example, extraction solvents) by high resolution mass spectrometry (Orbitrap) and gas chromatography mass spectrometry,and geolocation signatures, such as:
4/ the determination of oxygen isotope ratios in uranium oxides,
5/ the quantification of rare earth elements present at trace levels in uranium ore concentrates.

(184) **Discrimination of Uranium ore Concentrates from Several Countries by Chemometric Data Analysis**; Josette El Haddad[1], Aissa Harhira[1], Alain Blouin[1], Mohamad Sabsabi[1], Marvin Zaluski[2], Chunsheng Yang[2],Christopher Drummond[2], Slobodan Jovanovic[3], Tanya Hinton[3], Ali El-Jaby[3]; [1]National Research Council Canada - Energy, Mining and Environment; [2]National Research Council Canada - Information and Communications Technologies; [3]Canadian Nuclear Safety Commission

Canada is in the process of developing a National Nuclear Forensics Library (NNFL) cataloguing Canada's nuclear and other radioactive material holdings. A key component of Canada's NNFL development program is the development of data analytics approaches, methods and tools for the interrogation and interpretation of nuclear and other radioactive material data for purposes of conducting comparative assessments. The National Research Council (NRC) is leading the technical development of data analytics approaches, methods and tools under the Canadian NNFL development program.The program is pursuing an incremental approach by first demonstrating a technological and operational proof-of-concept using one material group. As such, the objective of the program is to develop, design and deploy a NNFL database prototype cataloguing a known population of uranium ore concentrate (UOC) material under Canadian regulatory control. UOC samples from eight geographic locations and twenty producers were measured for trace-element concentrations, as well as lead and uranium isotopic ratios using inductively coupled plasma mass-spectrometry (ICP-MS). The results of different chemometric methods, supervised and unsupervised, used to develop a predictive tool for the discrimination of the origin (geographic location and/or producer) of UOC material will be presented. The chemometrics analysis of the ICP-MS data revealed different geological formations as well as different chemical milling processes, which is a significant development in the field. These results improved the selection of predictors and the robustness of chemometric predictive methods. Excellent results on Identification of UOC material origins obtained without using the highest markers for discrimination (e.g., rare earth elements or the isotopic ratios) will also be presented.

(185) **DC Arc Spectroscopy – Plasma Characterization for Direct Solid Analysis of Nuclear Materials**; Benjamin T. Manard[1], John Matonic[1], Robert Jump[1], Dennis Montoya[1], Alonso Castro[1], Ning Xu[1]; [1]Los Alamos National Laboratory

Direct current (DC) arc spectroscopy, a well-established technique, has been employed over the last century and has been considered as the benchmark for the more commonly used inductively coupled plasma (ICP) technique. Recently, a resurgence of this plasma-based technique has been realized due to its ability to effectively excite elemental species from complex solid matrices. Applications can be seen ranging from the analysis of heat-source pellets which fuel space explorations, to potentially analyzing post-detonation debris from nuclear explosions, as DC arc can effectively excite species from very complex matrices including concrete.

With this resurgence in technology, commercially available systems have been incorporated into everyday research by Teledyne Leeman Labs. While the DC arc shows great promise for performing elemental analysis of solids in complicated matrices, plasma studies and characterization must be employed in order to enhance the instrument detection limits, sensitivities, and reproducibility. Presented here are in-depth plasma characterization studies by monitoring excitation temperatures and excitation/ionization efficiency while exploring different operating parameters/conditions. It is envisioned that with these studies, a more practical instrument can be realized and performing analyses of nuclear materials can be improved, particularly when monitoring trace elements in complex, solid matrices.

(186) **NIR With Problem Data Sets**; Franklin Barton[1], James de Haseth[1]; [1]Light Light Solutions Instruments, Inc.

There are many approaches to developing a data set for Near Infrared calibrations, some statistically valid, some not; but all with some interesting insights. There is the ideal situation with a large set of samples, ample laboratory resources for reference chemistry. Do you make a random selection or take every nth sample and scan and analyze? Do you start scanning and analyzing and build your calibration data set gradually as you go, with the big question? When do I stop? Yes, you can use the reference data to select a diverse range of samples insuring that the ends as well as the range are adequately sampled. Some chemometric software packages will choose the most diverse set of samples based on the spectra. This provides a stopping point for initial calibrations and limits the amount of reference chemistry you may have to do. However, it is often not the situations that arise in the NIR lab that provide the most strenuous challenges. Usually it is the real world samples from commercial operations that have their limitations based on the process. The bottom line is you get to work with what you are handed. We will examine some of these data sets and the unique opportunities for model development.

(187) **A New Look at the Derivative Quotient Method in Regression**; David Hopkins[1], Karl Norris[2]; [1]NIR Consultant, Battle Creek, MI, [2]NIR Consultant, Beltsville, MD

The original work of Karl Norris and William Hruschka in applying multiterm linear regression on derivative quotients has been reviewed using new software developed using Matlab. Using extensive graphical presentations, the function of the numerator and denominator terms can be demonstrated. The method starts with full spectra for a set of samples used for calibration, and results in one or more terms in a prediction equation where the numerators and denominators of each term are the values of derivatives at selected wavelengths. The derivatives are calculated by the Gap method pioneered by Norris, and each derivative is optimized for the gap, center wavelength, and width of 'boxcar' smoothing. We have extended the original work by utilizing derivatives up to fourth order. We have shown that the numerator and denominator derivatives do not have to be of the same order. It appears that the best models employ numerator and denominators that require differing levels of smoothing. Principles of developing calibration models and validating them will be presented, using NIR spectroscopy to measure protein in wheat in samples that exhibit a high degree of spectral variation due to particle size variability. The models involve only one to three terms developed from full spectral scans of the calibration samples, and therefore are very easy to implement. We believe that coupling the models with current chemometric methods for qualifying samples for analysis provide a powerful new technique for routine analysis of samples. This regression method appears to be capable of achieving greater accuracy than can be obtained utilizing the standard methods of pretreating the spectra and regression using PCR or PLS.

(188) **Ultra-Compact Smart Spectrometers For Food, Agriculture, and Pharmaceutical Applications**; Nada OBrien[1], Christopher Pederson[1], Peng Zou[1]; [1]JDSU Corporation

Recent innovations in optical designs have led to the realization of ultra-miniature spectrometers with excellent performance and low-power consumption enabling a new class of handheld NIR devices that are controlled by smart mobile devices. The benefits of such devices over conventional handheld instruments that contain an on-board computer and a display screen are numerous: lower cost, much smaller form factor and ease of model updates across a wired or wireless network, thus resulting in greater scalability and wider deployment. Another benefit is the ability to interconnect and network multiple such devices for a given application which allow for smart, real-time monitoring, and continual optimization of the analysis. This new class of NIR spectrometers is creating an ecosystem of ubiquitous NIR sensors that help farmers increase their yield, breeders optimize their biomass crop, and manufacturers reduce their costs and improve product quality.

In this presentation, we will talk about the technology behind a miniature NIR spectrometer that weighs 3 ounces and is less than 2" in diameter. The spectrometer covers the wavelength ranges of 950-1650nm in one configuration and 1150-2150nm in another. We will discuss specific fit-for-purpose applications of a handheld version of the spectrometer that is tethered to a small tablet computer for non-destructive on-farm nutrient content analysis of animal feed and show results of detecting fraud in adulterated chicken filets.

Other application examples in the pharmaceutical industry such as on-line analysis of active ingredient concentration on a tablet-press machine and end-point monitoring on a blending process will be presented. The spectrometer used on the rotating blender is battery-powered and relies on wireless communication for instrument control and data transfer. It weighs less than 3lbs and measures 6.3" x 5.37" x 5.37", the smallest NIR spectrometer ever utilized for industrial process analytical technology (PAT) applications making these spectrometers suitable for on-line process monitoring and control on continuous manufacturing processes. Additionally, their compactness allows for hassle-free deployment and minimum change to existing production lines.

We will therefore show how mobility, affordability, and simplicity of these smart spectrometers are democratizing the NIR spectroscopy field for a wide range of applications.

(189) **A Novel Configuration for Near-Infrared Analysis of LPG Composition and Quality Control in a Refinery Setting**; Susan Foulk[1], Shashi Mistry[2], Terry Todd[1], Nate Peters[2], Dian Wang[2]; [1]Guided Wave, Inc.; [2]Suncor Energy

Near-Infrared (NIR) spectroscopic process analyzers are commonly used in refineries for a variety of composition and physical property measurements such as octane number, aromatic content, and distillation properties of feed and finished products. Despite its wide use, there are few known instances of using NIR spectroscopy to measure the composition of LPG (liquefied petroleum gas). The composition analysis of LPG is typically carried out by accurate but expensive gas chromatography (GC). In this work, a Near-Infrared analyzer was installed on the Hydrocracker fractionation unit (HCU) on following columns:

1. Main fractionator unit (Naphtha)
2. Main fractionator unit (light distillate)

3. Main fractionator unit (Heavy distillate)
4. Depropanizer bottoms stream (Butane analysis)
5. Deethanizer bottoms stream (Propane analysis)

The Suncor Edmonton refinery had installed 90% (distillation) analyzers on the first three streams above and GCs on streams 4 and 5 above. Due to the need to improve on quality, uptime and to reduce analyzer maintenance costs, Suncor shifted to NIR technology for these LPG measurements. The LPG analyses must work for entire operating range of the HCU as well as assist in both liquid and vaporization modes. This paper will describe the installation and experimental work involved as well as provide a discussion of the LPG NIR data and process results. An HCU process unit-wide multi-variable controller will be incorporated for quality control using five NIR analyzers. Suncor intends to economically optimize the Hydrocracking unit using properties such as 90%, Viscosity, Cloud Point, and Flash Point as needs and seasons demand. The LPG stream properties are required to control the purity of both Butane and Propane for the export market as well as to stabilize the process unit. NIR allows Suncor to achieve speed of quality control at a much reduced cost over the long term.

(190) **Field Analysis of Fuel using a Portable Near-Infrared Spectrometer**; Wayne Smith[1], Carl Brouillette[1], Chetan Shende[1], Stuart Farquharson[1]; [1]Real-Time Analyzers, Inc.

Fuel quality is becoming increasingly important in many countries where fuel theft occurs in the form of diluting shipments using less expensive petroleum products. Diluted fuels with inferior chemical and physical properties lead to decreased engine performance and ultimately engine failure. These properties include aromatic, fuel system icing inhibitor, olefinic, naphthene, oxygenate, and saturate content; cetane and octane numbers; cloud, distillation, flash, freezing, and pour points; density (API gravity), Reid vapor pressure, and viscosity. Complete analysis of a suspect fuel requires multiple analyzers, typically one per property, and as much as 8 hours. Such analysis is not always performed. Consequently, there exists a need to rapidly verify fuel quality upon receipt. Often, such verification must be performed far from a lab containing standard fuel analysis instruments and apparatus. To satisfy this need, we have developed an inexpensive, compact (4x7x8", 6 lb) fuel analyzer based on near-infrared spectroscopy that employs chemometrics to determine the 16 properties listed above in 10 seconds, using only 3 mL of sample. This presentation will describe the analyzer and the chemometric analysis.

(191) **Recent Progress and Current Challenges in Using LIBS for Bacteriological Identification**; Steven Rehse[1], Dylan Malenfant[1], Derek Gillies[1], Vlora Riberdy[1], Anthony Piazza[1]; [1]University of Windsor

There is a well-known and urgent need in the fields of medicine, environmental health and safety, food-processing, and defense/security to develop new 21st Century technologies for the rapid and sensitive identification of bacterial pathogens. In only the last five years, LIBS has become a potential candidate technology for this application. LIBS' significant advantages over current as well as other competitive emerging technologies include speed (< 1 sec analysis), portability, robustness, lack of consumables, little to no need for sample preparation, no need for genetic amplification, no need for user expertise, and the ability to identify all bacterial pathogens without bias (including spore-forms and "viable but non-culturable" specimens).

In this talk I will present the latest achievements of our lab to more fully develop LIBS-based bacterial sensing; to increase our understanding of why a LIBS-based diagnostic is sensitive to bacterial elemental variation (and why it can fail); and to develop more physician-friendly or pathologist-friendly testing protocols. To date, we have uniquely identified species from over five bacterial genera with high-sensitivity and specificity. We have shown that bacterial identifications are unaffected by environment, nutrition media, or state of growth. We have shown that accurate diagnoses can be made on autoclaved or UV-irradiated specimens, although is currently a very active area of interest. Efficient discrimination of bacteria at the strain level has been demonstrated. A rapid urinary tract infection diagnosis has been simulated with no sample preparation. We have shown that efficient discrimination is possible in contaminated or "mixed" samples and a variety of autonomous multivariate analysis algorithms have been investigated with different library models constructed from the LIBS data in various ways.

I will then review the challenges facing us as we transition from laboratory "demonstration" experiments to more commercially-acceptable techniques and instrumentation. These challenges include: identifying a suitable substrate for mounting the bacteria, concentrating a number of cells for sufficient signal-to-noise from a clinical specimen, and introducing a limited number of cells reproducibly into the analytic plasma.

(192) **Laser-Induced Breakdown Spectroscopy for the Evaluation of Residual Catalysts in Pharmaceuticals**; Lydia Breckenridge[1]; [1]Bristol-Myers Squibb

Laser-Induced Breakdown spectroscopy (LIBS) has been evaluated as a technique to provide in-process support for the determination of residual catalysts in pharmaceutical products. Catalysts containing metals such as palladium, rhodium and copper are routinely used in pharmaceutical chemical development in order to facilitate a reaction. However, the catalysts must be sufficiently remediated in order to ensure that concentrations in the final active pharmaceutical ingredient (API) satisfy regulatory requirements, which are based on toxicological safety thresholds. LIBS has been demonstrated as a suitable tool to assess the presence and subsequent levels of a variety of different catalysts used during synthesis. Owing to the minimal sample requirements and little-to-no preparation afforded by the technique, results could be generated very rapidly, thereby allowing process chemists to make synthetic adjustments as needed in order to satisfy the established limits.

(193) **Identification of Meat Species by using Laser Induced Breakdown Spectroscopy**; Gonca Bilge[1], Banu Sezer[1], Hasan Murat Velioğlu[2], Kemal Efe Eseller[3], Halil Berberoğlu[4], İsmail Hakkı Boyacı[1]; [1]Hacettepe University, Department of Food Engineering; [2]Namık Kemal University, Department of Agricultural Biotechnology; [3]Atılım University, Department of Electrical & Electronics Engineering; [4]Gazi University, Department of Physics

Meat is one of the most consumed food product with a high nutritional value, However availability of meat products is limited due to high prices. Therefore, meat adulteration is widespread in meat industry. The most applied meat adulteration is addition of cheaper meat species such as pork and horse meat to costlier meat species such as beef. Consumers must be protected from these adulterations because of economical loses, health implications and religious beliefs. Many detection techniques have been developed for identifications of meat species. Analysis of DNA and protein is most common practices. Although genetic methods are quite sensitive and reliable, they need specialists and they are time consuming, expensive methods. In this study identification of meat species was performed by using laser induced breakdown spectroscopy (LIBS). There are several reports in the literature about elemental composition differences between meat species such as chicken, beef, fish and lamb. Studies show

that Ca, Mg, K, Na, Zn, Cu and Fe compositions varies between meat species. These differences were used for identification of meat species by using LIBS as a rapid and practical technique. For this purpose various meat species such as pork, calf and chicken from different regions of animals such as breast, hind and back were received from different sources (six animal for each species). LIBS spectra of meat samples were analyzed by multivariate data analysis technique, principal component analysis (PCA), and identification of meat species was conducted successfully. After that for the quantitative study and obtaining of calibration curves, controlled adulterated meat samples (pork in calf and chicken in calf at different ratios) were prepared and LIBS spectra of these samples were analysed with partial least square (PLS) method. Elemental compositions of the meat samples were confirmed with reference methods such as atomic absorption spectroscopy and inductively coupled plasma – mass spectrometry. According to the results, potential using of LIBS system for routine analysis of meat samples' quality control is high compared to other methods because it is rapid, in situ, reliable and free from sample preparation method.

(194) **Study of Plasma and Identification of Hazardous Elements in the Polystyrene using Laser Induced Breakdown Spectroscopy**; W. Aslam Farooq[1]; [1]King Saud University

W. A. Farooq, A. S. Al-Johani, Walid Towfik, Rabia Qindeel. Laser-induced breakdown spectroscopy (LIBS) is one of the analytical techniques which have vast application in the material analysis. We have applied the technique for the elemental analysis to find impurities and hazardous elements in polystyrene, material from one of the Saudi industries which are being used in manufacturing of water and food containers. The study was carried out in high vacuum chamber. Fundamental frequency from Q-switched pulsed Nd:YAG laser was used to generate the plasma of the sample placed on rotating stage in the vacuum chamber. The emitted light from the plasma was collected with Optical fiber and spectra were recorded after passing the light through the spectrometer equipped with ICCD camera. We have identified Al, Si, P, Ca, Mg, N elements in Polystyrene. We have also detected molecular lines of CN, CO, C2 and CH in the sample. Presence of Al, Si, and P might be harmful to the human body. LIBS plasma temperature was determined using Boltzmann plot and found to be 8000 K. The number density was 1.7x1018 cm-3 which was estimated from the stark broadened profile of the spectral line.

Ref: 1- L.J. Radziemski, Spectrochim. Acta Part B 57, 1109 (2002). 2- W.T. Mohamed, Opt. Appl. 37, 5 (2007)

(195) **Application of Low Frequency Raman During the Crystallization Process**; John Wasylyk[1], Ming Huang[1], Robert Wethman[1]; [1]Bristol-Myers Squibb Co.

Raman spectroscopy using low frequency (200-8 cm-1) spectral regions is used to study the various polymorphs. The low frequency spectral region provides access to collective vibrations of molecules in the crystalline and amorphous state and yields valuable insight when differentiation of various forms is quintessential. Quantitative analysis of the mixtures of polymorphs, as well as qualitative analysis of amorphous levels in bulk crystalline material, has been successfully demonstrated in the low frequency range. Studies involving low frequency Raman bands provide greater sensitivity for detecting the onset of crystallization and has allowed differentiation of crystal types when multiple forms are possible. Recent applications of in-line crystallization processes provided insight into more than one crystallization event leading to one final desired crystal form. Studies suggest that different responses for low-frequency Raman bands occur for the micro-crystalline domains present during the initial crystallization process. As a results of our studies, low frequency Raman has proven to be a valuable tool for at-line and on-line monitoring of active pharmaceutical ingredient crystallization.

(196) **The Contribution of the Low-Frequency Raman Spectroscopy to the Structural Description of Disordered Molecular Systems and Their Transformations: Application to Pharmaceuticals**; Alain Hedoux[1], Laurent Paccou[1], Yannick Guinet[1]; [1]University Lille 1, UMET - UMR CNRS 8207

The technology improvements in the domains of notch filters and laser diodes have led to the development of routine spectrometers giving access to the low-frequency domain. Typical molecular motions with correlated information analyzed in the Low-Frequency Raman Spectrum (LFRS), and the low-frequency data processing will be presented. The capabilities of the LFRS to provide structural descriptions of molecular disordered systems, and to monitor phase transformations will be shown. A special attention will be given to the analysis of the first stages of crystallization, with the demonstration that the LFRS is very sensitive to detect and identify the first traces of crystallization, and to discriminate a disordered mixing nanocrystals/undercooled liquid system from an amorphous state.

Pharmaceuticals exposed to various manufacturing stresses provide unusual polymorphic transformations including disorder-disorder phase transformations, leading to driven disordered states characterized by physical properties different from disordered states reached by mere temperature or pressure changes. The influence of dehydration, freeze-drying, mechanical grinding, compression on pharmaceuticals was analyzed by in-situ Raman spectroscopy investigations. Selected analyzes will be presented to show the capabilities of Raman spectroscopy to describe the mechanisms of transformation, and to analyze the structural description of driven disordered states by combining low and high-frequency Raman data.

(197) **Chemical Imaging of Crystalline Components in Pharmaceutical Dosage Forms by Using Low Frequency Raman Spectroscopy**; Toshiro Fukami[1], Motoki Inoue[1], Hiroshi Hisada[1], Tatsuo Koide[2]; [1]Meiji Pharmaceutical University, [2]National Institute of Health Sciences

Raman spectroscopy has been widely recognized as useful analytical method and used for an essential technique in pharmaceutical industry. In particular, microscopic Raman spectroscopy can visualize a distribution of components in pharmaceutical dosage forms with high resolution. Recently, low frequency region (ca. 10-200 cm-1) of Raman spectra can be obtained by the much progress in its device, though the region has been difficult to be measured so far. In this region, the spectra indicate the information about intermolecular interaction in crystalline active pharmaceutical ingredients (API). Thus, this technique is easily expected to be useful for the evaluation of polymorphism and crystallinity in APIs. In this study, we attempted to evaluate a molecular state of crystalline components and visualize their distribution in the dosage form. Workstation (Kaiser Optical Systems Inc., MI) and attached SureBlock XLF-MICRO-976 (Ondax Inc., CA) was used for the measurement of conventional and low frequency region of Raman spectra, respectively. Microscopic Raman measurement was carried out with mapping mode and then image analysis was performed by using Isys software. Pharmaceutical cocrystal consisting of caffeine (CAF) and 4-hydroxy benzoic acid (molar ratio at 2:1) were mixed with microcrystalline cellulose (MCC) and compressed to prepare tablets used as model dosage forms. Although the spectrum of cocrystal was almost identical with that of its physical mixture (PM) consists of CAF and 4-HBA in conventional region, highly distinguishable pattern was observed around 60 cm-1. Raman scattering derived from MCC was very weak in low frequency region and it also suggested the advantage of this technique. Microscopic imaging analysis was able to visualize the distribution of 2.5% of PM and 7.5% of cocrystal contained in MCC tablet.

(198) **Calibration of a Terahertz Analyzer for Predicting Solid Fraction in Roller-Compacted Ribbons and Tablets in a Small-Scale Piloting Study to Facilitate Pharmaceutical Formulation Development**; Mark Sullivan[1], Elaine Harrop Stone[2], Monwara Hoque[2], Xiao Hua Zhou[1], Richard McKay[1]; [1]Advantest America Inc; [2]Merlin Powder Characterisation Ltd

Pulsed terahertz spectroscopy provides a fast, non-destructive means to measure critical properties of pharmaceutical powder compacts including density, solid fraction, porosity and tensile strength (from breaking force). For a given powder composition the terahertz refractive index (RI) is a direct measure of sample porosity because the pulse retardation time depends on void volume and is not affected by attenuation of pulse amplitude. In this talk we present a strategy for incorporating the calibration of terahertz RI as part of a small scale piloting study using a compaction simulator which, in this case, was performed to evaluate direct compression and roller compaction dry granulation processes for formulation development. The results gave a quantitative measure of how much tensile strength was reduced in tablets with similar solid fractions as a result of increasing compaction pressure for roller compaction. This information is important for optimizing the granulation properties (flow, tendency to segregate) in relation to tablet tensile strength. Two important benefits were obtained from the use of non-destructive testing on tablets. First, the results showed a high degree of correlation between terahertz RI and tensile strength for individual tablets, thereby obviating the need to do statistical sampling on a large number of tablets. Second, the samples were available for subsequent performance testing, such as disintegration and dissolution. Prediction of solid fraction in ribbons can also be applied to assure comparability of material properties in process scale-up and tech transfer, as well as, for PAT applications in continuous processes.

(199) **Low Wavenumber Raman Spectroscopy Applications in API Phase Discovery and Characterization**; Courtney Maguire[1], Andrew Brunskill[1]; [1]Merck Research Laboratories

The low frequency, a.k.a. Terahertz, region of the Raman spectra is a newly chartered territory in pharmaceutical spectroscopy. While the fingerprint region has long been studied, the 0-300 wave number region offers a wealth of opportunities for extended characterization. The application of low frequency Raman in combination with other analytical tools, such as powder and single crystal x-ray diffraction, allow for a more in depth understanding of the polymorphic and material properties of pharmaceutical solids. Several cases studies highlighting the application of low frequency Raman, from early discovery to development, will be presented. Case studies will focus on areas of study not thoroughly probed by existing characterization tools, such as detection of amorphous in a crystalline solid, characterizing isostructural solvates, and identifying metastable phases.

(200) **Recent Advances in Tip-Enhanced Raman Spectroscopy**; Richard Van Duyne[1]; [1]Northwestern University

During the last few years, there has been an explosion of interest and activity in the field of plasmonics. The goal of plasmonics is to control and manipulate light on the nanometer length scale using the properties of the collective electronic excitations in noble metal films or nanoparticles, known as surface plasmons. An improved understanding of the interactions between adsorbed molecules and plasmonic nanostructures (i.e., molecular plasmonics) is having a significant impact in a number of research areas including electrochemistry, surface science, catalysis for energy conversion and storage, the materials science of nanoparticles, biomedical diagnostics, art conservation science, and nanolithography.

I will focus in on four recent advances in tip-enhanced Raman spectroscopy (TERS) which illustrate the power of this nanoscale vibrational spectroscopy. First, new insights into the nature of the relative intensity fluctuations in single molecule tip-enhanced Raman spectroscopy (SMTERS) will be discussed. Second, our current understanding of the adsorbate surface interactions involved in the low temperature (LT), ultrahigh vacuum (UHV) TERS of the Ag tip/Rhodamine 6G (R6G) /Ag(111) system will be described. Third, the achievement of 4 nm spatial resolution in the imaging of a dynamic 2D phase boundary in the 1,7-bis(3‘,5‘-di-t-butylphenoxy)perylene-3,4:9,10-bis(dicarboximide) (PPDI)/Ag(111) system. Finally, I will describe a new study of TERS and optically excited tip-enhanced fluorescence (TEF) of a self-assembled porphyrin monolayer on Ag(111). Through selectively exciting different Q-bands of meso -tetrakis- (3,5-di-tertiarybutylphenyl)-porphyrin (H2TBPP), chemical information regarding different vibronic excited states is revealed by a combination of theory and experiment. The observed TEF spectra suggest a weak coupling of H2TBPP to the substrate due to the bulky t-butyl groups and a possible alternative excited state decay path. This work demonstrates the potential of combining TERS and TEF for studying surface-mounted porphyins on substrates, thus providing insight into porphyrin-sensitized solar cells and catalysis.

(201) **Resolution and Enhancement in TERS Microscopy**; Satoshi Kawata[1], Atsushi Taguchi[1]; [1]Osaka University

Tip-enhanced Raman scattering microscopy has become one of the most promising tools for imaging and analyzing advanced nano-devices and nano-materials. However, the reproducibility of TERS experiments with probes has not been yet satisfactory for TERS users in many applications. In this presentation, I would like to discuss on the resolution and enhancement with TERS systems. We show through simulations that the enhancement varies with the number of grains and the gap distance among grains. The effectiveness of multi-grain probe for TERS imaging is demonstrated with experimental results.

(202) **Molecular Structure Changes on the Nanometre Scale Investigated and Induced by TERS**; Volker Deckert[1,2]; [1]University of Jena; [2]Leibnitz Institute of Photonic Technology

Tip-enhanced Raman spectroscopy (TERS) since its discovery has been used to investigate the molecular structure of all kind of different sample surfaces. The unique combination of sensitivity, specificity and spatial resolution allows to obtain structure related information from minute sample amounts and relate this information to a specific surface location with unprecedented accuracy. The whole range from inorganic materials to biological molecules has been investigated, utilising specifically the analytical capabilities of the technique. Recently the technique was also used to investigate chemical reactions and even induce them. TERS as a passive analytical tool potentially allows to address specific reaction sites for instance on a heterogeneous catalyst and relate the morphological information with site specific reactivity data. As an active catalyst probe it allows to specifically induce reactivity at arbitrarily selected surface positions also with nanometer resolution. In our presentation we will discuss the current state of reactivity studies using TERS and will specifically focus on the lateral resolution issues of the method. In view of recent sub nanometer TERS experiments, either the mapping single molecules or line scan experiments on bio-molecules, new attempts to explain this unexpected resolution will be also discussed.

(203) **Application of Vibrational Spectroscopy to Further the Understanding of Drug Product Stability, Dissolution, and Exposure**; Don Pivonka[1], William Rocco[1], Dilip Modi[1]; [1]Incyte Inc.

Vibrational spectroscopy has evolved as a very important tool in the analysis of solid state properties for both “Drug Substance” and formulated “Drug Product”. This lecture will present several case studies involving analyses throughout the pharmaceutical development progression to

include: initial analyses of the synthetic API, polymorph stability under elevated temperature/ humidity, and excipient compatibility. Work will also highlight understanding of salt forms, at the molecular level, in relation to dissolution processes and agglomeration for both neat API and Drug Product. Finally, within the drug development process, vibrational spectroscopy was used to identify and understand a situation in which exposure of formulations dosed in animals exhibited extreme dependence on polymorphic form throughout dissolution. The ability of infrared and Raman spectroscopy to identify kinetics of conversion from thermodynamically less favorable forms, which produced good exposure, to forms with greater thermodynamic stability, which exhibited little to no exposure, has proven critical throughout the formulation and dosing process.

(204) **Quantification of Residual Crystallinity in Pharmaceutical Formulations using Transmission Raman Spectroscopy**; Mark Mabry[1], Julia Griffen[1], Matthew Bloomfield[1,2], Andrew Owen[1], Darren Andrews[1], Pavel Matousek[3]; [1]Cobalt Light Systems, Ltd; [2]Cobalt Light Systems, Inc; [3]Central Laser Facility, STFC Rutherford Appleton Laboratory

The efficacy of many active pharmaceutical ingredients (API) is dependent on the crystallographic form and whether it is crystalline or amorphous. The bioavailability of many APIs is lower in the crystalline state, meaning that more API is required in the tablet or capsule for the same effect as when the API is amorphous. Amorphous compounds often exhibit poor long term stability, especially at increased levels of heat and humidity, and can revert to a more stable crystalline form. Monitoring this efficiently and with low limits of detection is important in formulation development and stability testing; however, with traditional methods can be highly intensive on both resource and materials.

Traditional methods for quantifying amorphicity are powder XRD and solid state NMR (ssNMR), both of which require powdered samples and may sub-sample the individual dose. XRD suffers from inherently poor sensitivity for materials with poor or no crystalline structure. Also, sample preparation may induce phase transformations. Although more sensitive than XRD, ssNMR is not a routine technique applicable to production environments, is very time-consuming and requires high capital expenditure. Both are destructive techniques.

Raman spectroscopy is known to be sensitive to polymorphic form. Conventional Raman surface imaging methods for pharmaceutical tablets can be effective at 'mapping' the distribution of different polymorphic forms; however, they are limited to one plane (i.e. the exposed surface) within the tablet and the preparation of that flat surface can lead to transformation of the physical form. Other problems, such as 'smearing' of the data or the distribution throughout the tablet of localised crystalline regions, can affect quantification significantly.

Transmission Raman spectroscopy (TRS) offers the potential to rapidly quantify both the proportions of polymorphic form and the ratio of crystalline to amorphous API in tablets and capsules. TRS requires no sample preparation, works with intact samples and through most coatings and capsule shells. Crucially, TRS is a true volumetric method and does not suffer from the limitations of conventional Raman imaging. We present data on the technique's ability to quantify polymorphs and also residual crystalline API in a formulation of amorphous API with sensitivity >10x that of XRD and comparable to ssNMR.

(205) **A Directly Correlated Raman and uHPLC-MS Content Uniformity Method for Dry Powder Inhalers Developed through DoE, Chemometrics and Mathematical Modeling**; Lauren Seabrooks[1], Nicole Canfield[1], Justin Pennington[1]; [1]Merck

Content uniformity (CU) is a critical quality attribute measured and monitored throughout the development and commercial supply of pharmaceutical products. As per national and international regulatory guidance's this analysis is ideally conducted on a sample size equivalent to a single unit dose. The Twisthaler®, Merck's proprietary dry powder inhaler (DPI) platform, delivers approximately 2 mg of agglomerates per unit dose. Agglomerates are approximately 500 µm and formed from the binding of smaller micronized particles of an excipient and active(s), making a unit dose from the Twisthaler® equivalent to approximately 50-80 agglomerates. Previously, ultra high performance liquid chromatography-mass spectrometry (uHPLC-MS) with mathematical modeling was utilized to assess CU on unit dose blend samples and on 50 to 80 individual agglomerates rather than on the average of 50-80 agglomerates. Herein we now report the development of a directly correlated Raman and uHPLC-MS method for CU analysis of unit dose blend samples and individual agglomerates by leveraging a series of mathematical algorithms. Raman spectroscopy is non-destructive, obviates the need for sample preparation, and is sensitive enough to detect down to the single agglomerate level. Model samples included blends of caffeine and lactose; albuterol and lactose; and albuterol and lactose agglomerates. First, design of experiments was employed to understand the influence of power, time, and averages on Raman spectra. This analysis revealed power and time were negatively correlated when optimizing albuterol and caffeine spectra but positively correlated for lactose. Second, multivariate curve resolution was utilized on 1:1 sample blends to assess Raman method robustness. This technique highlighted regions in which small adjustments to power and time resulted in a 4-10% change in concentration prediction. Third, mathematical modeling allowed samples to be scanned first for Raman spectra then diluted for uHPLC-MS analysis. This approach obviated the need for sample weights and provided direct sample to sample correlation between methods. Next, a partial least squares model was developed for both caffeine and albuterol blends with a root-mean square error of prediction (RMSEP) of 0.05 for each blend sample. Finally, single agglomerate CU was successfully determined from the established model and yielded an RMSEP of 0.07.

(206) **Screening of Antibiotics Using Portable Spectrometers**; Jason Rodriguez[1], Latevi Lawson[1], HIrsch Srivastava[1], Megha Mohan[1]; [1]FDA

The availability of safe and effective drugs is crucial in the event of a national emergency such as a pandemic. Currently, chromatographic techniques are the prevailing methods used to assess drug quality. While highly accurate, these methods are time consuming and require destruction of the sample. Here we present our efforts to expand the use of portable instrumentation to screen antibiotics. Portable spectroscopic instruments are capable of screening samples at a much higher throughput than can be achieved by laboratory analysis. These tests can be carried out in the field and can typically be conducted in less than a minute without destroying the sample, which is important should further analysis be necessary. Raman and near infrared spectroscopies are two complimentary techniques that may be used to screen for counterfeit or substandard drug products. We report our efforts to build both qualitative and quantitative models that can be used with portable Raman and near infrared instruments in the field to predict critical product attributes such as identity, dissolution rate and assay.

(207) **Prediction of Bead Coating Thickness using Raman Quantitative Model**; Hanzhou Feng[1], James Drennen[1], Carl Anderson[1,2]; [1]Duquesne University, Graduate School of Pharmaceutical Sciences; [2]Duquesne University

Background: Small particles coating processes are increasingly in pharmaceutical industry. This type of dosage form enables the subdivision of active ingredient into partial doses, improving the uniformity of gastric transit time, predictability of drug absorption rate, and overall the safety in use. The small particles coating processes are usually conducted in fluid bed coater. For a coating system of the diffusion-limited drug release

mechanism, the coating film layer can determine the release profiles. Therefore, it is widely accepted that the coating thickness is one of the most important critical quality attributes (CQAs) for the quality assurance of coating products. However, characterizing the thickness for small particles is a challenge in current industry. Conventional film thickness analyses suffer from many limitations, such as unreasonable assumptions, time-consuming and costly measuring procedures. It is important for pharmaceutical industry to find an alternative approach to control the quality of coating products.

Methods: Raman spectroscopy has attracted substantial attention in the pharmaceutical industry over the past few years as a novel process analytical technology (PAT). It was hypothesized in the present work that the chemical changes of the coating system associated with the coating phenomenon can be quantitatively detected by the Raman spectroscopy. A conventional bench-mark optical microscopy analysis was utilized as a reference analysis for small particles coating thickness determination. The Raman scattering spectral signal caused by the coating process was related to the corresponding thickness value provided by the reference method. A quantitative Raman model was generated using Partial Least Squares regression.

Results: The model statistics demonstrate the small particles coating thickness can be reliably predicted by the developed Raman calibration. The prediction result of an independent coating batch also indicates the capability of the Raman for assessing the coating batch performance. The Raman spectroscopy shows its promising as an alternative technique for quality control and performance assurance of coating products.

(208) **Wanderlust and the Traveling (Female) Scientist**; Sarah Maurer[1]; [1]Central Connecticut State University

Since the fall of 2002, I knew that I wanted to study the abiogenesis, and would go where the science led me, initially at the University of California, Santa Cruz, then in Los Alamos National Laboratory. When the researched moved to Denmark, during my second year of grad school, I went with it. Each new location had different expectations for women, scientists, and people in general that taught me how to navigate my academic career and, ultimately, to be happy. Several moves and a PhD later, I find myself in Connecticut, and I am not sure I ever want to move again. I am also an advocate for scientific crafting, STEM outreach, and building a diverse community of scientists.

(209) **Are You the Only Professional Woman in the Organization?**; Ellen Miseo[1]; [1]Hamamatsu Corp.

The question came from one of my students when I was giving them a tour of the contract R&D organization that I was working for. It made me stop and think because much of my career was in environments where I was the only technical/professional woman in the organization. And it also made me think about the part that mentors and role models played in my career. Why?

Starting a scientific career in a contract Research and Development organization, where most of the professionals were mid-career and had gotten there after spending time in industry made me unusual. But I was there because I was recognized as a subject matter expert in an area where the organization had no skills. So I looked outside the organization to build a peer network that supported me. Moving to an instrument company, I suddenly found that the peer network I had set up before, were now my colleagues and friends and continued to support me as I championed the introduction of a new product concept to the commercial instrument market.

Then came a number of corporate buy-outs and changes in roles (some not good) brought on by politics and cultural differences. Through this, the peer mentor network supported me. And what did this peer mentor network have in common with the question above? It was not composed of women in more senior roles. The lack of mentors did not stop my mentoring and helping to develop talent in technical fields. And by recognizing the lack of traditional mentors I can help make sure it does not happen to others.

(210) **A Nontraditional Career Path**; Emily Monosson[1]; [1]Independent/Ronin Institute

We make many choices during our careers. In my case the most important choice was to maintain the career I love (toxicology) while working part-time so that I could be home for my two children. As a result, over the years I have done field research, taught, consulted and most recently, have been writing books about science for a general readership; while maintaining a more scholarly interest in evolutionary toxicology.

(211) **Discussion Panel**; Fred LaPlant[1]; [1]3M

Fred is currently a supervisor at 3M in the Corporate Research Analytical Laboratory, managing a diverse group of people and technologies. He has applied spectroscopy in pharmaceuticals, automotive, and on-line monitoring. He received his PhD in Analytical Chemistry from Purdue and BS from San Diego State. He has also been involved in governance for the SAS and FACSS organizations.

(212) **Diversity in the STEM fields: Increasing Participation and Visibility of Women and other Underrepresented Minority Groups in Research and Science-Related Careers**; Colin Ingram[1]; [1]Andor Technology

The participation of women and underrepresented minorities in STEM fields is increasing every year. This is partly due to efforts aimed at raising awareness of the obstacles facing this group in the pursuit of a STEM based career. Despite advances in woman and minority participation, challenges continue to persist. This is especially true in institutions, industries and academic fields that are traditionally less diverse and are disproportionately white or Asian males.

Although the circumstances facing women and each underrepresented minority group are unique, the framework for solutions have many similarities. The goal of uniform proportional representation of both women and minorities in STEM fields will only be achieved if the entire community is aware of both the unique challenges these individuals may face and the unique perspective each individual brings to any organization, regardless of sex, gender, and race. Increasing awareness of opportunities and implementing successful career strategies will allow us to make greater strides in increasing numbers of women and minorities in STEM based careers.

Colin Ingram obtained his B.S. in Biochemistry from Montana State University in 2003 and his PhD in Chemistry from the University of Wisconsin-Madison in 2010. He is currently working as an Application Scientist for Andor Technology. Colin has participating in efforts to increase minority representation at the University of Wisconsin-Madison Chemistry department. He is particularly interested in efforts to encourage our young women and minorities to consider STEM careers at an early age.

(213) **Characterizing Working Catalysts with Correlated Electron and Photon Probes**; Eric Stach[1]; [1]Brookhaven National Laboratory

Heterogeneous catalysts often undergo dramatic changes in their structure as the mediate a chemical reaction. Multiple experimental approaches have been developed to understand these changes, but each has its particular limitations. Electron microscopy can provide analytical

characterization with exquisite spatial resolution, but generally requires that the sample be imaged both ex situ and ex post facto. Photon probes have superior depth penetration and thus can be used to characterize samples in operando (i.e when they are actively working). But they generally lack spatial resolution and thus give only ensemble average information.

We have taken advantage of the recent developments in closed-cell microscopy methods to develop an approach that allows us to successfully combine electron, x-ray and optical probes to characterize supported nanoparticle catalysts in operando. By measuring the reaction products at each stage of the reaction, we can directly correlate the information that can be obtained from each approach, and thus gain a deep insight into the structural dynamics of the system.

I will describe how we can use this approach to correlate scanning transmission electron microscopy, x-ray absorption spectroscopy (both near-edge and extended fine structure), and infrared microspectroscopy to understand how Pt and Pd nanoparticles supported on silia undergo structural changes during the room temperature hydrogenation of the ethylene, and how we can direct measure and describe the reaction products on the surfaces of the nanoparticles as the reaction proceeds. By combining these approaches, we can track the interplay between nanoparticle reduction, coarsening, and the specific surface species at different stages of the reaction. We will also show how this approach can be used to understand the partitioning that occurs in bimetallic nanoparticles during oxidation and reduction at elevated temperatures, with a focus on the NiPt system.

The presentation will focus on the development and application of experimental methods, including the high temperature atmospheric pressure electron microscopy, the direct measurement of reaction products using gas chromatography–mass spectrometry and the ability of a newly developed electron microscope for operando microscopy (based on the FEI Talos platform) to characterize bimetallic nanoparticles through energy dispersive x-ray spectroscopy.

(214) ***In situ* Probing of Environmental Liquid Surfaces and Interfaces using Microfluidics: Toward Multimodal and Mesoscale Imaging**; Xiao-Ying Yu[1], Zihua Zhu[2]; [1]Fundamental and Computer Sciences Directory, [2]W. R. Environmental Molecular Science Laboratory

The surfaces of aqueous phases and films have unique kinetics and thermodynamics, distinct from the bulk. However, major surface analytical techniques are mostly vacuum-based and direct applications for volatile liquid studies are difficult. We developed a vacuum compatible microfluidic interface, System for Analysis at the Liquid Vacuum Interface (SALVI), to enable direct observations of liquid surfaces and liquid-solid interactions. The unique aspects of this R&D 100 award winner include the following: 1) the detection window is an aperture of 2-3 μm in diameter allowing direct imaging of the liquid surface, 2) surface tension is used to hold the liquid within the aperture, and 3) SALVI is portable among multiple analytical platforms. SALVI is composed of a silicon nitride (SiN) membrane as the detection area and a microchannel made of polydimethylsiloxane (PDMS). Its applications in time-of-flight secondary ion mass spectrometry (ToF-SIMS) as an analytical tool were evaluated using a variety of aqueous solutions and complex liquid mixtures, some of which contain nanoparticles. SALVI was also used to investigate the solvent structure of switchable ionic liquids. Recently, we demonstrated in situ probing of the electrode-electrolyte solution interface (or solid-electrolyte interface, SEI) using a new electrochemical SALVI. It provides the first direct observation of the surface and diffused layer of SEI in a liquid with chemical speciation using dynamic ToF-SIMS. Moreover, SALVI was extended for studying biofilm growth and single mammalian cells using correlative imaging by more than one spectroscopy and microscopy technique, each offering different spatial and temporal scales. That is, collecting data on different information level from an identical area in the same sample ideally could lead to a more holistic view of the hierarchical structural organization of complex systems in the real world. Selected results from our latest development will be presented, showcasing new directions and applications of multimodal imaging of environmental surfaces and interfaces and studying chemistry from the bottom up, all based on microfluidics. SALVI, a portable microfluidic reactor, sets the analytical foundation toward chemical imaging of complex phenomena occurring in multiple time and length scales, or the mesoscale, underpinning chemical changes at the molecular level.

(215) **Surface of Oxide-Based Catalysts during Catalysis Tracked with Ambient Pressure XPS and Its Correlation with Catalytic Performances**; Franklin (Feng) Tao[1], Shiran Zhang[1], Luan Nguyen[1], Junjun Shan[1]; [1]University of Kansas

Oxide is one important type of heterogeneous catalysts for chemical and energy transformations. Compared to metal catalysts, oxide catalysts are less studied due to their complexity of surface chemistry and structure including surface composition, oxidation states of cations on surface, defects of oxygen vacancies, and structural and compositional restructuring. The flexibility in surface structure and chemistry of oxide catalysts has made in-situ studies of surfaces of oxide catalysts necessary. Photoelectron spectroscopy is one main technique for examining surface of an oxide catalyst. In this talk, in-situ photoelectron spectrometers with a reaction cell for catalysis studies will be introduced. I will focus on the progress in the in-situ studies of oxide catalyst surfaces with ambient pressure photoelectron spectroscopy under reaction condition (referred to one reactant) and during catalysis (referred to all reactants of a catalytic reaction). Catalytic performances CoO for reduction of NO with CO, of Co3O4 for preferential oxidation of CO in H2, and CeO2-based catalysts for water-gas shift will be presented. Correlation between surface chemistry and structure of these catalysts during catalysis and their corresponding catalytic performances will be discussed.

(216) **Vibrational Spectroscopic Imaging of Living Systems: Emerging Platform for Biology and Medicine**; Ji-Xin Cheng[1]; [1]Purdue University

Current medical imaging modalities rely on differences in tissue properties rather than on chemical-content changes deep inside the body. Consequently, a biopsy is routinely needed for ultimate diagnosis of a malignancy. Thus, we need noninvasive imaging technologies that can be used in vivo and can determine in real time the chemical content of deep-tissue. Vibrational spectroscopy has been a powerful tool for quantitative analysis of molecules in gas phase or solutions and has been deployed for label-free analysis of excised biological tissues. Yet, applying vibrational spectroscopy to non-invasive in vivo imaging, from single live cells to the human body, is still difficult. I will present our persistent efforts in developing in vivo spectroscopic imaging platforms that have been enabling groundbreaking biological discoveries and paradigm-shifting diagnosis strategies. Specifically, I will present coherent Raman scattering microscopy for real-time spectroscopic imaging of living cells and vibration-based photoacoustic endoscopy/tomography for bond-selective imaging of deep tissues.

(217) **UV Resonance Raman Spectroscopic Studies of Protein Structure and Dynamics**; Sanford Asher[1], David Punihaole[1], Elizabeth M. Dahlburg[1], Ryan S. Jakubek[1], Zhenmin Hong[1]; [1]University of Pittsburgh

UV Raman excitation into the ~200 nm peptide bond electronic transitions enhance peptide bond amide vibrations of the backbone. A particular band (the amide III_3) reports on the Ramachandran psi angle and peptide bond hydrogen bonding. This band is Raman scattered independently by each peptide bond with insignificant coupling between adjacent peptide bonds. Isotope editing of a peptide bond (by replacing the C_{alpha-} H with C_{alpha-} D) allows us to determine the frequency of individual peptide bonds within a peptide or protein to yield their psi angles. Consideration of the Boltzmann equilibria allows us to determine the psi angle Gibbs free energy landscape along the psi (un)folding coordinate that connects secondary structure conformations. The psi angle coordinate is the most important reaction coordinate necessary to understand mechanism(s) of protein folding. We have also discovered an analogous correlation for the primary amide sidechain of Gln. This allows us to monitor the hydrogen bonding and structure of this sidechain.

We examine the details of peptide folding conformation dynamics with laser T-jumps where the water temperature is elevated by an 1.9 μM IR nsec laser pulse and we monitor the ~200 nm UV Raman spectrum as a function of time. These spectra show the time evolution of conformation. We will discuss the role of salts on stabilizing conformations in solution.

(218) **icpTOF: Advantages of Sensitive Simultaneous Detection for Analysis of Nanomaterials**; Olga Borovinskaya[1], Martin Tanner[1]; [1]TOFWERK AG

ICP-MS is already applied for the analysis of nanomaterials in toxicological, biological, and environmental studies. In a more classical way, it is used to determine the bulk elemental composition of dispersions either directly or after size separation. However, in the last few years the detection of single particles gained a great attention from different scientific communities and industry. So-called single particle (sp)-ICP-MS [1] can determine particle size and concentration not only in liquids but also in solid samples, when coupled to laser ablation [2]. Most likely its application will be extended in a near future also to airborne particles.

The measurement of single particles implies the detection of extremely short signals (100-500 μm) and requires sensitive and fast instrumentation. Sequentially scanning instruments based on quadrupole or sector-field technology cannot determine more than one isotope per particle and the elemental composition of particles has to be known prior to sp-ICP-MS. This information is not readily available, especially for environmental samples or online experiments. Besides, the element ratios of single particles would be extremely useful for particle identification and for studies on particle transformation [3].

We present a new icpTOF mass spectrometer, which provides simultaneous detection of the whole mass range of elements at μs-time resolution [4] and with >3000 mass resolving power. These features render the determination of multi-element composition of single nanoparticles possible. The advantages of fast simultaneous detection for characterization of nanomaterials will be discussed. The performance of the icpTOF and size-equivalent detection limits in combination with different sample introduction systems will be shown. Methods to analyze particles composed of elements which suffers from polyatomic interferences and some applications will be presented.

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(219) **Analytical Insights into Human Risk Assessments of Noble Metal Nanomaterials**; Petra Krystek[1]; [1]VU University Amsterdam

Many engineered, inorganic nanomaterials are used for consumer and personal care applications. Therefore, the biological behavior and the safety of nanomaterials must be studied during risk assessments. For such evaluations, the bio distribution and the clearance of nanoparticles in vivo is increasingly important in nano-toxicology. Especially for material sizes below 100 nm, various tissues, skin layers and human body fluids after exposure are investigated for tracking the inorganic constituents of nanomaterials [1].

In vitro cell exposure testing has been put forward as a faster approach to screen nanomaterials on their toxic potential but also to study the possible cellular uptake which depends on the characteristics of the nanomaterial; e.g. elemental composition, size, shape, surface modification. Additional aspects like e.g. the use of serum proteins added to the cell culture medium, which affect aggregation of nanomaterials and this in turn may affect exposure levels, are of great relevance [2].

Next to the total quantification of the elements of interest by inductively coupled plasma mass spectrometry (ICPMS) [3,4], the hyphenation of asymmetric flow field flow fractionation (AF4) [2] to ICPMS delivers new insights into the understanding of nanomaterials in complex media of in vivo and in vitro studies. Selected examples in relation to risk assessments with noble metal nanomaterials will be discussed during this presentation.

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(220) **Single Particle ICP-MS in a Multitechnique Approach to Elucidate the Fate of Silver Nanoparticles in Burnt Patients**; Marco Roman[1,4], Chiara Rigo[1], Vincenzo Vindigni[2], Hiram Castillo-Michel[3], Warren R.L. Cairns[4]; [1]University Ca; [2]Burns Center, University Hospital of Padua; [3]European Synchrotron Radiation Facility (ESRF), Grenoble; [4]Institute for the Dynamics of Environmental Processes (IDPA-CNR), Venice

The topical administration of silver nanoparticles (AgNPs) is increasingly used in the treatment of burns to prevent infections and favour the regeneration of the tissue. Beside the antibacterial efficacy, we have documented that AgNPs can penetrate into the dermis, be up taken by the fibroblasts and affect mitochondrial activity, but no direct observations are currently available on their subsequent chemical transformation and fate in vivo in the human body, particularly concerning their potential to reach the circulatory system.

We developed a method for the simultaneous determination of dissolved Ag and the characterization of AgNPs in human plasma and blood, based on hydrodynamic chromatography hyphenated to ICP-MS in single particle detection mode, and combined with a dedicated algorithm for data treatment. From a single analysis, the method provides the concentration of dissolved Ag and the distribution of AgNPs according to the hydrodynamic diameter, mass-derived diameter, number and mass concentration. The method was applied to investigate the transformations of AgNPs standards and an AgNPs-coated dressing in human plasma, supported by synchrotron radiation μXRF imaging and μXANES speciation, showing that chlorides and protein thiols co-participate in the partial dissolution of AgNPs on an hourly time scale through dynamics driven by kinetic factors. Our methodology was then implemented within a multitechnique approach to study the transformations of AgNPs after topical administration to real burnt patients in hospital conditions. Of the 30 to 80 ng mL-1 of total Ag found in the whole blood of patients, the dissolved fraction was not statistically different from the total level of the metal, while NPs or aggregates were not detected. Combined with the Ag distribution and speciation in skin biopsies from corresponding patients, studied by laser ablation-ICP-MS, μXRF and μXANES, the work provided new and complete insights into the metallomics of AgNPs in humans and in vivo. Overall, our results support the hypothesis that the systemic mobilization of the metal after topical administration of AgNPs is mainly driven by their dissolution in situ.

(221) **HPLC-ICP-MS for the Determination of Gold Nanoparticles in Biological Tissues**; Jörg Bettmer[1], Juan Soto-Alvaredo[1], Carlos López-Chaves[2], Maria Montes-Bayón[1], Cristina Sanchez-González[2], Juan Llopis-González[2]; [1]University of Oviedo; [2]University of Granada

Nanomaterials (NMs) have gained great importance in our daily life as many consumer and industrial products contain NMs due their unique properties. According to "The Project on Emerging Nanotechnologies" more than 1,800 products are listed which contain nanoscale materials. Due to their increasing use concerns exist with respect to their distribution in the environment and biological systems and their potential toxicological impact. Analytical methods are therefore required to monitor and characterize these NMs in different matrices.

In the case of metallic or metal-containing NMs, inductively coupled plasma-mass spectrometry (ICP-MS) has become a very powerful technique. High sensitivity and selectivity are important properties in order to analyze the sought species, either qualitatively or quantitatively. Typically, ICP-MS can be used in combination with a separation or fractionation technique or in the so-called singe particle mode (spICP-MS).

Within this work main focus will be set on the development of a method based on HPLC-ICP-MS [1, 2]. Following basic method developments examples are shown on the analysis of the sought species (gold nanoparticles) in incubated cell cultures and rat tissues. The results obtained indicate that ICP-MS as detection system can provide some very useful information in order to better characterize NMs in biological systems. However, complementary techniques like other mass spectrometric and electron microscopic techniques are suggested for a more detailed picture of these NMs.

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(222) **Cloud Point Extraction for Silver Nanoparticle and Ion Quantification**; Nicole Hanks[1], Joseph Caruso[1], Peng Zhang[1]; [1]University of Cincinnati

Silver has been a widely applied antibacterial for medical and consumer products since ancient times. With advances in nanotechnology, silver nanoparticles are now the most vastly applied particle for consumer products today, finding their way into coatings, cleaners and water purification. Due to their mass production and use in the consumer market, there has been an increase of silver entering the environment due to mass used of these items. However, while quantification of total silver is easily accomplished, methods of quantifying the concentration of silver nanoparticles and silver ion separately is more difficult. Current speciation methods use liquid chromatography that require expensive columns with large pore sizes and long analysis time for one sample to be analyzed. Many of these separation methods use large amounts of organic mobile phase and dilute the sample before it can be quantified. This research shows the development and use of a cloud point extraction method to separate a sample's nanoparticle content from its ion counterpart and inductively coupled plasma mass spectrometry for quantification. Cloud point extraction is an environmentally friendly and cheap method for separating and pre-concentration nanoparticles from their ion counterparts.

(223) **Developing Single Particle Orientation and Rotational Tracking for Understanding Endocytosis and Intracellular Transport**; Ning Fang[1], Kuangcai Chen[2], Ashley Augspurger[2]; [1]Georgia State University; [2]Iowa State University

A cell can be conceived as a factory containing a hierarchical network of nanomachines. Fully understanding the working mechanisms of these nanomachines requires knowledge of both translational and rotational dynamics, and their coupling. The knowledge of rotational dynamics in and on live cells remains highly limited and requires further experimental advances through the use of new innovative tools. The differential interference contrast (DIC) microscopy-based Single Particle Orientation and Rotational Tracking (SPORT) technique has been developed in my laboratory to offer high spatial, angular, and temporal resolutions simultaneously for visualizing rotational motions of anisotropic plasmonic gold nanorods. The SPORT technique is capable of extracting important information (including rotational rates, modes, and directions) on the characteristic rotational dynamics involved in complex biological processes, such as endocytosis and intracellular transport.

(224) **High Speed Molecular Imaging by Phosphorescence Lifetime Multiphoton Microscopy**; Scott Howard[1]; [1]University of Notre Dame

Multiphoton microscopy (MPM) is now a widely used tool for three-dimensional, sub-micron, *in vivo* imaging deep in scattering tissue. To further empower MPM, fluorescent/phosphorescent lifetime imaging microscopy (FLIM/PLIM) for dissolved gas molecular imaging and

intrinsic molecular imaging will be presented for dissolved oxygen imaging of specific disease states. We will specifically describe new technology to overcome the fundamental limitations of MPM lifetime microscopy imaging rate by using multifocal multiphoton modulation microscopy (M4), a frequency multiplexed imaging technique that increases PLIM acquisition by two orders of magnitude. We will also discuss easily-prepared probes for molecular imaging of oxygen by MPM-PLIM and label-free whole tissue imaging of sickle cell disease, a hemoglobin oxygen-carrying deficiency disease, by MPM.

(225) **Ultrafast Nanoscopy of Energy and Charge Transport**; Libai Huang[1]; [1]Purdue University

The frontier in solar energy research now lies in learning how to integrate functional entities across multiple length scales to create optimal devices and advancing the field requires transformative experimental tools that probe energy transfer processes from the nano to the meso lengthscales. To address this challenge, we aim to understand multi-scale energy transport across both multiple length and time scales, coupling simultaneous high spatial, structural, and temporal resolution. In my talk, I will focus on our recent progress on visualization of exciton transport in organic materials from the nano to mesoscale employing ultrafast optical microscopy.

(226) **Gigapixel Fluorescence Histology for Rapid 'No-Cut' Surgical Pathology**; J. Quincy Brown[1]; [1]Tulane University

A number of clinical situations would benefit from a more timely and complete assessment of tissue pathology at the point-of-care, as compared to labor- and time-intensive destructive methods such as frozen section analysis. For example, high-throughput microscopy of intact core needle biopsies at the patient's bedside would indicate if additional biopsies are necessary to prevent a false negative procedure. Rapid non-destructive screening of tumor content would enable clinicians to most efficiently utilize precious cancer tissues for downstream molecular and laboratory analysis, particularly important as new strategies for personalized medicine emerge. Finally, intraoperative imaging of entire surgically-resected tumors would enable a rapid determination of the presence of residual cancer at the resection margin, in time to correct the surgery and improve post-surgical outcomes. Our laboratory is seeking to address these challenges with the development of ultra-high-throughput optical sectioning microscopy, combined with novel strategies for fluorescence histochemistry, to contribute practical and clinically-adoptable solutions for point-of-care pathology. In this talk I will describe the development of these technologies, and their application to rapid gigapixel imaging and diagnosis of point-of-care pathology tissues, including the entire surfaces of prostate and renal tumor resection specimens.

(227) **TERS Characterization of Membrane Receptors**; Zachary Schultz[1], Hao Wang[1]; [1]University of Notre Dame

Tip enhanced Raman scattering (TERS) is a chemically specific method that provides increased sensitivity and spatial resolution for optical microscopy. Electromagnetic enhancement of the Raman signal is generated from molecules within the evanescent field of a plasmonic nanostructure on the end of an atomic force microscope tip, providing Raman images with spatial resolution proportional to the tip curvature. The field is further enhanced when the TERS tip is near a metal surface, or a second nanostructure. In this presentation we will discuss how that small molecules with specific binding affinity for membrane receptors can direct the adsorption of nanoparticles onto intact cellular membranes to provide chemical information about the receptor protein. The functionalized nanoparticle serves a double purpose: 1) providing a beacon on the membrane landscape to identify the location of the receptor and 2) increasing the TERS response of the amino acids near the nanoparticle. The signals we have observed indicate the protein does not have to reside in the gap junction for high sensitivity detection. The observed TERS signal is sensitive to signal amino acid polymorphisms. Further the reproducibility of the signal provides insight into the ligand-receptor specificity. This methodology has potential to investigate the chemical properties associated with cell signaling, adhesion, and drug delivery.

(228) **Pharmacometabolomics Enabling Tools for Systems Pharmacology and Precision Medicine**; Rima Kaddurah-Daouk[1,2]; [1]Duke University Medical Center; [2]On behalf of the Pharmacometabolomics Research Network

Metabolomics provides analytical tools for a systems biochemical approach for the study of human diseases and treatment outcomes. Small molecules are building blocks for pathways and networks and capture net effects of execution of genetic, epigenetic, and proteomic programs to define a metabolic state of disease. Over the past decade we have pioneered applications of metabolomics for the study of neuropsychiatric diseases and have identified novel pathways implicated in disease pathogenesis highlighting common and unique mechanisms among these disorders. Through the effort of a national consortium we have started to illustrate the power of metabolomics as an enabling tool for the study of drug effects. It is now possible to define metabolic signatures of drug exposure that can identify pathways involved in both drug efficacy and adverse drug reactions. In addition, the "metabotype," the metabolic "signature" of a patient, is a unique identity that contains information about drug response and disease heterogeneity. We will illustrate from the study of drugs used for the treatment of cardiovascular and neuropsychiatric disorders. The application of metabolomics for the study of drug effects and variation in drug response is creating "pharmacometabolomics," a discipline that will contribute to Precision Medicine in several important ways.

(229) **Stable Isotope Resolved Metabolomics (SIRM) on Fresh Human Tissues as a Preclinical Drug Testing Platform**; Andrew Lane[1], Teresa Fan[1], Alexander Belshoff[2], Richard Higashi[1], Jeremiah Martin[1], Michael Bousamra[2]; [1]University of Kentucky; [2]University of Louisville

All preclinical drug testing models have advantages and drawbacks. We have been using SIRM to evaluate metabolic reprogramming of lung cancer cells in monoculture, in mouse xenograft/explant models, and in NSCLC patients in situ (1). We are able to determine the influence of the tumor microenvironment using these models. We have now extended the range of models to fresh human tissue slices, similar to those originally described by O. Warburg (2), which retain the original tissue architecture and heterogeneity with a paired benign versus cancer design under controlled cell culture conditions. This platform offers a human tissue model for preclinical studies on metabolic reprogramming of human cancer cells in their tissue context, and response to drug treatment (3). As the microenvironment of the actual target human tissue is retained and individualized response to drugs is obtained, this platform promises to transcend current limitations of drug selection for clinical trials or treatments. The overall approach will be described and illustrated with examples of glucose and glutamine metabolism, and the differential metabolic responses of tumor versus non-tumor tissue to inhibitors.

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(230) **Personalized Medicine in Human Space Flight**; Michael A Schmidt[1]; [1]Sovaris Aerospace, LLC

Space flight is one of the most extreme conditions encountered by humans. Advances in Omics methodologies (genomics, epigenomics, transcriptomics, proteomics, and metabolomics) have revealed that unique differences exist between individuals. These differences can be amplified in extreme conditions, such as space flight. In this session, we explore the role of targeted and untargeted omics in describing how converging variables can aggregate across molecular networks to affect astronaut health, safety, and performance on space exploration missions. We further explore how integrated omics can be used to accelerate discovery of space-based countermeasures for missions with short timelines, such as the first human mission to Mars in 2022. This includes an examination of how such personalized measures are relevant before, during, and after space missions.

(231) **Metabolomic Applications in Nutritional Research**; Lorraine Brennan[1]; [1]UCD Institute of Food and Health

The applications of metabolomics has expanded across biological disciplines in recent years; the present abstract will focus on applications in nutrition research. In general, the applications in the nutrition field can be divided in three categories: (1) Dietary intervention and phenotyping studies (2) dietary biomarker studies and (3) diet related diseases.

With respect to dietary biomarker studies metabolomics has resulted in identification of new biomarkers of certain foods. Metabolomics based approaches have suggested putative biomarkers for citrus fruit, red meat, fish and dairy products. Incorporation of these biomarkers into strategies for assessment of dietary intake will be an important step in the future.

Metabolomics can reveal detailed information on the metabolic phenotype (metabotype) which in turn describes the metabolic state of an individual. There is an expectation that assigning individuals to a particular metabotype will provide valuable information in future nutrition research and in particular will play a role in personalised nutrition. We have developed some strategies for the identification of metabotypes and will illustrate their potential use in personalised nutrition.

(232) **NMR as an Important Analytical Tool for Identifying Drug Metabolites in Support of Drug Discovery**; Yingzi Wang[1], Xiaoliang Zhuo[1], John Leet[1], Stella Huang[1], Joseph Cantone[1], Dieter Drexler[1], Kim Johnson[1], Benjamin Johnson[1], Michael Reily[1], Adrienne Tymiak[1]; [1]Bristol-Myers Squibb

Drug metabolites may be associated with pharmacological activity and can contribute to toxicity of drug candidates. Therefore, identifying the exact structures of unknown or unusual drug metabolites is a critical component of drug discovery. Specifically, it provides information to biotransformation studies that elucidate metabolic mechanisms, and identify sites of bioactivation that can be modified as an overall part of developing structure-activity relationships. NMR is a crucial analytical tool in this process of elucidating exact drug metabolite structures. However, there are challenges in analyzing metabolite samples isolated from in-vitro or in-vivo sources, including small sample amounts, interference from contaminants, and limited structural information available from biotransformation LC-MS/MS studies. In this presentation, recent efforts to identify metabolites by NMR in support of various drug discovery programs will be showcased. The examples demonstrate the value of NMR in characterizing metabolic pathways and informing structure-property relationships.

(233) **Pattern Recognition Studies of Serum N-Linked Glycans obtained by MALDI-IMS-MS Profiling**; Barry Lavine[1], Maissa Gaye[2], David Clemmer[2], Tao Ding[1], H Shion[3], W. Chen[3],A. Hussein[3], Y. Hu[4], S. Zhou[4], Yehia Mechref[4]; [1]Department of Chemistry, Oklahoma State University; [2]Department of Chemistry, Indiana University; [3]Waters Corporation, Pharmaceutical Life Sciences; [4]Department of Chemistry & Biochemistry, Texas Tech University

Advances in chemical instrumentation produce massive amounts of chemical data of unusual form that continues to drive research in the field of chemometrics. Currently, we are investigating delineation of disease phenotypes associated with esophageal adenocarcinoma by MALDI-IMS-MS analysis of serum N-linked glycans extracted from sera of patients and healthy individuals. MALDI-IMS-MS data were collected in duplicate for 58 serum samples obtained from individuals diagnosed with Barrett's esophagus (BE, 14 individuals), high-grade dysplasia (HGD, 7 individuals), esophageal adenocarcinoma (EAC, 20 individuals) and from normal controls (NC, 17 individuals). A combined mobility distribution of 9 N-linked glycans established for 90 MALDI-IMS-MS spectra (training set) is analyzed using a genetic algorithm for feature selection and pattern recognition. Phenotype delineation was achieved and as a result, the four phenotypes (BE, HGD, EAC and NC) could be unequivocally differentiated. Discriminants were developed that correctly classified all serum samples in the training set, and they were successfully validated against 26 blind samples. Although these discriminants were applied to a limited number of spectra from blind samples, this methodology appears promising as a means of discovering molecules from serum that may have the potential to serve as disease markers.

(234) **Medical Applications of Multivariate Statistical Process Control**; Lionel Blanchet[1,2], Jasper Engel[3], Frederik-Jan van Schooten[1]; [1]Department of Toxicology, Maastricht University Medical Center, the Netherlands; [2]Top Institute Food and Nutrition (TIFN), Wageningen, the Netherlands; [3]NERC Metabolomics Facility, School of Biosciences, Birmingham University

Data analysis in -omics is generally approached as a classification problem opposing a cohort of patients affected by a specific disease to another cohort (either of healthy volunteers or representing another disease). Besides its tremendous successes, this method suffers from a number of limitations. First and foremost, representative cohorts must be accessible. Enrolling sufficient number of patients is feasible for common diseases, but all too often impossible for rare diseases. Going further such "two-populations" approach is, in essence, incompatible with personalized medicine. Secondly, each disease requires its own specific diagnostic model, which is incompatible with the current clinical practice.

We demonstrate here that multivariate statistical process control (MSPC), as routinely used in industry; offer a successful alternative viewpoint of health. The sensor data routinely used in process control can advantageously be replaced by -omics technologies of various biofluids in a medical context. The analysis of biofluids provides hundreds or thousands of quantitative readouts directly reflecting the status of human metabolism. Moreover, a number of these biofluids can be collected non-invasively e.g. urine or breath.

Based on metabolomics data, two MSPC strategies can be envisioned. The first consist in using the general population to define the Normal Operating Conditions (NOC) [1]. This reference population can obviously be stratified based on gender, age, BMI, *etc*. A second approach

consists in defining personalized NOC based on longitudinal data from a single individual. In both cases, any deviations from the NOC can be efficiently detected. Identification of the fault, *i.e.* here the disease, make the most of the multivariate structure of the data and provide the most relevant clues to clinicians for establishing a definitive diagnostic.

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(235) **Sparse Deconvolution of High-Density Super-Resolution Images**; Cyril Ruckebusch[1], Romain Bernex[1], Siewert Hugelier[1], Olivier Devos[2], Johan de Rooi[2],Paul Ailers[2]; [1]LASIR CNRS Université de Lille, France; [2]Department of Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands

Fluorescence microscopy is about the only technique that can provide structural and dynamic information on live cells at the nanoscale. Previously limited to a spatial resolution of a few hundred nanometers due to diffraction, the recent development of sub-diffraction or so-called super-resolution fluorescence imaging has allowed direct access to the tens of nanometers length scale. Some of the functional super-resolution microscopy techniques are based on single-emitter fitting and localization algorithms (PALM1, STORM2) whereas others rely on the statistics of temporal fluctuations of the pixel signals (SOFI3) or on the dissimilarity between these pixel signals (MAPPIX4). However, the development of methodologies capable of handling high density of activated probes on each camera frame remains challenging.

In the last few years, high-density imaging has turned out to be a valuable alternative for the analysis of super-resolution microscopy data with overlapping fluorophore signals. Valuable algorithms were developed using sparse representation of the signals that adopts a L1-norm penalty (e.g., CSSTORM5). Here we present a new approach based on a L0-norm penalty that imposes a constraint on the number of non-zero elements in the vector containing the positions and intensities of all the single-emitters.6 As for other sparsity-promoting methods, we introduced a discrete formulation and reconstruct the high resolution image on a pre-defined sub-pixel grid to describe the positions of single-emitters. We validated our algorithm and compared our approach named SPIDER to others. In this work, we demonstrate functional super-resolution using data sets recorded on live cells.

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(236) **Multivariate Curve Resolution of Mass Spectrometry Imaging (MSI) of Biological Tissues**; Roma Tauler[1], Carne Bedia[1], Joaquim Jaumot[1]; [1]IDAEA-CSIC

Multivariate Curve Resolution is proposed for the analysis of Mass Spectrometry Imaging (MSI) data 1. Recently, developments on ionization of samples have dramatically expanded the number of applications of MSI due to the possibility of collecting the mass spectrum for each pixel of a considered surface in a reasonable time. Using this method, both spatial distribution and spectral information of analyzed samples can be obtained. However, there are major drawbacks inherent to MSI related to the high complexity of the data obtained from real samples and to the extremely huge size of the data sets generated by this technique. Therefore, the potential of chemometric tools in different steps of the analysis process is unquestionable, from data compression to data resolution of the different components present at each pixel of the image. In this work, this data analysis is carried out by means of the Multivariate Curve Resolution method. The benefits of the application of this method are shown for some examples consisting on data sets obtained from the literature and of an experimental data set obtained from plant tissues submitted to contamination stressing effects. Results will show that Multivariate Curve Resolution allows obtaining distribution maps of different components and their identification from resolved pure high-resolution mass spectra

1 Potential use of Multivariate Curve Resolution for the analysis of Mass Spectrometry Images. J.Jaumot and R.Tauler. The Analyst. 2015, 140, 3, 837-46; DOI: 10.1039/C4AN00801D

(237) **Investigations on the Analysis Workflow for Biomedical Application of Raman Spectroscopy**; Thomas Bocklitz[1], Jürgen Popp[1, 2]; [1]University of Jena, Institute of Physical Chemistry, [2]Institue of Photonic Technology

Raman spectroscopy features a unique potential as biomedical diagnostic tool since it measures molecular vibrations within the sample. These molecule specific vibrations result in characteristic “molecular fingerprint” of the sample under investigation and this fingerprint can be utilized to monitor changes within the sample. Due to the intrinsic weakness of the Raman effect and subtle changes occurring within the progress of a disease the application of chemometrical modeling is necessary. The combination from Raman spectroscopy and chemometrics results in a label-free, non-invasive diagnostic procedure, which can be used to identify bacteria, detect drugs or other molecules and support the diagnosis of a disease.

In order to receive robust and optimal performance of the models, the computational procedures have to be designed wisely. In this contribution we summarize our recent studies on the analysis of Raman spectra for biomedical tasks. Thereby, we evaluated the spike correction, investigated the spectrometer calibration, the background correction and the interplay of dimension reduction technique and cross-validation. With the result of these studies almost all tasks of the pre-processing and modeling of the Raman spectra can be constructed in an automatic manner, which would be the long term goal of the presented studies. Additionally the resulting chemometric models are more stable against corrupting effects, such as cosmic spikes and artefacts from the spectrometer. The optimized pre-processing and modeling routines allow the use of the full potential of Raman spectroscopy for biomedical applications.

(238) **Detection of Truly Dissolved and Gaseous Flame Retardants in the Lower Great Lakes Region using Polyethylene Passive Samplers**; Carrie McDonough[1], Rainer Lohmann[1]; [1]Graduate School of Oceanography, University of Rhode Island

Coupling passive sampling techniques and highly sensitive analytical techniques like gas chromatography coupled with mass spectrometry in the negative chemical ionization mode (GC/MS-NCI) is a promising approach for measuring time-integrated concentrations of trace-level gaseous and dissolved hydrophobic organic contaminants that has, as of yet, rarely been applied to the measurement of emerging flame retardants. Brominated flame retardants (BFRs) and organophosphate esters (OPEs) are pollutants that enter the aquatic environment via wastewater effluent, river runoff, and atmospheric deposition. Measuring the truly dissolved and gaseous fraction of these contaminants is crucial to our understanding of diffusive air-water exchange and bioavailability to aqueous organisms. In this study, polyethylene passive samplers (PEs) were used to sequester the dissolved and gaseous fraction of in-use and recently banned flame retardants from water and air. PEs were deployed by a network of citizen scientist volunteers throughout May – November of 2011 to 2014. They were deployed simultaneously in air and water to calculate air-water exchange fluxes. Samples from 2013 were analyzed by GC/MS-NCI for brominated and chlorinated flame retardants and in electron ionization (EI) mode for OPEs. In 2012, the most frequently detected brominated diphenyl ether (BDE) congeners were BDE 28, 47, 49, 100, and 99. Average gaseous BDEs ranged from 0.6 to 21 pg/m^3. Average dissolved Σ_{5BDEs} ranged from below detection at some offshore sites to 42 pg/L at the mouth of Niagara River, suggesting the river is a major source of BDEs to Lake Ontario. Six OPEs were frequently detected in atmospheric PEs. Gaseous OPEs were present at much greater concentrations than BDEs, with an average total concentration of 322+/-506 pg/m^3. The occurrence and spatial trends of emerging flame retardants will be described, and the advantages and disadvantages of using PEs coupled with GC/MS to measure trace-level flame retardants will be discussed.

(239) **A Great Lakes Perspective on Flame Retardants: Lessons from the Integrated Atmsopheric Deposiiton Network.**; Marta Venier[1], Amina Salamova[1], Todd Nettesheim[2], Ron Hites[1]; [1]Indiana University; [2]Environmental Protection Agency Great Lakes National Program office; [4]Indiana University

Despite the significant progress achieved over the past few decades, toxic contaminants continue to persist in the Great Lakes ecosystem and remain a threat to human health and the environment. Contaminant levels in Great Lakes fish have declined over the years; however, levels are still high enough to warrant fish consumption advisories throughout the basin. The atmosphere represents the major pathway by which most of these toxic contaminants enter the Great Lakes. The intent of the Integrated Atmospheric Deposition Network (IADN) is to measure and evaluate pollutant concentrations in the atmosphere (air-borne vapor, airborne particles, and precipitation) at a regional level of detail. Historically, IADN focused on legacy pollutants such as chlorinated pesticides, and polychlorinated biphenyls (PCBs). More recently, the focus was expanded to a new class of industrial chemicals, such as flame retardants. After an initial screening of 2004 samples, polybrominated diphenyl ether (PBDEs) flame retardants have been included in the list of analytes routinely measured by IADN starting in 2005. With time, new and alternative flame retardants have been added to the list of target chemicals. This relatively large dataset allows us to look at temporal trends to determine if legislative actions and/or manufacturers' decisions on production and use of these chemicals are affecting their environmental levels.

(240) **Emerging Flame Retardants in North American Aquatic Ecosystems**; Da Chen[1], Rebecca Sutton[2], Jeremy Moore[3], Doug Adams[4], Yan Wu[1], Hillary Marler[1],Hillary Marler[1]; [1]Southern Illinois University; [2]San Francisco Estuary Institute; [3]US Fish and Wildlife Service; [4]Cape Canaveral Scientific, Inc.

Flame retardants represent a group of halogenated and non-halogenated substances (i.e. over 70 compounds) widely added to thermoplastics, textiles, building materials, electronics and automobile parts to reduce their flammability. As most FRs are not chemically bound to the polymers that contain them, a fraction may escape during production, use, disposal and recycling processes, via volatilization or physical processes such as abrasion or weathering. Ultimately these FRs may enter aquatic environments. Polybrominated diphenyl ether (PBDE) flame retardants have been subject to the most extensive studies worldwide in terms of their sources, fate, environmental distribution and toxicity. While the production and usage of commercial PBDE mixtures has been phased out in North America, restrictions on PBDE applications have resulted in increased use of alternative flame-retardant chemicals to meet flammability standards. However, knowledge on the environmental presence and distributions of these alternative flame retardants remains limited to date. In this study, we screened for a suite of 65 emerging flame retardant chemicals in abiotic and biological matrixes from the San Francisco Bay, the Great Lakes, and the Atlantic. In the San Francisco Bay, a number of halogenated and non-halogenated organophosphate flame retardants (OPFRs) were detected in water of up to 4,800 ng/L. OPFRs were also detected in sediments, invertebrates, and harbor seal blubber from the Bay. Additional FRs, such as 1,2,5,6-tetrabromocyclooctane (TBCO), 1,2-bis-(2,4,6-tribomophenoxy)ethane (BTBPE), bis(2-ethyl-1-hexyl)tetrabromophthalate (BEHTBP), and dechlorane plus (DP)-related compounds (e.g. anti- and syn-DP, Dec-602 and Dec-604), were also detected in the Bay sediments and biota. In Lake Michigan, OPFRs were detected in water and sediments at concentrations of up to 290 ng/L and 180 ng/g dry weight, respectively. TBCO, BTBPE, BEHTBP, and DP-related compounds, as well as a several others, were also frequently detected in Lake Michigan sediments. They were also frequently detected in the Atlantic sharks of different species. Overall, our study revealed that a number of alternative FRs have been widely distributed in the North American aquatic systems. Some of them have demonstrated substantial bioavailability. Investigations on their sources, spatiotemporal distributions, fate and toxicity are urgently needed.

(241) **Strategies and Techniques for Identifying Unknown Compounds in Environmental Samples**; Eric J Reiner[1,3], Karl J Jobst[3], Miren Pena-Abaurrea[1], Anne L Myers[1], Li Shen[2], Alina Muscalu[2], Ralph Ruffolo[2], Xavier Ortiz[2], Paul A Helm[2]; [1]University of Toronto; [2]Ontario Ministy of the Environment and Climate Change; [3]McMaster University

There are approximately 100,000 industrial chemicals or chemicals of commerce used currently, but only a small fraction are monitored routinely. The Stockholm Convention on persistent organic pollutants (POPs) targets 24 halogenated organic compounds or compound classes. Environmental samples can contain thousands of non-targeted compounds, some of which may pose a risk to the environment or human health. Their identification can be very challenging because most routine analytical techniques are blind to non-targeted compounds. (Ultra)High resolution mass spectrometry (MS) and multidimensional gas chromatography (GCxGC) are complementary analytical techniques for non-targeted analysis of environmental samples. This contribution deals with the strategies implemented by our group to identify halogenated compounds in a range of challenging environmental samples.

Multidimensional chromatography offers unparalleled separating power which often displays thousands of peaks and the task of reviewing and interpreting such enormous data sets can be extremely laborious and time-consuming. One strategy is to use automated software (scripts) to rapidly and automatically identify halogenated compounds in complex GCxGC-TOF chromatograms using isotope patterns of Cl and Br.

Another approach for the identification of halogenated compounds exploits the negative mass defects of F, Cl and Br. (Ultra)high resolution mass spectra of environmental samples often display thousands of peaks. Distinguishing halogenated and non-halogenated peaks is can be a significant challenge, but is made straightforward by constructing a mass defect plot: the nominal mass of each mass spectral peak is graphed vs. the corresponding mass defect. Examples of complex environmental samples that have been characterized using one or a combination of the approaches described above will be presented.

(242) **Detection of TNT and RDX Based on Gold Nanoparticles Molecular Imprinted Matrix by SPR and SERS**; Geneviève Granger[1], Nathalia Bukar[1], Jean-François Masson[1], Andreea R. Schmitzer[1]; [1]Département de Chimie, Université de Montréal, Montréal, Canada

Over the last few years, energetic materials such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitroperhydro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) have shown an environmental impact on military bases, the surrounding population, fauna and flora, caused by military training involving ammunitions. On shooting ranges, soils near the firing positions and around targets require special monitoring, since the quantities of explosives residues found are significant, and these compounds can be transported to surface water and groundwater by precipitation. However, the current procedure to detect energetic materials in natural water is complex, long and poorly adapted. These operations require highly specialized personnel and increase the risk of cross contamination. Therefore, it is difficult to ensure a fast and continuous monitoring of the contaminants. The current objective is to develop a technique for identifying and quantifying explosives and their degradation products in natural water. Also, this test has to be in-situ, inexpensive and fast. Surface plasmon resonance (SPR) and surface-enhanced Raman spectroscopy (SERS) have been extensively used to probe energetic materials. A bis-aniline-cross-linked gold nanoparticles (AuNPs) matrix is used as a molecular imprinted polymer (MIP) on gold film to selectively capture the target compound. The association of the target such as TNT or RDX to the MIP with π-donor-acceptor interactions have allowed the detection of explosives by following SPR refractive changes. Plasmon coupling effects between the AuNPs and the gold film could also increase the SPR and SERS signals. The utilisation of MIP based essay will provide a tool for the extraction and pre-concentration of TNT or RDX on the detector's surface and will allow the detection of lower concentrations in natural water.

(243) **Generation and Quantitation of Parts per Quadrillion Levels of TNT and RDX**; Braden Giordano[1], Benjamin Andrews[2], Adam Lubrano[2]; [1]U.S. Naval Research Laboratory; [2]Nova Research, Inc.

The Trace Explosives Sensor Testbed relies on aqueous solutions of nitroaromatic and nitramine explosives as the point source for trace explosives vapor generation. Typically, trace vapors are quantified using TDS-PTV-GC-μECD systems, where the analyte is trapped on a Tenax-TA filled thermal desorption tube, desorbed and trapped on a Siltek-deactivated glass liner, and subsequently desorbed for GC-μECD analysis. Mass limits of quantitation for TNT and RDX are approximately 100 picograms, which translates to sampling times near 4 hours for accurate quantitation of 500 parts per quadrillion vapor (100 mL per minute sample rate) [1-3]. At first approximation, this is a two order-of-magnitude difference in time between vapor validation and sensor duty cycle. It is desirable that the time scale for vapor validation more closely match the time scale associated with sensors being evaluated.

Clearly, the large mass limit of detection needs to be significantly reduced to improve vapor validation duty cycle for parts per quadrillion vapor concentrations. This can be achieved with a combination of improvements, including eliminating the dependence of Tenax TA for analyte preconcentration and transitioning to a more sensitive detection mode. Quantitation of parts per quadrillion TNT and RDX vapor was achieved using an on-line PTV inlet fitted with a Siltek-deactivated glass liner coupled to a GC-MS using negative mode CI (methane reagent gas). Sampling rates improve to 165 mL/min (due to eliminating the need for Tenax TA), giving rise to detector limits of quantitation as low as 0.25 picograms. Consequently, a modest validation method duty cycle of only 10 minutes (includes sampling and GC run time), permits limits of quantitation in the tens of parts per quadrillion range for TNT and RDX vapor.

(244) **Analysis and Delivery of Vapor from Binary Explosive Mixtures for Instrumental and Canine Detection**; Susan Rose-Pehrsson[1,2], Lauryn DeGreeff[1], Frank Lucus Steinkamp[3], Braden Giordano[1], Christopher Katilie[1,2]; [1]U.S. Naval Research Laboratory; [2]Nova Research, Inc.; [3]National Research Council

Binary explosive mixtures are commonly found in improvised explosive devices (IEDs) which have become increasingly commonplace in the Middle East. The components of such mixtures often have common, innocuous uses independent from each other and thus are only of concern when they are combined. Binary explosives require an oxidizer and a fuel component. A common oxidizer is ammonium nitrate (AN), and examples of fuels include, but are not limited to aluminum powder and fuel oil. Detection protocols often focus on the detection or sensing of the oxidizer alone. Canines, for example, are often trained on solely AN, though recent canine evaluations have shown that the canines perform better when trained to the mixed components. Hazards associated with binary explosive mixtures often limit or preclude analytical research and other testing using the mixtures themselves. For this reason, it is important to establish differences, if any, between explosive components and component mixtures.

Qualitative and quantitative differences in the headspace of the individual fuel and oxidizer components and the mixtures were compared. As AN is a salt, the reported vapor pressure is a result of its dissociation into ammonia and nitric acid in ambient humidity. Collection and analysis of AN headspace by ion chromatography (IC) was carried out and has shown that the ratio of ammonia and nitric acid in the headspace is humidity dependent with dry conditions favoring a 1:1 ratio. Humidified conditions sequester the nitric acid, thereby reducing the amount of nitric acid observed and biasing the ratio towards ammonia. Additional techniques were used to quantitate ammonia in the headspace of AN alone and when mixed with a fuel, as well as to determine the headspace components of the fuels, mixed and unmixed. Overall, results showed minimal differences between individual explosive components and mixtures with little change with time.

This knowledge was applied to the development and testing of an odor delivery device for canine training for IED detection, as well as for instrumental detection and vapor generation. The device was shown to accurately portray the headspace of binary explosive mixtures while housing the mixture components separately, and thus, safely.

(245) **Trace Explosive Detection using Zinc Oxide Nanowire Catalysts**; Zachary Caron[1], Otto Gregory[1]; [1]University of Rhode Island, Department of Chemical Engineering

The detection of trace levels of explosives has been a priority for the Department of Homeland Security for more than a decade. By monitoring the vapor space of jetways and other high traffic area venues for energetic materials used by terrorists such as TATP, potential threats to the

traveling public could be mitigated. Current methods for explosive detection include the use of "drug sniffing" dogs, colorimetric methods and ion mobility spectrometry (IMS). Although dogs have low limits of detection for these analytes, they are expensive to train and cannot continuously monitor areas without sufficient rest. Research has focused on developing a robust, orthogonal sensor platform for the continuous monitoring of both nitrogen and non-nitrogen based explosives. Metal oxide catalysts play a significant role in the sensor platform, with zinc oxide and tin oxide showing the greatest response to TATP and DNT, respectively. Our sensor platform utilizes two microheaters; one coated with a metal-oxide catalyst and an uncoated microheater. By subtracting the signals from the two microheaters, extraneous heat effects are subtracted out, producing a response that is only due to catalytic activity. To transition from our current sensor platform to a MEMs based platform, it was necessary to significantly increase the surface area of the catalyst. Therefore, zinc oxide nanowires were hydrothermally grown to increase the surface area of the catalyst that covers the microheaters. Using this approach, we were able to discriminate between target molecules of interest with both high selectivity and sensitivity. Recent data has shown that the use of zinc oxide nanowires in conjunction with a sputtered catalyst film, greatly increases the response of our sensor compared to our sensor with only a sputtered catalyst. The dual purpose of the zinc oxide nanowires, increasing surface area as a catalyst support as well as being a unique catalyst, will increase the functionality of our continuous monitoring system for explosives and explosive precursors.

(246) ***In-situ* detection of energetic materials based on surface plasmon spectroscopies**; Thibault Brulé[1], Geneviève Granger[1], Natalia Bukar[1], Marc Vidal[1], Jean François Masson[1]; [1]Département de Chimie, Université de Montréal

A lot of occidental states are implicated in the limitation of the environmental impact of energetic materials used in military context. Groundwaters are mainly afflicted by such use of these materials. The main energetic materials are nitroaromatics (e.g. TNT) or nitramines (e.g. RDX and HMX). These last ones are the most problematic in groundwater because they are only marginally degraded in organic matrix of ground and migrate to groundwater reserves. Low concentrations are problematic, such that specific environmental criteria in water are associated to the different materials. TNT and RDX should not be over concentrated than 2 ppb, i.e. around 10 nM. The detection of these molecules at concentrations under these criteria is very challenging to improve groundwater control and reduce the environmental impact of their use.

In this aim, we choose to develop plasmon based techniques to enhance the optical response of these specific molecules. Selectivity of the target molecules is a significant challenge and thus, different strategies are tested. First, the development of Dynamic Surface Enhanced Raman Spectroscopy (D-SERS) combines the selectivity of Raman spectroscopy with the sensitivity involved by the dynamic approach. Second, the coupling of a Surface Plasmon Resonance (SPR) sensor with Molecular Imprinted Polymers (MIP) improves the selectivity based on the imprint recognition and the sensitivity enabled by the SPR approach. These strategies involve developments of specific plasmonic substrates to couple both approaches. Results are obtained on flat metallic films, on metallic nanoparticles and on nanostructured metallic surfaces.

In this contribution, we report how our innovative developments in surface plasmon spectroscopies in terms of substrate synthesis and also in detection approach for in-situ measurements of energetic materials in groundwater for concentrations of 1 pM, clearly below the environmental criteria.

(247) **AFM-IR Applications in Bio-Molecules Production**; Rolando Rebois[1], Ariane Deniset-Besseau[1], Delphine Onidas[1], Alexandre Dazzi[1]; [1]Laboratoire de Chimie Physique - Université Paris-Sud

AFM-IR (1) (coupling an Atomic Force Microscope with an IR laser) is simple and efficient technique to make infrared spectroscopy and imaging at nanometer scale. The nanoIRTM has been marketed, as a commercial version of the AFMIR invention, by Anasys Instruments company (CA, USA). Using this technique, we were able to obtain exciting data showing the detection of bio-molecules such as bio-polymer produced by Rhodobacter (2,3) and size estimation of triacylglycerol vesicles inside dried Streptomyces bacteria (4).

There is now a real focus in the use of cells to make fuel. Bio-fuel appears to be a way to avoid dependency on mineral oil (politics, price and environment) and really attractive because of its renewable energy properties. There are many types of cell (Streptomyces bacteria, Algae, yeast) that have shown their ability to produce triacylglycerol, a glycerolipid that can be converted in biodiesel by trans-esterification. Our work is to use infrared analysis to monitor this production on living cells by changing or adding the culture medium (N deprivation, glucose substitution, etc..) and testing different mutants. The major advantage of this study is that the triacylglycerol can be detectable specifically without a tag using infrared imaging because of its carbonyl band at 1740 cm-1 making easy the detection of the "fattiest" bacteria.

The production of bio-plastic is still challenging because their properties are not always optimum for industrial process as polymer from petrochemistry. (PHB) poly(3-hydroxybutyrate) has been receiving more interest because of its thermo-plastic and mechanical properties similar to those of synthetic polymer (isotactic polypropylene). However, the crystallinity of this polymer is often an issue for specific applications. Using the nanoIR, we have estimated the amorphous and crystal phases of PHB granules directly inside bacteria (Rhodobacter) and study the effect of solvent on these granules.

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(248) **AFM-IR Spectroscopy and Imaging of Polymer Nanofibers and Thin Films at the Nanoscale**; John Rabolt[1], Liang Gong[1], Isao Noda[1,3], Bruce Chase[1], C. J. McBrin[1], Curtis Marcott[1,2]; [1]Department of Materials Science and Engineering, University of Delaware; [2]LightLightSoluions; [3]Department of Materials Science and Engineering, University of Delaware

The combination of atomic force microscopy (AFM) and infrared (IR) spectroscopy is an extremely powerful tool that can provide topographic information that can be correlated with chemical, conformational and molecular orientation information at a spatial resolution of 50-100 nm. Using an AFM-IR instrument, we have explored the correlation between structure, processing and chain orientation/crystallinity in biodegradable and biocompatible poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (PHBHx) electrospun nanofibers and thin films and tested the hypothesis that different processing protocols can alter the concentration of the stable α-crystalline form and the metastable β-crystalline form.

The ability to obtain IR spectra at high spatial resolutions has allowed us to probe, for example, crystalline populations as a function of nanofiber diameter and as a function of location within the fiber and we have observed, for the first time, the existence of a core-shell structure in a single electrospun nanofiber. In addition, the spectroscopic imaging capability of the AFM-IR instrument has been utilized to investigate poly(tetrafluoroethylene) (PTFE)/Ni composite thin films created on stainless steel during abrasion. The tribological implications of the chemical nature of the film transferred will be discussed.

(249) **Correlated Nano-Chemical and Nano-Mechanical Imaging of Protein Nanoribbons Involved in Dental Enamel Formation**; Martin Wagner[1], Karina Carneiro[2], Stefan Habelitz[2], Thomas Mueller[1]; [1]Bruker Nano Surfaces; [2]University of California, Preventive and Restorative Dental Sciences

Infrared spectroscopy can give valuable information on chemical composition, but far-field techniques such as FTIR spectroscopy are limited in spatial resolution. S-SNOM is a well-established near-field technique [1] that can overcome this diffraction limit, allowing an improvement in spatial resolution down to 10 nm.

Our s-SNOM instrument is based on an atomic force microscope whose tip is illuminated with a quantum cascade laser. Field-resolved detection of the scattered light measures absorption [2]. We have combined the instrument with peak-force tapping, a technique that allows pN-level force control between tip and sample. Besides being able to image fragile material systems, one can extract valuable nano-mechanical information such as adhesion or modulus with molecular resolution [3].

Here, we study an amelogenin sample. Amelogenin is a protein that is critical to dental enamel formation [4,5]. In the presence of calcium and phosphate ions it self-assembles into ordered, self-aligned nanoribbon bundles. Since the ordering is similar to the one observed in phosphate-based apatite crystals that comprise dental enamel, it is likely that the bundles form a template for these crystals. To help clarify that open question, we map the distributions of phosphate and hydroxyapatite nanocrystals within the bundles consisting of <30 nm narrow nanoribbons.

We present correlated topography, near-field and nano-mechanical data. While the presence of phosphate could be identified using s-SNOM absorption maps, no apatite nanocrystals with higher modulus than the ribbons were observed in peak-force tapping. This indicates that for these in vitro preparation conditions apatite crystals have not formed yet.

In summary, using a novel combination of near-field imaging and peak-force tapping we study the phosphate distribution and crystallization in protein samples. Our findings help to understand the formation processes of dental enamel and the role of amelogenin protein.

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(250) **Introducing nano-FTIR – Imaging and Spectroscopy at 10nm Spatial Resolution**; Tobias Gokus[1], Andreas Huber[1], Florian Huth[1]; [1]Neaspec GmbH

Neaspec's near-field optical microscopy systems (NeaSNOM) allow to overcome the diffraction limit of light enabling optical measurements at a spatial resolution of 10nm.

Scattering-type Scanning Near-field Optical Microscopy (s-SNOM) [1] employs an externally-illuminated sharp metallic AFM tip to create a nanoscale hot-spot at its apex. The optical tip-sample near-field interaction is determined by the local dielectric properties (refractive index) of the sample and detection of the elastically tip-scattered light yields nanoscale resolved near-field images simultaneous to topography.

Development of a dedicated Fourier-transform detection module for analyzing light scattered from the tip which is illuminated by a broadband laser source enabled IR spectroscopy of complex polymer nanostructures (nano-FTIR) [2]. Identification of individual contaminants has been demonstrated. Other applications show characterization of embedded structural phases in biominerals [3] or organic semiconductors. The patented modular system design allows for tailored system configurations where the ultimate spectral coverage can be achieved by using synchrotron-based broadband IR light sources [4]. Use of material-selective frequencies for near-field measurements enable to fully characterize local morphology of organic semiconductors [5] or to analyse individual protein complexes at the nanoscale [6].

Extending the concept of broadband-sSNOM spectroscopy to the THz-spectral range, we demonstrate nanoscale material characterization at THz-frequencies by coupling the free space beam of a THz-TDS system to a s-SNOM microscope [7,8]. Material specific near-field spectra of metals and highly doped semiconductors in the spectral range between 0.5 - 2.5 THz as well as near-field images (at fixed delay) can be obtained. The presented results open the door to study nano-scale light-matter interaction in the THz-spectral range for all materials systems that can be investigate by conventional AFM measurements.

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(251) **Characterization of a Polyethylene–Polyamide Multilayer Film Using Nanoscale Infrared Spectroscopy and Imaging**; Mauritz Kelchtermans[1], Michael Lo[2], Eoghan Dillon[2], Kevin Kjoller[2], Craig Prater[2], Curtis Marcott[3]; [1]ExxonMobil Chemical Europe, Belgium; [2]Anasys Instruments; [3]Light Light Solutions

Atomic force microscopy (AFM) and infrared (IR) spectroscopy have been combined in a single instrument (AFM-IR) capable of producing IR spectra and absorption images at sub-micrometer spatial resolution [1]. Using such an instrument, cross sections of multilayer films can be spectroscopically characterized at levels not previously possible. In particular, it was possible to observe nanoscale IR spectroscopic differences, as well as thermal and mechanical property differences in the sub-micrometer thick tie-layers located between individual polyethylene and polyamide layers of a multilayer film. It also could be confirmed that a two micrometer thick barrier layer between two polyamide layers near the center of the laminate consists of a copolymer of ethylene and vinyl alcohol. Mechanical stiffness and thermal property differences are also observed between the various layers in the film. This powerful capability should prove generally useful for reverse engineering complex unknown multilayer films, as well as in aiding the intelligent design and development of superior multilayer materials.

(252) **Single Particle LIBS Analysis in Optical Traps. Imaging, Multielemental Analysis, and Detection Power**; Javier Laserna[1]; [1]Universidad de Malaga

Single particle characterization still constitutes a challenge to contemporary chemical analysis. Considerable effort worldwide is being devoted to conceive experimental strategies providing detection capabilities compatible with the extremely low mass of micro- and nano-particles and the ability to determine the chemical composition of the individual entities.

The notion of using optical levitation to trap individual particles was demonstrated in the past century. Recently we have proposed the multielemental analysis of individual nanoparticles in optical traps using LIBS. In this talk, the experimental methods for sample preparation and optical detection will be discussed. An analysis of the photon budget involved in our approach will be presented.

(253) **Pathways Towards High-Resolution Chemical Analysis and Imaging with Femtosecond LIBS**; Vassilia Zorba[1]; [1]Lawrence Berkeley National Laboratory

Laser Induced Breakdown Spectroscopy (LIBS) is a versatile analytical technique for elemental material analysis. Today, LIBS is predominantly used as a macro- and meso-scale spectroscopic tool (spatial resolution ~hundreds to tens of micrometers). Extending LIBS capabilities towards the nano-scale is a very important step towards enabling a number of currently inaccessible applications, including analysis and chemical imaging of advanced materials systems (e.g. energy applications such as generation, conversion, storage). For these cases, the resolution of the LIBS analysis needs to be commensurate with the characteristic lengthscales of the features that need to be chemically resolved. Transitioning to better resolution is challenging due to issues with the detection of LIBS emission as we scale down in ablated mass. In this talk we provide an overview of the strategies we are currently using for improving resolution (spatial and axial) towards nano-meter scale LIBS. Femtosecond (fs) lasers, which offer reduced thermal effects and enhanced non-linear effects (e.g. multiphoton absorption), are used as the basis for high-resolution material sampling. Some of the approaches that will be presented include the use of fs lasers in tight-focusing conditions (high numerical aperture objectives), orthogonal ultraviolet fs/ns double-pulse configurations, and near-field fs-laser excitation/near-field detection schemes. Results of high-resolution chemical imaging in energy systems will also be presented, including Li-ion batteries and interfacial layers in electrochemical energy storage.

(254) **New Hybrid Calibration-Free/Artificial Neural Networks Approach for Quantitative Analysis**; Vincenzo Palleschi[1]; [1]National Research Council

The classical approach to LIBS quantitative analysis of materials is based on the use of calibration curves, obtained from a number of reference samples. Unfortunately, the LIBS signals are extremely sensitive to any change in laser-sample coupling, produced by fluctuations in the laser irradiance on the samples or by matrix effects. Consequently, the analytical figures of merit of LIBS place the technique at the lower end with respect to other more established laboratory elemental techniques, such as X-Ray Fluorescence (XRF) or Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), for example. For overcoming this drawback several years ago we have developed a Calibration-Free approach, based on the knowledge of the chemical/physical processes in the laser-induced plasma, which is very effective for correcting all the effects that would prevent the use of a calibration approach. CF-LIBS analysis overcomes the matrix effect allowing in principle very accurate measurements, but for exploiting at best the potential of the technique, the CF-LIBS algorithm should be applied on the single spectra, eventually averaging the concentrations if several measurements have been performed on the same sample. Usually, the reverse is done and for practical reasons the CF-LIBS method is applied on the average spectrum, instead, which is conceptually wrong. On the other hand, an alternative approach to LIBS quantitative analysis, based on the use of Artificial Neural Networks has been recently proposed which is much faster than the Calibration-Free method. The artificial neural networks are extremely fast and flexible, they operate on single spectra but suffer of the problems associated to all the methods based on calibration curves and, in particular, the matrix effect.

In this communication, we will show how the basic equations of Calibration-Free LIBS can be embedded in an Artificial Neural Network algorithm, thus combining the advantages of the two methods, i.e. the speed of the Neural Network and the precision of the Calibration-Free analysis.

(255) **New Methodology for Quantitative Laser-Induced Breakdown Spectroscopy Based on CSigma Graphs. Application to Fused Glass Samples**; Carlos Aragon[1,2], Jose Antonio Aguilera[1, 2]; [1]Departamento de Fisica, Universidad Publica de Navarra; [2]Institute for Advanced Materials, Public University of Navarre

In this work, we present a new approach for quantitative laser-induced breakdown spectroscopy, which we have called Cσ-LIBS. The core of this approach for LIBS is the Cσ graph, a generalized curve of growth which allows including lines from several elements in a given ionization state. Cσ graphs are obtained from the line intensities in the measured spectrum, together with the plasma temperature and electron density, making use of a database of atomic data. The laser-induced plasma is described using a simple so-called homogeneous double model, in which the plasma is assumed to contain neutral and singly-charged ions. Due to their different spatial distribution in the laser-induced plasma, the two ionization stated are treated separately, their emissions being described by distinct apparent parameters, including the temperature, the electron density, the columnar total number density of neutral atoms and ions, and a multiplicative parameter. The equation of radiative transfer is integrated over a

single region for each ionization state, leading to two calculated Cσ curves. To account for the true complex spatial distribution of parameters, a model limit is introduced.

Within the Cσ-LIBS framework, we have developed three methods with different objectives, namely plasma characterization, measurement of transition probabilities and quantitative elemental analysis. In quantitative analysis by Cσ-LIBS, conventional calibration curves are replace with only one or two Cσ graph, which include all the elements. In the present work, we describe the Cσ-LIBS methodology, as well as experiments performed for testing and validation of this new approach. In the experiments, we have used fused glass samples prepared from pure compounds and also from certified reference materials.

(256) **Development of a Process Analytical Solution for Real-Time Monitoring of Continuous Flow Reactors**; Brian Marquardt[1], Thomas Dearing[2], Michael Roberto[3], Olav Bleie[4]; [1]University of Washington; [2]MarqMetrix Inc; [3]Infometrix; [4]Univ. of Bergen, Norway

Continuous flow reactors (CFRs) are flow cells that are optimized for the continuous production of a target chemical compound. Currently, production of pharmaceuticals relies on large-scale batch processes that are intensified from the laboratory scale. Compared to batch reactors, CFRs have greater energy efficiency due to their superior mixing schemes and heat transfer properties. Due to this efficient heat transfer, reactions that require cryogenic temperatures in batch, such as the Swern oxidation, can frequently be performed at much milder temperatures in CFRs, significantly reducing temperature control costs. Though these features make CFRs attractive to process-intensive industries, they are currently limited by a lack of on-line analytical technologies suitable for small-volume, high-throughput measurements.

Recently, a program was initiated to evaluate the use of process analytics (PAT) and NeSSI sampling systems for the validation of CFRs performing a Swern oxidation. A four reagent sampling system is interfaced to a continuous low-flow reactor enabling reaction monitoring using a four channel process Raman spectrometer. Real-time process control of the Swern reaction was achieved by monitoring a series of process control parameters that included flow rate, stoichiometry, reactor temperature and pressure. Establishing control of the process allowed for optimization, minimizing the production of "off spec" product and removing the need for off-line quality control analytics. Real-time chemical modeling was performed to determine product quality using multivariate statistical methods. Results from initial experiments demonstrated that the oxidation, which requires cryogenic temperatures in batch, can be performed at cost effective temperatures in continuous flow.

(257) **Recent Advances in Automatic Continuous Online Monitoring of Polymerization reactions (ACOMP)**; Wayne Reed[2], Michael F. Drenski[1]; [1]Advanced Polymer Monitoring Technologies, Inc.; [2]Tulane University

ACOMP has been applied to many different types of polymerization reactions in the past two decades. After a brief overview of principles and basic operation, three new directions are highlighted in this presentation; 'Second generation ACOMP' (SGA) is being developed to monitor the onset and evolution of polymer stimuli responsive behavior during synthesis, by using multiple, independent light scattering and viscosity detector stages, with the ability to change both temperature and solution conditions between detection stages. Early SGA results on monitoring LCST in copolymers with NIPAM of continuously varying comonomer composition are presented. Preliminary results on the response of copolymeric polyelectrolytes to stepped levels of ionic strength during synthesis are shown.

First steps in using ACOMP to both monitor and control polymerization reactions are being taken. Ideal reaction trajectories are posited for polymerization reactions in order to produce endproducts of desired properties, then providing active control to steer reaction trajectories towards the ideal ones.

Finally, ACOMP has now been installed, for the first time, in the full scale industrial reactor milieu. Some features of this technology transfer program are highlighted.

(258) **Development of NIR Methodology for Process Monitoring and Control using an Offline Calibration Approach**; Evan Hetrick[1], Zhenqi Shi[1], Lukas Barnes[1], David Myers[1], Bryan Castle[1], Salvador Garcia Munoz[1], Ian Leavesley[1]; [1]Eli Lilly and Company

Near infrared (NIR) spectroscopy has become a useful means for monitoring various processes common to pharmaceutical development and manufacturing. One such application is for monitoring blend uniformity for dry product formulations. As companies throughout the pharmaceutical industry move towards continuous drug product manufacturing, a challenge that has developed is demonstrating consistency of the blend throughout an entire continuous manufacturing run. Furthermore, in-process spectroscopic controls should be capable of detecting 'process upsets' within relevant limits between at-line unit dose testing. These challenges are compounded by variability from sources that can be attributed to the process itself, the material (formulation), instrumentation, and the environment. This presentation describes a method whereby a NIR calibration was developed using an offline approach designed to mimic the continuous manufacturing process as closely as possible. The offline approach enabled a more efficient calibration and provided the flexibility needed to account for potential sources of variability that may impact the calibration model. Potential sources of variability were identified via a thorough risk analysis followed by a screening DOE. Finally, the performance of the calibration was tested via an independent run on the full-scale continuous manufacturing platform.

(259) ***In situ* ATR-FTIR: A Technological Shift in Continuous Processing**; Dom Hebrault[1]; [1]Dom Hebrault

Real-time analytics, such as in situ ATR-FTIR, is becoming widely utilized in academia and industry to gain a deeper insight into organic synthetic processes, leading to increased efficiency and quality of research, development, and manufacturing. It is complementary to offline analysis such as HPLC and NMR, and, thanks to its high molecular specificity, used to study reaction progression in situ, analyze reaction kinetics and to elucidate reaction mechanism and pathway. It provides a highly specific and comprehensive information set. Case studies relating the use of real time in situ FTIR to enhance continuous chemical processing will be presented. More specifically, we'll examine how ATR-FTIR combined with chemometrics, provides real-time composition analysis of complex molecules in a flow mode, and facilitates reaction rate and conversion improvement, which leads to fewer side-reactions and permitted facile isolation and purification of the final compounds.

(260) **Getting More Out of Process Measurements with Diode Array Spectrometers: Instrumental and Analysis Approaches**; Robert Lascola[1], Patrick O'Rourke[1], Elizabeth Evans[1], Edward Kyser[1]; [1]Savannah River National Laboratory

Absorption spectroscopy is a well-explored method for process measurements, and the continued size and cost improvements for diode array-based instruments seemingly makes them plug-and-play devices. This status encourages using the instruments without necessarily understanding their limitations. We have found that rigorous characterization of intensity nonlinearities and wavelength calibration can greatly extend the

accuracy and dynamic range of these instruments. Here, we describe how we have implemented these improvements in the context of monitoring chemical reprocessing of nuclear materials using off the shelf instrumentation. The absorption spectra of plutonium complexes are sensitive to oxidation state, solution acidity (nitric acid), and temperature. Oxidation state can change during some processing conditions, and the concentration range exceeds the linearity range of typical spectrometers (~1.5 AU). However, corrections for stray light, order sorting, and diode readout permit linear response approaching 3 AU. Wavelengths are calibrated in real time using Xe lamp emission lines. These actions allow spectra obtained on different instruments to be transformed to a virtual instrument basis with a high degree of similarity. We thereby address two cardinal concerns of process monitoring, calibration transfer between instruments and long-term instrument/calibration stability. For the Pu monitoring application, we further extend the monitoring capability by spectral classification based on absorbance levels and solution acidity (determined by partial least squares model), with subsequent application of localized models to determine total Pu and relative proportions of up to three Pu valence states. This scheme expands the measurement range by >3x. Methods for making and characterizing standard solutions, for some of which the Pu valence states are unstable, will be described. The combined advances greatly enhance the suitability of absorption spectroscopy for nuclear materials process monitoring and safeguards applications and could be exploited in other monitoring uses.

(261) **Semiconductor-enhanced Raman Scattering: Towards Applications in Highly Robust SERS Sensing**; Yukihiro Ozaki[1], Wei Ji[1], Yue Wang[2], Ichiro Tanabe[1], Bing Zhao[2]; [1]Department of Chemistry, School of Science and Technology, Kwansei Gakuin University; [2]State Key Laboratory of Supramolecular Structure and Materials, Jilin University

Conventional surface-enhanced Raman scattering (SERS) sensing employsmetallic substratesand has achieved numerous promising results. However, the robustness of SERS sensor is still one of the main drawbacks that restrict their practical applications.Recent advancements in semiconductor-enhanced Raman scattering spectroscopy offer new approaches to and new possibilities for development of novel SERS sensors. Their fundamental mechanism is still under investigation, preliminary evidences have shown that the charge-transfer process plays a key role for semiconductor materials with small dimensions (less than 100 nm).Thus, most of SERS in semiconductors shown relatively low enhancement factors. But in theory,semiconductormaterials are superior to metals for design of SERS sensors due to their thermal and chemical stability, which can provideusthe stable and reproducible SERS signal. In this presentation, we will show some examples of quantitative analysis of inorganic ions, such as chromium(VI) and phosphates,by taking advantage of semiconductor-enhanced Raman scattering spectroscopy. Meanwhile, the stability and reproducibility of semiconductor-based SERS sensor will also be discussed. The results indicate that semiconductor material have great potential for highly robust SERS sensing.

(262) **Coherent Raman Spectroscopy with Optical Frequency Combs**; Takuro Ideguchi[1,2], Simon Holzner[2], Birgitta Bernhardt[2], Guy Guelachvili[3], Theodor Hänsch[2,4], Nathalie Picqué[2,3,4]; [1]The University of Tokyo; [2]Max-Planck-Institut für Quantenoptik; [3]Institut des Sciences Moléculaires d'Orsay, CNRS; [4]Ludwig-Maximilians-Universität München

The invention of optical frequency comb made a huge step in the field of precision spectroscopy. The laser provided a way to measure absolute optical frequency with unprecedented accuracy. Since then, it has been used as a standard tool for precision measurements. In the last decade, other applications of the optical frequency comb have been demonstrated beyond the original purpose. Broadband molecular spectroscopy is one of those. Among broadband molecular spectroscopy with frequency comb, dual-comb Fourier transform spectroscopy provides unprecedented measurement speed with precision and accuracy.

Dual-comb spectroscopy uses two frequency combs. The laser combs have slightly different repetition frequencies so that the delay between pulses emitted from two lasers is automatically scanned by pulse-pair to pulse-pair. This automatic delay-scanned pulses work as a robust fast-scanning interferometer without mechanical moving part. The dual-comb interferometer can be used for fast Fourier transform spectroscopy. The much faster measurement capability than a conventional way with a Michelson interferometer opens up new opportunities to investigate fast phenomena observed by broadband molecular spectroscopy.

Recently, we have extended the concept of dual-comb spectroscopy to the nonlinear regime. A successful demonstration of coherent Raman dual-comb spectroscopy showed a broadband Raman spectrum in the molecular fingerprint region (200-1400 cm-1) within tens of microseconds. We also demonstrated a hyper-spectral imaging with a raster-scan microscope. The label-free molecular vibrational spectroscopy and imaging could provide new diagnostics in a variety of scientific and industrial fields. In this talk, we introduce the coherent Raman dual-comb spectroscopy and imaging.

(263) **Functionalised Nanoparticles for the Detection of Explosives and Small Molecule by SERS**; Karen Faulds[1], Rachel Norman[1], Duncan Graham[1], Neil Shand[2]; [1]University of Strathclyde; [2]Defence Science and Technology Laboratory

Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity. SERS produces a unique 'fingerprint' spectrum in which the peaks are narrow, this has advantages for multiplexing, allowing for the detection of multiple target analytes. We have made great progress in the development of SERS as a quantitative analytical method. Here we report on a SERS based assay which has been developed using highly specific fluorescently labelled monoclonal antibodies which are conjugated to silver nanoparticles for the direct detection of small molecules. An antibody which has a high affinity for a target analyte is an attractive capture probe as it will specifically bind. Therefore, silver nanoparticles were functionalised with antibodies which, upon binding of the target analyte, allow for a specific SERS spectrum to be obtained due to the analyte being brought close to the nanoparticles' surface. Proof of concept was demonstrated by using TNT and RDX. The unique aspect of this approach is that it is the intrinsic SERS signal from the explosive molecule that is detected as the antibody brings the explosive into contact with the metal surface.

TNT and RDX are small nitroaromatic molecules which are commonly used as concealed explosives. Therefore, a simple and rapid test for explosives which has an unambiguous result, is affordable and can be widely deployed is highly desirable. We have developed a direct SERS detection assay which is quantitative, fast, simple, highly specific and selective towards targeting molecules. This assay has resulted in an observed limit of detection of 10 nM of TNT and 600 pM of RDX. Furthermore, with the use of multivariate analysis, we can also detect and distinguish both targets from within a complex matrix. This approach demonstrates the use of sensors for fast, sensitive and direct detection of small target analytes.

(264) **Raman Spectroscopy of Single Electrospun Nanofibers**; Marie Richard-Lacroix[1], Christian Pellerin[1]; [1]University of Montreal

Electrospinning is a widely used technique to produce nano- to microscale continuous fibers by applying a high voltage on concentrated polymer solutions. Electrospun fibers are known to exhibit unusual and tunable properties, including an exponential increase in modulus with a decreasing diameter. Molecular orientation and chain disentanglement are believed to be critical factors, but are extremely challenging to probe at the single fiber scale. We have previously developed a method enabling the study of orientation in single electrospun fibers by confocal Raman spectroscopy. [1] Here, we use it to quantify molecular orientation in more than 100 individual atactic polystyrene (PS) fibers. We show the exceptional level of orientation reached by the smallest fibers and the high correlation between the drastic increase of the orientation of the chains and the improvement of the modulus as a function of the diameter reduction. We have also demonstrated that partial disentanglement in electrospun PS fibers could be probed by vibrational spectroscopy due to the appearance of bands associated to a conformation made possible by disentanglement. [2] Here, we show our capability to probe disentanglement-related Raman bands in individual fibers, to localize them in the shell of the fibers and to quantitatively correlate their amplitude with the diameter reduction. Combining these results allows us to draw a statistically meaningful picture of the detailed chain organization in PS electrospun fibers.

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(265) **Spectroscopy on Mars - Searching for Signs of Life**; Ian Hutchinson[1], Richard Ingley[1], Howell Edwards[1], Nick Waltham[2]; [1]University of Leicester; [2]Rutherford Appleton Laboratories

In 2018, the first Raman spectrometer to be deployed in an extraterrestrial environment will start its nine month journey to Mars onboard the ExoMars rover. The instrument will provide detailed information on the molecular composition of the Martian surface by analysing crushed samples extracted from depths of up to 2m using a drill and Sample Preparation and Distribution System (SPDS). The laser based spectrometer (Raman Laser Spectrometer - RLS) incorporates a 532 nm excitation source, a transmissive holographic grating and a CCD based camera system. A small optical head equipped with an auto-focus mechanism will deliver a 50 micron laser spot to the surface of the crushed material and the sample distribution system will scan the sample past the spot so that specific transects can be analysed.

The design of the flight instrument is almost complete and a number of prototype versions of the spectrometer are currently being utilised to verify the performance of each individual sub-system and the overall scientific capabilities of the instrument (e.g. sensitivity limits). The data obtained from these studies are also being used to optimise instrument operating modes and sampling strategies and a range of natural samples have been recovered from analog field sites in recent months to facilitate the investigation and optimisation of key performance parameters (i.e. resolution, dynamic range & sensitivity). Here we describe a number of such studies relating to the characterisation and optimisation of the detector/camera system. We also describe the current status of the camera development programme for the RLS instrument and report the mineralological and astrobiological capabilities of the system. In addition, we also compare and contrast the data that the RLS it is likely to acquire with the complementary Raman based instruments proposed for NASA's Mars 2020 mission (i.e. SuperCam and SHERLOC).

(266) **Nanoplasmonic Analysis of Norovirus on Structured Lipid Membranes**; Andreas Dahlin[1]; [1]Chalmers University of Technology

Plasmonic nanopores have been used in various sensing applications during the last 10 years. The structures consist of nanoscale apertures in thin films where at least one material is metallic, thereby supporting surface plasmon modes. For nanopore arrays, optical excitation of propagating surface plasmons is possible at normal incidence using visible or near infrared light. The detection principle for sensing applications is refractometric, i.e. the local increase in refractive index due to molecular binding on the surface gives a wavelength shift of the resonances observed in the transmission spectrum. However, it is hard to find suitable applications of plasmonic nanopores in biomolecular interaction analysis since conventional surface plasmon resonance (SPR) instruments normally provide a better resolution in terms of surface coverage.

In recent work we and others have focused on a few particular sensing applications where plasmonic nanopores can provide unique advantages over SPR. This includes flow-through configuration and temperature control by resistive heating. Here I present a new example of such an application. A certain type of plasmonic nanopores is used to analyze the influence from negative surface curvature for interactions on lipid membranes. I will show how the monitoring of multiple spectral resonances provides information about where in the nanostructure binding occurs, as the electromagnetic field is not homogenous and varies with incident wavelength. Clearly such information is not possible to achieve with SPR which is based on a planar metal surface and the monitoring of a single resonance. As a model system we investigate norovirus binding to glycosphingolipids and show how this multivalent interaction depends on the curvature of the membrane.

(267) **Head-to-Head Comparison of the Performance of SERS and ELISA Diagnostic Tests for Infectious Disease**; Marc Porter[1], Lars Laurentius[1], Nicholas Owens[1], Alexis Crawford[1], Jennifer Granger[1]; [1]University of Utah

Surface-enhanced Raman scattering (SERS) has emerged as an intriguing approach to the creation of ultrasensitive assays for a wide range of diagnostically important biolytes. One variant of this approach uses a sandwich-style assay in which captured antigens are selectively tagged by a gold nanoparticle engineered to incorporate both a monolayer of a Raman reporter molecule and a selective molecular recognition element like an antibody. Results have shown, for example, that this type of heterogeneous immunoassay often, but not always, has a limit of detection notably lower (~100 times) than that of an enzyme-linked immunoassay (ELISA). This presentation describes the findings from an in-depth set of experiments that were designed to carry out an assessment of the factors that dictate the performance of both assay architectures. This presentation describes the results of this work and the implications with respect to the possible origins of the difference in detection capabilities of these assays and how to fine tune performance.

(268) **Single Nanoparticle SPRI Microscopy and Plasmonic Nanocone Arrays For Biosensing**; Robert M. Corn[1], Adam Maley[1], Millie Fung[1]; [1]Dept. of Chemistry, University of California-Irvine

The real-time adsorption of single nanoparticles onto chemically modified gold thin films is measured with near-infrared (814 nm) surface plasmon resonance microscopy (SPRM). Nanoparticle adsorption creates point diffraction patterns in real-time SPRM differential reflectivity images that can be counted to obtain a digital adsorption binding curves. The intensity of the single nanoparticle point diffraction pattern depends upon the size and refractive index of the nanoparticle. Single nanoparticle SPRM measurements are used to quantify (i) the sequence-selective adsorption and enzymatic desorption of DNA-functionalized gold nanoparticles and (ii) the uptake of potential drug molecules like the bioactive peptide melittin into solvent-swollen hydrogel nanoparticles. In a separate project, the fabrication of unique nanotextured surfaces such as

plasmonic nanocone arrays that can be used to create simple biosensors that exhibit unique optical properties, superhydrophobicity and catalytic activity will also be discussed. Similar surfaces exist in nature on the surfaces of lotus leaves, moth eyes and the wings of butterflies.

(269) **Microdialysis SPR: Sensing in Whole Blood**; Jean-Francois Masson[1]; [1]Département de Chimie, Université de Montréal, Montréal, Canada

Blood is the primary biofluid for diagnostics and drug delivery. The performance of materials, drugs, sensors and devices exposed to blood is limited due to its molecular complexity, nonspecific interaction with surfaces, clotting and optical interference due to turbidity, fluorescence and absorption. New methods to decrease complexity of blood, while retaining the possibility of injecting blood directing in a sensing device is of high importance. Dialysis relies on the faster migration of metabolites and small molecules through a porous membrane. This concept was applied to surface plasmon resonance (SPR) biosensing. Size filtering and diffusion through a microporous membrane restricted the access of blood cell and larger biomolecules to a SPR sensing chamber and smaller, faster diffusing biomolecules migrated preferentially to the sensor limiting interference from blood and serum. The microfluidic of a small and portable SPR instrument was modified with a diffusion-gated side-by-side chamber separated by a microporous membrane (0.4 μm pore diameter). The affinity of a small peptide (EP80317) with anti-atherosclerotic activity and targeting type B scavenger receptor CD36 was successfully monitored at micromolar concentrations in human serum and in human blood without any pre-treatment of the sample. This concept could be applied to establish biomolecular interaction and quantify molecules directly in whole blood in point-of-care diagnostics, disease treatment monitoring and pharmacokinetic studies of drugs.

(270) **Poly(n-isopropylacrylamide): Growth Kinetics in Grafting from and Thickness Change upon Temperature Induced Brush Collapse in Water**; Gustav Emilsson[1], Kunli Xiong[1], Andreas Dahlin[1]; [1]Dept. of Applied Physics, Chalmers University of Technology

Responsive polymers are macromolecular nanostructures which undergo some conformational change due to an external stimuli, such as pH or temperature. Their responsive properties are relevant for and opens up new possibilities in e.g. the fields of nanoactuation, bioseparation and biosensors. One example is the combination of responsive polymers with novel nanostructures, such as plasmonic nanoholes and nanopores in thin metal films; which could possibly offer new applications in e.g. bioseparation and biosensors.

Poly(N-isopropylacrylamide) (PNIPAM) is a thermoresponsive polymer which undergoes a characteristic collapse at 32 °C in water resulting in, among other things, a decrease in thickness (if attached to a surface). This conformational change has been used to create "smart" surfaces which can e.g. be used to regulate cell adhesion. To our knowledge real time measurements of the surface initiated polymerization of PNIPAM has so far not been conducted and the application of PNIPAM to plasmonic nanohole arrays still remains to be investigated.

We here present real time monitoring of the growth rate of PNIPAM, via surface initiated polymerization, using Surface plasmon resonance (SPR). Providing quantitative data on growth kinetics. Further characterisation of the formed brushes is also performed using "non-interacting molecules" in SPR, quartz crystal microbalance with dissipation, and Fresnel models. Revealing information about the polymer in both its extended and collapsed state. We also investigate the possibility to apply the method to plasmonic nanohole arrays. Proving that the method is suitable for combining plasmonic nanohole arrays with PNIPAM.

The current results are a promising step towards some potential applications gained by functionalizing nanostructures with responsive materials. For example, controlled passage of molecules through membranes using temperature. We are currently exploring different such applications on a range of nanostructures; in particular plasmonic nanoholes and nanopores.

(271) **Development of SRM 3232 Kelp for Dietary Supplement Measurements**; Lee Yu[1]; [1]National Institute of Standards and Technology

Kelp has been a part of human diet for centuries. In recent years, kelp has been consumed in the US as a dietary supplement because it is a good source of folic acid and vital elemental nutrients such as iodine. To support FDA food safety and nutrition measurements program, NIST is developing a standard reference material (SRM) 3232 Kelp. The value assign measurements for nutritional elements Ca, I, and P and toxic elements Cd and Pb were made with two independent analytical methods and one primary method, respectively. However, the 34 mg/kg arsenic found in the SRM is misleading when use in safety assessment. In an effort to make the SRM more suitable for assessing the safety of the dietary supplement, arsenic species in kelp extract were investigated, and a procedure was developed to extract arsenic reproducibly from the kelp powder. Arsenosugars with molar mass of 328, 482, and 392 and dimethylarsinic acid (DMA) were identified in the extract through a combination of ion trap time of flight mass spectrometry (IT-TOF) and the retention time in LC-ICP-MS measurements. As part of the arsenic value assignment for the SRM, arsenosugars in the extract were measured. To circumvent the challenge of lacking arsenosugar calibration standards in the quantification measurement, fractions of arsenosugars were collected after anion and cation LC separation of the kelp extracts spiked with monomethylarsonic acid (MMA) and trimethylarsine oxide (TMAO) internal standards, respectively. The arsenosugars were quantified as arsenic by INAA using the internal standards MMA and TMAO as the calibrants. Results of nutritional and toxic elements in the candidate SRM will be discussed.

(272) **Identification of Stroke Metalloprotein Biomarkers and Metal Profile in Human Blood Plasma**; Keaton Nahan[1], Julio Landero[1], Opeolu Adeoye[2], Joseph Caruso[1]; [1]University of Cincinnati, McMicken College of the Arts and Sciences; [2]University of Cincinnati, Medical Center

In 2012, stroke caused a total of 6.7 million deaths worldwide according to the World Health Organization. Stroke is defined as a nuerological injury as a result of cell death caused by a lack of blood flow or hemorrhaging to the central nervous system. Significant risk factors for stroke include high cholesterol, smoking, and high blood pressure (hypertension). Metalloproteins and metals play an important role in the regulation of hypertension. Metalloproteins such as the sodium potassium pump, as well as the Na/K/2Cl cotransporter, cause an increase in intracellular Na+ and K+ ions that result in vasoconstriction induced hypertension. Considering the CDC has classified hypertension as one of the three high risk factors for stroke, metals as well as metalloproteins are a selective choice for stroke biomarker identification. Differentiations between stroke mimics, ischemic and hemorrhagic stroke victims have been identified based on the metalloproteins as well as the grouping based on PCA of the metal profile of human blood plasma. Biomarker identification will lead to fast paced diagnosis in order to provide selective care whether they are a victim with stroke symptoms without cell death induced nuerological injury (stroke mimic) from ischemic and hemorrhagic stroke victims.

(273) **Determination of Calcium, Magnesium, and Aluminum in Pine from the Southern Appalachians**; David Butcher[1], Alyssa Bailey[1]; [1]Western Carolina University

Conifers have been affected by acidic deposition at various locations throughout the world. A proposed mechanism for conifer decline by acidic deposition involves decreased availability of calcium and magnesium (essential minerals) accompanied by increased exposure to aluminum (a toxic mineral). This presentation reports the determination of calcium, magnesium, and aluminum in pine foliage and surrounding soil from Jackson County, North Carolina, in the Southern Appalachians, United States. Statistical treatment will be employed to characterize tree health and possible effects of acidic deposition. This work is part of a continuing study of conifers in this region.

(274) **Calculation of Ion Beam Formation behind the Skimmer Cone of an ICP-MS**; Ross Spencer[1]; [1]Brigham Young University

When the streaming plasma in an Inductively Coupled Plasma Mass Spectrometer passes through the skimmer cone it is still quasineutral (the Debye length is small compared to the size of the skimmer cone). To turn the plasma into a beam it is necessary to remove the electrons and focus the ions. This is done by placing a relatively large negative potential in the path of the plasma behind the skimmer cone. If done properly, this should make a sheath-edge in the plasma beyond which the electrons cannot pass and through which the ions stream into the focusing and analyzing section of the device. This paper shows how this sheath-edge may be computed by using a combination of particle streaming for the ions and electrostatic plasma equilibrium for the electrons, assuming that the electron density obeys Boltzmann statistics. The calculation is carried out on an axisymmetric grid in cylindrical geometry using a banded-matrix direct solver for Poisson's equation. The behavior of this electrostatic sheath as the stopping potential is varied will be discussed.

(275) **Determination of Residual Unbound Cr(III) and Cr(VI) in a Cr(III)-EDTA API by HPLC-ICP-MS**; Qiang Tu[1], Tiebang Wang[1], Xiaoyi Gong[1]; [1]Merck & Co., Inc.

Measuring intestinal permeability using orally ingested Cr(III)-EDTA has been established as a non-invasive approach to the assessment of intestinal damage in animals and humans. For human clinical trials, impurity levels of Cr(III)-EDTA will need to be accurately determined before it can be used as an active pharmaceutical ingredient (API). Especially, Cr(VI) is a known carcinogen and has a very low concentration limit in an oral drug. While Cr(III) is relatively non-toxic, the presence of unbound Cr(III) in Cr(III)-EDTA may affect accuracy of the assay in clinical trails. In this work, an anion exchange HPLC-ICP-MS method is developed with carefully controlled conditions for the determination of Cr(VI) and unbound Cr(III) in the Cr(III)-EDTA API. At pH 9.3, negatively charged Cr(VI) and Cr(III)-EDTA can be separated and quantified, while unbound Cr(III) is lost due to precipitation. At pH 3.5, Cr(VI) is converted to unbound Cr(III) and the total amount can be quantified after HPLC separation with Cr(III)-EDTA. If both Cr(VI) and unbound Cr(III) are present, the unbound Cr(III) can be calculated as the difference of results obtained at the two pH values. The method has been successfully applied to the analysis of samples being prepared for clinical trails.

(276) **Challenges in Trace Element Analysis of Cobalt Precursors**; Lisa Milstein Mey-Ami[1], Phil Clancy[1], Fuhe Li[1], Hugh Gotts[1]; [1]Air Liquide Balazs Nanoanalysis

Cobalt based thin films, as deposited by chemical vapor deposition and atomic layer deposition, are becoming increasingly important in the manufacture of integrated circuits (e.g. microprocessor, logic and memory based devices). In particular, cobalt carbonyl complexes have been used for this purpose and these semiconductor precursor compounds' performance is influenced by trace elements (sub ppb and ppt levels).

The high concentration of cobalt in the samples poses molecular interference challenges for the most advanced elemental analysis techniques. ICPMS cannot resolve 59Co16O from 75As. Analytical methods were investigated to resolve CoO from arsenic and will be presented. In addition, methods for determining other trace metals and non-metals (e.g. S and P) will be discussed.

(277) **Elemental Quantification of Carbon via Production of Polyatomic Ions in Plasma Assisted Reaction Chemical Ionization (PARCI)**; Peter Josef Haferl[1], Haopeng Wang[1], Kaveh Jorabchi[1]; [1]Georgetown University

Elemental ionization methods have been invaluable in non-targeted and compound independent chemical analysis through the implementation of plasma ionization sources. Elemental carbon quantification in particular is valued in pharmaceutical, biological, and environmental arenas for its use in the study of metabolic and degradation pathways. However, carbon quantification has proven difficult due to inefficient ionization with conventional methods. Recent development of a plasma-assisted reaction chemical ionization method (PARCI) has shown to effectively ionize non-metals at trace levels, such as halogens, in negative mode. Here we investigate the potential of PARCI for carbon quantification via production of CN-, CNO-, and CO3- from molecular analytes. These three ions show decreasing background intensity in order of CNO-, CN-, and CO3- using a pure helium plasma. Interestingly, analyte detection sensitivity follows the order of CN-, CNO-, and CO3- suggesting that the CN- ionization pathway is favored for analytes. We have initially observed a limit of detection at 14 pg for carbon and a variation of carbon response of less than 20% among analytes with various structures and halogenation. Further studies are underway to improve CN- formation via optimization of plasma chemistry.

(278) **Absorption Spectroscopy of 238U in Laser-Induced Plasma**; Jason Becker[1], Brian Brumfield[1], Nicole LaHay[1], Patrick Skrodzki[1], Mark Phillips[1], Sivanandan Harilal[1]; [1]PNNL

Laser ablation coupled with laser absorption spectroscopy (LA-LAS) provides real time, non-invasive identification and quantification of solid, liquid, or gaseous targets with no sample preparation. LA-LAS is particularly pragmatic in special nuclear material (SNM) sensing as it holds the ability to simultaneously detect bulk and trace SNMs, such as U, in atmospheric conditions. This study examines the ability of LA-LAS to measure trace levels of 238U in a N2 environment, providing a discussion of plasma persistence, time-resolved line shapes, and plasma characterization. The results indicate the capability of LA-LAS to provide SNM trace detection in solid samples at atmospheric conditions.

(279) **Development and Validation of a New Method to Measure Activity of the Na+, K+ ATPase Using ICP-MS QQQ**; Cory Stiner[1], Julio Landero[1], Judith Heiny[1]; [1]University of Cincinnati

The sodium-potassium pump (Na+, K+ ATPase) is a vital trans-membrane protein present in all eukaryotic cells. It creates and sustains a step trans-membrane gradient of Na+ and K+ ions that is used for secondary transport processes. The Na+, K+ ATPase is also the receptor for cardiotonic steroids, a class of compounds that can block its transporting activity, increasing vascular tone in muscles and is used for the treatment of congestive heart failure. Endogenous cardiotonic steroids are also present at low levels in various tissues, organs, and blood. These compounds play an important role in blood pressure regulation. The presence of un-characterized cardiotonic steroids in tissues present a relevant area for research within this field of interest.

One of the tools to assess the activity of the Na+, K+ ATPase is the measurement of rubidium uptake, as a substitute for its natural potassium target. The current method used to quantify Rb/measure the Na+, K+ ATPase activity in tissues and cells require the use of radioactive materials. The cells are bathed in a physiological saline that contains a trace amount of 86Rb, and the Na+, K+ ATPase is activated by various physiological stimuli. The amount of 86Rb transported into the cells by the Na+, K+ ATPase per unit time is quantified to determine the turnover rate of the enzyme.

The instrument used for the current method to detect and measure the ionizing radiation from 86Rb is called a scintillation counter. The new method developed using ICP-MS QQQ does not require the use of radioactive 86Rb. Instead natural abundance 85Rb and 87Rb, can be used to quantify Rb within the cells to determine the activity of the Na+, K+ ATPase. The new method provides greater precision and statistical power with conventional tracer flux measurements. This method is also widely applicable to studies of other metal ion transporters and metal-dependent processes in a range of cell types and conditions.

(280) **A Green and Fast Approach to Arsenic Speciation**; Maria C. Hespanhol da Silva[1,3], Julio A. Landero[2], Joseph A. Caruso[2]; [1]Universidade Federal de Viçosa; [2]University of Cincinnati; [3]Conselho Nacional de Desenvolvimento Científico e Tecnológico

A green and fast method based on aqueous two-phase system (ATPS) combined with High Performance Liquid Chromatography coupled to Inductively Coupled Plasma-Mass Spectrometry (HPLC-ICP-MS) was developed for the speciation of arsenic. Arsenic species were extracted from samples and introduced into the ATPS where they partitioned into the two phases. ATPS is an excellent alternative for the selective extraction of species because the systems are composed mostly of water and other components that are nontoxic, nonflammable, biodegradable, and recyclable. The possibility of linear scale-up, ease of use, low-cost, and rapid phase separation without the formation of stable emulsions are advantages of the ATPS. The ATPS can be formed by the mixture of aqueous solutions of: (i) certain electrolytes and a polymer, (ii) two types of water soluble polymers or (iii) two types of salts, under specific thermodynamics conditions. ATPS have been used in the partition of different solutes, including cellular organelles, proteins, DNA, antibodies, nanoparticles, dyes and ions. However, the application of ATPS to metal speciation has not been explored extensively.

Five As-species (Arsenocholine (AsC), arsenobetaine (AsB), dimethyl arsenic acid (DMA), monomethyl arsenic (MMA) and arsenate (As(V)) have been separated via HPLC with isocratic elution in less than 5.5 minutes. The separation was performed on a reverse-phase C8 column with a mobile phase containing tetrabutylammonium hydroxide (TBAH) as ion-pairing reagent, sodium phosphate buffer and methanol at pH 5.5. The detection limits of arsenic species with HPLC–ICP-MS were 1.69, 1.09, 1.66, 1.32 and 1.41 ppb of arsenic for AsC, AsB, DMA, MMA, and As(V), respectively. Recovery of the species was investigated and optimized using the fish protein Certified Reference Material (DORM-3). Two different ATPS were used for speciation: L35+ ammonium sulfate + water and PEO1500+ ammonium sulfate + water. AsB concentration was found in DORM-3 were 7.03 and 6.25 mg kg-1, for L35+ ammonium sulfate + water and PEO1500+ ammonium sulfate + water ATPS, respectively.

(281) **Effect of Laser Wavelength and Ambient Pressure on Late-Time Bulk Particle Emission**; Niral Shah[1], Patrick Skrodzki[1], Brian Brumfield[1], Nicole LaHaye[1], Sivanandan Harilal[1], Mark Phillips[1]; [1]Pacific Northwest National Laboratory

Laser produced plasma is a highly complex, transient process consisting of interdependent mechanisms such as laser target heating, material ablation, laser-plasma coupling, shockwave formation, plasma target heating, late-time bulk particle emission, nucleation and condensation, etc. Consequently, the parameter space for laser ablation depends on multiple factors. Here, the effect of ambient gas pressure, ablation laser fluence, and ablation laser wavelength on late-time bulk particle emission from a glass sample containing 1.3% (w/w) U is investigated using focused shadowgraphy and absorption spectroscopy. Controlling bulk particle emission is of particular importance for applications such as pulsed laser deposition, EUV lithography, absorption spectroscopy, and laser ablation ICP-MS. A nitrogen ambient environment is held at 1, 100, and 700 torr, the laser fluence is varied from ~30 J/cm2 to ~1500 J/cm2, and both the fundamental (1064 nm) and fourth harmonic (266 nm) of an Nd:YAG laser are studied. Based on the focused shadowgraphy results, pressure dependence on late-time bulk particle emission is found to be negligible, higher fluences lead to greater particle generation, and 266 nm generates fewer and smaller sized particles.

(282) **Method Development and Validation for the Analysis of Polyacrylic Lithography Reagents by ICPMS**; Phil Clancy[1], Hugh Gotts[1], Scott Anderson[1]; [1]Air Liquide-Balazs NanoAnalytical

For over fifty years now, semiconductor manufacturers have used the technique of lithography to pattern silicon substrates. As feature size decreased and packing density increased, the pressure on the manufacturers of lithography equipment and materials to keep up with this progress has increased dramatically. Not only must these materials meet the stringent demands of shrinking feature size, they must also conform to ever-increasing demands of purity, especially regarding trace metal contamination. As demands for higher purity have increased, analytical laboratories have likewise been under pressure to develop more and more sensitive instruments and techniques to measure lower and lower levels of contamination, typically 0.1-1 ppb in these materials.

In response to the need expressed by suppliers and users of lithography reagents to measure very low levels of trace metal contamination in polyacrylate materials, Balazs NanoAnalytical has applied the technology and procedures described by Perkin Elmer for analyzing solvents (IPA, PGMEA) to the analysis of polyacrylate materials.

The materials dealt with in this paper typically consist of a proprietary acrylic ester, hydrocarbon acrylate, crosslinking agent and photoinitiator. They are moisture-sensitive and will polymerize if exposed to water or acids, the preferred matrix in analysis by ICPMS. Perkin Elmer describes a procedure for the analysis of solvents directly. They offer an “Oxygen Upgrade” for the NexIon 300S whereby oxygen is introduced directly into the spray chamber of a Peltier-cooled sample introduction system. Cooling of the spray chamber condenses much of the solvent, preventing it from entering the interface area, and the oxygen gas helps to keep the carbon oxidized, preventing it from depositing on the cones.

We at Balazs, have prepared dilutions of polyacrylate materials in PGMEA and aspirated the organic phase directly into a NexIon 300S equipped with the oxygen upgrade. The procedure has been thoroughly validated and has been successful at analyzing contaminants in these materials as sup-ppb levels. Spike recoveries and repeatability studies will be presented that demonstrate the sensitivity and reliability of this procedure.

(283) **Combining Statistics and Chemometrics for Guidance of Continuous Improvement Efforts**; Mark Henson[1]; [1]Shire Pharmaceuticals
The quality of pharmaceutical actives and final dosage units is regularly monitored and statistically trended via capability metrics such as the P_{pk}. Such metrics give an indication of risk of failure and contain contributions from both the process and analytical method. When there is insufficient capability, investigations into the root cause often rely upon prospective designed experiments such as a measurement systems analysis. Such approaches are time and resource intensive and impractical for systemic improvement initiatives involving an extensive portfolio of products each with a number of unique analytical test methods. An alternate method of applying statistical decomposition of historic product testing data was developed to rapidly identify the greatest sources of long-term and short-term variability in the product/method portfolio. The patterns of results then allow for targeted application of chemometric modeling efforts to increase method or processes understanding and more efficiently identify root causes for improvement.

(285) **Multivariate Analysis of Absolute and Complex Number Microwave Spectra Measured on Pharmaceutical Formulations**; Olof Svensson[1], Halldis Thoroddsen[1, 2], Álvaro Díaz-Bolado[1], Anders Sparén[1], Mats Josefson[1]; [1]AstraZeneca R&D Mölndal, Mölndal, Sweden, [2]Chalmers University of Technology, Göteborg, Sweden
Pharmaceutical products may be sensitive to water and therefore, it is important to measure and control the water content of a formulation during manufacturing and storage of pharmaceuticals. The traditional method for determining water content is Karl Fischer titration, but this method is both time consuming and sample destructive. Microwave spectroscopy using resonators is a fast and non-destructive alternative to Karl Fischer titration. Microwave measured data are generally evaluated in a univariate way by calculating the ratio between the frequency shift and the peak width for a resonance mode using the amplitude of the microwave signal. A calibration between the moisture content and the microwave ratio is then calculated using a second degree polynomial. On the other hand, microwave measurement equipment generally provides both amplitude and phase information of the measured signal. This additional information could be exploited by modifying the current multivariate data analysis techniques to handle complex measured variables.

In this work, we investigated the use of multivariate analysis to evaluate both real and complex number microwave spectra and compare the results with the traditional univariate evaluation. Simulated data were used to investigate and understand how the multivariate methods work and should be interpreted for data consisting of complex numbers both for transmission/reflection and resonance microwave spectroscopy. The multivariate methods were also used to analyze real microwave spectroscopy data from a moisture content calibration for tablets and a study to investigate the interaction between water and other polar solvents in liquid phase using resonant microwave spectroscopy measurements.

(286) **Fluorescence Excitation Spectroscopy and Imaging Multivariate Optical Computing for the Characterization of Natural Phytoplankton Populations**; Shawna Tazik[1], Joseph Swanstrom[1], Cameron M. Rekully[1], Stefan T. Faulkner[1], Nicholas S. Viole[1], Timothy J. Shaw[1], Tammi L. Richardson[2], Michael L. Myrick[1]; [1]University of South Carolina, Department of Chemistry and Biochemistry; [2]University of South Carolina, Marine Science Program and Department of Biological Sciences
A quantitative characterization of phytoplankton cell size and taxonomic composition is essential for understanding marine biogeochemical cycles, quantifying carbon export, and for predicting the ocean's response to future climate change. Our labs have been developing a new instrument for the detailed characterization of the cell size and taxonomic composition of phytoplankton communities that combines fluorescence excitation spectral information with an all-optical approach to multivariate statistics called multivariate optical computing (MOC). The instrument, known as the SSIMOC (Shipboard Streak Imaging Multivariate Optical Computing) photometer, is a simple filter photometer that images the chlorophyll a fluorescence response of individual phytoplankton cells after exciting them with a broadband source modulated by a spinning filter wheel. The wheel contains specially designed interference filters that have been encoded with detailed spectral information; these filters are called multivariate optical elements (MOEs). The application of the combined imaging, fluorescence excitation, and MOC methods to phytoplankton samples collected in the ocean at Martha's Vineyard Coastal Observatory (MVCO) will be discussed. The performance of the SSIMOC photometer with regards to cell detection efficiency, sampling and detection rates, and the fraction of the phytoplankton population sampled will be examined.

(287) **A Convex Optimization Approach to Calibration Transfer**; Thomas Boucher[1], Melinda Dyar[1, 2], CJ Carey[1], Stephen Giguere[1], Sridhar Mahadevan[1]; [1]University of Massachusetts Amherst, [2]Mount Holyoke College
In all spectroscopic applications, there is a need to ensure that possible differences in instruments, environment, or experimental conditions are mitigated. Calibration transfer (CT) is a technique for transferring a calibration curve from one instrument to another using a transfer function calculated from a small subset of standards recorded on both instruments. CT can also be used to transfer a calibration curve of an instrument from one set of environmental conditions to a differing set of conditions. For example, CT provides an excellent solution to the task of reconciling data for inter- and intra-lab comparisons on Earth and in extraterrestrial applications or for alleviating specific differences such as varying standoff distance or changing instrument performance in remote locations.

In this work, we introduce an entirely new framework for CT based on recent advancements in the field of convex optimization. Traditionally, a series of piecewise functions is defined over windowed wavelength ranges to calculate a CT transfer map. This approach, known as piecewise direct standardization (PDS), is cumbersome and time-consuming, ignorant to global structure of the data, and often inaccurate for non-smooth spectra (e.g., LIBS, Raman). PDS-based methods also often over-fit the training standards, resulting in a CT function that is not generalizable. In our approach, we instead optimize a single convex loss function that contains a series of penalty terms that target specific behaviors, spectroscopies, and task types. By adding and removing penalty terms, a customized loss function is made specific for the data and task. This global loss function is also decomposable, so by design these methods are easily parallelizable for fast large-scale distributed computing. After constructing the loss function, we use state-of-the-art algorithms from optimization, like alternating direction method of multipliers and incremental proximal descent, to efficiently minimize it. To demonstrate the effectiveness of our framework, we compare it against many competing CT methods across multiple datasets and forms of spectroscopy, including NIR and LIBS. To promote the use of our framework, a complete open source implementation in Python will be made available for download from the author's website.

(288) **A Framework for Fully Customized Baseline Removal**; Stephen Giguere[1], M. Darby Dyar[2], CJ Carey[1], Thomas Boucher[1], Sridhar Mahadevan[1]; [1]College of Information and Computer Sciences, University of Massachusetts, Amherst, [2]Department of Astronomy, Mount Holyoke College

In all types of spectroscopic data, it is desirable for peaks to be the only regions of a spectrum with nonzero intensity values, but this is typically not the case in practice. Various underlying physical phenomena cause the peaks to be superimposed onto a slowly varying baseline (also called background or continuum). This signal may vary dramatically even among individual spectra from the same data set, and must be removed prior to quantitative analysis. This complication, known as the baseline removal (BLR) problem, is common to nearly all types of spectroscopy.

In the past two decades, many solutions to the BLR problem have been proposed for different applications. While this has resulted in many creative and effective approaches to baseline removal, it also poses its own challenges. Namely, users are often presented with a long list of BLR methods to apply with very little intuition given about how they work, how they are similar, and which is most effective for their application. To date, there has been no significant attempt to unify these methods in a way that succinctly describes their features while reducing the effort required to best apply them.

We propose a framework that identifies the common semantic steps that comprise almost all baseline correction methods. In particular, BLR is modeled as an arrangement of high-level subtasks that form a template. Because each subtask represents a semantic operation, an implementation, or algorithm for completing a subtask, must be chosen for each. Distinct correction algorithms can be formed by varying the template, or by selecting different implementations for each subtask.

In addition to allowing simpler and more insightful comparisons between existing methods, this framework also supports the automatic construction of BLR techniques that are customized to specific applications and for different types of spectroscopy. This is accomplished by fixing a template and varying the choice of implementations until a combination is found that best accomplishes the chosen task. This eliminates the need for users to spend valuable time learning the internals of existing approaches simply to determine which method is best for their application.

(289) **Characterizing Calibration Data Sets by Fusion of Dissimilarity Merits Including Outlier Detection**; Brett Brownfield[1], John Kalivas[1]; [1]Idaho State University

Being able to explicitly describe a particular calibration space is necessary for many multivariate applications. There are several merits to characterize a space including Mahalanobis distance, Q-residual, and k-nearest neighbors. However, these and other merits require a tuning parameter. Sum of ranking differences (SRD) and merit fusion processes are new tools that simultaneously evaluate multiple merits. Therefore multiple sets of tuning parameters for multiple merits can be evaluated simultaneously. This provides a more robust characterization of a sample space. Two applications requiring a thorough breakdown of the calibration space are outlier detection and classification. Outliers are difficult to detect in high dimensional space. Confounding this problem is some outlier merit values are influenced by the outlier being detected. Therefore, using multiple merits reduces these effects. Examples of merits for outlier detection are Procrustes analysis, distance measures, angles, and analyte prediction errors. Combination of multiple merits across multiple tuning parameters deals with some of the effects of masking and swamping. In addition, new validation samples can be evaluated to determine if they belong in the calibration domain. Similar approaches can be used for classifying samples to several different calibration domains. Results are presented for different spectral data sets including NIR, Raman, and X-ray fluorescence.

(290) **Investigation of Cyclodextrin Complexes with PAHs using Steady-State Fluorescence and Parallel Factor Analysis**; Joseph Chiarelli, Jonathan Kenny; [1]Tufts University

Cyclodextrin complexes with polycyclic aromatic hydrocarbons (PAHs) are interesting in various fields of study, such as environmental work. Our work uses the advantage of excitation-emission matrices (EEMs) in conjunction with PARAFAC to investigate these complexes. This method allows for us to produce separate spectra and concentration data, score values, for the free molecule and its complexes. Part of this work focuses on several complexes of β-cyclodextrin and naphthalene. Specifically 1:1 complexes and 2:2 complexes are investigated. Score values are used to calculate equilibrium constants for the various complexes and compared to literature data. Other PAHs are investigated as well. Our method also provides the possibility of investigating mixtures of multiple PAHs with cyclodextrins and results of current work are discussed.

(291) **MATSA: A User-Friendly Software Program for Magnetic Audio Tape Spectral Analysis**; Nathan C. Fuenffinger[1], Brianna M. Cassidy[1], Zhenyu Lu[1], Michael L. Myrick[1], Eric M. Breitung[2], Stephen L. Morgan[1]; [1]University of South Carolina; [2]Library of Congress

For both personal and professional purposes, magnetic audio tapes experienced widespread use for decades as a recording medium and are an invaluable source of cultural and historical information. Unfortunately, the data stored on magnetic audio tapes is at risk of being destroyed due to chemical deterioration. In recent years, a massive effort has been put in place by many cultural heritage institutions to transfer the media from the magnetic tapes to a digital format, thereby preserving the endangered information for future generations. The first step in the digitization process involves examining the collection of tapes and removing any degraded tapes which, during playback by an audio engineer, may be further impaired or cause damage to the tape recorder. A fast, objective, and non-destructive method of assessing a tape's playability is ideal, and previous work in our laboratory has shown that combining attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) and multivariate statistics can be used for this purpose.

Here, we have developed a user-friendly software package which would enable tape custodians to visualize spectral data and determine the playability of magnetic audio tapes. Among the methods currently implemented in MATSA are two feature extraction techniques, principal component analysis (PCA) and partial least squares (PLS), in addition to six classification algorithms including artificial neural networks, decision trees, naïve Bayes classification, support vector machines (SVM), and linear and quadratic discriminant analysis. The program was built using a reference collection of ATR FT-IR spectra from 95 quarter-inch audio tapes. An internal validation accuracy of 93% was achieved after applying PCA-SVM classification to the reference data. A separate test set consisting of spectra from 50 tapes was classified with 88% accuracy. PCA loadings plots, also accessible in MATSA, suggest that carboxylic acid formation due to hydrolysis of the polyester-urethane elastomer is the most significant predictor of playability in the IR region.

SciX 2015 ABSTRACTS

(292) **An Effective Approach to Building a Calibration Matrix for a Multi-Component Mixture**; Huggins Z. Msimanga[1], Mihyang Song[2], Newsha Tavakoli[3], Truong Thach Ho Lam[4]; [1]Kennesaw State University; [2]Mercer Univeristy College of Pharmacy; [3]Georgia Institute of Technology; [4]Philadelphia College of Osteopathic Medicine

A protocol for developing a calibration matrix is proposed and evaluated using Children's Dime Tap, a syrup cough remedy that contains at least five ultraviolet-active components. Fundamentally, a calibration matrix must account for all the components, chemical or physical, that are in the sample being analyzed. In other words, composition of the sample and relative concentrations of all detector-active components must be known prior to designing a successful calibration matrix. With no knowledge about the sample composition, a common approach is to use simulated model mixtures and test their predictions on known test samples, or on the basis of package labels. In this study, high-performance liquid chromatography/photodiode array detector (HPLC/PDA) was used to identify all the sample components that were ultraviolet-active, and later, to provide reference amounts of the actives in Children's Dime Tap. A spiking technique was used to compensate for any mismatch between the sample and calibration solutions. Based on the HPLC/PDA findings, several calibration models were built using ultraviolet spectrometry and target factor analysis. Model predictions were tested using Children's Dime tap samples. For the three actives listed in Children's Dime tap, the final calibration matrix predicted concentrations of 210mg/L +- 3 %RSD for brompheniramine maleate, 506 mg/L +-1 % RSD for phenylephrine hydrogen chloride, and 1040 mg/L +- 4 %RSD for dextromethorphan hydrogen bromide. HPLC results in the same order were 203 mg/L +- 4% RSD, 511 mg/L+- 4 %RSD, and 993 mg/L +- 4 %RSD. Preliminary use of HPLC/PDA before trying out different calibration matrices was a quick way of discovering the underlying structure of Children's Dime Tap formulation.

(293) **Clustering in Spectroscopy - How Important is the Review Process?**; Michael Boruta[1]; [1]ACD/Labs

Grouping or cluster data is useful in many areas of analysis. Whether we are looking at samples for competitive analysis, formulated products, attempting to determine the suitability of raw materials, or looking for salts and polymorphs - having a method to group similar samples can speed up the analysis of the materials. The method to group the data can be a manual process or an algorithmic one such as PCA. However the usefulness of either method relies strongly on the reliability of the resulting clusters.

There are many factors that can affect the reliability of the clusters. This poster will look at some of those factors and some methods that can help improve the reliability of the resulting groups

(294) **Comparison of Metal Concentrations in Soil with LIBS, XRF, and ICP-MS**; Jay Clausen[1]; [1]US Army Corps of Engineers ERDC-CRREL

Site characterization has typically involved shipping of samples to fixed based analytical laboratories. The analytical methods for metal involve lengthy sample preparation requiring digestion with acids resulting in hazardous waste generation. In addition, the process of sample collection, shipping, preparation, and analysis is time consuming and costly. In the last several years, man-portable technologies such as x-ray fluorescence (XRF) or x-ray diffraction (XRD) have been utilized for site characterization. However, these technologies are not well suited for all elements. In the past several years, an environmentally friendly, optically based technology has been introduced for analytical analysis and is referred to as laser induced breakdown spectroscopy (LIBS). LIBS can be effectively used for site characterization to detect metals and other substances. Soil samples collected from a variety of military sites as well as analytical standards were analyzed with LIBS, XRF, and inductively coupled plasma, optical emission spectrometry (ICP-OES), and ICP-mass spectrometry (MS). The analytical results of these different methods are compared, along with analytical precision, and instrument sensitivity.

(295) **Optimization of Liquid Jet System for Laser-Induced Breakdown Spectroscopy Analysis**; Pavel Porizka[2], Katarina Skocovska[1], Jan Novotny[2], David Prochazka[2], Karel Novotny[2,3], Jozef Kaiser[2]; [1]Faculty of Mechanical Engineering, Brno University of Technology; [2]CEITEC BUT - Central European Institute of Technology, Brno University of Technology; [3]CEITEC MU - Central European Institute of Technology, Masaryk University

Laser-induced breakdown spectroscopy (LIBS) enables direct in-situ analysis of samples in any state of matter. This capability of LIBS enables its utilization in vast variety of applications, for instance bioapplications and online monitoring. We report on the optimization of a system for direct elemental analysis of samples in liquid phase using LIBS technique. This system consists of a peristaltic pump and a thin specially designed nozzle producing a thin flow of liquid solution/suspension. Such arrangement was used to reduce splashes of liquid and sedimentation of suspension and thus to improve the repeatability of an experiment. Firstly, stepping frequency of the peristaltic pump was synchronized with a flashlamp of ablation laser source. Using such synchronization, changes in pressure and hence volume of liquid along the step of the peristaltic pump were mitigated. Changes in the liquid flow volume affect the laser ablation process in the sense of so-called effective volume function. Other phenomenon affecting LIBS signal fluctuations, moving breakdown, was also studied. Afterwards, single pulse (SP; 1064 nm Nd:YAG laser pulse) and double pulse (DP; 1064 nm and 532 nm Nd:YAG laser pulses) LIBS systems were optimized to obtain best possible signal-to-noise ratio. The performance of SP and DP LIBS in a detection of traces of heavy metals was estimated. As a result, significant improvement in sensitivity (limits of detection) of DP LIBS system for analysis of Cu and Pb was observed.

(296) **Multivariate Classification and Quantification of Sedimentary Rocks Analyzed using Stand-Off Laser-Induced Breakdown Spectroscopy System**; Pavel Porizka[1], Jan Novotny[1], Gabriela Vitkova[1], David Prochazka[1], Jakub Klus[1], Michal Brada[1],Ales Hrdlicka[1,2], Karel Novotny[1, 2], Jozef Kaiser[1, 2]; [1]CEITEC BUT - Central European Institute of Technology , Brno University of Technology; [2]CEITEC MU - Central European Institute of Technology, Masaryk University

In this study we present a newly designed robust Laser-Induced Breakdown Spectroscopy (LIBS) device capable of stand-off (from 6 to 20 meters) as well as remote sensing. All the samples were measured from the distance of 9 meters in collinear arrangement; axes of the laser beam and the collecting optics are identical. The plane of the sample surface is placed perpendicular to the analytical axis of the stand-off system, allowing the best conditions for LIBS analysis. Samples of 28 sedimentary rocks were analyzed in the form of pressed pellets. The sample set contains four different sample matrices. During quantitative analysis the emphasis is given to the detection of copper and nickel. Afterwards, obtained data set was treated with multivariate algorithms. For classification, algorithms based on least squares method (principal component analysis, partial least squares discriminant analysis) were utilized and their performance compared to that of artificial neural network algorithm (Kohonen maps). In the case of quantification, results obtained using principal component regression and partial least square regression were compared.

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(297) **The Effects of Laser Pulse Energy, Spot Size, and Wavelength on Laser Produced Plasmas in Transverse Magnetic Fields**; Payson Dieffenbach[1], Michael Marino[1], Prasoon Diwakar[1], Ahmed Hassanein[1]; [1]Center for Materials Under Extreme Environment, School of Nuclear Engineering, Purdue University

Laser-produced plasmas (LPP) are used for various applications including laser induced breakdown spectroscopy (LIBS), extreme ultraviolet (EUV) lithography, pulsed laser deposition (PLD), and nanoparticle generation. A magnetic field is able to manipulate LPP properties such as plasma confinement, debris mitigation, plasma focusing, emission enhancement, etc. In this study, the influence of a transverse magnetic field, varying wavelengths and spot sizes, and pressure conditions on nanosecond laser-produced plasma is studied and presented. To generate a nanosecond plasma, a Quantel Q-Smart 850 mJ pulse laser (λ: 1064, 532, 266 nm and FWHM: 7 ns) is used to ablate three different samples which include aluminum, copper, and tungsten. A permanent magnetic trap was used with nearly uniform magnetic field of 0.8 T to control plasma expansion dynamics. These samples were ablated using varying fluences (spot size, energy, and wavelength) in vacuum and atmospheric conditions. Three different plasma diagnostics were used to study the dynamics of the transient plasma. Optical time-of-flight (TOF) measurements showed that atomic mass of the target material played important roles in determining the reduction in velocity and expansion of the plasma. Secondly, fast photography was performed using an intensified charged coupled device (ICCD) to study the plume dynamics. In the presence of a transverse magnetic field, the plume expansion was confined; the plume front traveled a shorter distance as compared to the free expanding plasma. The same effect occurred under ambient air conditions as the plume was not freely expanding as it would in a vacuum. Finally, optical emission spectroscopy (OES) was used to determine the temperature, electron density, and ionization rate of the plasma plume. In the presence of a transverse magnetic field and in vacuum conditions, enhancement in the emission of key ionic lines and plasma temperature was found. It was observed that spot sizes also had varying effects on the plume dynamics. Additionally, different laser wavelengths affected key ionic lines as well as neutral lines observed from the spectral analysis. The role of spot size and ambient air conditions on plasma characteristics under the influence of a magnetic field will be addressed in detail.

(298) **Analyzing Ice with LIBS**; Jay Clausen[1], Richard Hark[1, 2], Alexander Bol'shakov[3], John Plummer[4]; [1]USACE ERDC CRREL; [2]Juanita College; [3]Applied Spectra Inc.; [4]JR Plumer Associates LLC

Over the past decade our research team has evaluated the use of commercial-off-the-shelf laser-induced breakdown spectroscopy (LIBS) for chemical analysis of snow and ice samples under polar conditions. One avenue of research explored LIBS suitability as a detector of paleo-climate proxy indicators (Ca, K, Mg, and Na) in ice as it relates to atmospheric circulation. LIBS results revealed detection of peaks for C and N, consistent with the presence of organic material, as well as major ions (Ca, K, Mg, and Na) and trace metals (Al, Cu, Fe, Mn, Ti). The detection of Ca, K, Mg, and Na confirmed that LIBS has sufficient sensitivity to be used as a tool for characterization of paleo-climate proxy indicators in ice-core samples. Techniques were developed for direct analysis of ice as well as indirect measurements of ice via melting and filtering. Pitfalls and issues of direct ice analysis using several cooling techniques to maintain ice integrity will be discussed. In addition, a new technique, laser ablation molecular isotopic spectroscopy (LAMIS) was applied to detection of hydrogen and oxygen isotopes in ice as isotopic analysis of ice is the main tool in paleoclimatology and glaciology studies. Our results demonstrated that spectra of hydroxyl isotopologues 16OH, 18OH, and 16OD can be recorded with a compact spectrograph to determine hydrogen and oxygen isotopes simultaneously. Quantitative isotopic calibration for ice analysis can be accomplished using multivariate chemometric regression as previously realized for water vapor. Analysis with LIBS and LAMIS required no special sample preparation and was about ten times faster than analysis using ICP-MS. Combination of the two techniques in one portable instrument for in-field analysis appears possible and would eliminate the logistical and cost issues associated with ice core management.

(299) **Spectroscopic Analysis of Cerium, Cesium and Strontium (Nuclear Surrogates) using Laser Induced Breakdown Spectroscopy (LIBS)**; Charles Ghany[1,2], Hervé Sanghapi[1, 2], Chet Bhatta[1,2], Bader Alfaraj[1,2], Fang Yueh[2,3], Jagdish Singh[2,3]; [1]Mississippi State University; [2]Institute for Clean Energy Technology; [3]JPS Advanced Technology R&D, LLC

Samples containing cerium, cesium and strontium were made with known concentrations and analyzed using laser-induced breakdown spectroscopy (LIBS). Rapid and in situ analysis of powder samples, including nuclear surrogates helps in providing more information for quicker analysis of suspicious materials, environmental samples and forensic applications with respect to powder specimens. Powder samples are more challenging to analyze using LIBS than pellets. That is why it is of crucial importance, since many samples found in nature are not in lab pellet form. Spectra resulting from these samples under various experimental conditions were compared for possible interferences and other properties, placing particular interest in Ce II 418.65 nm, Cs I 852.11 nm and Sr I 460.73 nm atomic emission spectra lines, which are outstandingly strong and persistent lines of the above mentioned elements respectively, with relatively high intensities according to National Institute of Standards and Technology (NIST) data base. Peak intensities, normalized areas, limit of detection amongst others were compared.

(300) **Comparative Study of Elemental Nutrients in Organic and Conventional Vegetables by Laser Induced Breakdown Spectroscopy (LIBS).**; Chet Bhatt[1,2,3], Charles Ghany[1, 2, 3], Bader Alfarraj[1,2,3], Fang Yueh[1,2], Jagdish Singh[1,2,3]; [1]Mississippi State University; [2]Institute of Clean Energy Technology (ICET); [3]Department of Physics and Astronomy, MSU

In this study, LIBS technique is used to compare the presence of major nutrient elements (Ca, Na, K, and Mg) in organic and conventional vegetables. Different parts of Cauliflower and Broccoli were used as working samples. Optimum values of laser energy, gate delay, and gate width were used to acquire the LIBS spectra from those samples. The intensity ratios of different elemental lines present on the similar location of the organic Cauliflower and Broccoli samples were compared with those of conventional ones. The intensity ratio of elemental lines between different parts of the Cauliflower and Broccoli are also compared. The detailed analysis of the elemental nutrients in Cauliflower and Broccoli will be presented.

(301) **Overview of Some Theoretical Modeling of LIBS Emission Spectra**; David Kilcrease[1], Heather Johns[1], James Colgan[1], Beth Judge[2], James Barefield II[2], Roger Wiens[3],Sam Clegg[4]; [1]Theoretical Division, Los Alamos National Laboratory; [2]Chemical Diagnostics and Engineering, Los Alamos National Laboratory; [3]Space and Remote Sensing Division, Los Alamos National Laboratory; [4]Physical Chemistry and Applied Spectroscopy, Los Alamos National Laboratory

We give an overview of our recent modeling efforts of LIBS emission spectra carried out by our team at Los Alamos National Laboratory. We have been concerned with the modeling of both high-resolution laboratory experimental spectra as well as spectra from the ChemCam instrument that is on the Mars Curiosity Rover. We model the LIBS plasma using the Los Alamos suite of atomic physics and atomic kinetics modeling codes. We use the Hartree-Fock atomic structure code of Cowan (CATS) [1] to generate sets of atomic data. The plasma kinetics code ATOMIC

[2,3] is then used to calculate atomic level populations in either local thermodynamic equilibrium (LTE) or non-LTE. ATOMIC then calculates the resultant emission spectrum. To account for the effects of line self-absorption we model the transport of radiation through a series of spherical shells each of which may be at a different temperature and density [3] to simulate the LIBS plasma. The electron collisional broadening of the lines is treated with an improved model, which we compare with an older hydrogenic model. The resultant emission spectrum is then compared with experiment. Of particular interest are the spectra of mixtures of a number of elements. As an example we self consistently model the spectra of basalt consisting of its thirteen major elemental components including a CO_2 atmosphere and show good agreement with LIBS experimental spectra [3]. We also show some modeling and experimental comparisons for other mixtures such as iron oxide and calcium fluoride. We have undertaken this modeling effort with the intent of increasing the understanding of the physics taking place in LIBS plasmas in order to more thoroughly understand the experimental spectra.

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(302) **A Study of Wheat Flour Tortillas using Laser Induced Breakdown Spectroscopy (LIBS)**; Charles Ghany[1,2], Hervé Sanghapi[1, 2], Chet Bhatta[1,2], Bader Alfaraj[1,2], Fang Yueh[2,3], Jagdish Singh[2,3]; [1]Mississippi State University; [2]Institute for Clean Energy Technology; [3]JPS Advanced Technology R&D, LLC

Wheat flour tortillas of different colors and composition were studied with the aid of laser induced breakdown spectroscopy (LIBS). Due to its simplicity, fast response, little or no sample preparation, LIBS is presented as an effective tool for rapid in situ sample analysis of food samples with emphasis on tortillas. Spectroscopic analysis of the plasma generated by Nd:YAG laser irradiation of tortilla was carried out. A careful selection of spectral lines of Ca, Na and K which do not suffer from spectral interference was made. Spectral properties of the above mentioned elements such as peak intensities, intensity ratios, and area under spectral lines were analyzed. Optimization of laser pulse energy, detection gate width and gate delay for well resolved spectra with high signal-to-noise ratio was done and other parameters studied. Among the spectral lines selected for analysis, the Na I 588.99 and 589.59 nm doublet were found to show interesting properties. Tortillas manufactured by Gruma Corporation of brown, orange, green and white colors as well as homemade tortillas were studied in this work and the results from both dried and undried samples will be presented.

(303)) **Laser Ablation Molecular Isotopic Spectrometry of Rare Isotopes**; A.A. Bol[1], X.L. Mao[, 2], J.J. Gonzalez[1,2], R.E. Russo[1,2]; [1]Applied Spectra Inc; [2]Lawrence Berkeley National Laboratory

Laser Ablation Molecular Isotopic Spectrometry (LAMIS) is a direct and rapid technique that measures optical emission in laser plasmas for isotopic analysis. LAMIS exploits relatively large isotope shifts in spectra of transient molecular isotopologues formed in laser induced plasma when the ablation plume cools. LAMIS can be performed at atmospheric pressure in open air or inert buffer gases. Solid samples can be directly analyzed in their original unaltered condition, without preparation. Gases, vapors, aerosols, and liquids can be analyzed as well. Isotopic depth profiling, two- and three-dimensional mapping are possible. A spectrometer with modest spectral resolution can be suitable for both LIBS and LAMIS techniques, and thus elemental and isotopic measurements can be accomplished on the same instrument. To date detection of several rare isotopes (H, B, C, N, O, Cl, Sr, and Zr) in laser ablation plumes was demonstrated. Quantitative measurements have been realized using multivariate regression models that relate the spectral intensities of the isotope-specific molecular spectra to the original abundances of isotopes in the sample. This calibration is based on measuring spectra of known reference samples. In contrast, standardless quantification has been achieved through fitting a simulated sum of the spectra of relevant isotopologues to the experimental emission spectra. Precision in LAMIS measurements was within 9‰ for the 10B/11B ratio determined with confidence of 95%. Accuracy depended on quality and homogeneity of the reference standards or quality of spectral simulation. Simultaneous determination of isotopes of different elements was shown to be physically possible, while determination of several isotopes of the same element was successfully demonstrated (Sr, Zr). Double-pulse LAMIS and femtosecond LAMIS indicated further prospects for improving accuracy and sensitivity in this technique. The ability of LIBS/LAMIS for simultaneous elemental and isotopic analysis makes it a useful tool for multiple applications anticipated in industrial, laboratory, and field operations, potentially at a standoff distance to the sample. Commercial LIBS/LAMIS instrumentation is expected to emerge in the future.

(304) **Evaluation of Optical Depths of Sr Emission Lines in Laser Induced Breakdown Spectroscopy (LIBS)**; Bader Alfarraj, Herve Sanghapi, Charles Ghany, Chet Bhatt, Fang Yueh, Singh Jagdish; [1]ICET-MSU

Laser induced breakdown spectroscopy (LIBS) is widely used laser spectroscopic techniques in various fields, such as material science, forensic science, biological science, and the chemical and pharmaceutical industries. In most of LIBS work, the analysis is performed using radiative transitions from the atomic emissions. In this work, the focus is on the determination of plasma characterization, such as plasma temperature to obtain the optical depths of Sr lines. A binary mixture of strontium nitrate and aluminum oxide of different concentrations in powder form was used as sample. LIBS spectra were collected by varying various parameters, such as laser energy, gate delay, and gate width to optimize the LIBS signals. The atomic emission from Sr lines observed in LIBS spectra of different sample composition were used to characterize the laser induced plasma and evaluate of optical depths of LIBS. The details of this study will be presented in this paper.

(305) **Femtosecond Laser Ablation: A Molecular Dynamics Study**; Alexander Miloshevsky[1], Mark Phillips[, 2], Gennady Miloshevsky[1], Sivanandan Harilal[2]; [1]Purdue University; [2]Pacific Northwest National Laboratory

The laser ablation of materials due to a femtosecond laser pulse is a very complex phenomenon, which depends on both the material properties and the laser parameters and is of great interest in the areas of science and engineering. The molecular dynamics (MD) method is applied to study the non-equilibrium process of ultrafast laser-material interactions at an atomic level. In classical MD simulations, the atoms interacting through inter-atomic potentials are only considered with no explicit electrons taken into account. However, the deposited laser energy is initially absorbed by the electrons, and then it is transferred to lattice atoms through electron-phonon collisions. As temperature of atomic lattice is rising, the material is heated, melted and ablated. The duration of ultrashort laser pulses (10's of femtoseconds) is much shorter than the time required for the electron-phonon equilibration (1-10 picoseconds). To solve this problem, a custom made PLUME fix implementing the Momentum Scaling Model (MSM) into the Large-scale Atomic/Molecular Massively Parallel Simulator (LAMMPS), developed and distributed by Sandia National Laboratories, is utilized to describe the absorption of laser light by atoms. Laser pulses with a fluence of 500 J/m², 1500 J/m², 3000 J/m², and

5000 J/m² are simulated and size distributions, velocities, mass fractions, and 2D contour plots of lattice temperature and mass density are investigated. Our results show the laser fluence influences the nanoclusters size distribution, magnitude and direction of velocities, polar angle etc. greatly. The results also show the nature and behavior of materials in a highly non-equilibrium state, microscopic mechanisms of melting, and disintegration of material.

(306) **Simultaneous Measurement of Conserved Scalars in Flames using LIBS**; Wendong Wu[1], Richard Axelbaum[1]; [1]Washington University in St.Louis

Measuring different scalars simultaneously in a broad range of combustion systems with a suitable spatial and temporal resolution is critical for the validation of rapidly developing numerical models. However, the Raman Spectroscopy approach, which has been successfully used in hydrogen and diluted-methane diffusion flames, is not effective for higher-hydrocarbon fuels. In this study, a new approach to measure conserved scalars utilizing laser induced breakdown spectroscopy (LIBS) is introduced. The atomic ratios were measured by LIBS in ethylene-air counter-flow diffusion flames across the flame, from the fuel to the oxidizer side. The elemental mass fraction distributions for C, N, H, O elements were thus obtained and the mixture fraction was calculated based on the measured elemental mass fractions. Numerical results were compared to experimental results to study the influence of preferential diffusion on determination of mixture fraction. This study demonstrates the potential of LIBS as a simultaneous multi-scalar measurement method in a broad range of combustion systems.

(307) **Adaptive Multi-Sensor Data Fusion Model for *In-situ* Mars Exploration**; Tajana Schneiderman[1], Pablo Sobron[1, 2]; [1]The Ohio State University, [2]The SETI Institute

Laser Raman spectroscopy (LRS) and laser-induced breakdown spectroscopy (LIBS) can be used synergistically to characterize the geochemistry and mineralogy of potential microbial habitats and biosignatures. The value of LRS and LIBS has been recognized by the planetary science community: (i) NASA's Mars2020 mission features a combined LRS-LIBS instrument, SuperCam, and an LRS instrument, SHERLOC; (ii) an LRS instrument, RLS, will fly on ESA's 2018 ExoMars mission. The advantages of combining LRS and LIBS are evident: (1) LRS/LIBS can share hardware components; (2) LIBS reveals the relative concentration of major (and often trace) elements present in a sample; and (3) LRS yields information on the individual mineral species and their chemical/structural nature. Combining data from LRS and LIBS enables definitive mineral phase identification with precise chemical characterization of major, minor, and trace mineral species.

New approaches to data processing are needed to analyze large amounts of LRS+LIBS data efficiently and maximize the scientific return of integrated measurements. Multi-sensor data fusion (MSDF) is a method that allows for robust sample identification through automated acquisition, processing, and combination of data. It optimizes information usage, yielding a more robust characterization of a target than could be acquired through single sensor use. We have developed a fuzzy logic adaptive MSDF model aimed towards the unsupervised characterization of Martian habitats and their biosignatures using LRS and LIBS datasets. Here, we discuss the performance of our novel MSDF model and demonstrate that automated quantification of the salt abundance in sulfate/clay mixtures is possible through data fusion of collocated LRS and LIBS data.

(308) **Application of Low and Mid Frequency Raman for Characterization of Amorphous-Crystalline Indomethacin.**; Michaella Raglione Raglione[1], John Wasylyk[1, 2], Peter Larkin[3]; [1]The University of Delaware; [2]Bristol Myers Squibb; [3]Cytec

Vibrational (IR and Raman) spectroscopy has been utilized for decades in identifying compounds using the mid-frequency range, which is characteristic of the atom's vibrations within the molecule. These spectroscopic techniques probe the molecular structure and local environments providing valuable insight into both the amorphous and crystalline states of molecular substances. Improvements in technology over the last several years have improved the quality and availability of low-frequency Raman (LFR) instrumentation. The LFR spectral region provides insight into the lattice vibrations of molecular crystals providing the scientist a tool for directly monitoring the intermolecular interactions in the solid-state. The LFR spectral region can also provide some information on the short range order that exist in solution and the solid amorphous state. The more intense LFR bands provide greater sensitivity for detecting the onset of crystalline formation in an amorphous matrix. This study utilized the model system indomethacin (IND), whose different crystalline forms are well characterized. The LFR spectra of these forms showed very distinct and intense bands for the crystalline and amorphous forms. The observations from this study, demonstrated that LFR is better suited than the mid-frequency range for monitoring changes in form and amorphous content.

(309) **High Throughput Integrated Raman Probe with Elongated Core Collection Fiber-optic**; Robert Chimenti[1]; [1]Innovative Photonic Solutions

Fiber-optic probes are an increasingly popular method for the collection of Raman spectra. These probes are used in various applications such as process monitoring, art / archeological investigations, and in vivo tissue analysis, as well as for general purpose tools in university chemistry labs. Traditional fiber-optic Raman probes consist of two fiber-optic cables, one for coupling the excitation source into the probe (typically 105µm core diameter) and one for coupling the scattered light into a spectrometer (typically 200µm core diameter). While effective, this design suffers from high coupling losses during excitation and collection, decreasing the throughput compared to free space Raman systems.

We developed a novel fiber-optic Raman probe maintaining the flexibility of a traditional fiber-optic Raman probe with throughput comparable to typical free space Raman setups. This design integrates an open beam wavelength-stabilized diode laser into the probe in order to eliminate the coupling losses associated with the excitation fiber. The free space laser configuration allows for shaping the laser output into a collimated elliptical beam, forming an elliptical spot at the sample when the beam is focused. This maintains the étendue of the laser since the active area of the diode has an elliptical cross-section. The sample then emits Raman scattered photons from the elliptical spot which, when re-collimated by the collection optics, again forms an elliptical beam. Finally, the collection beam is coupled into a custom fiber-optic cable with an elongated core that is rotationally aligned to the entrance slit of the spectrometer. In the end, the étendue is maintained from the active area of the diode to the sample and back through the collection optics to the entrance slit of the spectrometer.

We have shown this configuration has several advantages (both economic and technical) over traditional fiber-optic Raman probes. The primary technical benefit of this design is a throughput enhancement of between 3 to 5 times compared to a traditional approach. Additionally, this design eliminates fiber coupling loses in the excitation source, allowing more power at the sample or the diode to run at a lower current, increasing its useful lifetime without changing diodes.

(310) **Improved Material Identification in the Field using a Long Wavelength Handheld Raman Spectrometer**; Claire Dentinger[1], Claude Robotham[1], Eric Roy[1]; [1]Rigaku Raman Tehcnologies

Handheld Raman analyzers are very well suited for material identification outside the analytical laboratory and they are becoming ever more accepted and used for portable measurements in such diverse areas as the pharmaceutical industry, safety and security professionals and academic institutions. The primary advantage of using handheld Raman is to take the analysis to the sample rather than bringing samples to an analytical laboratory, improving turnaround time and efficiency. However, doing analysis at the sample is only viable if the handheld Raman analyzer can reliably measure the materials of interest. Using a long Raman excitation wavelength, 1064 nm, allows a wider range of materials to be investigated. This is due to reduced fluorescence for many materials when measured with a longer excitation wavelength. Instrumental design will be discussed then feasibility studies will be shown investigating different applications areas. Common pharmaceutical excipients and finished pharmaceutical products are looked at in terms of analysis with different Raman wavelengths and the specificity of Raman to identify closely related materials. An investigation of how some homemade explosives and chemical warfare agents (CWA) that are often found with colored impurities perform on the long wavelength handheld Raman spectrometer will also be discussed.

(311) **Calorimetry-Derived Vectors to Resolve Pure Raman Spectral Components of Phospholipid Vesicle Phase Transitions**; Jay Kitt[1], Joel Harris[1]; [1]University of Utah

Vibrational spectroscopy is a well-established technique for investigating composition variation during a chemical process. As a process proceeds, changes in molecular structure or sample composition result in correlated variation in spectroscopic peaks. Thus by measuring spectra as function of an experimental parameter (e.g. time, temperature), an understanding of structural changes during the process can be gained. The challenge of this type of analysis is discovering the relationship between spectral changes and the variable dimension of the process. In this work, we propose to meet this challenge for a temperature dependent process using a novel method for resolving spectra through composition vectors derived from differential scanning calorimetry. These composition vectors are then used to determine component spectra through a thermal phase transition by matrix least squares analysis. More specifically, the phase transitions of unilamellar DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) vesicles were investigated. Raman spectra of DPPC vesicles were measured as a function of temperature using optical-trapping confocal Raman microscopy. An aliquot of the same vesicle suspension was measured using differential scanning calorimetry where enthalpy changes corresponding to each phase transition of DPPC melting were determined by integrating calorimetric heat-capacity profiles and used to construct a series of 'concentration' vectors where the relative concentration of each component is proportional to the enthalpy change from the previous phase. A matrix least-squares approach was used to resolve pure component spectra corresponding to gel, intermediate, and liquid-crystalline phases of DPPC vesicles. Resolution of pure components was verified by comparing least-squares resolved spectra to measured spectra at locations along the temperature dimension where only one component is present; fitted spectra agree with less than 10% deviation from measured values at any point along the spectral dimension. This method for deriving composition vectors could be easily extended to other processes where evolution of heat or changes in heat capacity can be independently determined, for example, polymerization, glass transition, or melting of heterogeneous materials.

(312) **Advances in Kaiser Raman Analyzers for *in situ* Studies of Small Volume Liquid-phase Reactors**; Ian Lewis[1], Sean Gilliam[1], Lisa Ganster[1]; [1]Kaiser Optical

In the last 15 years Raman spectroscopy has emerged as an important in situ analytical and process control tool. The early years of this period were dominated by improvements in Raman spectrometer components including the development of high power, small footprint NIR lasers, high performance holographic laser rejection filters, and low-noise CCD array detectors. The later years have seen a significant increase in the type and quantity of published in situ application successes. At the core of this emergence has been developments in sampling and sampling interfaces. The ability to flexibly configure the optical sampling interface using fiber-optic delivery allows Raman analyzers to be integrated to reactors ranging from the micro-scale to large volume manufacturing reactors. In this presentation, examples of improvements in sampling for in situ liquid-phase Raman applications will be shown, as well as applications of Raman spectroscopy for the study and control of small reactor systems including sealed microwave systems, continuous flow reactors, NeSSI platform devices, and small volume thermal reactors.

(313) **State of the Art Microanalysis using Raman Microscopy**; Peng Wang, Thomas Tague[1], Sergey Shilov[1]; [1]Bruker Optics Inc

Raman spectroscopy is one of the most common techniques for materials identification and characterization. Based on inelastic scattering of a monochromatic excitation source, Raman measures vibrational, rotational and other low-frequency modes in a system. It's not only characteristic for functional groups, but also sensitive to backbone and crystalline structures. Therefore, Raman technique finds extensive applications in various fields including but not limited to pharmaceuticals, polymers, forensics, cultural heritage & art, life science, mineralogy, materials science and so on. Especially for a system that requires microanalysis, Raman spectroscopy offers further advantages such as minimum sample consumption, minimal sample preparation, sub-micron spatial resolution, confocal measurement and many more. Senterra Raman microscope is a compact system that combines numerous novel and patented features. Controlled by the user friendly OPUS software that incorporates wizard guided measurement and interactive 3D display and evaluation tools, microanalysis was made easy and fun.

(314) **High-Speed Compressive Raman and Fluorescence Imaging of Pharmaceutical Composites**; Owen Rehrauer[1], Bharat Mankani[1], Greg Buzzard[1], Brad Lucier[1], Dor Ben-Amotz[1]; [1]Purdue University

The recently developed Optimized-Binary Compressive Detection (OB-CD) is here extended to simultaneously image pharmaceutical composites containing both Raman and fluorescence features. These studies extend previous demonstrations of high-speed chemical classification, quantitation,and powder imaging, with integration times per pixel as low as 100 µs. Here we demonstrate high-speed OB-CD Raman imaging of powdered mixtures of model pharmaceutical compounds and demonstrate that Raman components can be quantified in the presence of fluorescent excipients with integration times as low as 50 µs/pixel.

(315) **Impact of Radiation Environment on the Performance of Analytical Instrumentation for Planetary Missions**; Arthur Smalley[1], Ian Hutchinson[1], Richard Ingley[1], Melissa McHugh[1]; [1]University of Leicester

Raman Spectroscopy, X-ray Fluorescence (XRF) and X-ray Diffraction (XRD) are leading candidates for inclusion as analytical instruments in future planetary mission payloads. While a Raman system has not yet flown, the deployment of the first spectrometers are imminent, as they are included onboard ESA/Roscomos' ExoMars and NASA's 2020 missions. Conversely, XRF is a well-established planetary technique with a proven record on Mars and an XRD was included on MSL.

While XRF allows the atomic composition of a sample to be determined, XRD and Raman are sensitive to a material's molecular structure. In some cases and in particular for planetary applications, it is important to combine atomic and molecular spectroscopic techniques in order to thoroughly characterise science targets. The abundance of various minerals can be determined to help understand geological history. As well as mineralogical information, Raman Spectroscopy is a powerful tool for identifying potential biomarkers in the search for past or present life.

These techniques are commonly used in terrestrial research and commercial applications; however developing a space qualified instrument requires extensive design considerations and rigorous qualification testing. The overall design of the instrument is subject to extremely tight budgets regarding power consumption, mass, volume and data, while there is also a requirement to withstand harsh mechanical, thermal and radiation environments associated with launch, cruise and landing.

The harsh radiation environment, primarily caused by galactic cosmic rays and solar energetic particles, can significantly degrade instrument performance. To ensure an instrument can operate and deliver on the scientific goals of a mission, thorough radiation damage modelling and testing is an essential part of the instrument design phase. Work typically focuses on the effects that radiation has on the instrument's detector, and the methods utilised to minimise and compensate for them.

Studies investigating the effects of both ionising and non-ionising radiation have been performed by applying radiation to detectors with doses equivalent to those expected during the cruise stage to Mars. Data obtained from the devices are reported and used to provide comment on optimum operating parameters as well as achievable performance in terms of signal to noise ratio and energy resolution.

(316) **Transmission Raman Spectroscopy using a Spatial Heterodyne Raman Spectrometer**; K. Alicia Strange (Fessler)[1], Kelly Paul[1], S. Michael Angel[1]; [1]The University of South Carolina

Transmission Raman spectroscopy (TRS) is well suited for the quantitative analysis of heterogeneous turbid samples [1]. It has been suggested that TRS of heterogeneous materials would benefit from the use of a defocused laser beam and a large field-of-view spectrometer[2], and to this end, fiber optic Raman probes have been used to increase the field-of view[3]. A spatial heterodyne Raman spectrometer inherently has a large field-of-view and should be useful for TRS of heterogeneous samples. In this paper, we demonstrate for the first time transmission Raman spectroscopy using a spatial heterodyne Raman spectrometer.

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(317) **Raman Analysis of Ancient Carbonaceous Matter of Relevance to Martian Geology**; Richard Ingley[1], Cédric Malherbe[2], Ian Hutchinson[1], John Parnell[4]; [1]University of Leicester; [2]University of Liège; [3]University of Leicester; [4]University of Aberdeen

Raman spectrometers are included in the science payloads of the upcoming ESA/Roscosmos 2018 and NASA 2020 rover missions to Mars. While they will be the first Raman systems to fly, flight prototypes have been at an advanced stage for many years. They have demonstrated their ability to discern minerals and molecules of interest to planetary scientists, while meeting the engineering constraints required for deployment in space.

The search for existing or extinct life on Mars is of particular scientific interest, and discussion around the ability of Raman spectrometers to detect so-called bio-markers is abundant in the literature. While Raman spectroscopy is routinely used to characterise biotic molecules in terrestrial applications, the ExoMars Raman instrument has a mainly geological remit; if bio-molecules are present in the martian regolith, their concentration will almost certainly be below the detection threshold of these miniaturised, radiation-hardened instruments. Ancient, fossilised life would more likely present itself as reduced carbon, likely to be an interesting target for in-situ Raman analysis on Mars. Indeed, martian meteorites have been shown to contain reduced carbon of magmatic origin. While Raman analysis cannot by itself discern reduced carbon from abiogenic or biogenic processes, the study of reduced carbon on Mars will likely be of great interest. In addition to the potential link with extinct organisms, the nature of carbonaceous deposits can yield information about the geological history of the bulk material and its suitability as a bio-habitat.

The team has completed work on a number of mars-analogue samples containing reduced carbon, including some bearing similar geology to the martian Nakhla meteorite. These include milled shales of varying thermal maturity from a non-marine basin containing organic matter precipitated by extinct algae, and upper ordovician pillow lava formed on a marine bed and containing veinlets of quartz with traces of carbon. Here the authors present the final piece of analysis of these carbon-bearing samples, conducted on core samples extracted from the former region. Raman spectra of this most recent sample are presented to show the nature of the carbon present and its relationship to the rest of the parent material.

(318) **Wide-Field, Hyperspectral Raman Spectroscopy Using a Fiber Array Spectral Translator Coupled with a Spatial Heterodyne Spectrometer**; Nathaniel Gomer[1], Matthew Nelson[1], S. Michael Angel[2]; [1]ChemImage Sensor Systems; [2]University of South Carolina

Raman spectroscopy is a valuable tool for the investigation and analysis of explosive, biological, and geological analytes because it provides a unique molecular fingerprint that allows for explicit target identification. Typically, Raman systems offer very low throughput (due to narrow entrance slits), are physically large and heavy, and can only probe an area the size of a focused laser spot, which hinders the ability of the system to investigate large areas. These limitations are directly related to a system's spectrometer, which is typically dispersive grating based and requires a very narrow slit width and long focal length optics to achieve high spectral resolution. To address these problems, ChemImage Sensor Systems (CISS), in collaboration with the Angel Research Group at the University of South Carolina, are developing a wide-field Raman hyperspectral imaging system capable of providing wide-area, high resolution measurements with greatly increased throughput in a small form factor. The concept couples a fiber array spectral translator (FAST) fiber array, a two-dimensional fiber for hyperspectral imagery, with a spatial heterodyne spectrometer (SHS), a slit-less, grating based Michelson interferometer. The fusion of these technologies creates a wide-field, high throughput Raman hyperspectral imaging system capable of yielding high spectral resolution using defocused excitation, giving the system a greater area coverage than traditional Raman systems. This presentation will provide an overview of spatial heterodyne Raman spectroscopy and FAST hyperspectral Raman imaging, and discuss the development of the system.

(319) **How Low Can You Go? Modelling a Raman Spectrometer to Determine how Instrument Parameters Affect Lower Sensitivity Limits**; Liam Harris[1], Ian Hutchinson[1], Richard Ingley[1], Howell Edwards[1]; [1]University of Leicester

Raman spectroscopy is ideally suited to both geochemical investigations and the search for signs of extinct or even extant life. A Raman spectrometer has not yet been flown on a space mission, however the value of the technique is now recognised by most space agencies. The advantages of Raman spectroscopy are that it requires relatively short integration times, it is nondestructive and no physical or chemical sample preparation is necessary, all of which mean it is particularly appropriate for planetary exploration applications. There are currently three Raman instruments baselined for inclusion in Mars missions: the Raman Laser Spectrometer on the European and Russian Federal Space Agencies' ExoMars rover, due for launch in 2018, and both the SuperCam and SHERLOC instruments on NASA's Mars 2020 rover. Since a Raman spectrometer has not yet operated on the surface of another planet, there is a great deal of preparatory work necessary prior to the successful deployment and operation of these instruments. A key aspect of this work is the assessment of the sensitivity limits of the proposed instruments to a range of minerals and compounds of interest in order to establish their lowest detectable concentrations. The instrument sensitivity is also a function of its operating parameters, so a sensitivity analysis of these parameters is required as well. To enable this, an end-to-end model has been developed in software which simulates target composition (material concentration, distribution and cluster size), laser beam parameters (footprint size and power density), choice of sampling mode and optical resolution. The model is validated by comparing numerically generated spectra to laboratory data with a number of operating modes and sample types. Here we present simulations of the analysis of crushed rock samples with a distribution of grain sizes with the ExoMars Raman Laser Spectrometer. Values for the lowest detectable concentrations of some of the materials which are of great interest to the ExoMars mission are provided and optimal sampling strategies are discussed.

(320) **Optimising the Performance of a Stand-Off Raman Spectroscopy Instrument For Planetary Exploration Applications**; Melissa McHugh[1], Ian B. Hutchinson[1], Richard Ingley[1], Nick Nelms[2], Howell G.M. Edwards[1]; [1]University of Leicester; [2]European Space Research and Technology Centre, European Space Agency

Significant advances in the miniaturisation and robustness of technologies needed to build remote Raman systems have led to the recent selection of a stand-off Raman instrument (SuperCam) for the NASA 2020 Mars mission payload. Stand-off Raman Spectroscopy provides a way of retrieving detailed information from a remote distance, allowing rovers to access regions of interest (that might normally be inaccessible) without the need to re-locate and use limited rover resources.

Instrument designs are limited by the challenging mass, power and data rate constraints associated with deploying such a system on a rover platform. In addition, stand-off Raman systems require complex modes of operation, which are often required to minimize the impact of the various sources of background noise, including ambient light, sample fluorescence and detector dark current. The many inter-relating parameters, complicated trade-offs and their impact on instrument performance are investigated using a radiometric model. Such a model is essential to fully understand the performance of each aspect of a stand-off system and to optimise the overall capability of the system, given the restraints imposed by a particular application (and the demanding environment that it must operate in).

Here we describe the development of a radiometric model for stand-off Raman instrumentation. The model has been used to assess the theoretical performance of three separate instrument configurations for nine different sample/environment scenarios. We present the signal-to-noise ratios achieved for a carefully selected set of samples which exhibit significantly different levels of fluorescence emission in low, medium and high levels of ambient light.

(321) **Synthesis and Characterisation of Novel SERS Active Phosphate Capped Gold Nanoparticles**; Peter White[1], Wassie Mersha[1], Mark Baron[1]; [1]University of Lincoln

A phosphate coated gold colloid (AuNPs) having very good stability and strong SERS activity was synthesised by reduction of tetrachloroaurate with hydroxylamine phosphate salt in alkaline condition at room temperature. This fast and reproducible method of gold nanoscale colloid synthesis was optimized by considering different conditions. Accordingly, colloids which have different size and Surface Enhanced Raman Spectroscopy (SERS) activity were obtained by varying the volumes of the precursor reagent using a fixed volume of reducing agent.

The colloidal nanoparticles were characterized by UV-Vis absorption spectroscopy, Zeta seizer, fluorescence spectroscopy, and SERS. The surface plasmonic properties study using UV-Vis spectroscopy shows that the newly synthesised AuNPs have a surface plasmon resonance band expected from gold nanoparticles. Zeta potential values of AuNPs of approximately -45 mV were recorded which is expected for stable negatively charged nanoparticles.

Fluorescence quenching efficiency was studied by using rhodamine-6G dye (R6G) as a probe and the results found confirms that phosphate coated AuNPs are efficient quenchers of R6G fluorescence. The SERS properties of the colloids were studied at 532 nm and 633 nm and the results indicate that the newly synthesized AuNPs give excellent enhancement of the Raman scattering from positively charged R6G dye with superior enhancement obtained with 633 nm laser excitation.

SERS with AuNPs was applied to the analysis of the antibacterial drug nitrofurantoin resulting in an excellent Raman response.

(322) **The Analysis of Blue Solvent Dyes by SERS using Treated Silver Nanoparticles**; Peter White[1], Thomas Purbrick[1], Mark Baron[1]; [1]University of Lincoln

Solvent dyes are utilised on their own and in combination as colorants in multiple industries for a range of products such as; fuels, plastics, lubricants, waxes and inks. The variety of their applications means they are often encountered within a Forensic context and the accurate determination of a dye could be an important part of the analytical procedure involved.

Surface Enhanced Raman Spectroscopy (SERS) has been successfully used in the identification of multiple fuel markers that are similar to solvent dyes which are routinely added to a variety of fuels types around the world, to prevent laundering practices and other associated crimes. Gas oil is dyed using multiple solvent blue dyes across Europe, depending on its intended use and geographical location.

A SERS method is proposed using a treated silver nanoparticle based colloid which allows for the detection of and discrimination between closely related when compared with a standard SERS method.

(323) **Ambient Ionization and a Decade of DART**; Robert Cody[1]; [1]JEOL USA, Inc.

Following the introduction of Desorption Electrospray Ionization (DESI) and Direct Analysis in Real Time (DART) a decade ago, the field of ambient ionization has seen rapid growth. A surprisingly wide variety of new atmospheric pressure ion sources have appeared, with an "alphabet soup" of acronyms. Several ambient ionization sources are commercially available and are in use in government, industrial, and academic laboratories. The first book on ambient ionization appeared in print this year.

The majority of work in our lab has been centered on DART ionization. Qualitative analysis is well established, but quantitative analysis with DART is also quite common. Our most recent efforts and collaborations have focused on chemometrics for species identification and classification of materials.

Although DART is a very versatile ionization method, no single ion source is universal. Therefore, we have explored alternative ambient ion sources that can complement DART and can be easily implemented without major hardware changes. Among these are inlet ionization, paper spray, and O2- attachment chemical ionization.

(324) **Laser Induced Plasma for Ambient Ionization**; Jens Riedel[1]; [1]BAM Federal Institute for Materials Research and Testing

Only a few years after the invention of the laser, Knox et al. introduced the concept of laser probe mass spectrometry, a technique which employed intense laser radiation for ion generation. In these early studies at excessive irradiation microplasma formation could be observed to be an effective channel for both atomic and molecular ions. This plasma generation in vacuum led to undesired distortions of the mass analyzers and, thus, was discarded as an analytical ion source. Under ambient conditions, the surrounding air effectively cools the plasma cloud, making the plasma more controllable. The resulting laser induced plasma are nowadays commonly used in laser induced breakdown spectroscopy (LIBS) applications as excitation source for optical emission spectroscopy experiments. However, little effort has been made to introduce LIBS plasma as an ion source for ambient mass spectrometry. The main hindrance is the transient character of laser induced plasmas that typically only have a lifetime on the order of several microseconds. This drastically reduces the duty cycle of these plasma sources. After these microseconds, the generated ions recombinate to uncharged atoms and even newly bound molecules, making them inaccessible to mass-to-charge analyzers. The advent of high repetition lasers together with the ever growing knowledge about manipulation of charged species at atmospheric pressures allow overcoming these obstacles. This presentation will introduce several ionization schemes using laser induced plasma as the primary ion source. We believe that these novel ionization strategies will pave the way for future applications in both, atomic and molecular mass spectrometry.

(325) **Expanding Analytical Frontiers of the Solution-Cathode Glow Discharge**; Andrew Schwartz[1], Kelsey Williams[2], Jacob Shelley[2], Steven Ray[1], Gary Hieftje[1]; [1]Indiana University, Department of Chemistry; [2]Department of Chemistry and Biochemistry, Kent State University

Since its development in 1963, the inductively coupled plasma (ICP) has risen to the forefront of atomic spectrometry instrumentation. Though well recognized for its analytical merits, ICP-based instrumentation comes with significant drawbacks. The constraints of large, bulky instrumentation, high radiofrequency power (1−2 kW), significant gas consumption (>15 L/min Ar), and the necessity to nebulize sample solutions all limit the use of the ICP to well-funded laboratories, and preclude any opportunities for field deployment. As a result of these constraints, development of alternative plasma sources for atomic spectrometry remains a topic of considerable interest.

Recent years have witnessed the development of solution-electrode glow discharges, atmospheric-pressure glow discharges that make use of flowing liquids as one or both of the electrodes. Among the several versions of these devices, the solution-cathode glow discharge (SCGD) has garnered significant interest due to both its advantages and analytical performance for optical emission spectrometry (OES). Unlike traditional plasmas for solution analysis, the SCGD is compact, inexpensive, operates in the ambient atmosphere (requiring no compressed or flowing gases), utilizes low, direct-current power (~70 W), and samples directly from a flowing stream of sample solution, obviating the requirement for a nebulizer and greatly reducing dispersion of transients and memory effects. Moreover, the SCGD yields OES performance comparable to that of larger, more expensive sources; detection limits and precision are generally on par with those of radially viewed ICP-OES.

Here, we present a survey of the analytical aspects of the SCGD as an alternative spectrochemical source. Its advantages and its analytical performance will be compared to those of traditional, ICP-based methods with emphasis on temporal response, limits of detection, precision, and linear dynamic range. Finally, work to expand the role of the SCGD beyond the frontiers of OES will be presented, especially the potential role of the SCGD as an ion source for atomic, molecular, and ambient mass spectrometry.

(326) **Laser Ablation Sample Transfer for Tissue Proteomics and Genomics**; Kermit Murray[1], Fabrizio Donnarumma[1]; [1]Louisiana State University

The goal of our research is to develop ambient sampling methods based on laser ablation of material from tissue and other biological samples. The ablated material is collected for further analysis by mass spectrometry or by genomic sequencing. An infrared laser operating at 3 μm is used to ablate biological material from a cell or tissue sample. The ablated material is aspirated and collected on a filter from which the biological material is extracted into solution. For proteomics analysis, the extracted material is deposited on a target for matrix-assisted laser desorption ionization or injected into a nanoflow liquid chromatography-mass spectrometry instrument for chromatographic separation of the captured material before mass spectrometry analysis. The approach allows ablation of selected regions of interest from tissue sections mounted on microscope slides. Laser ablation sample transfer and nanospray MS/MS analysis was demonstrated using an ion trap mass spectrometer. Infrared laser ablated material was captured in a chromatographic stationary phase pipette tip that was extracted and injected into the LCMS system. Initial studies have focused on the detection of tryptic peptides obtained from in situ digestion. Parallel genomic studies use the same IR laser ablation and collection scheme, but focus on DNA and RNA analysis of the ablated material with regards to expression quantification, sequencing and modification detection. Initial studies have demonstrated the ablation and collection of plasmid DNA 3200 base pairs in length. Sequencing analysis as well as gel electrophoresis demonstrated that the ablated DNA samples were transferred completely intact and with the chemical structure of each base pair preserved. Ongoing studies are directed at collection of ablated material from rat brain tissue with PCR amplification of the collected genomic material and detection of modifications such as methylation. The overall goal is the combination of genomic and proteomic sampling with spatially resolved regional sampling. This approach will complement mass spectrometry imaging methods by adding genomic information and LC-MS/MS derived protein ID assignment to the raw imaging data.

(327) **Correlation-based Technique to Facilitate Detection, Identification, and Differentiation of Many Analytes in Direct Mass Spectrometry Approaches**; Jacob Shelley[1], Yi You[1], Sunil Badal[1], Allyson Beechy[1]; [1]Department of Chemistry and Biochemistry, Kent State University

Over a century ago, J.J. Thomson realized a method to separate and detected chemical species based on their mass by converting them to ions and manipulating them in vacuum with electric and/or magnetic fields. Mass spectrometry provides the distinct analytical advantage of very rapid, high-resolution separation and detection. As Thomson noted, complex samples do not necessarily need to be purified, pretreated, or separated prior to mass-spectrometric analysis. Recently, this benefit has become significantly more practical with improvements in vacuum technology and the advent of atmospheric-pressure desorption/ionization sources. The resulting field, called Ambient Desorption/Ionization Mass Spectrometry (ADI-MS), aims to eliminate sample preparation by relying on the source to volatilize/ionize analytes and on the mass spectrometer to separate/detect these species. Though this field has experienced significant success over the past 10 years, non-targeted detection and identification of species in complex mixtures is still rather time/labor intensive and requires expertise in mass-spectral interpretation. Furthermore, the presence of multiple ions from a single species clutters the spectra and complicates spectral analysis.

Here, we introduce a correlation-based algorithm as a means of classifying and, ultimately, identifying species in ambient mass-spectrometric experiments. Time traces for two ions from an ADI-MS analysis are cross-correlated and the shape of the resulting cross-correlogram yields information about relative origin of the species. Ions resulting from the same molecule (e.g., molecular ion, isotope, fragment, adduct, etc.) exhibit highly correlated time-dependent fluctuations and the correlogram can be easily distinguished from background ions. Ions resulting from two unique chemical species in the sample produce a shift in the correlogram due to differing desorption/ionization effects. A computer program has been developed to automatically perform this function, process the correlogram, group related ions, and categorize them as being background-, molecular-, fragment-, isotopic-, or adduct-ions. This processing approach will be shown for many complex samples and multiple sources including DART and FAPA. Factors which could affect the ability to differentiate chemical species, including mass-spectral resolution, spectral acquisition rate, sample introduction, and sample/spectral complexity will also be explored. Finally, prospects for expanding this processing tool to include automated elemental composition determination and fragment-ion characterization will be discussed.

(328) **Probing Low Frequency Vibrational Excitations and Their Effect on Electron and Proton Transport in Proteins**; Paul Champion[1]; [1]Northeastern University

Electron and proton transport in proteins have been studied with vibrational coherence spectroscopy, resonance Raman spectroscopy, and ultrafast kinetic measurements. Cytochrome c (cyt c) and green fluorescent protein (GFP) and are employed as model systems for electron and proton transport, respectively. Recent studies have demonstrated how distortions along the “ruffling” mode of the heme group in cyt c can control the electron transport rates by over two orders of magnitude. In GFP, a large and temperature dependent kinetic isotope effect (KIE) demonstrates that room temperature tunneling is taking place along the O-H---O elements of the GFP “proton wire”. The thermally accessible vibrational mode of the donor (D) and acceptor (A) oxygen atoms is included in the tunneling reaction coordinate and it is shown how the Duschinsky mixing of the D-A mode with the much higher frequency O-H oscillator delocalizes the tunneling wavefunction and offers shorter tunnel pathlengths, which control the underlying transport rate. We conclude that specific low frequency vibrational modes in biomolecules have evolved to extract energy from the thermal bath and efficiently utilize this energy for barrier crossing or tunneling reactions.

(329) **Plasmonically Enhanced Raman Spectra of Cells and Body Fluids: SERS Applications in Diagnostics and Forensics**; Lawrence Ziegler[1]; [1]Boston University

Surface enhanced Raman spectroscopy (SERS) provides valuable and unique spectral signatures of bacterial cells, red blood cells, cancer cells and human body fluids for rapid diagnsotics of a wide variety of diseases and in the field of forensic science. For example, a SERS based methodology can be used for rapid, antibiotic specific bacterial urinary tract infection (UTI) and STD diagnostics. The SERS bacterial signals on both Au and Ag substrates are found to principally arise from purine degradation products and closely related co-enzymes. When combined with multivariate data analysis techniques and reference libraries, this SERS based platform results in rapid, growth-free bacterial detection and identification with antibiotic susceptibility specificity readily applicable for UTI diagnostics. Rapid distinction between gonorrhea and chlamydia, two very different bacterial types than UTI causative agents, can be accomplished by use of Ag and Au SERS substrates. The effects of in vitro blood aging, also attributable to purine degradation biochemical pathways, are readily seen in the SERS spectra of whole blood. In addition, SERS spectra of red blood cells provides a possible rapid malaria diagnostic. Furthermore, the SERS spectra of trace amounts of human body fluids allows this Raman methodology to be exploited for forensic analyses at crime scenes. In contrast to existing confirmatory forensic identification procedures, SERS provides a single, sensitive, specific, portable rapid platform for human body fluid (blood, semen, vaginal fluid, saliva) bringing novel capabilities to the field of forensic science.

(330) **Predictability and Sensitivity of ROA Spectroscopy for Structure Elucidation of Protein Therapeutics**; Rina Dukor[1]; [1]BioTools Inc

Structure characterization of protein therapeutics is ‘required’ by regulatory agencies for both innovator and ‘biosimilar’ versions of a drug. A large number of techniques are available for secondary and tertiary structure (known as higher order structure - HOS), among which is spectroscopy. In particular, the use of FT-IR spectroscopy as a probe of secondary structure is now widespread throughout the biopharmaceutical industry. And Raman spectroscopy, although a well-established technique in analytical sciences, is now gaining some popularity for structure elucidation of biologics. Its advantage is ability to detect the conformation of disulfide bonds and gain information from side-chains, in addition to secondary structure. More recently, several studies have shown an enhanced sensitivity of ROA (Raman Optical Activity) with differences observed when none are observed with any other spectroscopic techniques. In this presentation, we will discuss advances in four forms of vibrational spectroscopy (FTIR, VCD, Raman and ROA) as applied to structural studies of proteins, with emphasis on predictability (and thus sensitivity) nature of ROA.

(331) **Enhanced Vibrational Optical Activity: Making Small Big**; Laurence Nafie[1]; [1]Syracuse University

Vibrational optical activity (VOA) consists of infrared vibrational circular (VCD) and vibrational Raman optical activity (ROA). [1] Because VOA intensities are typically small relative to their parent infrared (IR) or Raman intensities, in the range of four to six orders of magnitude smaller, any methods of making VOA intensities larger is of interest, both theoretically and for practical applications. The main reason that small VOA intensities are difficult is the limitation it imposes on sample concentrations, relative high, and collection times, relatively long. The variety of ways in which VOA intensities can be enhanced are somewhat different for VCD and ROA, not all of which are understood quantitatively. For

VCD, enhancement has been observed for molecules with low-lying infrared electronic states, spray-dried films amino acids and simple peptides, and supramolecular chirality in protein amyloid fibrils. For ROA, there is resonance with single or multiple electronic states, rare earth complexes with nearby electronic states and supramolecular assemblies. These various ways of VOA enhancement will be described and all will be shown to be worthy of further study and exploration.

1. L. A. Nafie, Vibrational Optical Activity: Principles and Applications, Wiley, Chichester, (2011)

(332) **Raman Spectroscopy of Amyloid Fibrils**; Igor Lednev[1], Valentin Sereda[1]; [1]University at Albany, SUNY

The conversion of proteins from their soluble functional states into structures referred to as amyloid fibrils has been implicated in a large number of diseases. Atomic-level structural characterization of amyloid fibrils is challenging because of the limitations of solution NMR and X-ray crystallography when applied to insoluble, noncrystalline protein aggregates. We have demonstrated that Raman spectroscopy is uniquely suitable for structural characterization of amyloid fibrils. Specifically, deep ultraviolet excitation allows for resonance enhancement of Raman scattering by amide chromophores, building blocks of the polypeptide backbone. The latter data reports on the distribution of the backbone dihedral angles and, consequently, the three-dimensional protein conformation according to Asher's semiempirical approach (*J Phys Chem B* 2006). In combination with hydrogen-deuterium exchange and two-dimensional correlation analysis, ultraviolet Raman spectroscopy became a powerful tool for studying protein fibrils' structure and dynamics. Most recently, we utilized polarized Raman spectroscopy for probing amyloid fibrils aligned with high efficiency in an anisotropic sample. We used a simple approach, based on the so-called "coffee stain" phenomenon, for preparing well-oriented insulin fibrils. Raman spectra were measured while the angle between the fibril sample and the excitation beam polarization was changed gradually. No polarizer was used for the collection of scattered light. A dramatic change in the amide I band intensity was observed by varying the polarization orientation, suggesting a good alignment of fibrils in the sample. We developed a method for the quantitative evaluation of the orientation of selected chemical groups, relative to the main fibril axis, using polarized Raman spectroscopic data. This method involves calculating the order parameters of the most probable orientation function of the Raman tensor. The preferred orientation of the principal axis of the Raman tensor with respect to the fibril axis was also calculated. Knowing the relationship between the principal axis of the Raman tensor and the corresponding chemical moiety, it is possible to obtain orientation information for the latter. The orientation of selected chemical groups in bovine and human insulin fibrils probed with polarized Raman spectroscopy will be discussed.

(333) **PAT and Multivariate Condition Monitoring for Drug Product Continuous Process**; Yang (Angela) Liu[1]; [1]Pfizer Worldwide Research & Development

Continuous process has gained great traction in pharmaceutical industry. Besides the quality and economical drivers, one of the critical technical advantages is the integration of the state of art engineering design and the real time process analytical technology (PAT). The integration at the infrastructural level generates big data consisting of real time process variables and process analytical information. It enables the possibility of obtaining deeper process understanding to achieve the robust control strategy at the process and quality level. Multivariate process analysis examples on an integrated solid oral dosage continuous process will be presented. Multivariate process condition monitoring using both PAT and process variables for process understanding and fault detection will be demonstrated.

(334) **Validation Of Bioanalytical Methods: DoE Methodology**; Roujian Zhang[1], Benhur Ogaby[1], Binbing Yu[1], Lingmin Zeng[1], Roujian Zhang[1]; [1]MedImmune

The number of recombinant proteins, especially monoclonal antibodies approved as new therapeutics has increased significantly in the past decade. The complexity and heterogeneity of these new biological drugs poses unique challenges to the analytical methods used to monitor and control the drugs' quality and potency. Many regulatory guidelines, industry practices and compendial chapters stem from the traditional small molecule pharmaceuticals and are challenging to directly apply to biologics. This presentation will discuss the typical challenges encountered during analytical method development, qualification, validation and transfer. A case study of validation of a purity method following ICH Q2 guideline using Design of Experiment (DoE) approach will be discussed. Different approaches of determining linearity, precision, and limit of quantitation as well as setting pre-determined acceptance criteria associated with them will be discussed.

(335) **Screening Soy Hydrolysates for the Production of a Recombinant Therapeutic Protein in Commercial Cell Line by Combined Approach of NIR and Chemometrics**; Guiyang Li[1], Zai-qing Wen[1], Guoxiang Chen[2]; [1]Amgen Inc; [2]MedImmune, LLC

Soy hydrolysates are widely used as the major nutrient sources for cell culture processes for industrial manufacturing of therapeutic recombinant proteins. The primary goal of this study was to develop a spectroscopy based chemometric method, a partial least squares PLS, to screen soy hydrolysates for better yield of protein production (titers) in cell culture medium. Harvest titer values of 29 soy hydrolysate lots with production yield between 490 mg/L and 1350 mg/L were obtained from shake-flask models or from manufacture engineering runs. The soy hydrolysate samples were measured by Near Infrared (NIR) in reflectance mode using an infrared fiber optic probe (IFOP). The fiber optic probe could easily enable in situ measurement of the soy hydrolysates for convenient raw material screening. The best PLS calibration has a determination coefficient of R2=0.887 utilizing no spectral preprocessing, the two spectral ranges of 10,000- 5376 cm-1 and 4980-4484 cm-1, and a rank of 6 factors. The cross validation of the model resulted in a determination coefficient of R2 = 0.741 between the predicted and actual titer values with an average standard deviation of 72 mg/L. Compared with the resource demanding shake flask model, the combination of NIR and chemometric modeling provides a convenient method for soy hydrolysate screening with the advantage of fast speIed, low cost and non-destructive.

(336) **Utilizing CoA and Spectroscopic Data to Aid Model Maintenance of Real Time Release Methods**; Dongsheng Bu[1], Yan Zhang[1], Dimuthu Jayawickrama[1], Gary McGeorge[1]; [1]Bristol-Myers Squibb

We have developed and implemented a Real Time Release testing (RTRt) method for a pharmaceutical tablet assay. Multivariate models incorporating near infrared (NIR) spectroscopy were applied, and NIR model results may change with time as new sources of variability are introduced. To ensure consistent model performance over time an outlier detection approach is required. By examining spectral residuals indicates different spectral features possibly from lactose raw material change. Lactose, which accounts for over half of the total weight in the drug product, is capable of forming a number of polymorphs. It is critical to understand the relationship of the polymorphs to the NIR spectra used for the RTRt methods. This work investigates the significance of lactose variability with respect to NIR-based methods for drug product RTRt. FT-NIR, FT-IR, and Raman spectra were acquired in parallel for multiple lots of lactose from two different manufacturing sites. Co-existing forms could not be clearly determined by a single spectroscopic technique. Two-dimensional correlation spectroscopy and Principal

Component Analysis (PCA) were used to understand correlation between CoA data and three types of vibrational spectroscopy signatures. It is clear that lactose characteristics vary across the vendor changing manufacturing sites.

(337) **Development and Validation of API Characterization Methods via On-line Raman Measurements for Real-Time Release Testing**;
John-David McElderry[1], Chunsheng Cai[1], Justin Pritchard[1], Frank Qi[1], Kelly Swinney[1]; [1]Vertex Pharmaceuticals

Vertex has developed continuous manufacturing (CM) and real-time release testing (RTRT) on a comprehensive equipment train for making drug product. The real time release strategy utilizes process analytical techniques and chemometric modeling. These multivariate models are developed to be robust against material and process variation following a quality by design (QbD) approach. For drug product physical forms, a limit test method is developed to detect small amounts of the undesired form in a tablet. The test uses Raman data from conforming and non-conforming tablets to calibrate a partial-least squares discriminant analysis (PLS-DA) model. Robustness is built into the model by including variability in material lot, process conditions, and manufacturing sites into the calibration set. The model is also chemically robust as demonstrated by the fact that features in the PLS factor loadings correspond to spectral features from pure physical forms. The Hotelling's T^2 and Q-residual limits are established to ensure that the analyzed samples belong to the design space that the calibration set represents, and thus the model prediction results are valid. The model is validated for specificity and detection limit following ICH Q2(R1) guidelines. The limit test is implemented on the CM process to automate the data acquisition and data processing using PAT data management software.

(338) **The Role of Polyfluoroalkyl Substances in Understanding Perfluoroalkyl Acid Contamination at Aqueous Film-Forming Foam Impacted Sites**; Christopher Higgins[1], Simon Roberts[1]; [1]Colorado School of Mines

Poly and perfluoroalkyl substances (PFASs) are constituents in aqueous film-forming foam (AFFF) used to extinguish fires. Substantially elevated PFAS groundwater concentrations have been observed at firefighter protection training areas, where co-contaminants such as chlorinated solvents and fuel hydrocarbons are also commonly present. Though regulatory efforts are focused on perfluoroalkyl acids (PFAAs), polyfluorinated species (i.e., PFAA precursors), many of which behave differently than PFAAs, are potential sources of PFAAs at AFFF-impacted sites. For example, a recent field investigation suggested significant conversion of polyfluoroalkyl chemicals to PFAAs in situ due to natural and enhanced remediation of petroleum hydrocarbons at an AFFF-impacted site. Bench-scale research into the fate and transport potential of PFASs at AFFF-impacted sites will be presented, with a particular focus on the role of PFAA precursors. These bench-scale studies include examinations of the effects of various chemical oxidants, typically employed via in situ chemical oxidation (ISCO), on PFAA fate and transport. In addition, future challenges in addressing PFAS contamination at AFFF-impacted sites will be discussed.

(339) **Comparison of Online and Offline Solid Phase Extraction Methods for Analysis of Perfluoroalkyl Acids in Water using Liquid Chromatography Tandem Mass Spectrometry**; Xianming Zhang[1], Andrea Weber[1], Cindy Hu[1], Wenlu Zhao[2], Minggang Cai[2], Pete August[2],Rainer Lohmann[2], Chad Vecitis[1], Elsie Sunderland[1]; [1]School of Engineering and Applied Sciences, Harvard University; [2]Graduate School of Oceanography, University of Rhode Island

As a sample preparation technique to remove interfering substances and enrich target analytes, solid-phase extraction (SPE) is essential for analysis of trace-level perfluoroalkyl acids (PFAAs) in water samples. Conventionally, SPE is conducted using sorbent filled cartridges. Lately, automated systems of online SPE linked with liquid chromatography mass spectrometry (LC-MS) are commercially available and gaining popularity. Offline and online SPE methods have their own pros and cons in terms of analytical performances and time/monetary cost, which need to be considered when deciding which SPE methods to use for environmental trace analysis. With the purpose of achieving the best analytical results for trace-level PFAAs in natural and drinking water samples, we compared offline SPE using an Oasis Wax cartridge with an automated online SPE system (Agilent's 1290 FlexCube with Zorbax SB-Aq SPE columns) linked with an Agilent 6460 LC tandem MS system. This presentation will cover our evaluations on the procedure blanks, recoveries, limits of detection and quantification, accuracy, and reproducibility as well as time/monetary cost of using online and offline SPE methods for water sample preparation and PFAA analysis. Using the two SPE methods, we analyzed PFAAs in surface water sampled from 25 locations in Rhode Island. Measured PFAA concentrations and derived per-capita emissions of PFAAs in Rhode Island will be presented. The rationale of selecting one of the SPE methods for analysis of PFAAs in water samples will be discussed.

(340) **Analytical Challenges on Newly Identified Commercial Fluorosurfactants and Extractable Organofluorine in Human**; Leo Yeung[1], Scott Mabury[1]; [1]University of Toronto - Department of Chemistry

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are anthropogenic chemicals that have been used in various industrial and daily applications since the 1970's. A previous study showed that quantifiable PFASs accounted for approximately 48% on average (range: 30-90%) of the extractable organofluorine (EOF) in Chinese blood collected in 2004, indicating significant amount of organofluorine remained unidentified.

Polyfluoroalkyl phosphate diesters (PAPs), perfluoroalkyl phosphonates (PFPAs), perfluorinated phosphinates (PFPiAs), and fluorotelomer sulfonates (FTSAs) are commercial fluorosurfactants that have been newly identified and reported in different environmental matrices. These chemicals might account for a portion of the unidentified organofluorine.

In the present investigation, a single LC-MS/MS method using a water/methanol gradient containing 0.1% aqueous ammonium hydroxide was developed to analyze a suite of 48 known PFASs in blood plasma (n=112) from two German cities (Münster and Halle) for the period of 1982-2009. PFPAs and monoPAPs that had been previously reported as suffering severe tailing on a C18 reverse phase column were separated using this method. An ion pair method was used to extract PFASs in blood samples; quantifiable PFASs and EOF were analyzed using LC-MS/MS and Total organofluorine - combustion ion chromatography (TOF-CIC), respectively. Among the newly identified commercial fluorosurfactants, diPAPs (6:2, 6:2/8:2, and 8:2) and FTSAs (6:2 and 8:2) were detected in 40% of the German samples. Increases in the proportion of unidentified organofluorine were observed in the plasma samples, from 8% in 2001 to 48% in 2009 and from 5% in 2001 to 39% in 2008 in the Münster and Halle samples, respectively. These increasing trends of unidentified organofluorine in plasma samples suggested humans are being exposed to new and unidentified fluorinated products.

(341) **Perfluorophosphinates and Other Perfluorinated Acids in Northern Pike and Double-Crested Cormorants**; Amila De Silva[1]; [1]Environment Canada

Perfluorophosphinates (PFPIA), perfluorophosphonates (PFPA), and polyfluorophosphoric acid diesters (diPAP) were measured in plasma of northern pike (Esox lucius) and double-crested cormorants (Phalacrocorax auritus). Pike were sampled at two locations upstream and

downstream of Montréal in the St. Lawrence River, Canada. Cormorants were sampled from colonies throughout the lower Great Lakes in Ontario: Pier 27 and Farr Island (Hamilton Harbour, Lake Ontario), Scotch Bonnet Island (Lake Ontario), Mohawk Island (Lake Erie), Lake Nipissing and Bergin Island (St. Lawrence River). The PFPAs and diPAPs were below detection limits in every sample. In pike and cormorants, PFPIA were measured with a detection frequency of 100%. To our knowledge, this is the first report of PFPIA in fish and bird plasma. Concentrations were statistically higher in fish from Ilet Vert (IV), a site downstream of the island of Montréal and its wastewater treatment plant (WWTP) outfall compared to the upstream reference site, Lac des Deux Montagnes (LDM). The dominant congeners were 6:8 PFPIA (0.27 ±0.097 ng/g in LDM and 0.56 ±0.36 ng/g in IV) followed by 6:6 PFPIA (0.058 ±0.031 ng/g in LDM and 0.20 ±0.14 ng/g in IV) with less frequent detection and lower concentrations of 8:8 and 6:10 PFPIA. There are few reports of PFPIAs in wildlife for comparison; however, PFPIA in pike plasma from this study were higher than in lake trout fillet from Lake Ontario and Lake Erie. The PFPIA levels in cormorants were greater than in pike. In Hamilton Harbour, total PFPIA (∑PFPIA) was 5.9 ± 0.88 ng/g (1.8± 0.37 ng/g 6:6 PFPIA and 3.6 ±0.48 ng/g 6:8 PFPIA). The lowest cormorant concentrations were from Bergin Island, 0.51 ± 0.083 ng/g ∑PFPIA. The determining source of PFPIAs in these organisms is unknown. Cormorants are obligate piscivores, whereas pike are more opportunistic and their diet is not limited to fish. However, lab studies have suggested that PFPIAs are not bioaccumulative and can be readily metabolized and excreted by organisms. Therefore short-term dietary habits and point sources may be factors and less so, trophic position. For example, PFPIA concentrations were significantly correlated with carbon stable isotope ratio 13C/12C (δ13C) for pike in LDM, suggesting elevated PFPIA with benthic feeding strategies. This research indicates that PFPIAs are widely present in fish and birds albeit at concentrations lower than perfluorocarboxylates and perfluorosulfonates.

(342) **Pilot Whales as an Indicator of Temporal Patterns in PFASs in North Atlantic Seawater**; Elsie Sunderland[1], Bjarni Mikkelsen[2], Maria Dam[3], Rosanna Bossi[4]; [1]Harvard University; [2]The Faroese Museum of Natural History; [3]Environment Agency, Faroe Islands; [4]Aarhus University, Faculty of Science and Technology

Perfluorinated alkylated substances (PFASs) are a broad class of contaminants produced in large quantities as surfactants for consumer products and and manufacturing. These compounds are ubiquitously detectable in wildlife and humans globally and have been associated with severe immunotoxicity in children and a variety of metabolic and developmental disorders. Industry has been replacing some of the more bioaccumulative long-chained PFASs by shorter and longer chained compounds in recent years but the timescales of biological responses to shifts in production are not well characterized. Here we examine the temporal trends and composition of PFASs in pilot whale samples from the North Atlantic spanning the early 1990s to 2013. We also investigate differences among sex and size in the most recent sampling years to better characterize inter-individual variability. Our analysis of changes in PFAS composition and concentrations over time focuses on young male whales to minimize this variability. The presentation will discuss the rate of change in most abundant compounds (PFOS, PFOSA) and their relation to chemical production as well as the emergence and detection of newer compounds.

(343) **Development Of A Portable, Ion Trap, Mass Spectrometer With Multi-Interface Support For Analyte Sampling**; Yang Cui[1], Eric Bergles[1], Mike Chai[1], Charlie Zhang[1], William Yang[1]; [1]BaySpec, Inc.

The safety and security of in-field personnel is enhanced by the development of analytical instruments that can rapidly detect and identify hazardous and/or illicit chemical compounds such as explosives, chemical warfare agents (CWAs), narcotics and pesticide residues. A rugged, portable, ion trap mass spectrometer that supports multiple methods of ion sampling to determine the presence and identity of chemical targets has been developed. As a core technology, this instrument utilizes a miniaturized, linear ion trap (LIT) mass analyzer which allows mass analysis to be completed in the sub-mTorr pressure regime. Operation at elevated pressures allows the instrument to be coupled to a highly miniaturized vacuum system that significantly reduces instrument weight and power consumption. Designed for portability, this instrument has a small footprint, is lightweight, and can be easily transported, setup, and operated by one person in the field. This instrument is compatible with ambient ionization techniques and supports analyte sampling at atmospheric pressures. Although simple in design, this technology is highly versatile and with little modification can be used in broader applications such as screening for toxic industrial chemicals (TICs), pesticides, and other environmental contaminants. A thermal desorption electrospray ionization ion source has been developed for food inspection application with the instrument.

(344) **You Can't Tell a Book by Its Cover: Analytical Adventures in Anthropodermic Bibliopegy**; Daniel Kirby[1], Anna N. Dhody[2], Beth Lander[2], Richard R. Hark[3,4]; [1]Peabody Museum of Archaeology and Ethnology; [2]The College of Physicians of Philadelphia; [3]Brown University, John Hay Library; [4]Juniata College, Department of Chemistry

The practice of binding books in human skin, known as anthropodermic bibliopegy, is not in vogue so much nowadays, but was au courant well into the 19th century. A 2006 article in The Harvard Crimson reported that no fewer than three such volumes were on the shelves of the Harvard University Library System. However, provenance supporting claims of human skin binding was sketchy and difficult to authenticate. For example, an inscription in one of the books declared: "the bynding of this booke is all that remains of my dear friende Jonas Wright, who was flayed alive by the Wavuma on the Fourth Day of August, 1632. King Mbesa did give me the book, it being one of poore Jonas chiefe possessions, together with ample of his skin to bynd it. Requiescat in pace."

Some books bound in human skin were used by physicians as a means to honor the memory of their patients. The College of Physicians of Philadelphia owns three books on the history of women's health that were purportedly bound in the tanned skin of "Mary L__," a 28 year old Irish widow who died in 1869 while under the care of Dr. J. Stockton Hough.

Except for inconclusive visual examination and unsuccessful DNA testing, no fruitful analyses had been done on any of the supposed "human skin bound" books, but the itch to learn the truth of the matter, one way or another, remained alive. This presentation will describe the use of PMF (peptide mass fingerprinting) to finally set the record straight.

PMF, a standard bioanalytical method, can be used to identify mammalian sources of collagen, the primary proteinaceous component of vertebrate connective tissues, including skin, the source of parchment. For PMF, enzymatic digestion is used to cleave collagen at specific amino acid sites forming a mixture of peptides. The amino acid sequence of each protein is unique, thus the resultant mixture of peptides is unique. The peptide mixture is analyzed by MALDI resulting in a mass spectrum of characteristic marker peptides or a "peptide mass fingerprint." Small evolutionary variations in collagen's amino acid sequence result in species-specific markers–unique fingerprints–which, in the case of anthropodermic bibliopegy, allow us to distinguish human parchment or tanned leather from other mammalian sources.

(345) **GC-MS and GC-IR Studies on Substituted Cathinones: Bath Salt-type Aminoketone Designer Drugs**; Randall Clark[1], Jack DeRuiter[1], Younis Abiedalla[1], Karim Abdel-Hay[1]; [1]Auburn University

This presentation will describe our research efforts to evaluate the structure-retention, structure-fragmentation and other structure-property analytical relationships for a large series of substituted aminoketone drugs of abuse (cathinone derivatives).

Our research has focused on the development of regioisomer specific methods for the identification of ring substituted aminoketone compounds (cathinone derivatives). The work includes the chemical synthesis of all regioisomeric forms of selected aromatic ring substituted aminoketones; generation of analytical profiles for each compound; chromatographic studies to separate/resolve all regioisomeric aminoketones having overlapping analytical profiles, and design and validation of confirmation level methods to identify individual compounds.

Based on the structure of the unsubstituted cathinone molecule, designer modifications are possible in three distinct regions of the molecule: the aromaric ring, the alkyl side chain and the amino group. All three of these areas of possible designer modification are currently being explored by clandestine chemists. Additionally, the carbonyl group itself may be the target of designer modifications in the future. Legal control of a specific molecule often provides the driving force for clandestine development of additional substituted cathinone designer molecules.

The synthesis, GC-MS, MS/MS, GC-IR and related spectroscopic properties will be presented for several series of substituted cathinone derivatives.

(346) **Probabilistic Detection of Firearms Discharge Residue on Skin Using Ion Mobility Spectrometry and Neural Networks**; Suzanne Bell[1]; [1]West Virginia University

The discharge of a firearm produces a wealth of chemical and physical evidence. To date, forensic analysis has focused on particulates formed from primer residues that are characterized using scanning electron microscopy and x-ray spectroscopy. Because particulate evidence is subject to loss via secondary transfer, the value of firearms discharge evidence could be enhanced by including organic constituents that originate from the propellant. In addition, the organic fraction can be targeted for screening assays as long as the analysis is rapid, probabilistic, and minimally destructive. These requirements can be met through careful design of sampling protocols and media. This presentation will describe the use of commercial ion mobility spectrometers for the analysis of hand swabs obtained across a characterized general population (n~ 200). Samples were analyzed using two instruments across several months and by different operators to characterize the ruggedness/robustness of the method. Figures of merit associated with the validated sampling collection, storage, and instrument procedures will be summarized.

Across the data set (thousands of spectra), it was not possible to define a fixed set of mobility peaks capable of differentiate shooters from non-shooters to any significant degree. This is not surprising given the numerous variables that contribute to the instrument response including time from discharge to sampling, sample storage conditions, firearm type, number of rounds, and environmental conditions. To address this complexity, pattern recognition algorithms are the better alternative to a peak-based identification scheme. Here, back-propagation neural networks were constructed that could differentiate shooters from non-shooters in > 80% of the cases. The resulting outputs can be treated as probability distribution functions and the results interpreted as such within the confines of similar populations. Data collection, pre-processing and network structures and performance will be described. Particular attention will be paid to samples in which false positives/false negatives would be expected such as from law enforcement personnel or hunters. Examples of how such a screening assay could be utilized in the forensic setting as part of a comprehensive forensic characterization of firearms discharge residue will be presented.

(347) **A Case Study in the Determination of Geographical Origin for Dalbergia, a CITES Listed Wood Species**; James Jordan[1], Michael Doughten[2], Tyler Coplen[2], Haiping Qi[2], Ed Espinoza[3]; [1]National Geospatial-Intelligence Agency; [2]U.S. Geological Survey; [3]U.S. Fish & Wildlife Forensics Laboratory

Illegal logging is one of the leading causes of deforestation today. Sadly, tree species indigenous to regions critical to maintaining Earth's ecological diversity also possess desirable properties, which leads to exploitation. In 1992, in response to the threat of logging, Brazilian rosewood (Dalbergia nigra) became the first ever tree species to be listed on Convention of International Trade of Endangered Species of Flora and Fauna (CITES), prohibiting international trade in the timber or other products from this species. Despite its inclusion on Appendix 1 of CITES, the species continues to be logged illegally and traded internationally. However, this does not prevent this and other protected species from entering the market through illegal channels.

For decades, the archeology community explored methods to uncover hidden relationships between chemical data (i.e., trace element abundances and/or isotope ratios) and the geographical provenance of discovered artifacts. Similarly, ecological studies have explored the geochemical relationships between local geology and the flora and fauna to study migration and species distributions. While the analytical community has sought to further develop these methods for ascertaining the provenance of banned substances, very few methods provide for the unambiguous determination of geographical provenance. In the course of this investigation, we obtained independently authenticated wood samples and analyzed them via high precision Thermal Ionization Mass Spectrometry (TIMS) for 87Sr/86Sr and then examined the results alongside stable light isotope analyses of 15N/14N, 13C/12C, 18O/16O and 2H/1H as determined by Thermal Combustion Elemental Analysis (TC/EA) followed by Isotope Ratio Mass Spectrometry (IRMS) and additional measurements of selected crustal, trace, and rare Earth element abundances determined by Inductively Coupled Plasma Optical Emission (ICP-OES) and Inductively Couple Plasma Mass Spectrometry (ICP-MS) measurements. Applications of non-parametric (i.e. chemometrics) approaches often require some fundamental statistical assumptions to be met, such as the effective treatment of missing values or left-censored data, scaling, and heteroskedasticity. Preliminary analysis of the data demonstrated that classical methods such as partial least squares discriminant analysis (PLSDA) performed after judicious application of pre-processing enabled us to classify correctly the geographical location of about 95 percent of exotic wood samples. However; we have learned that hierarchical approaches (i.e. tree structured models) which incorporate multiple levels of PLSDA permit greater variable selection/reduction, less preprocessing time, and more robust results. This investigation seeks to consider the natural variability among a dense collection of samples from within a region to that of the broader hierarchical geospatial model.

(348) **Measuring Correlated Composition and Optical Properties at the Nanoscale with the PTIR Technique: Application to Perovskites Solar Cells**; Andrea Centrone[1]; [1]NIST, Center for Nanoscale Science and Technology

Organo-metal trihalide perovskite (OTP) is an emerging class of photovoltaic materials that attract attention because they combine the high efficiency typical of inorganic semiconductors with the low material cost and ease of fabrication of emerging technologies. The improvement of OTP solar cell efficiency has been unprecedented, skyrocketing from 3.8 % to 20.1 % in less than 5 years. However, the knowledge of how the local material properties, such as the chemical composition, the bandgap and the defect density are related to OTP devices operation is still limited.

Photo Thermal Induced Resonance (PTIR) is a novel, technique that employs an AFM tip as a local detector to locally transduce the thermal expansion of the sample induced by light absorption into large cantilever oscillations. Our PTIR setup combines an AFM microscope with three lasers providing wavelength-tunability from 500 nm to 16000 nm, thus extending PTIR to the visible range for the first time. Local absorption spectra (electronic or vibrational) and maps are obtained with a wavelength-independent resolution as high as 20 nm. In this work the PTIR, technique is used to answer some open questions regarding OTP materials.

1) To provide a photovoltaic effect, solar cells typically require electrodes of different materials; however, for OTP films sandwiched between two identical electrodes the photovoltaic effect can be switched on and off by applying a small bias for few seconds. Ion electro-migration was suggested to be responsible for this effect but no direct evidence was provided. Here, PTIR is used map, in situ, the distribution of the methyl ammonium ion component of the perovskite film under applied bias, providing direct evidence of ion electron migration.

2) When chlorine ions are present in the precursor solution of mixed Cl/I OTPs they provide several beneficial effects to the perovskite film; however, it is not clear whether those effects are related to chlorine incorporation or just to better crystallization conditions. Here, PTIR is used to map the local bandgap distribution and chemical composition in the OTP film. After in situ annealing the bandgap distribution become more homogeneous and the Cl content fall below the limit of detection.

(349) **Assessing the Chemical, Mechanical and Structural Properties of Shale at Nanoscale**; Jing Yang[1], Andrew Pomerantz[1]; [1]Schlumberger-Doll Research Center, Schlumberger

Over the past decade, the boom in oil and gas production from shale has revolutionized the energy landscape in the U.S.. Shale is a sedimentary rock, composed of dispersed organic matter (OM) scattered in the mineral framework. Assessing the fundamental properties of shale, such as its composition, structure, mechanics, holds the key for understanding the processes of shale oil and gas generation, storage and transport. Yet the structural heterogeneity and compositional variability in multi-scale create substantial challenges for shale analysis.

Recent development of the AFM-IR (coupling the Atomic Force Microscopy and IR laser) technique provides a platform to probe the chemical and mechanical properties of shale at nanoscale. The spatial resolution of AFM-IR is much finer than the classical characterization methods such as Fourier transform infrared spectroscopy (FTIR) imaging and the indentation technique.

In this study, the combination of AFM and IR is implemented to perform spatially resolved chemical and mechanical analysis of shale. These results show the great diversity in chemical composition, mechanical properties in shale. Meanwhile, scanning electron microscopy (SEM) reveal the nanoscale structure of shale, visualizing the pores, kerogen (major component of the OM in shale), clay networks at high resolution. Assessing the chemical composition, mechanical and structural properties of shale at nanoscale is essential for development of robust shale reservoir evaluations.

(350) **IP-Enhanced Infrared Photoexpansion Nanospectroscopy in Air and Aqueous Solutions**; Mikhail Belkin[1], Mingzhou Jin[1], Feng Lu[1]; [1]The University of Texas at Austin

Mid-infrared absorption spectroscopy in the “molecular fingerprint” region is a powerful technique for chemical analysis. The mid-infrared photoexpansion nanospectroscopy was developed recently to measure absorption fingerprint spectra of samples on nanoscale [1]. In this technique, light absorption is detected by measuring associated sample thermal expansion with an atomic force microscope (AFM). Initially, this approach required high-power laser pulses to induce sample heating by 10-50 degrees and required sample thickness of at least ~100 nm to produce detectable signal. Recently, we demonstrated that the sensitivity of this technique may be improved to take nanoscale spectra of samples as thin as molecular monolayers with better than 30 nm spatial resolution [2]. This was achieved sending low-power laser pulses at a repetition frequency that is tuned in resonance with the mechanical vibrational frequency of the AFM cantilever and by employing tip-enhancement of the optical field below a sharp gold-coated AFM tip. Here, we will present our preliminary results on photoexpansion spectroscopy in water that requires mitigation of liquid damping of cantilever vibration and liquid absorption of infrared light by water.

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(351) **High Speed Infrared Nanospectroscopy with Sub-Monolayer Sensitivity**; Craig Prater[1], Eoghan Dillon[1], Qichi Hu[1], Honghua Yang[1], Curtis Marcott[3], Feng Lu[2],Mingzhou Jin[2], Mikhail Belkin[2], Kevin Kjoller[1]; [1]Anasys Instruments; [2]The University of Texas at Austin; [3]Light Light Solutions

The combination of infrared spectroscopy with atomic force microscopy (AFM) has enabled critical breakthroughs in extending chemical analysis to the nanoscale. This presentation will focus on two techniques: (1) photothermal AFM-based infrared spectroscopy (AFM-IR) and; (2) scattering-type scanning near-field optical microscopy (s-SNOM). These complementary techniques offer chemical analysis and chemical and optical imaging with spatial resolution down to a few nanometers for both soft and hard matter applications. AFM-IR and s-SNOM have already been successfully applied to many applications, including complex polymeric materials, graphene and other 2D materials, cells, tissues, proteins, photonic devices, and fuel cell membranes.

This contribution will focus on recent key advances in AFM-IR and s-SNOM that have dramatically improved sensitivity, spatial resolution, measurement speed and the range of samples that can be measured. Sensitivity and spatial resolution improvements have been achieved through resonance enhanced techniques that dramatically amplify the efficiency of excitation of the sample and the detection of absorbed IR radiation. These advances have achieved sub-monolayer sensitivity IR nanospectroscopy and chemical imaging with sub-20 nm spatial resolution and enable spectroscopic analysis on extremely small amounts of biological and other organic materials.

We have recently also made dramatic improvements in measurement speeds, such that AFM-IR spectra can be acquired in a few seconds. These innovations significantly improve measurement throughput and pave the way for high resolution IR hyperspectral imaging. We have also recently demonstrated the capability with the s-SNOM technique to perform rapid point spectroscopy and chemical/optical imaging with the same laser source.

In this presentation we will overview and contrast AFM-IR and s-SNOM techniques, discuss recent advances and show measurement examples in materials and life sciences applications, including advanced polymers, fibers, biological cells, amyloids, protein secondary structure and other areas.

(352) **Resonance Tracking in Resonance Enhanced Infrared Nanoscopy**; Georg Ramer[1], Anna Balbekova[1], Andreas Schwaighofer[1], Bernhard Lendl[1]; [1]Vienna University of Technology, Institute of Chemical Technologies and Analytics

Resonance enhanced atomic force microscopy infrared nanoscopy (RE-AFMIR) is an analytical technique that allows non-destructive chemical analysis of solid-state samples at better than 50 nm spatial resolution. This technique has been applied to a range of problems spanning from biology, medicine and pharmaceutics to polymer- and material science, synthetic chemistry and plasmonics. In each of these fields the technique has been shown to provide important information that is not easily accessible with other methods. Our own research is focused on the extension and improvement of the RE-AFMIR method itself. In our self built setup consisting of a Daylight Solutions external cavity – quantum cascade laser (EC-QCL) and a Keysight 5400 AFM we have recently demonstrated a method for time-resolved analysis using RE-AFMIR, whereby a repeating or non-repeating spectral change – such as the switch between two secondary structures of a polypeptide film – can be followed at the spatial resolution provided by RE-AFMIR and a speed of one spectrum every 1.5 seconds.

One of the main challenges in RE-AFMIR is keeping the repetition rate of the excitation laser at the contact resonance frequency of the cantilever. Since this frequency depends on parameters of the cantilever (force constant, vibration mode, setpoint) as well as of the sample (Young's modulus, surface properties) imaging across different materials leads to shifts in the contact resonance. To the operator these shifts are only visible as changes in the signal amplitude. For tracking resonance frequencies in scanning probe microscopy several techniques such as phase-locked loops (PLL), dual-frequency excitation and probing of the amplitude around the resonance have been proposed.

While these published methods can serve as guide for the selection of a resonance tracking technique they were usually developed for sine-wave excitation which can not always be easily translated to the pulse-train excitation provided by lasers used in RE-AFMIR. For direct comparison of different methods regarding their capability to accurately track the contact resonance in a RE-AFMIR system a flexible controller has been designed that allows to implement different tracking methods on the same sample position. We are thus able to experimentally select the most suitable resonance tracking method.

(353) **3D Carbon-electrode Dielectrophoresis in Sample Preparation**; Rodrigo Martinez-Duarte[1]; [1]Clemson University

Although microfluidics is now a de facto analysis tool in a number of assays, there is still a critical need for automated sample preparation. This important step in any biomedical assay is currently made in a central laboratory and can represent a staggering 95% of the time, complexity and cost of a medical assay. Thus, the aim is at miniaturizing and automating sample preparation to fully unlock the potential of microfluidics in point-of-care and clinical diagnostics. The goal is a sample-to-answer system where a raw sample is introduced and results obtained with the touch of a button.

Here, I present a collection of recent independent advances on the use of Carbon-electrode Dielectrophoresis (carbonDEP) in different sample preparation steps. In cell purification, carbonDEP has been validated as a tool for selective trapping and washing of M. smegmatis cells towards the development of new drugs to treat tuberculosis. 3D Carbon electrodes were used for high throughput lysis of mammalian cells as an on-chip alternative to chemical lysis. Lastly, CarbonDEP was demonstrated in the isolation of lambda-DNA from solution, validating the potential use of these devices for molecular trapping and purification. In all cases, the use of 3D carbonDEP allows for higher throughput than traditional DEP techniques. Current work is on integrating these functions in an individual device such that targeted cells are selectively trapped, lysed and their intracellular components purified, all in a single chip.

(354) **Experimental Evidence of Deterministic Absolute Negative Mobility for Organelles and Colloids**; Alexandra Ros[1], Jinghui Luo[1], Katherine Muratore[2], Edgar Arriaga[2]; [1]Arizona State University; [2]University of Minnesota

Efficient particle separation techniques in the micron- and submicron range are required for the various analytical challenges related to nanotechnology and microbiology. Especially the separation by size is demanding since traditional methods can often not resolve within species size variations. Here, we demonstrate a counter-intuitive, yet efficient migration mechanism for µm- and sub-µm particles, taking advantage of particle transport in a nonlinear post array in a microfluidic device under the periodic action of electrokinetic and dielectrophoretic forces. We reveal regimes in which particle migration opposite to the average applied force occurs for larger particles, whereas normal response is obtained for smaller particles – a typical signature of absolute negative mobility (ANM). The combination with dielectrophoresis induces a deterministic migration component which allows the application for sub-µm sized (bio)particles in microdevices fabricated with standard optical lithography methods. This deterministic ANM (dANM) was characterized in numerical modeling revealing optimized driving parameters for µm-sized beads. Furthermore, we experimentally prove dANM with colloidal particles in excellent agreement with the employed numerical model. The observed dANM is characterized by improved migration speed at least two orders of magnitude higher compared to previous ANM systems with colloids. In addition, we were able to induce dANM for mouse liver mitochondria representing the first demonstration of ANM migration with biological species. We envision that the efficient size selectivity (migration into opposing directions) can be advantageously employed for nanotechnological applications, in organelle sub-population studies or in fractionating protein nanocrystals in the future.

(355) **DNA Fractionation using Surface Dielectrophoresis**; Ghislain Tchantchou[1], Jeremy Buhain[1], Sagnik Basuray[1]; [1]New Jersey Institute of Technology

Here a new approach for fractionating biomolecules using surface Dielectrophoresis (DEP) is described with significant enhancement in resolution of size based separation, without using the standard topological restrictions of the separation media. To visualize the separation dynamics, we have used electromagnetics and molecular dynamics simulations in conjunction with experimental studies to investigate the translation of various size ranges of DNA on different surfaces. We have developed a new, simple, clean-room independent and rapid lab-on-a-chip device fabrication technique using screen printed electrodes and additive manufacturing that uses a high resolution stereolithography 3D Printer. Initially the effects of frequency spectrum, electrodes configuration, channel size, buffer conductivity and the DNA size on the DNA fractionation are numerically investigated using COMSOL software. A fluorescent labelled DNA ladder, adsorbed on the surface of the microchannel, on translation up on application of a non-uniform AC field reveals multiple peaks from the fluorescence intensity plot over distance. This suggests separation of the different sizes of DNA in the ladder, each peak corresponding to different DNA size. The peaks are further resolved (optimized) using an in house developed numerical convolution technique relying on well-established chromatography techniques in conjunction with the COMSOL simulations looking at the individual and coupled effects of adsorption and electric field. As the friction between the surface and the DNA molecules is an important factor controlling DNA fractionation, different mobility regimes are expected due to the conformation change adopted by DNA molecules up on the combined effect of adsorption and AC field. To understand how the parameters affected changes in the DNA conformations and controlled DEP fractionation of DNA under application of non-uniform AC field three limiting cases were chiefly identified for more scrutiny: a weakly adsorbing surface, a moderately adsorbing surface and a strongly adsorbing surface. Subsequent experiments of different sized DNA fragments on various surfaces clearly show that it is possible to optimize the surface energy for efficient separation of a wide range of DNA size with enhanced spatial resolution. In future, we are planning an optically active surface (plasmonic/photonic/raman) integrated with device architecture fabricated by additive manufacturing tools on paper microfluidics.

(356) **Ultrafast Immunoassays by Coupling Dielectrophoretic Biomarker Enrichment on Nano-Slit Device with Electrochemical Detection**; Nathan Swami[1], Walter Varhue[1], Bankim Sanghavi[1], Kuo-Tang Liao[2], Chia-Fu Chou[2]; [1]Electrical Engineering, University of Virginia; [2]Institute of Physics, Academia Sinica, Taiwan

Heterogeneous immunoassays usually require long incubation times to promote specific target binding and several wash steps to eliminate non-specific binding. Hence, signal saturation is rarely achieved at detection limit analyte levels, leading to significant errors in analyte quantification due to the extreme sensitivity of the signals to incubation time and methodology. Additionally, conventional immunoassay methodologies are not well suited for working with the sub-nanoliter level sample volumes often encountered within point-of-care diagnostic and therapeutic applications, such as for the serial monitoring of cells from liquid biopsies. The poor binding kinetics of immunoassays at detection limit levels can be alleviated through creating a highly concentrated analyte plug that is localized in the vicinity of immobilized capture probes. A commonly investigated methodology for achieving highly concentrated analyte plugs from dilute samples is through electrokinetic enrichment due to ion depletion at the micro/nanochannel interface for coupling with pre-immobilized antibodies. An alternate methodology to enhance biomarker binding kinetics is by immobilizing antibodies inside nano-slit channels, since this device geometry significantly reduces the extent of the diffusion layer. However, no prior work has applied electrokinetic enrichment of the biomarkers inside nano-slit device geometries with immobilized capture probes, to explore the enhancement in analyte binding kinetics obtained by combining these two approaches. Herein, we present a device to achieve this outcome by coupling frequency-selective dielectrophoretic enrichment of the target biomarker in physiological media to capture probes immobilized in a nano-slit channel to enable ultrafast immunoassays that exhibit near-instantaneous (< 2 minutes) signal saturation with dilute biomarker samples (picomolar levels) within ultra-low sample volumes (picoliters). In this manner, by coupling dielectrophoretic biomarker enrichment with electrochemical detection methods, we are able to eliminate the need for wash steps to remove excess secondary antibody or reporter molecules, since electron transfer rates steeply drop-off for electroactive molecules localized beyond a few nanometers from the electrode surface. Based on the reduced signal variations at early time points with detection limit levels of Prostate Specific Antigen, and the relative insensitivity of the signals to incubation time at later time points, we envision a method for carrying out more accurate immunoassays using analyte enrichment in nano-slits.

(357) **Particle Separation Employing Dielectrophoresis**; Blanca Lapizco-Encinas[1]; [1]Rochester Institute of Technology

Microfluidics is a dynamic and rapidly growing field with potential applications in numerous areas, from food and water safety, to environmental monitoring and clinical analysis. Working on the microscale offers attractive advantages such as shorter processing times, low sample and reagent consumption and the possibility of portable systems with a high level of integration. Manipulation and analysis of biological particles is one of the fields that has been most benefited with the marriage of microfluidics and analytical sciences. Many of these applications require fast response methods able to handle/analyze high value biological products, in a gentle manner, without leading to cell damage or bioproduct denaturation.

Important research efforts are being devoted to the development of analytical techniques that can be used with microfluidic devices. Electrokinetics, electric field driven techniques, such as electrophoresis, electroosmosis and dielectrophoresis, are widely used in microfluidic devices, offering new ways to assess, manipulate and analyze bioparticles. Dielectrophoresis (DEP) is an electrokinetic transport mechanism driven by polarization effects when a dielectric particle is exposed to a spatially non-uniform electric field. DEP has great flexibility, since it can be used with charged and neutral particles employing AC or DC electric potentials. DEP offers more versatility than electrophoresis since it has the capability for significant particle enrichment, up to three orders of magnitude. DEP also offers means for effective continuous particle sorting.

This work is focused on insulator based DEP (iDEP), a dielectrophoretic mode that uses insulating structures between two external electrodes to create electric field gradients and generate dielectrophoretic forces on particles. This study includes experimental and mathematical modeling work. Particle mixtures, including biological cells, were separated and analyzed employing different variations of iDEP. The results demonstrate that iDEP has great potential as a bio-analytical technique.

(358) **Portable LIBS from Research to Reality**; Francois Doucet[1], Lutfu Ozcan[1]; [1]ELEMISSION Inc.

Field portable technologies and methods are sought to provide simple, inexpensive, and fast analysis of materials in the mining, construction, and other industries. However, the level of portability needed for this particular application imposes some restrictions on the choice of many of the

core components used in a low cost LIBS handheld sensor. This means that relatively low-performance components, such as a low-energy laser source and a low cost, low resolution spectrometer, and working conditions in ambient air at atmospheric pressure, must be considered to fulfil these conditions. In addition, the market price of such a portable device should be affordable or as low as possible to increase the breadth of potential end users and allow the deployment of multiple units for security enhancement. The present paper describes an overview of the research made in the field of LIBS over the past decade.

(359) **Handheld LIBS for Metal Alloy Analysis**; Phillip Tan[1], Greg Petersen[1], Jacob Scheckman[1]; [1]TSI Incorporated

The elemental analysis of cast metal, scrap metal, and installed metal parts in finished goods has traditionally been conducted by x-ray fluorescence (XRF) and Arc/Spark optical emission spectrometry (OES). With the recent availability of miniature and cost-effective laser spectrometers, handheld laser-induced breakdown spectrometry (LIBS) offers a new tool for field analysis of metals. The main advantages of handheld LIBS over XRF is its ability to analyze light elements like Al, Mg and Si in about 1/10th of the time, i.e. 3 seconds, while being substantially smaller in weight and size than portable OES. In this presentation, calibration examples, usability testing and performance results of a handheld LIBS metals analyzer will be presented, and results will be interpreted in context of competitive technologies and market needs.

(360) **A Novel Handheld LIBS Analyzer and Its Applications**; Sean Wang[1], Jing Li[1], Katherine Bakeev[1], Qun Li[1]; [1]B&W Tek, Inc.

We present a new type of handheld laser-induced breakdown spectroscopy (LIBS) spectrometer, NanoLIBS, for developing mobile solutions for real world applications. A micro diode-pumped passive Q-switched solid-state laser with high repetition rate of well above 1 kHz in comparison to 1-10 Hz as used in a traditional LIBS instrument is employed to produce a train of laser pulses. The laser beam is further fast scanned over a pre-defined area, hence generating several hundreds of micro-plasmas per second at different locations. Synchronized miniature CCD array spectrometer modules collect the LIBS signal and generate LIBS spectra. The NanoLIBS weighs less than 2 kg and is operable for more than 4 hours with integrated batteries. The limit of detection (LOD) is in the range of 1 to 1,000 ppm, which is element and matrix dependent. The typical measurement time is within 1 second. The handheld LIBS spectrometer has a Linux based operation system, NanoLIBS Operating System (NOS). NanoLIBS is an open platform for developing mobile LIBS solutions. For a custom analyzer for certain specific applications, the user will a) collect the LIBS data using NanoLIBS; b) export the collected LIBS data to a PC; c) use the chemometrics software for model and calibration development; d) convert model under NOS with custom graphic user interface (GUI) to be embedded in the handheld unit. Examples of successful application development will be presented.

Key words: laser-induced breakdown spectroscopy (LIBS), high repetition rate micro-pulse laser

(361) **Advances in Handheld LIBS Instrumentation for Soil and Geochemical Monitoring**; Brendan Connors[1], Morgan Jennings[1], Justin Spott[1], David Day[1]; [1]SciAps, Inc.

Field-portable handheld LIBS analyzers are now available that offer many features commonly found in large bench-top systems including variable time gating, argon purge, high resolution, wide spectral range, sample rastering, and video targeting. In this presentation we will discuss how *in-situ* use of handheld LIBS analyzers can affect in-field decision making for applications such as environmental soil monitoring and geochemical analysis. We will also discuss how *in-situ* analysis affects software and calibration requirements as compared to traditional bench-top systems.

Results will be shown from both univariate and multivariate calibration models. As LIBS is well-suited to testing of light elements, and other in-field testing options for these are limited, results for low Z elements such as lithium, beryllium, and boron will be highlighted. High resolution spatial mapping of veins and inclusions in ore faces will also be discussed.

(362) **Metals Analysis - When to Use Portable XRF, LIBS, or OES, a Presentation by Oxford Instruments**; David Clifford[1]; [1]Oxford Instruments

Portable analyzers have become an essential tool in the quality process to provide elemental analysis and identification of alloys for material verification across a broad-range of industries. Today's quality managers have a choice of techniques when it comes to alloy analysis and may need help identifying what technology is appropriate for their application. X-Ray Fluorescence (XRF), Laser Induced Breakdown Spectroscopy (LIBS), and Optical Emissions Spectroscopy (OES) all have their place in this field and each has its advantages and limitations. Oxford Instruments will present an overview of these topics in a manner that will allow a user to identify which technique is the most appropriate for their application needs.

If you are a Quality Manager, an Engineer, a Purchasing Manager or Safety Professional, you know the importance of improving efficiencies and costs while adhering to local and global standards. Incorporating any or all of these methods into your quality control process can assist to ensure alloys and components conform to standards at any phase of the quality process from incoming inspection through to the final quality check. These methods help improve process efficiencies, reduce costs, and most importantly can assist a company in avoiding costly recalls or catastrophic failures.

(363) **Ultra-miniaturized Hyperspectral Imager**; William Yang[1]; [1]BaySpec, Inc.

We present a self-contained, miniaturized hyperspectral imager (HSI) in handheld or ultra-compact form for both push-broom and snapshot types (as light as 180 g). It is realized by an innovative design that integrates image sensor with spectral dispersing elements in an extremely compact form factor. It eliminates the need for expensive, moving, bulky and complex optics that have been used in conventional hyperspectral imagers for decades. The result is a spectral imager that can be used everywhere easily such as simply point-and-shoot, on small, low-cost UAVs, or conveyor belts in production lines, with dramatically increased convenience and reduced cost. Small and low-cost devices pave new avenues to numerous applications such as precision agriculture, remote sensing, quality control, and eventually be adapted for much broader use such as in point-of-care diagnostics and other new in-field applications.

(364) **Developing Screening Methods for Drug Compounds Using Portable Ion Mobility Spectrometry**; Connie Ruzicka[1]; [1]US Food and Drug Administration

As the global sources of both pharmaceutical products and dietary supplements expands to include more countries and different manufacturers, the potential risk for counterfeit, adulterated or substandard products to enter the US marketplace increases. To help ensure the safety of the American drug supply, the FDA is developing rapid and reliable screening methods to assess the quality of drug products and dietary

supplements. Ion mobility spectrometry (IMS) is a rapid sensitive technique that requires minimal sample preparation, thereby making it a viable screening tool for testing drug products and supplements. Portable IMS instruments are widely available and have been used mainly for illicit drug, explosive, and chemical warfare detection. With the availability of portable IMS instruments, samples can be analyzed on-site with analysis times on the order of minutes. This presentation will describe in detail the necessary steps required to develop IMS screening methods for drug products. It will discuss the procedures used for validation as well as highlight FDA's continuing effort to conduct on-site field screening of drug products at import facilities.

(365) **Portable/Handheld Infrared Spectrometers Becoming a Reality for the Food Industry**; Luis Rodriguez-Saona[1]; [1]The Ohio State University

Portable instrumentation for use in out-of-lab applications are uniquely positioned for rapid on-site identification and non-destructive authentication of incoming ingredients because of its speed, ruggedness, compactness, ease of use and transportation. We will present information on the feasibility of portable infrared systems in applications relevant to monitoring authentication and adulteration in high-risk foods. We have evaluated the performance against benchtop systems directed at developing fingerprinting strategies for rapid and specific analysis of high-risk foods (i.e. fats, olive oil, milk), providing reliable tools for assessment of food tampering and safety. This technology can enable the food manufacturer for real-time and field-based measurements to control the raw material stream, addressing safety and brand equity.

(366) **The Versatility of Portable Raman in Process Development**; Thomas Padlo[1], Katherine Bakeev[1], Philip Zhou[1], [1]; [1]B&W Tek, Inc.

Raman Spectroscopy is a well suited spectroscopic technique for process development and control within development labs as well as chemical, pharmaceutical and other industries. We will show real life examples including the synthesis of a privileged structure for medicinal chemistry. This work demonstrates the utility of a high resolution portable Raman spectrometer as a simple and versatile tool for monitoring the progression of a reaction using univariate analysis such as peak trending, as well as multivariate analysis approaches to predict the concentration of the chemical system being studied. Using portable Raman systems allows the end user to be able to make measurements in the lab, but also provides the ability to make measurements at-line or on-line in small scale pilot plants or be implemented in large scale production sites. For known reactions which are repetitively performed, or for continuous online process monitoring of reactions, the present approach provides a convenient alternative to bench top systems.

(367) **Progress in Portable Visible Spectrometry**; Alexander Scheeline[1]; [1]SpectroClick

Stacked transmission gratings generate hundreds of diffraction orders best observed using multi-megapixel cameras. Why not use the long-established linear array and a plane reflection grating operating in first order instead? This presentation contrasts the behavior of single order and multiorder visible spectrometry, emphasizing the trade offs in resolution, throughput, camera dynamic range vs. instrument dynamic range, and performance in uncontrolled environments (variable temperature, dust). Changes to cell phone camera firmware may be required if visible spectrometry is to become a standard feature of these consumer devices. Algorithms for camera linearization and spectral extraction are addressed, as are user interfaces both standard and novel.

(368) **Raman Spectroscopy for Enantioselective Analysis of Chiral Systems**; Johannes Kiefer[1]; [1]Universitaet Bremen

Differentiating between the enantiomers of chiral substances and their quantification is an analytical challenge. In the past, a number of analytical approaches including Raman Optical Activity (ROA) and Vibrational Circular Dichroism (VCD) have been developed for this purpose. Conventional Raman spectroscopy, however, has been judged incapable of distinguishing enantiomers for many decades. This presentation proposes a Raman spectroscopic method for overcoming this limitation. Advantage is taken of the polarization properties when Raman scattering occurs in an optically active medium. It is shown that inserting a half-wave retarder to rotate the signal polarization by a fixed angle enables the efficient and universal enantiomeric discrimination. A systematic investigation of the influences of selected experimental key parameters is carried out. Furthermore, a concept for quantification of the data, e.g. to determine the enantiomeric ratio is introduced and implications for experimental realization are discussed.

(369) **Surface Enhanced Raman Optical Activity as a New Chirally-Sensitive Nanoprobe**; Ewan Blanch[1,2], Saeideh Ostovar pour[1, 2], Lisa Rocks[3], Karen Faulds[3], Duncan Graham[3], Vaclav Parchansky[4],Petr Bour[4]; [1]RMIT University; [2]University of Manchester; [3]University of Strathclyde; [4]Charles University

Chirality gives stereochemically attuned nanosensors the potential to revolutionise the study of biomolecular processes. Such devices may structurally characterise the mechanisms of protein-ligand binding, the intermediates of amyloidogenic diseases and the effects of phosphorylation and glycosylation. We demonstrate that single nanoparticle plasmonic reporters, or nanotags, can enable a stereochemical response to be transmitted from a chiral analyte to an achiral benzotriazole dye molecule, in the vicinity of a plasmon resonance, from an achiral metallic nanostructure. The transfer of chirality was verified by the measurement of mirror image surface enhanced resonance Raman optical activity (SEROA) spectra for the two enantiomers of each of ribose and tryptophan. Computational modelling confirms these observations and reveals details of the novel chirality transfer mechanism responsible.

This is the first report of colloidal metal nanoparticles in the form of single plasmonic substrates displaying an intrinsic chiral sensitivity once attached to a chiral molecule. These startling results also raise the prospect of developing chirally-attuned nanosensors using SERS.

(370) **Raman Spectroscopy Detects Invasive Brain Cancer Cells in Humans**; Kevin Petrecca[1]; [1]McGill University

Grade 2-4 gliomas are primary brain cancers that invade into the surrounding normal brain. In contrast to well-circumscribed non-invasive primary brain cancers (grade 1 pilocytic astrocytomas) and secondary brain cancers (metastases), the boundary limits of invasive gliomas are not detectable by magnetic resonance imaging (MRI) intraoperative inspection, or any other technique. Two sets of contemporary studies show that the residual glioma cells not resected at surgery decrease survival time. 1) Eighty-five percent of grade 4 gliomas (glioblastomas) recur at the previous resection cavity margin. 2) The volume of residual cancer is inversely proportional to survival time; patients with no post-operative MRI-detectable contrast-enhancing glioblastoma live more than 3 times longer than patients with greater than 1.5 cm of residual glioblastoma. This inability to visualize the entirety of an invasive glioma, in the absence of another suitable biomarker or curative adjuvant therapies, limits the utility of surgical treatment leading to a median recurrence time of 6 months and an overall survival time of 14.6 months.

With these detection deficiencies in mind, high performance optical approaches are being developed for enhanced cancer detection and guidance because of their molecular profiling capabilities. We describe here the capabilities of Raman spectroscopy (RS), a non-invasive label-free surface imaging modality that gives spectral information based on the vibrational and rotational modes of molecular species in tissue. We found that RS can detect glioma in the background of normal brain during surgery with a sensitivity of 93% and specificity of 91% and that RS can detect glioma invasion beyond the extent of disease detected by T1-contrast-enhanced and T2-weighted MRI in humans during brain cancer surgery.

The appreciation that current imaging technologies cannot detect all brain cancer cells is critical because this limitation profoundly reduces the effectiveness of the surgical treatment of glioma. RS has potential to function as an intraoperative guide during brain cancer surgery that can minimize the number of cancer cells remaining following surgery, increasing the effectiveness of surgery and directly lengthening survival.

(371) **Deep UV Raman and TERS Microscopy**; Satoshi Kawata[1]; [1]Osaka University

Raman scattering spectroscopy is a promising technology for detecting and analyzing biomolecules in a cell without any labeling. When combined with microscopy, it becomes a label-free imaging technique to reveal molecular distribution in a cell [1]. However, the cross-section of Raman scattering at biomolecule is extremely weak compared with infrared absorption. If Deep-UV is employed as the excitation of Raman scattering, much larger cross-section and much richer spectral information are obtained. due to the resonance Raman scattering. We have built resonant Raman microscope with deep UV lasers and have shown the distribution of nucleotide bases in cells [2]. We have also developed a tip-enhanced Raman scattering (TERS) microscope in DUV [3]. The spatial resolution for the plasmic tip is typically 10-15 nm [4,5]. Al and In are good materials for plasmic probe in DUV [3,6]. High reproducibility and large enhancement were obtained [7-8]. Photodegradation of a specimen under DUV exposure will also be discussed [7].

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(372) **Selective-Sampling Raman Micro-Spectroscopy for Tissue Diagnosis**; Ioan Notingher[1]; [1]University of Nottingham

Raman micro-spectroscopy is a powerful technique that can discriminate between tumours and healthy tissues with high accuracy, based entirely on intrinsic chemical differences. However, raster-scanning Raman micro-spectroscopy is a slow imaging technique that typically requires data acquisition times as long as several days for typical tissue samples obtained during surgery (1×1 cm2) - in particular when high signal-to-noise ratio spectra are required to ensure accurate diagnosis.

However, tissue samples have high spatially-correlated regions and often raster-scanning is not the most optimal sampling technique. In general, the basic problem in sampling is to estimate some characteristic of a population by observing only a part of the population.

An efficient sampling strategy for detection of tumours in tissue resection specimens is stratified sampling. In this technique, the sample is divided in regions (or strata) based on a given criterion and then sampling points are selected within these regions (e.g. in a survey, human population may be stratified on the basis of geographic area, socioeconomic factors, sex, age, etc). In the case of tissue samples, stratification can be achieved by using other imaging techniques, such as auto-fluorescence imaging to determining the optimal locations of the sampling points for Raman measurements. Further, stratified sampling can be combined with adaptive cluster sampling to optimize the diagnosis for tissue samples based on histological subtypes.

To increase the diagnosis speed even further, stratified sampling technique can be integrated with multiplex Raman micro-spectroscopy techniques. We have developed novel techniques based on spatial light modulators to measure simultaneously tens of Raman spectra from sampling points arranged in arbitrary patterns. This technique overcomes the restrictions of other multiplex Raman techniques in which the sampling points are restricted to pre-defined rigid patterns (limitations caused mainly by the spectrometer slit, or arrays of optical fibres and slits). By integrating this new multiplex Raman microscopy technique with stratified sampling based on autofluorescenc-Raman spectroscopy, it is possible to reduce the diagnosis of tumours in large tissue specimens to only few minutes.

We will present examples of using Raman micro-spectroscopy based on the selective sampling strategies for diagnosis of skin and breast carcinomas.

(373) **Three-Dimensional Raman Imaging of Ion-Exchanged Waveguides**; David Tuschel[1]; [1]HORIBA Scientific

Segmented channel waveguides have been fabricated in single-crystal $KTiOPO_4$ through a topotactic process of partial cation exchange. The ion-exchanged waveguides maintain the high nonlinear susceptibility of $KTiOPO_4$ to function as frequency doubling laser light sources. We applied three-dimensional confocal Raman imaging to understand and characterize the changes to the chemical bonding and crystalline structure as well as measure the volumetric structure of the waveguide segments.

(374) ***In situ* Analysis of Materials Under Mechanical Stress: A Novel Instrument for Simultaneous Nanoindentation and Raman Spectroscopy**; Chris Michaels[1], Yvonne Gerbig[1], Robert Cook[1]; [1]NIST

Instrumented indentation or "nanoindentation" is a method that is widely used in the study of the mechanical deformation of materials on small length scales (~ micrometer). Raman spectroscopy is a technique that provides insight into the molecular or crystallographic level processes involved in the mechanical deformation of materials, such as strain build-up, phase transformations and variations in crystallinity. Typically these

approaches have been used separately wherein the spectroscopic analysis of the material might take place prior to and after the end of a mechanical transformation. Of course, there is significant interest in in situ analyses of materials during mechanical transformation as such an approach promises a richer understanding of the underlying physics than is likely possible with analysis limited to pre- and post-transformation. For example, the ability to follow the path of phase transformations rather than just the endpoints is certainly desirable. Consequently, significant effort has been directed toward the coupling of indentation instruments with various in situ analysis capabilities.

This talk describes the design and operation of a nanoindentation instrument that is coupled with a laser scanning Raman microscope to conduct in situ spectroscopic analyses of mechanically deformed regions of optically transparent materials under contact loading. The force transducer of the device allows adjustment of crucial experimental parameters, such as indentation loads and loading rates. An incorporated displacement sensor allows for collection of force-displacement curves comparable to conventional nanoindentation instruments. The device is mounted on the sample stage of an inverted optical microscope that is configured for Raman microscopy, allowing optical access to the mechanically deformed regions of transparent samples. The capabilities of this novel instrument will be demonstrated by in situ studies of the indentation-induced phase transformations in an epitaxial silicon-on-sapphire (SoS) thin film, in both a microspectroscopy and a laser scanning Raman imaging configuration.

(375) **Confocal Raman Microscopy Investigation of Solute Accumulation into Individual C18 Particles**; David Bryce[1], Jay Kitt[1], Joel Harris[1]; [1]University of Utah

Given the structural stability, high surface area and ease with which it can be chemically modified, porous silica is used as a support in a variety of analytical applications including chromatographic separation and solid-phase extraction. Because the efficiency of these applications is significantly impacted by transport dynamics in the interior of the silica support, developing a deeper understanding of the nature of molecular capture by porous silica is essential to advancing these applications. In this work, the dynamics of molecular accumulation within individual, C_{18}-functionalized chromatographic silica particles are measured using confocal Raman microscope. Specifically, accumulation of pyrene from a 2 µM solution in 80-20 water-methanol was measured in a series of individual particles having radii that range of 2.2 to 7.3 µm. The measured accumulation times into the center of the particle increase linearly with particle radius, and thus do not correspond to within-particle diffusion-limited accumulation, where the time for accumulation would increase with the square of the radius. Furthermore, the concentration in the center of particle rises exponentially with no time delay after the change in concentration in the external solution. The absence of observed diffusive delay and the linear dependence of the time constant on particle radius suggests that accumulation is rate-limited by a partitioning barrier at or near the outer particle boundary followed by rapid surface diffusion into the interior of the particle. A partitioning barrier near the particle boundary predicts time constants for accumulation that increase linearly with particle radius, because the capture rate that depends on the interfacial area ($4\pi r^2$) but offset by the particle capacity that is proportional to volume ($4\pi r^3/3$). These measurements were used to develop a model to characterize the kinetics of accumulation. Using this model, the accumulation velocity for pyrene of $4.8\pm0.1x10^{-3}$ cm/sec across the interfacial boundary was determined. These results can be compared to adsorption kinetics measured on planar C_{18} functionalized silica surfaces to examine changes in capture efficiency for pyrene due to the pore structure of chromatographic materials.

(376) **AFM and Raman Mapping of Neural Stem Cells Before and After Differentiation**; Radu Alex Boitor[1], Faris Sinjab[1], Ioan Notingher[1]; [1]The University of Nottingham

A wide range of imaging modalities are available for studying single cells. Fluorescence-based techniques are the most commonly used, but these require labelling prior to measurements, and offer little quantitative information. Conversely, imaging by Raman micro-spectroscopy (RMS) and atomic-force microscopy (AFM) require no labelling and reveal more detailed, complementary information. RMS imaging allows the spatial distribution of particular biomolecules to be imaged within cells, while AFM can map the topography, and thus the volume of cells. For these sorts of measurements performed on the same sample positions, quantitative information can be deduced about the cell contents, such as concentrations of particular biomolecules.

We have used a combination of AFM and RMS to investigate neural stem cells before and after differentiation. Particular attention was paid to the distribution of RNA in the cells imaged by Raman microscopy, which has previously been shown to be a highly specific and sensitive means of discriminating the differentiation status of the cells[1].

The quantification of the RNA concentration in the absence of cell volume measurements has previously relied on assumptions about the uniform concentration of the 1450 cm^{-1} CH_2 vibrations within the cell. However, this may vary across a Raman map as lipid-rich regions may contain higher concentrations of molecules producing this Raman band. In order to investigate this, we have correlated the RNA distribution with the AFM topography at the single cell level. This has allowed us to directly compare the Raman signal of RNA and the cell volume being sampled, at the same acquisition point.

We have also used the combined AFM-Raman dataset to investigate potential improvements for Raman image normalization, which can be correlated with the AFM map, and thus the cell volume. With improved normalization techniques, quantification of biomolecule concentrations becomes more accurate, which is not only useful for the differentiation of neural stem cells, but also potentially for other cell-based systems.

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(377) **Coherent Anti-Stokes Raman Scattering Correlation Spectroscopy and Imaging**; Karen Antonio[1], Zachary Schultz[1]; [1]University of Notre Dame

Although fluorescence correlation spectroscopy (FCS) and fluorescence cross-correlation spectroscopy (FCCS) are widely used to investigate dynamic processes in living systems, these techniques are limited by target-specific dyes and burdened by sample preparation. Coherent anti-Stokes Raman scattering (CARS) is an underutilized detection method for correlation spectroscopy (CS). Unlike FCS measurements, CARS has the ability to monitor fluctuations in vibrational signal without the use of exogenous agents, which allows for the examination of a broader range of biomolecules in an unperturbed environment. We use a multiplex CARS microscope, synchronizing a picosecond pulsed beam for increased spectral resolution with a broadband Stokes beam to obtain multiplex CARS spectra. The nonlinear properties of CARS and point-by-point scanning capabilities allow for three-dimensional imaging of the sample. In this study, we investigate the molecular mobility of various analytes

at different chemical interfaces. CARS-CS has the potential to provide localization and diffusion dynamic information in a label-free, nondestructive approach to understanding chemical interactions in cellular systems.

(378) **New Hybrid Plasmonic Mode and Applications to Bimodal SPRI / SERS Interrogation Sensing System**; Michael Canva[1,2]; [1]LCF, Laboratoire Charles Fabry - Institut d; [2]LN2, Laboratoire Nanotechnologies Nanosystèmes - U. de Sherbrooke / CNRS

Plasmonic imaging sensors are becoming widely used for characterization of biomolecular interaction dynamics as well as to assess the presence of given molecular targets in a solution to analyze. For the latter application and in certain cases, three points mainly remain to be improved: 1) clearly assessing the presence of the given target, 2) getting better limits of detectability for low concentration analysis and 3) getting a better dynamical range of concentration characterization.

We have recently showed that the latter point can be solved using spectral monitoring. Concerning lowering the detection limits in terms of captured molecular targets requires doing better that the limits given by classical propagating plasmon and therefore points towards micro-nano structuration of the substrates instead of the conventional gold thin film. As for the former point, the SPR signal cannot asses the target composition or structure and its identity relies solely on the specificity of the biofunctionalized probe sensing surface. Much can be done on this biochemistry requirements but it does have some limits within biomolecules with common binding sites. For this point another answer can be to actually analyze the capture targets. This has been investigated, in particular using coupled techniques such as SPRI/MALDI etc. We intend adding SERS which also requires structuring the plasmonic substrate.

We are therefore investigating the possibility of using the same nano-micro-structured plasmonic substrates for parallel SPRI and SERS characterization that would be performed in a common bimodal instrumental system.

One of the main result we recently achieved is the understanding and demonstration that substrates possessing both a metallic thin film able to support propagating plasmon mode, as well as, directly binded onto it, arrays of nanoparticles able to support localized plasmon modes, (the array also lead to Bragg modes) can exhibit (new) hybrid plasmonic modes possessing advantages of both types: enhanced field localization and mode spectral dispersion leading to high sensitivity.

We showed that this type of structure can be optimized for both the SPRI and SERS detection therefore opening the way to quantification and identification of biomolecular targets in optical plasmonic instrumental systems.

(379) **Optics, Plasmonics and SharpEdgeOnics in Novel Nanoarchitectures**; Michael J. Naughton[1]; [1]Boston College

We discuss our research activities on optical and plasmon excitation and propagation in micro- and nano-architectures, with potential utility in display, biosensing, optogenetics, and photovoltaic technologies. One architecture, the plasmonic halo, forms a novel optical color filter as well as a potential molecular sensor, while another yields nearly reflectionless optical coupling into highly directional plasmon propagation, and another, the nanocoax, offers a multitude of applications. Finally, a plasmonic metamaterial scheme for hot electron extraction in ultrathin solar cells is discussed.

Work in collaboration with K. Kempa, M.J. Burns, J.M. Merlo, F. Ye, A. Rose, Y. Calm, N. Nesbitt, J.R. Naughton, C. Yang, L. DiImperio, J. Kong, X. Wu, J. Christianson, J. Varela, M.M. Archibald, A. Valera, T. Connolly and T.C. Chiles.

(380) **Plasmonic Gold Nanohole Arrays for Surface-Enhanced Raman Scattering Biosensing**; Nianqiang (Nick) Wu[1], Peng Zheng[1], Xuefei Gao[1]; [1]West Virginia University

The presentation shows how to tune the plasmonic properties of gold nanohole array patterns. Both the localized surface resonance plasmon (LSPR) and the propagating surface plasmon polaritron (SPP) modes can be excited in the gold nanohole array patterns. Both the modes can be tuned by the size and pitch of nanoholes. Surface enhanced Raman scattering (SERS) enhancement is dominated by the absorption from the 'gap' defects formed within the array pattern. Furthermore, the gold nanostar-based nanoparticles have been coupled to the gold nanohole array as the SERS substrate for biosensing, which enables highly sensitive detection of heavy metal ions.

(381) **Mapping the Extracellular Space Using Ion-Selective Core-Shell Luminescent Nanoparticles**; Denis Boudreau[1,2], Jérémie Asselin[1, 2], Philippe Legros[1,2], Mazeyar Pavinzadeh Gashti[1], Rihab Bouchareb[3], Jesse Greener[1],Patrick Mathieu[3]; [1]Department of Chemistry, Université Laval; [2]Center for optics, photonics and lasers, Université Laval; [3]Quebec Heart and Lung Institute, Université Laval

The development of nanomaterials displaying enhanced luminescence properties is an active field of research. In particular, it is known that the photophysical behavior of molecular dyes can be controlled by placing them at precise distances from metal nanoparticles, which is proving useful for the design of sensitive sensing strategies for various chemicals and biomolecules. Reproducible improvements in detection sensitivity and photostability can be obtained with core-shell nanoparticles composed of a nanometer-size metal core coated by multiple layers of silica. Furthermore, by carefully controlling the spacing between the core and fluorophores arranged in concentric layers, these nanostructures can be used to enhance Förster resonant energy transfer (FRET) efficiency and range between donor-acceptor pairs localized on these multilayer composite NPs. These multilayer core-shell nanoparticles present many of the features required of an ideal self-supported sensing platform: they offer high optical detection sensitivity, excellent chemical and photophysical stability, high dispersability in water, and facile surface functionalization. In the work presented herein, composite nanoparticles incorporating ion-sensitive fluorophores have been grafted onto solid substrates for realtime quantitative imaging of cell and bioflm samples and on optical fibers for *in vivo* ion sensing in neurons.

(383) **Particle-Image-Velocimetry Analysis of Aerosol from a Solution-Cathode-Glow-Discharge**; Allen White[1], Andrew Schwartz[, 2], Steven Ray[2], Gary Hieftje[2]; [1]Indiana University, Rose-Hulman Institute of Technology; [2]Indiana University, Department of Chemistry

The solution-cathode—glow-discharge (SCGD), an atmospheric-pressure, direct-current (DC) glow discharge that is sustained directly upon the surface of a flowing liquid cathode, is an alternative plasma source for atomic optical emission spectroscopy (OES). Unlike traditional plasmas employed for the analysis of solutions, such as the inductively coupled plasma (ICP), the SCGD is compact and inexpensive, consumes little power (~70 W), operates in the ambient atmosphere (requiring no compressed or flowing gases), and, owing to the fact the plasma samples directly from a stream of flowing sample solution, obviates the need for sample-solution nebulizers, greatly reducing memory effects and temporal broadening of transient peaks. Moreover, the SCGD offers OES performance comparable to that of more complex, expensive sources, including radially viewed ICP-OES.

Although the analytical performance of the SCGD has been well characterized, the fundamental mechanics of the source are a topic of debate. Among these uncertainties, the fluid mechanics of the air and analyte mixture surrounding the discharge are not well understood. The present study employs particle-image-velocimetry (PIV) to analyze the interaction of the glow discharge with the surrounding air and air/analyte mixture. Results will be compared to earlier schlieren-imaging studies and conclusions which can be drawn regarding analyte solution-to-plasma transport discussed.

(384) **Measurements of Solvated Electrons at a Plasma-Liquid Interface via Optical Absorption Spectroscopy**; Paul Rumbach[1], David Bartels[1], R. Mohan Sankaran[2], David Go[1]; [1]University of Notre Dame; [2]Case Western Reserve University

Since its discovery in 1962 by Hart and Boag, the hydrated electron has remained a fascinating research topic. Even after half a century of work, many questions remain unanswered regarding the structure and kinetics of the solvated electron. In this work, we present a novel method for generating solvated electrons using an atmospheric-pressure plasma and measuring them via optical absorption spectroscopy using a laser diode in a reflection geometry [1]. Our results indicate that free electrons in the plasma penetrate approximately 2.5 nm into solution before reacting away. Using known electron scavengers such as nitrite, nitrate, hydrogen peroxide, and sulfuric acid, we observe reaction kinetics for the near-interface electrons similar, but not identical, to that of bulk electrons. Using a series of laser-diodes, we measure their optical absorption spectrum and find that it is significantly blue-shifted from that of bulk electrons. We hypothesize that this is due to the intense electric field in the Debye layer of the plasma-liquid interface, which yields a Stark shift in the measured spectrum. Overall, our method provides a new and unique system for studying solvated electrons with many potential chemical applications.

[1] P. Rumbach, D.M. Bartels, R.M. Sankaran, and D.B. Go, Nature Comm. 6, 7248 (2015).

(385) **Design Modifications to a Solution Cathode Glow Discharge and Examples of Industrial Application**; Stuart Schroeder[1]; [1]Alberta Innovates Technology Futures

The solution cathode glow discharge (SCGD) is a direct current atmospheric pressure glow discharge that operates in the ambient atmosphere for the elemental analysis of aqueous samples. The SCGD offers a number of advantages over traditional ICP-AES in terms of simplicity, affordability, low power requirements, no need for an inert plasma gas, and improved long term stability. A variety of electrode designs and solution interfaces have been employed for the SCGD to maintain stable plasma emission, high sensitivity, and robust electrical contact to the flowing sample solution. In one design, a waste sample solution reservoir with electrical grounding has been used to produce a uniform electric field below the plasma which inhibits a glow-to-arc transition. This design, however, suffers from small fluctuations in the waste solution level which leads to plasma instabilities. In another design, the waste reservoir is eliminated and electrical contact to the plasma is maintained by a grounding electrode 3 mm beneath the cathode tip. This design can be difficult to stabilize with frequent glow-to-arc transitions. A new design will be discussed in this presentation that offers lower sample flow rates than previously described. Low sample flow rates are desirable for reduced sample and acid consumption. With this design, stable emission intensity is observed with improvements to sensitivity as the sample flow rate decreases.

The SCGD may fulfill the unmet need for on-line multi-element solution analysis for industrial process control applications. Examples of targeted industrial applications of the SCGD will be presented that take advantage of the unique characteristics of the SCGD.

(386) **Discharges with Liquid Electrode: Properties and Mechanisms**; Peter Bruggeman[1]; [1]University of Minnesota

In this talk, we will discuss dc driven glow discharges with water electrode and RF driven atmospheric pressure plasma jet (APPJ) in direct contact with liquids. The fascinating properties of these plasmas include: (1) self-organization of the anode spot at the plasma-liquid interface leading to beautiful patterns and structures, (2) extremely high OH densities up to $10^{23}m^{-3}$ while the electron density is not exceeding 10^{19} m^{-3} and (3) gas temperatures as high as 3000 K leading to exceptional large spatial gradients at the water-liquid interphase. We will review these properties and discuss the underpinning mechanisms of plasma-liquid interactions that are key in understanding these discharge properties.

(387) **Highly Sensitive Elemental Analysis for Cd by Solution-Anode Glow Discharge Atomic Emission Spectrometry**; Zhenli Zhu[1]; [1]China University of Geosciences(Wuhan)

The contamination of air, drinking water supplies and foods with cadmium ions poses a serious threat to global health and environment. Accordingly, it is of great demand to determine trace cadmium in environmental samples. In recent years, liquid discharge microplasmas1 are especially promising for the development of portable instrument for elemental analysis due to their small size, low power consumption, cost effectiveness, and reduced/no inert gas requirement. Richmond et al.2 demonstrated that an atmospheric-pressure microplasma can act as a gaseous cathode, metal-free electrode to mediate electron transfer reactions in aqueous solutions. A novel solution-anode glow discharge atomic emission source was developed for Cd determination in aqueous solutions. In this approach, a glow discharge microplasma was generated between the tip of a tungsten bar and the liquid (i.e. electrolyte) surface, acted as a gaseous cathode. Qualitative and quantitative determinations of Cd in the sample solution were achieved by atomic emission measurements in the plasma. The effects of operating parameters such as electrolyte pH, discharge current, Ar gas flow rate and sample introduction rate were investigated. Under the optimal conditions, the limit of detection (LOD) was calculated to be 0.05 μg L−1; good repeatability (relative standard deviation (RSD)=1.7 %, n=5) was obtained for a Cd2+ 5 μg L−1 standard. Compared with other liquid discharge systems that operate at atmospheric pressure, the volatile molecular species of Cd can be generated efficiently at the plasma-liquid interface in this approach, which greatly increase the sample introduction efficiency, and thus result in the merits of high sensitivity, and low detection limit. It provides an alternative analytical method for the determination of elements in water samples.

(388) **Data Pre-Processing and Data Processing for Multivariate Spectral Analyses**; Max Diem[1]; [1]Northeastern University

Spatially resolved microspectral datasets collected either by infrared or Raman microspectroscopy, often consist of between 105 and 107 individual vibrational spectra. In particular for biomedical application, the variations between the individual spectra are superimposed on spurious systematic and random confounding features that may prevent meaningful multivariate analysis from being carried out successfully. A typical example of systematic confounding factors is the sample morphology dependent scattering in infrared microspectral datasets that can cause significant shifts in the shape and position of bands.

Pro-processing of spectral datasets is aimed at reducing systematic as well as random spectral perturbation to a level at which they no longer interfere with mathematical methods of analysis. The removal of cosmic ray spikes in Raman microscopy, the reduction of water vapor and resonance Mie contributions in infrared microscopy, and methods to reduce random noise from both datasets will be discussed.

(389) **Single Cell Raman Imaging: Problems and Pitfalls When Comparing Images for Quantitative and Qualitative Purposes**; Martin A. B. Hedegaard[1]; [1]University of Southern Denmark, Department of Chemical Engineering, Biotechnology and Environmental Technology

Single cell imaging have seen a huge development in recent years. It have been applied to a range of different problems such as drug uptake studies[1] and to compare cell populations[2].

For these purposes a group of analysis methods have been developed and optimised for Raman images. These include e.g. cluster analysis, SVM, spectral unmixing. The outcome of these methods does however depend heavily on the measurement conditions. The most important for single cell analysis includes measurement times, spatial resolution, substrate, laser focus, instrument variation and if the cells are hydrated during measurement. All these factors is something that we need to take into account, when comparing measurements across a number of cells and especially when comparing maps measured taken on different days with different configurations.

In the presented work I will focus specifically on the effect of normalisation of single cell images measured in water. The main problems here are usually twofold. The first and main problem is the water background that in most cases will distort normalisation. Secondly there will be significant differences in cell to water content depending on focus and substrate used.

To be able to directly compare individual images a correction first for the water content must be applied and secondly also take into account eventual substrate contributions. If these effects are removed it is possible to normalise and compare Raman images measured at different times with different acquisition conditions and apply a unified cluster analysis or spectral unmixing method for all the maps.

Data that have been corrected with Extended Multiplicative Scatter Correction with Spectral Interference Subtraction using reference spectra will be shown. For images without substrate interference it is also shown that no references is needed as long as the map includes background around the individual cell.

1. T. Chernenko, C. Matthaeus, L. Milane, L. Quintero, M. Amiji, M. Diem, ACS Nano, 3(11),3552-3559 (2009)

2. H. Autefage, E. Gentlema, E. Littmann, M. A.B. Hedegaard, T. von Erlach, M. O'Donnell, F. R. Burden, D. A. Winkler, M. M. Stevens, PNAS, 114(12) 4280-4285, (2015)

(390) **Sample and Model Selection for Local Modeling Utilizing Data Fusion Ranking Techniques**; Rachel Emerson[1,2], John Kalivas[1]; [1]Idaho State University; [2]Idaho National Laboratory

Large spectral libraries of calibration samples spanning numerous conditions can be useful for multivariate calibration model development. However, the prediction accuracy using large, variable global databases is often not sufficient for many applications. Local modeling techniques can be utilized to select a set of calibration samples from a global library specific to each individual prediction sample in order to build models meeting accuracy requirements. Most local modeling methods use spectral similarity measurements, such as the distance or angle between spectral vectors, to compare a prediction sample to library spectra. Few of these methods utilize more than one similarity measure at a time. This work explores methods of combining a group of similarity measures through data fusion in order to select calibration samples spectrally resembling each individual prediction sample. Another aspect of local modeling explored in this work is the use of "y-windowing" to further narrow the calibration samples used in the model by sorting the selected spectrally similar calibration samples into local ranges of respective prediction properties of interest. Models are then formed using these subsets or windows. The calibration window(s) most consistent with the prediction sample is deemed best for the final calibration samples to form the local model. In utilizing both of these localizing techniques, a reduced number of calibration samples is selected matching both spectrally and chemically for forming regression models. These models are substantially more accurate when compared to models formed using the entire global spectral library.

(391) **Issues in Hierarchical Modeling of Complex Chemical Data**; Steven Brown[1]; [1]University of Delaware

Building classification models for complex data involving many classes is problematic. Classification algorithms commonly used in chemometrics do not perform well with many classes, especially when some of the classes may overlap slightly. A hierarchical approach to modeling, where classes are established in a defined relationship such as a tree or other structure, may be beneficial in these cases because the class relationships can be incorporated in the modeling, either directly or indirectly.

This talk will compare conventional "flat" classification with hierarchical modeling, will consider how hierarchical models are constructed and assessed and will demonstrate the benefits of hierarchical models on complex data involving spatial information and related classes.

(392) **Feasibility of an End-of-Shift Monitor for the Determination of α-Quartz in Mine Dusts**; Peter Griffiths[1], Andrew Weakley[2], Arthur Miller[3], Emanuele Cauda[4]; [1]Griffiths Consulting LLC; [2]University of California, Davis; [3]National Institute of Occupational Safety and Health, Spokane; [4]National Institute of Occupational Safety and Health, Pittsburgh

The inhalation of α-quartz in respirable mine dusts is of particular concern due to the recent rise in cases of pneumoconiosis in miners. Currently, there is no field-portable instrument that can specifically measure airborne α-quartz and give miners timely feedback on their exposure. The U.S. National Institute for Occupational Safety and Health (NIOSH) is therefore conducting studies to investigate technologies capable of field-based measurement of airborne α quartz. In this talk, we will discuss the potential application of Fourier transform infrared (FT-IR) spectrometry for field-based monitoring. Air samples were collected by trapping respirable dust on a porous PVC filter after removing larger particles with a Dorr-Oliver cyclone. Most of the FT-IR measurements reported in this talk were carried out in the transmission mode. Transmission measurements on samples were done directly on the filter (without ashing or other sample treatment), and partial least squares (PLS) regression was applied to select regions of the spectra. Wavelengths essential to calibration were selected using a modified Monte Carlo unimportant variable elimination (MCUVE) procedure. Wavelength elimination using MCUVE helped to improve PLS prediction, reduce the number of required PLS factors, and identify additional silica bands distinct from the doublet near 800 cm^{-1} that is used in the conventional method of estimating α-quartz. PLS regression appeared robust against the influence of residual filter absorptions, as well as the absorptions from other minerals present in the infrared spectra of the collected samples (including kaolinite, which is the most common confounder in IR-based

approaches to the quantification of α-quartz in coal dust samples) and outperformed the manual calibration. For coal mine samples the material on the PVC filters produced a minimal signal in the 1600 cm^{-1} region, which would have the potential to affect the silica quantitation of quartz. This suggests that similar approaches could be used for samples collected in both coal and non-coal mines. Our results support the feasibility of quantifying respirable silica in both coal and non-coal mines using field-portable infrared spectrometers.

(393) **Using NMR Spectroscopy to Gain Novel Insights for Diabetes Drug Design**; Wolfgang Peti[1,2,3]; [1]Brown University School of Medicine; [2]Brown University; [3]University of Copenhagen

We have used biomolecular NMR spectroscopy to fully understand the catalytic mechanism of tyrosine phosphatases (PTPs) as well as how intrinsic disordered regulatory elements within PTPs allow for the modulation of the catalytic activity of PTPs. These experiments were performed on a variety of PTPs with a special focus on PTP1B, which has proven to be an ideal drug target for diabetes. We were able to leverage this data to develop novel routes of PTP1B inhibition, with high specificity, something that was so far impossible. Here we will present our recent advances in how to target intrinsically disordered regions of PTP1B with small molecules as well as present their modes of action.

(394) **Raman Spectroscopy Based Sensing of Alternative Glycemic Markers: Quo vadis?**; Rishikesh Pandey[1], Nicolas Spegazzini[1], Niyom Lue[1], Jeon Woong Kang[1], Gary Horowitz[2], Ishan Barman[3],Ramachandra Dasari[1]; [1]Massachusttes Institute of Technology; [2]Harvard Medical School; [3]Johns Hopkins University

Long-term glycemic markers notably glycated hemoglobin and glycated albumin reflect retrospective indices of integrated glucose value in the blood stream over the time span being unique to the biomarker. The secondary diabetic complications correlate more strongly with the elevated concentration of these biomarkers than the commonly used measurement of fasting glucose. Additionally, these biomarkers have gained traction is recent years for the prescreening of diabetic. While HbA1c measurement is affected by the conditions that alter erythrocyte survival, glycated albumin can be influenced by factors that affect albumin turnover. The combined determination of HbA1c and glycated albumin will provide a uniquely powerful metric in estimating the “true” glycemic history of a patient - a feature that is currently lacking in almost all clinical laboratories globally.

In this talk, we present a Raman spectroscopy based novel approach to simultaneously detect and potentially quantify both the glycemic markers. We believe that this sensing approach can provide a powerful tool for simultaneous determination of this panel of biomarkers in routine clinical diagnostics in the future.

(395) **Raman-based Blood Glucose Concentration Prediction by Structural Calibration**; Nicolas Spegazzini[1], Rishikesh Pandey[1], Jeon Woong Kang[1], Ishan Barman[2], Ramachandra Rao Dasari[1]; [1]Massachusttes Institute of Technology; [2]Johns Hopkins University

Raman spectroscopy has become a promising tool for the noninvasive measurement multiplexing components of blood - a pending problem in biophotonics. A novel analytical framework is proposed that enables longitudinal monitoring based on spectroscopy chemical concentration and identifying nonlinearity sources, as well as noise homoscedastic and heteroscedastic. In order to improve the prediction model calibration. The main idea is to propose a weight error-based strategy based on in the identification and modeling of the sources that contribute to the error measurement. We demonstrate the efficacy of the proposed approach as compared to conventional calibration methods. Using blood glucose monitoring by Raman spectroscopy as an example. The approach exhibits a reduction in the error over partial least squares regression when applied to a dataset acquired from human subjects undergoing oral glucose tolerance tests. This method can offers a new route at screening gestational diabetes and opens doors for continuous process monitoring.

(396) **Early Diagnosis of End Stage Renal Disease Due to Diabetes using IR Imaging**; Vishal Varma[1,2], Andre Kajdacsy-Balla[1, 4], Sanjeev Akkina[3,4], Suman Setty[1.4], Michael Walsh[1,2,4]; [1]Department of Pathology, University of Illinois at Chicago; [2]Department of Bioengineering, University of Illinois at Chicago; [3]Department of Medicine, University of Illinois at Chicago; [4]University of Illinois Cancer Center

The main treatment for end-stage renal disease (ESRD) is kidney transplantation, however close monitoring of post-transplant biopsies is required to identify subclinical complications. The most common cause of ESRD is due to diabetic nephropathy. We identified biochemical markers using infrared (IR) imaging that were associated with recurrent diabetic nephropathy. IR imaging is an emerging approach to obtain images of the biochemical composition of tissue biopsies in a label-free fashion. An initial study focused on identifying patients with no evidence of diabetic nephropathy and patients with advanced diabetic nephropathy. Serial sections were acquired and stained with PAS or imaged using chemical imaging. IR spectra were extracted to identify biomarkers associated with diabetic nephropathy progression. A second study identified transplant patients who underwent very rapid recurrent diabetic nephropathy and patients with no evidence of diabetic nephropathy after two years. Biomarkers were identified that were changed in renal structures associated with the progression of diabetic nephropathy, including increased levels of glycation. Using these two biomarkers alone, it is possible to diagnose the disease with high specificity and sensitivity. In comparison, using the histological parameter, mesangial fractional surface area, alone gave slightly lower specificity and sensitivity. These biomarkers were found to be increasing in the cohort of transplant patients that underwent rapid diabetic nephropathy recurrence. In addition, the early biopsies from the patients that underwent later diabetic nephropathy progression were biochemically different from the non-progressive patients, suggesting that chemical imaging may identify pre-histological biomarkers that will predict outcome. Furthermore, we present recent data using discrete frequency data collection using a QCL based imaging system. We have identified a number of biomarkers that are associated with the advancement of diabetic nephropathy and that we can track the early recurrence of diabetic nephropathy in surveillance biopsies. In addition, we have highlight a ‘biochemical-signature’ that may be predictive of the later progression of diabetic nephropathy recurrence.

(397) **Multivariate Analysis as a Tool to Extract Characteristic Bands Associated with Diabetic Retina Tissue using Synchrotron Infrared Spectromicroscopy**; Ebrahim Aboualizadeh[1], Christine Sorenson[, 2], Reyhaneh Sepehr[3], Mahsa Ranji[3], Nader Sheibani[4], Carol Hirschmugl[1]; [1]University of Wisconsin-Milwaukee, Department of Physics; [2]Department of Pediatrics, University of Wisconsin School of Medicine and Public Health; [3]Biophotonics Laboratory, University of Wisconsin Milwaukee, Department of Electrical Engineering and Computer Science; [4]Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health

Retinal layers (photoreceptors, bipolar cells and ganglion cells) have distinct chemical signatures, and can be easily detected with chemical imaging [1]. Hyperspectral imaging using the synchrotron source and focal plane array detector produces a 3-D cube of data (x,y,Abs(λ); # of pixels) that contains spatial and spectral information, where each pixel in the image plot contains a high signal-to-noise spectrum. Hyperspectral datasets of eye sections obtained from wild type (non-diabetic) and Akita/+ (diabetic) mice provide insight into specific chemical signatures associated with diabetic retinopathy, a major complication of diabetes. Male Akita/+ mice spontaneously develop diabetes by 4-weeks of age due

to a mutation in their insulin gene. Retinas from 6-week-old, and 6-month-old mice were evaluated for detection of chemical changes as a function of the disease.

To evaluate the hyperspectral data we use Principal Component Analysis (PCA) to compare chemical information and detected biomarkers employing both the spectral and spatial information. For example, it is known that the photoreceptors are affected early in the progression of diabetes. Thus, the chemical signatures (individual pixel spectra) from the photoreceptor layers are compared as a function of disease (WT vs. Akita) to detect potential biomarkers of the disease pathology. The PC loadings and scores reveal the spectral and spatial chemical variation, respectively. The PC loadings [2] are well known to give insight into the spectral components that are major contributors for that PC. An image representation of the PC scores associated with each pixel reveals which areas of an image are highly linked to the features in the loading. Significant differences attributed to glycation process in diabetes, the spectral regions related to lipid and nucleic acids and DNA bands are highlighted from PC loadings. From pixel to pixel comparison of image representation of the PC scores higher variations for diabetic samples were observed.

[1] M.Z. Kastyak-Ibrahim, et al. ,"Biochemical label-free tissue imaging with subcellular-resolution synchrotron FTIR with focal plane array detector, NeuroImage,60,1,2012, 376-383.

[2] Anna de Juan,et al., Chemometric Tools for Image Analysis" (Chapter) in Infrared and Raman Spectroscopic Imaging, by R. Salzer and W. S. Heinz; 585-618, Wiley-VCH Verlag GmbH & Co.,(2014).

(398) **Ambient Ionization in the Security Industry: Atmospheric Pressure Photoionization (APPI) for Explosives Trace Detectors (ETDs)**; Jack Syage[1], Karl Hanold[1], Andrey Vilkov[1]; [1]Morpho Detection, LLC

Ambient ionization/analysis has been used for decades in the security industry, beginning with the use of swabbing material inserted into thermal desorbers followed by ionization and ion mobility spectrometry (IMS) analysis. New generation explosives trace detectors (ETDs) are being developed improving on sample collection/delivery, ionization, and mass analysis (e.g., use of mass spectrometry).

Photoionization is gaining recognition and acceptance for its unique benefits in the ionization and detection of a broad range of compounds and specific classes of compounds that present problems to other ionization methods. Atmospheric pressure photoionization (APPI) has specific benefits that make it well suited to the detection of a wide range of explosives compounds, particularly the troublesome nitrate compounds and explains why it is being incorporated into the latest generation of ETDs . We will review these implementations to IMS and MS instruments and discuss the ionization mechanism including use of dopants to achieve highly specific detection of a broad range of explosives and narcotics.

(399) **Characterizing and Databasing Drugs and Drug Analogs to Stay Ahead of Clandestine Designer Drug Laboratories**; Kristina Williams[1], Guido Verbeck[1]; [1]University of North Texas

Clandestine laboratories are constantly creating new designer drugs faster than forensic laboratories can identify them and lawmakers can ban them. To help assuage some of the pressure from this fact, the Federal Analog Act was passed in 1986. This law makes it possible to schedule any substance that is "substantially similar" in structure or central nervous system effect. The ambiguous nature of this language has led to several courtroom disputes between scientists about similarities in structural backbone and functional groups based on a two-dimensional representation of the molecule. To avoid this, the establishment of a biologically relevant metric for comparison of these substances is needed.

Psychoactive drugs must cross the blood-brain barrier to affect the body in the desired way; therefore, an *in vitro* assay to determine blood-brain barrier (BBB) permeability has been adapted for use with controlled substances and derivatives and their analysis via electrospray ionization – mass spectrometry (ESI-MS). Parallel artificial membrane permeability assay (PAMPA) uses two stacked 96-well plates with the top wells submerged in the bottom well. The bottoms of the top wells are porous PVDF membranes (0.45 um pores). Coating the PVDF membrane in porcine brain lipids creates a stable lipid bilayer that effectively mimics the BBB. Monitoring the passage of drugs through this membrane can provide a measure of BBB permeability comparable to that of *in vivo* assays.

Once BBB permeability of illicit drugs is established, relative affinity for membrane-bound receptors in the brain must be assessed. A cell membrane chromatography – mass spectrometry (CMC-MS) stationary phase can be created by adhering homogenized Sprague-Dawley rat brain onto 5 um silica particles and packing them into a 50mm x 2.5mm stainless steel column. This can be used to compare retention times, and thus relative affinity for the membrane-bound receptors, of different substances.

To maximize the efficiency of this method to assess drug analogs, a 'shotgun' synthesis developed to obtain multiple tryptamine derivatives from a single reaction of tryptamine with 5 alkyl iodides. This facilitates the creation of a searchable mass spectrum database of potential drug analogs with ancillary data (BBB permeability, CMC retention, etc.).

(400)) **Mobilized Open Air Ionization: Detection of Explosives and Dangerous Supplements with a Compact DART-MS**; Frederick Li[2], Joseph Lapointe[1], Joseph Tice[1], Adam Hall[3], Brian Musselman[1]; [1]IonSense, Inc. Saugus, MA; [2]Boston University School of Medicine: Biomedical Forensic Sciences Program, Boston, MA; [3]Northeastern University: The Barnett Institute of Chemical and Biological Analysis and the Department of Chemistry and Chemical Biology, Boston, MA

Terrorists and criminals are opportunists who thrive on our inability to detect their efforts. In the case of terrorists, the weapon of choice is the explosives while the criminal world has shifted considerable interest to the adulteration of food and medicines as a source of income. Both terrorist and criminals recognize the need to avoid detection, leading to more and more sophisticated schemes for concealing their activity. In our laboratory we are developing an innovative field-deployable technology with the capability of detecting threats on-site or in situ and providing real time results regarding those threats. The technology is simple, rapid, sensitive, and reliable. In our detection system, a Direct Analysis in Real Time (DART) ambient ionization source has been interfaced to a compact Waters Acquity QDa mass spectrometer in order to create a rapid, sensitive and mobile platform for threat detection. Our analytical approach requires minimal sample preparation for the detection of a wide range of explosives and chemicals. Using the DART ionization source interfaced to this mobile single quadrupole mass spectrometer we had analyzed a wide range of explosive standards, including various high and low explosives. In-source fragmentation by using various cone voltages has been evaluated to determine the optimal voltages that provide the most characteristic fragmentation for each explosive. Explosives were also deposited onto various surfaces and collected using various collection methods to emulate the sample collection and analysis method associated with IMS.

Rapid inspection of food supplements laced with pharmaceuticals has also been investigated as the increase in frequency of this dangerous manipulation of food has been noted worldwide. Application of reverse search libraries to the detection of these threats will be discussed.

(401) **MALDI-MS as a Tool for the Characterization of Inks for Forensic Document Analysis**; Rhett Williamson[1], José Almirall[1]; [1]Florida International University

The ability to create a chemical profile for inks that can be used for the association of common inks and discrimination of inks from different sources is useful in forensic document analysis. Additionally, the capability to characterize individual inks in intersecting lines and/or mixtures on documents can aid in sequence determination and tracing back a questioned document to its source. Therefore, it is of great interest to forensic document examiners to develop chemical analytical techniques for the analysis of inks that is rapid, requires little-to-no sample preparation, is minimally destructive to samples, and ultimately allows for the association of common inks and discrimination of inks from different sources.

Matrix-assisted laser desorption coupled to mass spectrometry (MALDI-MS) is a surface sampling technique that allows for rapid, in-situ analysis of inks with little-to-no sample preparation. The addition of a matrix to the sample prior to analysis improves greatly the signal-to-noise ratio and reduces background interference of the acquired mass spectra while maintaining the visual integrity of the ink. Utilizing both positive and negative ion mode allows for characterization of different components of the inks including dyes/pigments and polymeric vehicle content.

In this study, four classes of printing inks: inkjets, toners, offset, and intaglio, were analyzed and it was shown that the information obtained from MALDI-MS analysis is useful for the association of inks from common sources and the discrimination of inks from different sources. A number of writing instruments including pen ink and stamp ink were also analyzed both individually and in mixtures. It was found that MALDI-MS is an extremely useful technique for the routine analysis of printing inks and the chemical information provided could prove useful in forensic document analysis.

(402) **Direct Sample Analysis Using Electrospray Ionization High Performance Ion Mobility-Mass Spectrometry**; Adam Graichen[1], Robert Jackson[1], Jianglin Wu[1], Ching Wu[1], Mark Osgood[1]; [1]Excellims Corporation

Ion mobility spectrometry (IMS) has traditionally been used as a screening tool to identify potentially hazardous materials including drugs, chemical warfare agents, and explosives. However, many IMS instruments suffer from relatively low resolving power, ultimately resulting in false positive detection for samples containing complex background materials. In the past decade, our research has focused on the development of IMS devices with demonstrated resolving powers equivalent to or greater than conventional high performance liquid chromatography (HPLC) systems. Due to the orthogonal nature and speed of mobility based separations, a growing number of analysts are able to limit their dependence on upfront HPLC methodologies. When used in combination with a mass spectrometer, combined ion mobility-mass spectrometry (IMMS) systems enable unequivocal identification of the ion species generated during sample introduction that may be otherwise impossible if using IMS alone. Specifically high resolution/mass accuracy instruments such as those utilizing orbitrap mass analyzers deliver superior confidence in compound characterization. The flexibility of our drift tube configuration allows additional modification of drift gases for fine tuning difficult separations, as well as for the introduction of dopant materials that may alter/promote ionization chemistry or ion transport processes.

Due to the slow ion injection/scan events of ion trap mass spectrometers (ITMS), dual gate ion mobility devices provide an effective means by which mobility-mass experiments can be accomplished. Ions can be targeted or preselected based upon their unique mobilities, although scanning the delay in the opening of the exit gate produces comprehensive correlated mobility-mass datasets. As this technique only transfers a relatively small portion of ions into the ITMS due to the low combined duty cycle of the two gates, we have developed a multiplexed method utilizing pseudo-random-binary-sequence (PRBS) modulation to significantly increase the duty cycle of our dual gate IMS. Full mobility period scan functionality can be accomplished on a 2 to 5 second time scale with this multiplexed modulation technique. This approach is shown to be quite useful in applications where mobility-mass analysis is required during the presence of a transient analyte signal, as often encountered in thermal desorption/ionization experiments.

(403) **Nanoscale Chemical Imaging of Phase-Separated Polymer Systems and Organic-Inorganic Films**; Mark Rickard[1], Gregory Meyers[1], Carl Reinhardt[1], Jamie Stanley[1]; [1]The Dow Chemical Company

Atomic force microscopy coupled to infrared spectroscopy (AFM-IR) is a relatively new technology that provides the spatial resolution of AFM with chemical information from IR spectroscopy. It has the potential to connect the nanoscale chemical structure of a material to its macroscopic properties and performance. AFM-IR is based on the rapid thermal expansion of a material, which is detected by an AFM tip, when it absorbs IR light. This approach provides spatially-resolved chemical information at length scales of < 50 nm, which greatly exceed the diffraction limit of light.

Applications of AFM-IR to phase-separated polymer systems and hybrid organic-inorganic films are described. For polyurethane systems, AFM-IR enabled direct characterization of the soft and hard segments. In a polyether-epoxy thermoset with spherical phase-separated morphology, AFM-IR revealed that the spherical phase contained more epoxy functionality and that the matrix contained more polyether functionality. The relative compositions of these phases were estimated from AFM-IR spectra and images. In addition, AFM-IR was used to characterize a hybrid organic-inorganic film.

(404) **NanoMineralogy of Extraterrestrial Samples Using AFM-tip Enhanced Infrared Spectroscopy**; Gerardo Dominguez[1], Alex McLeod[2], Zack Gainsforth[3], Priscilla Kelly[2], Fritz Keilmann[4], Andrew Westphal[3],Mark Thiemens[2], Dimitri Basov[2]; [1]California State University, San Marcos; [2]University of California, San Diego; [3]University of California, Berkeley; [4]Ludwig-Maximilians-Universität and Center for Nanoscience

For decades cosmochemists have used increasingly sophisticated instrumentation for the analysis of primitive planetary materials in order to piece together the formation history of our solar system as well as the origins of life itself. The successful return of cometary and interstellar dust grains by NASA's Stardust Mission in 2006 inaugurated a new era in planetary science and more sample return missions to several solar system bodies are expected in the years to come. A key advantage of sample return missions over remote astronomical observations and conventional spacecraft missions is that samples of extraterrestrial objects, even microscopic ones, can be analyzed with the most sophisticated analytical instrumentation available on the planet. However, the heterogeneous and submicron nature of sample return mission samples make their complete analysis challenging.

Several mature techniques exist that are capable of mapping the isotopic, elemental, and in some cases the functional group composition of solid samples with submicron spatial resolutions. Infrared spectroscopy, a powerful analytical technique sensitive to the nature of chemical bonds in samples, has traditionally suffered from an inability to resolve spectral contrast below the wavelength of infrared light. The maturation of AFM-tip based near-field imaging has now made it possible to perform infrared spectral imaging of samples with sub-micron spatial resolutions. Here we show how AFM-based infrared spectroscopy, made possible using a commercial NanoFTIR implementation (Neaspec) together with spectral deconvolution techniques we have developed, can be used to quantitatively analyze the spatial distribution of inorganic and organic functional groups in primitive meteorites and individual cometary dust grains. The results of infrared spectral scanning are corroborated using SEM and XANES and highlight the great potential that AFM-tip based NanoFTIR has in performing nano-mineralogy of natural samples and for understanding the origins of prebiotic molecules in the solar system.

(405) **Looking Inside Single Cells and Tissue using Nanoscale Infrared Spectroscopy**; Curtis Marcott[1], Eoghan Dillon[2], Qichi Hu[2], Kevin Kjoller[2]; [1]Light Light Soluitons; [2]Anasys Instruments

Infrared (IR) spectroscopy is a powerful tool for obtaining chemical information about materials. Unfortunately, the wavelength of light used to make the measurement limits the size of structures that can be reliably identified by IR spectroscopy. Diffraction typically limits the spatial resolution of IR microspectroscopy to 3-10 μm, making this technique problematic for identifying small structures in samples such as tissue sections and single cells. Atomic force microscopy (AFM), on the other hand, provides exquisite spatial resolution (as small as one nanometer), but this technique does not provide any chemical information. A new technique which combines AFM and IR spectroscopy is described. It is based on the combination of a tunable infrared laser with an atomic force microscope that can locally map and measure thermal expansion of nanoscale regions of a sample resulting from the absorption of infrared radiation. Because the AFM probe tip can map the thermal expansion on very fine length scales, the AFM-IR technique provides a robust way to obtain interpretable IR absorption spectra at spatial resolution scales well below the diffraction limit. Several types of tissue samples, including hair, skin, and bone cross sections have been examined by AFM-IR spectroscopy and imaging. The technique has also been shown to be useful for chemically characterizing structures inside single cells and for identifying secondary structure and orientation of sub-micrometer-diameter protein fibers.

(406) **AFM-IR Studies of Individual Electrospun Nanofibers: Structural Analysis and Mapping of Poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (PHBHx) Fibers**; Liang Gong[1], D. Bruce Chase[1], Isao Noda[1,2], C.J. McBrin[1], John Rabolt[1]; [1]Department of Materials Science and Engineering, University of Delaware; [2]Meredian Bioplastics

The combination of atomic force microscopy (AFM) and infrared (IR) spectroscopy is a powerful tool that provides chemical, conformational and molecular orientation information at a spatial resolution of 50nm. Using an AFM-IR instrument, we have explored the correlation between structure, processing and chain orientation/crystallinity and tested the hypothesis that improved chain orientation in electrospun nanofibers can increase modulus and tenacity even while crystallinity is disrupted by appropriate addition of longer side-chain comonomer compositions. Nanofibers and thin films of poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (PHBHx) have been produced with a wide range of crystallinity and molecular orientation. They have been characterized using polarized AFM-IR spectroscopy and imaging in order to assess the role of processing on orientation and morphology. This paper will highlight the use of AFM-IR spectroscopy and imaging to characterize orientation and crystallinity changes as a function of side-chain comonomer concentration and illustrate the importance of obtaining rapid spatial spectroscopic images in inhomogeneous materials.

(407) **Photothermal AFM-IR of Bacteria – Polyurethane Bilayers: Impact of Local Sample – Cantilever Damping on Quantitative IR Measurements**; Daniel Barlow[1], Justin Biffinger[1], Allison Cockrell[3], Michael Lo[2], Kevin Kjoller[2], Debra Cook[2],Woo Kyung Lee[1], Pehr Pehrrson[1], Wendy Goodson[4], John Russell[1]; [1]Chemistry Division, Naval Research Lab; [2]Anasys Instruments; [3]Nova Research, Inc.; [4]Nanostructured & Biological Materials Branch, Materials & Manufacturing Directorate, Air Force Research Laboratory

Exposure of synthetic polymers to microbial and other biological environments can result in chemical degradation, often by enzymatic activity. Quantitative assays are important to characterize degradation mechanisms and accurately correlate relationships with environmentally dependent microbial physiology. For microbial degradation of polyurethane films, conventional FTIR microscopy has been previously applied in quantitative assays with micron – scale spatial resolution. Photothermal AFM-IR offers the potential to extend this analysis to the nanoscale, allowing early degradation processes and mechanisms relative to single microbes to be quantified. As a first step towards this, we have used AFM-IR to characterize a known polyurethane degrading microbe (Pseudomonas fluorescens, Pf01) grown on films of a polyether – polyurethane (PU) formulation known to resist enzymatic degradation. This allowed us to conduct preliminary AFM-IR assessments with a relevant microbe and polymer, but without additional complications from biodegradation. Height images of air-dry samples showed the growth procedure in liquid media resulted in monolayer Pf01 biofilm clusters on top of the ~250 nm PU layer, providing a conducive model system for AFM-IR in an ATR configuration. Both bacteria and PU spectral signatures were detectable by AFM-IR spectroscopy and showed generally good agreement with FTIR. However, PU AFM-IR absorption intensities were observed up to 2x higher in regions covered by dried bacteria, versus uncovered regions, even though the PU thickness was uniform over the substratum. This was due to damping variations which were reflected in the cantilever ring-down and attributed to differences in loss modulus and tip – sample adhesion for the two materials. This shows that local cantilever damping can be an important property to assess in AFM-IR analysis of combined biological / polymer samples, a factor that has received little attention thus far. Analysis of the cantilever ring-down will be discussed regarding extraction of damping parameters for normalization of the IR signal.

(408) **Miniaturized Raman Spectrometers for Space Applications: The Detectability of Biomarkers in Geological Matrices Relevant to Mars Exploration**; Cedric Malherbe[1], Melissa Mchugh[1], Ian B. Hutchinson[1], Richard Ingley[1], Howell G. M. Edwards[1]; [1]University of Leicester, Department of Physics and Astronomy

A Raman spectrometer will be utilized on a planetary exploration mission for the first time when the ExoMars rover is launched in 2018. A Raman spectroscopy instrument was selected for inclusion on the ExoMars mission because of its ability to assess the habitability and to search for evidence of life on Mars. Indeed Raman spectrometers have the capability to detect geological substances which are inorganic molecules and inorganic molecular ions constituting the rocky surface of Mars. Information on the nature of rocks present on the surface of Mars will provide information about the habitability of the planet. Besides the detection of geomarkers, Raman spectrometers have the ability to detecting potential biological-derivative substances, often referred to as biomarkers. These biomarkers are organic molecules originating from extent or extinct living organisms. In preparation for ExoMars, it is crucial to study the detection capability of miniaturized Raman spectrometers (specifically

developed for space missions and therefore compromised by the associated challenging constraints such as minimal power budget, mass budget, data budget and overall envelope) on both lab synthetic samples and natural terrestrial analogues samples.

We present here a systematic comparison of the capability of a number of Raman spectrometer designs/configurations to detect biomarkers in geological matrices that are relevant to the Martian surface and subsurface. In particular, we will compare spectral images recorded with benchtop instruments and a Raman Laser Spectrometer prototype developed at the University of Leicester to optimize/characterize the camera system that will be used for the ExoMars mission. Several parameters such as the excitation wavelength, the number of spectra recorded per sample and selection of optimal operating modes will be discussed. As an example, the habitability of desert varnish in the context of planetary exploration will be discussed in detail. Desert varnish are mineral coatings comprising clay, iron oxide and manganese oxide which are associated with living organisms in many stable extreme environment on Earth. Desert varnish are recognized as terrestrial analogue samples for martian surface because similar mineral formation have been recently identified on Mars.

(409) **Sampling and Preconcentration of Volatile Organic Compounds Using Capillary Microectraction of Volatiles (CMV)**; Natasha Kreitals[1], Anamary Tarifa[1], Dnisha Hamblin[1], Jose Almirall[1]; [1]Florida International University

The analysis of volatile organic compounds (VOCs) in air samples has applications across a wide range of scientific fields. For example, monitoring of air pollutants in indoor air and outdoor environments is an important aspect of health and safety management. The analysis of headspace is also common practice in forensic chemistry, particularly for the detection of ignitable liquid residues from fire debris evidence. As VOC biomarkers are being discovered in breath samples of patients with medical conditions, the analysis of breath samples as a medical diagnosis tool may also become practice in the future. This paper will outline a novel sample collection and preconcentration device for the analysis of VOCs from air samples. The device known as a capillary microextractor of volatiles (CMV) is a new geometry of PSPME [1-4] and consists of a 2 cm glass capillary tube filled with a sorbent material. The CMV allows for dynamic sampling of VOCs, and has been designed to couple to a thermal desorption probe of a GC inlet. With an increase in surface area of 5000 times compared to a SPME fiber [5-6], the CMV offers improved capacity over the static sampling method of SPME. Further, the device offers a reduced cost compared to currently available sorbent tubes. The efficacy of VOC sampling using CMV will be demonstrated for a range of applications including detection of air pollutants, detection of nicotine in breath, and detection of ignitable liquid residues.

1. Patent US 8,668,873 B2 Awarded March 11, 2014 (Disclosed 2008)

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(410) **Sample Delivery of Biphasic Droplets Containing Protein Crystals for Serial Femtosecond Crystallography with an X-Ray Free Electron Laser**; Austin Echelmeier[1], Garrett Nelson[1], Bahige G. Abdallah[1], Uwe Weierstall[1], John C. H. Spence[1], Petra Fromme[1], Alexandra Ros[1]; [1]Arizona State University

We propose the utilization of aqueous droplets as an efficient method for protein crystal delivery into an X-ray Free Electron Laser (XFEL) for structure determination by serial femtosecond crystallography (SFX). SFX uses XFEL pulses short enough to outrun X-ray damage and attain diffraction from smaller crystals grown from complex proteins that cannot grow crystals large enough for traditional crystallography methods employing damage-inducing X-ray exposure durations. Thus, it becomes possible to determine the structure of small crystals, which possess fewer crystal lattice defects than larger crystals, of many poorly studied complex proteins with important biological functions in undamaged conformations. One limitation of this novel crystallography approach is the large volume of crystal suspension needed to obtain full data sets allowing structure determination with current data analysis methods. The current method of continuous sample injection into an XFEL requires milliliters of sample and wastes the majority of the protein crystals grown under resource-consuming conditions. Our method of delivering droplets containing protein crystals at a frequency matching that of the XFEL pulses reduces sample waste and would require about three orders of magnitude less sample volume to obtain a full crystallography data set.

We designed a microfluidic droplet generator capable of delivering aqueous droplets in an oil phase to reduce aqueous crystal suspension consumption. To introduce droplets into the XFEL, the droplet generator needs to be interfaced with an injector. With the current gas dynamic virtual nozzle (GDVN) injector, interfacing is accomplished via a fused silica capillary. We have successfully coupled a capillary to our microchip device, and droplets of both a fluorescent dye and photosystem I (PSI) protein crystals entered the coupled capillary, demonstrating the viability of droplets outside of the microchip environment for coupling to the downstream injector. The PSI crystal droplet generation frequency reached ~20Hz, with droplet volumes as low as 8-10 nL. Both aqueous and oil jets were produced using a GDVN, indicating the feasibility of the biphasic droplet delivery stream. We are further developing droplet generation conditions to match the current 120 Hz frequency of the XFEL laser to provide an efficient method of sample introduction for SFX.

(411) **Electrokinetic Biomarker Enrichment in Physiological Media by Coupling Dielectrophoresis with Ion Conductivity Gradients in Nanoslits**; Nathan Swami[1], Ali Rohani[1], Walter Varhue[1], Kuo-Tang Liao[2], Chia-Fu Chou[2]; [1]Electrical Engineering, University of Virginia; [2]Institute of Physics, Academia Sinica, Taiwan

The creation of an enriched plug of rare biomarkers is of great interest since biomarkers can be present within bio-fluids at billion-fold lower levels than common interfering proteins. A common methodology for achieving highly enriched analyte plugs from dilute samples is through applying an electrokinetic force balance in conjunction with ion conductivity gradients to create a trap due to the sharp spatial profile in the field. Typically, ion concentration polarization (ICP) effects at perm-selective nanochannels are used to cause sharp field gradients due to ion depletion at the anodic interface of microchannel to the nanochannel, and this is balanced against electro-osmosis within the microchannel to cause exceptionally high degrees of biomarker enrichment. However, the degree of biomarker enrichment can fall sharply within physiological media of high conductivity, due to a reduction in electro-osmosis and ICP effects. An alternate strategy is to generate the ion conductivity gradient

inside the nanochannel, rather than these prior methods that create ICP effects at the microchannel interface to the nanochannel. Herein, we achieve this by utilizing lateral constrictions of high surface charge non-uniformity inside a nanoslit channel for initiating ion depletion and ion accumulation regions under low levels of DC field (1.5 V/cm). The ensuing ion conductivity gradients are coupled with AC fields (~70 Vrms/cm at 1-6 MHz) at a critical frequency to enhance the degree and extent of negative dielectrophoresis for ultrafast biomarker enrichment within physiological media, since negative dielectrophoresis continues to be a significant force field within media of high conductivity. This presentation is focused on optimizing the device geometry, media conductivity, and applied field conditions to enhance the spatial extent for biomarker electrophoresis along the length of the nanoslit, as well as cause its localized frequency-selective trapping by negative dielectrophoresis for enabling biomarker enrichment in applications such as biomarker discovery, protein crystallization and for speeding binding kinetics of biosensors.

(412) **Using Pyrolyzed Paper for Electrochemical Detection in Microfluidic Paper-Based Analytical Devices**; Carlos Garcia[2], Elizabeth Evans[1], Jason Giuliani[1], Gema Duran[3], Angel Rios[3], Tomas Benavidez[1]; [1]UT San Antonio; [2]Clemson University; [3]University of Castilla-La Mancha

Aside from traditional examples such as glassy carbon and mesoporous carbon, carbon-based electrodes share a common structure composed of sp2-bonded atoms that supports electrical conductivity, the capacity to form charge-transfer complexes, unique optical properties, chemical reactivity, and the possibility to interact with a variety of biorecognition elements. Alternative materials such as carbon ink or pyrolytic carbon have the potential to enable operational electrochemical sensors, most of them offer limited surface area and their application is restricted by the resistivity of the material. To address these deficiencies, we report the possibility to prepare electrodes by pyrolysis of paper. The resulting electrodes, which can be fabricated from a variety of substrates, feature low resistivity, large surface area, and uniform thickness. The unique characteristics of the material allow its use for the development of biosensors by simply immersing them in a solution containing a selected enzyme or their modification by electrochemical means. More importantly, the material presents ideal characteristics for the integration of these electrodes to microfluidic paper-based analytical devices (µPADs).

(413) **Using LIBS to Determine Ground Water Quality Changes Due to Subsurface Activities**; Dustin McIntyre[1], Christian Goueguel[3], Cantwell Carson[3], Herve Sanghapi[3], Jinesh Jain[2]; [1]USDOE NETL; [2]AECOM/URS; [3]ORISE

The injection of CO2 into deep aquifers has the potential to affect the quality of groundwater supplies if leakage were to occur by adding contamination from the injection formation or fluids. Therefore, the detection of CO2 and/or entrained contaminants that migrate into shallow groundwater aquifers is important both to assess storage permanence and to evaluate impacts on water resources. Naturally occurring elements (i.e., Li, Sr) in conjunction with isotope ratios can be used to detect such leakage. We propose the use of laser induced breakdown spectroscopy (LIBS) as an analytical technique to detect a suite of elements in water samples. LIBS has a real time monitoring capability and can be applied for elemental and isotopic analysis of solid, liquid, and gas samples. The flexibility of probe design and use of fiber optics make it a suitable technique for real time measurements in harsh conditions and in hard to reach places. The laboratory scale experiments to measure Li, K, Ca, and Sr composition of water samples indicate that the technique produces rapid and reliable data. Since CO2 leak from saline aquifers may accompany brine solution we studied the effect of sodium salts on the accuracy of LIBS analysis. In addition to ground water, the applicability of the technique to issues with injection well leakage, monitoring wells, abandoned well location and monitoring, location and mitigation of improperly completed wells in the basin as well as bore holes with failed seals and wells will be discussed. This work specifically details the fabrication and application of a miniature ruggedized remotely operated diode pumped solid state passively Q-switched laser system for use as the plasma excitation source for a real time LIBS analysis. This work also proposes the optical distribution of many laser spark sources across a wide area for widespread leak detection and basin monitoring.

(414) **Analysis of Bakery and Dairy Products by Laser Induced Breakdown Spectroscopy**; Kemal Eseller[1], Gonca Bilge[2], İsmail Boyaci[2]; [1]Atilim University; [2]Hacettepe University

Laser induced breakdown spectroscopy (LIBS) is a rapid optical spectroscopy technique for elemental determination. Bakery products have been analyzed both quantitatively and qualitatively. The calibration involving atomic emission intensity and sample concentration, is still a challenge due to physical-chemical matrix effect of samples and fluctuations of experimental parameters. To overcome these problems, various chemometric data analysis techniques have been combined with LIBS technique. In this study, LIBS was used to show its potential as a routine analysis for Na measurements in bakery products. A series of standard bread samples containing various concentrations of NaCl (0.025%–3.5%) was prepared to compare different calibration techniques. Standard calibration curve (SCC), artificial neural network (ANN) and partial least square (PLS) techniques were used as calibration strategies. Among them, PLS was found to be more efficient for predicting the Na concentrations in bakery products with an increase in coefficient of determination value from 0.961 to 0.999 for standard bread samples and from 0.788 to 0.943 for commercial products.

To quantify of adulterated milk powder with adding whey powder, a rapid and in-situ method has been developed by using laser induced breakdown spectroscopy (LIBS). This method is which is based on elemental composition differences between milk and whey products. Milk samples were collected from five different sources and milk powder, sweet and acid whey powders were produced as standard samples. Milk powder was adulterated with sweet and acid whey at different ratios. Based on the LIBS spectra of standard samples and commercial products, identification was occurred with Principal Component Analysis (PCA) method which showed a good discrimination of milk, whey and demineralized whey powders. Partial Least Squares regression (PLS) quantification method has been applied. PLS analysis the correlation coefficient (R2) and root mean square error of prediction (RMSEP) in the best model were obtain for adulteration with sweet whey. Mineral compositions of the milk products were supported with Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) method.

A new method development for ash analysis has been in wheat flour by using laser induced breakdown spectroscopy (LIBS). Ash content is a measure of the total amount of minerals in flour. It is important for a number of reasons such as quality parameter, nutritional labeling, nutrition, processing and microbiological stability. For this reasons ash analysis is a significant routine analysis. In this study ash analysis in wheat flour was performed by LIBS which is multi elemental spectroscopy method as a quick and simple method. Wheat flour having different ash contents were analyzed by LIBS and spectra were evaluated with partial least square (PLS) method. Results were correlated with reference ash analysis method's results. Calibration graph showed good linearity between ash contents with correlation coefficient (R2). Limit of detection for ash analysis was calculated and results showed that LIBS is a potential reliable method for routine ash analysis in flour.

(415) **LIBS Analysis of Plant Samples – Advantages and Limitations**; Jozef Kaiser[1], Jan Novotný[1], David Prochazka[1], Pavel Pořízka[1], Aleš Hrdlička[1], Karel Novotný[1]; [1]Brno University of Technology, CEITEC - Central European Institute of Technology, Technicka 3058/10, 616 00 Brno, Czech Republic

Laser-Induced Breakdown Spectroscopy (LIBS) is an established analytical technique based on spectroscopic analysis of radiation, which is emitted by a micro-plasma induced on the analyte surface by a laser pulse [1]. LIBS in comparison to another advanced analytical techniques is cost-effective. LIBS can be used without or with a limited sample preparation for analysis of samples in any state of matter. It allows analysis practically in real-time and in-situ. This can be advantageous, when e.g. the use of another advanced laboratory-based techniques based on wet digestion of the samples, as inductively coupled plasma optical emission or mass spectrometry (ICP-OES/MS), due to their long turnaround times and analytical cost per sample is not suitable. Another advantage of LIBS is that it allows depth-profiling. Elemental analysis with appropriate depth resolution for selected samples using another advanced analytical techniques as e.g. X-ray fluorescence (XRF) can be problematic. If it is requested, remote or stand-off sensing [2] and elemental mapping [1] using LIBS is also possible. On the other hand, quantitative analysis using LIBS is not straightforward [1, 2]. Here we report on advantages and limitations of using LIBS for analysis of plant samples with a special emphasis on high-resolution elemental mapping of selected plant compartments.

Acknowledgements

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(416) **Laser-Induced Breakdown Spectroscopy: Application to Nuclear Waste Management**; Jagdish Singh[2], Fang Yu Yueh[1]; [1]Institute for Clean Energy Technology, Mississippi State University; [2]JPS Advanced Technology R&D LLC

This presentation will describe the experimental conditions associated with the slurry measurement to achieve good precision of the laser induced breakdown spectroscopy (LIBS) measurement. The goal of this project is to assist the Defense Waste Processing Facility (DWPF) at the Savannah River Site (SRS) in accelerating DWPF melter operations. The capability of direct analysis of slurry will significantly increase analytical throughput and will reduce waste generation in radiological analytical facilities, providing analyses suitable for waste acceptance and production records. Laser Induced Breakdown Spectroscopy (LIBS) will serve as the basis for the system for direct analysis of DWPF Sludge Receipt and Adjustment Tank (SRAT) product (slurry consisting of only sludge or waste). The analytical results will be used to support the determination of the appropriate amount of frit to be combined with the sludge in the melter. The main issues to be resolved with LIBS analysis of liquid samples are poor detection sensitivity and precision. Because water can quench the laser plasma and suppresses the LIBS signal, poor sensitivity may result. Large standard deviations for LIBS liquid data are due to the laser induced shock wave caused by turbulence on the liquid surface. Slurry samples contain a large amount of water and large particle sizes. The effects of water content and particle sizes on LIBS measurement will need to be studied. To improve LIBS' reproducibility and detection limits for slurry measurements, various experimental parameters which can affect LIBS' analytical figure of merit were studied. The study shows that by using the appropriate slurry sample handling systems, optimum experimental parameters and some data processing techniques, reasonable accuracy and precision (below 5%) based on 2.5% error confidence for the major elements can be achieved. For minor elements, we found the accuracy and precision are poorer (about 10-15%), Current work demonstrates that LIBS has the potential to be developed for on-line analysis and control of DOE wastes slurry processing. However, further work on improving signal sensitivity and data reproducibility is needed.

(417) **LIBS to the Extreme: High-dose Radiochemical Analyses where ICP Methods Cannot Follow**; Rodger Martin[1], Tom Hylton[1]; [1]Oak Ridge National Laboratory

The hazards of concentrated high-dose radiochemical process solutions pose unique challenges for quantitative analysis. Massive (million-fold) sample dilutions are required to reduce the radiological content of milliliter-sized samples for transfer from hot cell containment to glove boxes or radiological hoods for hands-on work. These dilutions seriously impact the quantitative analysis of impurities using ICP-based methods.

Previous quantification of beryllium impurity content in concentrated plutonium-238 process solutions is presented. After dilution, direct ICP-MS analysis can only determine Be content to some order-of-magnitude "less than" value. Two ongoing projects at Oak Ridge National Laboratory require the radiochemical separation of plutonium, primarily the Pu-238 isotope, from other actinide and nonactinide constituents. One intractable, legacy disposition project requires the dissolution and separation of solid plutonium−beryllium neutron source material to eliminate the intense neutron dose. Another USDOE project (the Pu-238 Supply Project) produces Pu-238 from in-reactor irradiation of neptunium-237, followed by Pu-238 purification for use by NASA as heat and power sources for interplanetary missions. Information on Be impurity levels within the Pu component is required for both projects.

For neutron source disposition, the ion exchange (IX) separation process reduces residual Be in the Pu solution from multi-gram to milligram levels. Re-use of Pu-238 requires quantification down to ppm by weight or to microgram levels. Industrial hygiene (IH) concerns of personnel exposure to Be require submicrogram measurement. For quantification at these levels, ICP-MS requires an additional step within the hot cell that strips out Pu-238 from the solution before analysis, but at added cost, effort, radioactive waste, and analytical uncertainty.

The results of ICP-MS analyses of Be and other trace impurities are presented. A review of past and present LIBS measurements of Be to nanogram levels is presented. Potential LIBS microsampling of Pu-Be separations without sample dilution is discussed and compared to earlier LIBS results for a concentrated process sample. Future LIBS use in IH-driven monitoring of Be contamination within the hot cells will be compared to existing analyses and to current needs in nuclear waste characterization and nuclear materials monitoring.

(418) **Transmission Raman Analysis of Bilayered Tablets**; Gary McGeorge[1], Yan Zhang[1]; [1]Bristol-Myers Squibb

Transmission Raman Spectroscopy (TRS) has become an increasingly applied technology in the analysis of pharmaceutical tablets for quality control purposes and developing formulation and process understanding. One area that has received only cursory attention to date is that of

bilayered tablets that represents an unusually challenging situation. A bilayered tablet is composed of two discretely unique formulations that are sequentially compressed, one after the other to create a layered composite tablet. Traditional absorption techniques are unable to quantitatively assess the tablet composition without imposing constraints on several of the tablets manufacturing variables.

This presentation will provide an understanding of the relationship of the API content and the transmission Raman spectral response in bilayered pharmaceutical tablets to facilitate development of quantitative models for the prediction of API content in bilayer tablets. The Raman intensity was considered as a function of the Raman photon generation and decay in a layer of interest and Raman photon decay from a second layer. To separate and understand the various contributions a variety of tablet configurations were studied and it was found that 1) with increasing the thickness of the non-API layer, the API Raman signal displayed an exponential decay as a function of the non-API layer thickness as well as the total tablet thickness; 2) when only changing API concentration, the Raman signal linearly responds to the API content; 3) when increasing the weight/thickness of the API layer and keeping the non-API layer constant, the Raman signal reaches a maximum at a particular thickness and then decays as tablets become thicker. The complex spectral response was effectively modeled according to a modified Schrader, Kubela-Munk model where both the Raman photon generation factor and photon losses were accounted for. Coupling the results of these studies together yields a comprehensive approach for modelling multi-component bilayer tablets. The addition of a beam enhancer on the bottom surface allowed for a selective over-enhancement of the bottom layer, which helps in the analysis of thin layers or coatings.

(419) **PAT Methods Development for the Pharmaceutical Industry**; Carl Anderson[1]; [1]Duquesne University

TBA

(420) **A NIR In-Process Control Method for Determination of API Concentration in Tablets Manufactured by a Continuous Process**; Frank Qi[1]; [1]Vertex

TBA

(421) **Monitoring Drying Performance of Pharmaceutical API by Raman Spectroscopy and Mass Spectrometry**; Ming Huang[1], Daniel Hsieh[1], Robert Wethman[1], John Wasylyk[1]; [1]Bristol-Myers Squibb Co.

The powder properties and morphology of active pharmaceutical ingredients (API) can be directly influenced by the drying protocol. During the pharmaceutical development cycle of API, we have used an integrated balance combined with mass spectrometry and Raman spectroscopy to collect information about weight loss, vapor composition and spectral changes in the API during the drying process. These changes are correlated to elucidate the drying mechanism and the structure changes upon drying. The experimental results are then compared with the theoretical approach including the use of diffusion model to predict the drying time as a function of temperatures. A proper understanding of the drying process led to the production of correct form, shorter drying time, improved particle size distribution and better powder property. The finalized drying protocol ensures consistent quality product throughout the manufacturing lifecycle. Case study using this approach will be presented.

(422) **To Find Needles in Haystacks, Use a Metal Detector; Pharmaceutical Materials Analysis by Nonlinear Optical Stokes Ellipsometry**; Garth Simpson[1], Paul Schmitt[1], Niraj Trasi[1], Lynne Taylor[1]; [1]Purdue University

The crystal form of an active pharmaceutical ingredient can profoundly impact both stability and bioavailability, with the need for new tools for rapidly screening new crystal forms. In this work, second harmonic generation (SHG) microscopy, based on the frequency doubling of light, is proposed for quantitative evaluation of the crystalline state of APIs, targeting applications in high-throughput screening and process monitoring. SHG is highly selective for chiral crystals, providing no significant background form disordered materials. The polarization dependence of SHG is directly connected to both the crystal orientation within the field of view and the local nonlinear optical properties of the crystals. Mathematical algorithms are described for disentagling these two contributions and recovering the local SHG tensor for each crystallite, which is now dependent only on crystal form. The combinations of tensor elements as measured by nonlinear optical Stokes ellipsometry can serve as a first-pass evaluation to identify new crystal forms. This approach has several key figures of merit: i) measurements can be performed at up to video-rate, consistent with the timeframes required for PAT and high-throughput screening, ii) image contrast is high, with signal produced only from crystalline material facilitating automated image analysis algorithms, iii) particle size distributions naturally emerge from the analysis to help inform kinetics analyses.

(423) **Chip Based Raman Analytics of Body Fluids**; Juergen Popp[1,2]; [1]Leibniz Institute of Photonic Technology; [2]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University Jena

Raman spectroscopy is an especially efficient analytical method since it probes molecular vibrations distinct for each type of molecule. A Raman spectrum can be seen as a characteristic “molecular fingerprint” of every sample. The ability to obtain specific chemical information labelfree makes Raman spectroscopy attractive for many clinical investigations of bodily fluids, pathogens, cells, and tissue biopsies. In this context chip-based sampling methods offering the opportunity to handle small sample volumes and to apply sample preparation steps. In this contribution, we will summarize our recent results in implementing Raman spectroscopy in chip-based sampling approaches. It has been recently shown that Raman spectroscopy in combination with innovative chemometric data analysis strategies is a powerful microbial analysis tool i.e. permits the quick identification of single bacteria cells without the need of time-consuming cultivation steps. Many application areas require fast and reliable pathogen diagnostics. We will show that this Raman identification approach is of great relevance for water-, air- and soil monitoring (e.g. identification of anthrax endospores embedded in complex matrices), for food analysis and most importantly for an early diagnosis and therapy of infectious diseases. Before Raman spectroscopy can be used to identify pathogens, they have to be isolated from the sample matrix (e.g. blood or other body fluids), which poses a great challenge. Here, we introduce innovative chip-based bacterial isolation strategies out of complex sample matrices (e.g. blood or urine) like e.g. a dielectrophoresis (DEP) Raman setup for the fast identification of pathogens from the urinary tract by capturing bacteria directly from dilute suspensions in microstructured regions using spatial non-uniform electric fields. By using this DEP Raman chip, only minimal sample preparation is required to apply the patient’s urine sample to the chip. Moreover, we report about the identification of circulating tumor cells by coupling Raman spectroscopy with microfluidics and micromanipulation approaches. In the last part we present lab-on-a-chip (LOC) Surface enhanced Raman spectroscopy (SERS), which is of particular interest for therapeutic drug monitoring together with their metabolites. Such a LOC-SERS approach has been used to determine the enzyme activity in lysed red blood cells.

(424) **Developing Deep UV Raman Standoff Spectrometer for Trace Explosives**; Sanford Asher[1], Sergei Bykov[1], Katie Gares[1], Kyle Hufziger[1]; [1]University of Pittsburgh

We are developing a deep UV resonance Raman standoff spectrometer to detect trace explosives. For this effort we are developing novel compact lasers, ultrahigh efficient spectrometers and imaging spectrometers. We are developing methodologies to detect explosives at standoff distances greater than 2 m. We are exploring the spectroscopic signatures of explosives as well as their photochemistry, which gives rise to specific dynamic spectral signatures.

(425) **An Application of SERS in Forensics: Hair Dyes**; Dmitry Kurouski[1], Richard Van Duyne[1]; [1]Northwestern University

Hair is one of the most common types of physical evidence found at a crime scene. Forensic examination may suggest a connection between a suspect and a crime scene or victim, or demonstrate an absence of such associations. Therefore, forensic analysis of hair evidence is invaluable to criminal investigations. Current hair forensic examinations are primarily based on a subjective microscopic comparison of hair found at the crime scene with a sample of suspect's hair. Since this is often inconclusive, development of alternative and more accurate hair analysis techniques are critical. We demonstrate that surface-enhanced Raman spectroscopy (SERS) can be used to detect the artificial dyes directly on hair. This spectroscopic technique is capable of a confirmatory identification of analytes with single molecule resolution, requires minimal sample, and has the advantage of fluorescence quenching. We shown that SERS can (1) identify whether hair was artificially dyed or not, (2) determine if a permanent or semi-permanent colorants were used, and (3) distinguish the commercial brands that were utilized to dye hair. Such analysis is rapid, minimally destructive, and can be performed directly at the crime scene. This discovery provides a novel perspective of forensic investigations of hair evidence.

(426) **Discrimination of Animal and Human Blood Using Raman Spectroscopy and Chemometrics**; Kyle C. Doty[1], Gregory McLaughlin[1], Igor K. Lednev[1]; [1]University at Albany, SUNY

The species identification of a blood stain is an important and immediate challenge for forensic science, veterinary purposes, and wildlife preservation. In particular, determining the origin of a bloodstain is a critical, yet overlooked, step in establishing its relevance to the crime. For identifying a stain as blood, a variety of presumptive and confirmatory tests exist. However, most of these tests are destructive to the sample and some presumptive tests can react with various substances, including blood from certain animals, to provide a false positive result. It is ultimately preferable to confirm the presence of blood, and the species of origin, before forensic DNA profiling. But this can be practically challenging because only a presumptive test, if any, is typically used for bloodstain identification. Therefore, time and money is wasted on analyzing nonhuman and non-blood samples and this should be avoided. Previously we showed that Raman spectroscopy can be used to reliably differentiate dried blood traces from three species: human, cat, and dog. We have since expanded upon that work to include a wider survey of animal species. This current study demonstrates that statistical analysis of near infrared Raman spectroscopic data can be effectively applied as a nondestructive technique for differentiating dried blood traces of humans (varying in age, race/ethnicity, and gender) from eleven different animal species. The methodology for differentiation, utilizing partial least squares-discriminant analysis (PLS-DA), is two-fold: human blood was distinguished from animal blood (I.) in a binary manner (i.e. human vs. animal) and (II.) for individual species (i.e. species specific). The developed approach does not require the knowledge of a specific (bio)chemical marker for each individual species class, but instead relies on a spectroscopic statistical differentiation of various components. A blind test and an external validation were used to confirm the discriminatory performance of both PLS-DA models. This approach resulted in remarkable classification ability even with intrinsically heterogeneous classes and samples. These findings further demonstrate great potential of using Raman spectroscopy in forensic serology as a nondestructive, rapid, and confirmatory method for species identification of a suspected bloodstain.

(427) **Forensic Analyses by Morphologically Directed Raman Spectroscopy**; Brooke Kammrath[1], Andrew Koutrakos[1, 2], Josemar Castillo[3], Joe Wolfgang[3], Deborah Huck-Jones[4]; [1]Henry C. Lee College of Criminal Justice and Forensic Sciences, Dept of Forensic Science, University of New Haven; [2]University of Verona; [3]Malvern Instruments Inc.; [4]Malvern Instruments Ltd

Morphologically Directed Raman Spectroscopy (MDRS) is a novel and reliable tool that can be applied to a variety of forensic evidence types, such as illicit drugs, counterfeit pharmaceuticals, hoax powders, soils and gunshot residues. It has the ability to provide criminalists with more information from forensic samples than is currently employed for investigations and adjudications. MDRS combines automated particle imaging and Raman spectroscopy into one instrument. Particle imaging is performed to determine particle size and shape distributions of components in a blended sample. Particle size is an important physical property of particulate samples because it has a direct influence on a variety of material properties such as reactivity or dissolution rate, suspension stability, efficacy of delivery, texture, feel, appearance, flowability, handling, viscosity, packing density and porosity that could be invaluable in the discrimination of two samples or the detection of excipient particles. Although not commonly employed in the forensic sciences, the measurement of particle size distributions is routinely carried out across a wide range of industries and is often a critical parameter in the manufacture and analysis of many products and substances. Raman spectroscopy is a useful tool in forensic science for determining molecular chemistry because it is rapid, reliable, can detect trace components in mixtures, allows analysis without contact and is non-destructive. Combining these two analytical techniques allows the individual components present within a blend or mixture to be independently characterized and compared for the purpose of source determination. This presentation will demonstrate how MDRS can be used to gain a better understanding of mixtures with significant importance in forensic science investigations.

(428) **Low-cost Bioanalytical Instrumentation for the Developing World**; Alex Nemiroski[1], Dionysios C. Christodouleas[1], Ashok A. Kumar[1], Jonathan W. Hennek[1], George M. Whitesides[1]; [1]Harvard University

The ability to perform electrochemical or photometric testing in the field, and in resource-limited environments, and to transmit data automatically to "the cloud" can enable a broad spectrum of analyses useful for personal and public health, clinical analysis, food safety, and environmental monitoring. Although the developed world has many options for analysis and web connection, the developing world does not have broad access to i) the expensive equipment necessary to perform these tests, ii) the components, personnel, or expertise required to maintain and operate the equipment, iii) the infrastructure (e.g., continuous and stable electrical power) necessary to keep them running, and iv) the advanced technologies required for network connectivity. In this seminar, I will present our recent work towards overcoming these challenges to providing health-care in resource-limited settings. Specifically I will discuss the recent development of electrochemical and photometric devices that are affordable, portable, and user-friendly. These devices have been validated in the detection of a broad range of important biochemicals, environmental toxins, and disease biomarkers, perform as well as standard laboratory equipment, and transmit the results of testing to the cloud from any phone, over any network, anywhere in the world.

(429) **Arsenic in Drinking Water: Promoting Awareness through Remediation and Measurement Projects for Students**; Julian Tyson[1], Ray Kronquist[2]; [1]University of Massachusetts, [2]Chemists without Borders

Several programs, organized by Chemists without Borders or the University of Massachusetts Amherst, in which secondary school or college students are introduced to the impact of arsenic contamination of the environment, and in particular of groundwater in Bangladesh, are described. A common feature is that students are recruited as members of a research group or investigative team and take ownership of the work by making relevant chemical measurements and participating in discussion of the implications of their findings. Leadership is provided in a hierarchical model in which, very often, more experienced students acting as near-peer mentors guide the activities of the newly recruited members of the groups. In some of the programs, the students work with teachers who have been trained by researchers on the university campus. Both in-school and out-of-school programs are described. A feature common to all is that chemical measurements are provided by low-cost field test kits based on the Gutzeit-Marsh reaction, the modification of which has provided a driving force for a considerable number of research projects for the college students. Currently, researchers affiliated with Chemists without Borders are critically evaluating several possible strategies for making the test more cost effective. Many hundreds of students have been impacted, and the programs, particularly that in Bangladesh, have considerable potential for empowering the students as agents of change in their communities as they not only take specific action as a result of their engagement but also educate other members of their families and communities about the potential hazards of consuming arsenic-contaminated water and rice and how these can be mitigated. Raising student awareness has also be achieved by incorporating relevant arsenic-related topics into undergraduate courses on the UMass campus, including the junior-year writing class, a large-enrollment, physical sciences general education class for non-scientists (taught in both face-to-face and online modes), and a faculty first-year seminar.

(430) **Three Tales from Vietnam**; Alexander Scheeline[1]; [1]SpectroClick

Vietnam is the 12th most populous country on earth. In 1989, it was war-ravaged, destitute, and isolated from much of the world. It has become one of the Asian Tigers, rapidly growing, the world's second largest rice exporter, and an economic dynamo. It still thinks of itself as Third World, and is striving mightily to become the next Korea, Taiwan, or Singapore. This talk discusses three vignettes of the interaction of analytical chemistry and Vietnam, one from the classroom, one from the environmental impact of non-investment in wastewater treatment, and one from the viewpoint of a water treatment laboratory. Because the author does not speak Vietnamese, all opinions are biased by an English language perspective on a complex Eastern culture.

(431) **Catalyzing Analytical Chemistry and Natural Products Drug Discovery Around the World**; Nina Dudnik[1]; [1]Seeding Labs

Drug discovery research is often thought of as the purview of high-tech industry. However a large community of scientists globally is engaged in tapping the power of medicinal plants to identify new medicines for a growing array of diseases. Unfortunately the costs of equipping and maintaining such a research program are often prohibitive for universities in low and middle income countries (LMICs). LMIC chemists have long had to rely on older techniques and equipment, which dramatically slows their research progress. In many cases lack of access to modern equipment for sample processing and analysis has blocked their research altogether.

Since 2008, Seeding Labs' flagship program, Instrumental Access, has been able to provide over $2M in equipment to scientists at 20 institutions in 13 countries in Latin America, the Caribbean and Africa. Natural products drug discovery is the most-well-represented area of research among these scientists. Our global partners are drawing on local resources from plants to marine fungi to address a wide range of health issues including malaria, flatworm infections, cancer, diabetes and wound healing. Instrumental Access has provided them with everything from new glassware to instrumentation for a range of chromatographic techniques. The research and educational activities catalyzed by this infusion of equipment lead to publications and online databases to disseminate their findings, and funding which can be re-invested into equipment, ensuring that universities stay up-to-date with technological advances to train the next generation and conduct research on a globally competitive scale.

(432) **Opitical and Sensing Properties of Coupled Nanoplate-Nanosphere Structures Formed with Regio-Selective Control**; Francis Zamborini[1], Prashant Jain[2], Aiqin Fang[1], Sarah White[2]; [1]University of Louisville, [2]University of Illinois

Our group has developed methods for selectively attaching Au nanospheres (NSs) to Au nanoplates (NPs) with control over the attachment location and the NS size. We measured the plasmon properties of hexagonal-shaped NPs (aspect ratio ~3) by obtaining dark-field scattering spectra of individual NPs before and after attachment of a Au NS or NSs and correlated the scattering spectra with scanning electron microscopy (SEM) images of the same structure. Before NS attachment, the NPs show one sharp peak with the wavelength of maximum scattering near 650-700 nm. For Au NP-NS dimers, attachment of a 25-30 nm diameter NS to the side edge of the NP led to a much larger shift in the wavelength (~22 nm) compared to a NS attached to the top terrace of the NP (2-3 nm shift). Also, the size of the NS played an important role. The shift was ~10 nm for a 10-15 nm diameter NS and 90-100 nm shift for a NS of 40-60 nm diameter. The shift increased with NS size up to about 40 nm, but then the shift remained fairly constant above 40 nm diameter. The size dependent coupling behavior fit with the size dependent of the electric field decay length from the NS surface. The attachment of multiple NSs to NPs showed more complex spectra and larger shifts in the wavelength of maximum scattering depending on the symmetry of the NSs arranged around the NP and size of the NSs. The shift in scattering wavelength was not linear with the number of NSs attached. Each NS led to a greater shift than the previous one. NPs of different shape (hexagonal, triangular, and circular) show different behavior in plasmon coupling with NSs and different sensitivity to the refractive index of the environment. Coupled NP-NS structures also show different refractive index sensitivity compared to individual NPs. Simulations confirm the experimental results in this work and offer new insights on the plasmon coupling between shape and size asymmetric nanostructures.

(433) **Identifying Uranium Speciation in Environmental Samples using Raman and SERS**; Amanda Haes[1], Grace Lu[1], Tori Forbes[1]; [1]University of Iowa

Uranium, a radioactive material with a long half-life (~1 billion yrs) accumulates in the environment in its oxidative form (uranyl) thereby contaminating soil and water. Uranyl, however, can form complexes with sulfate, phosphate, or carbonate, which complicates detection. Furthermore, uranyl speciation varies with pH. As such, methods that are capable of identifying trace uranyl species in complex samples are needed. Herein, localized surface plasmon resonance spectroscopy (LSPR), Raman spectroscopy and surface enhanced Raman scattering (SERS) serve as label-free and near real-time methods for identifying uranium species in solution. First, a straight-forward protocol for spectral analysis will be shown using Raman spectroscopy and aqueous uranium samples. Raman excitation wavelength, pH, and coordinating ions are systematically varied. The spectral analysis results are rigorously validated using uranyl speciation models. Next, plasmonic nanomaterials are used to enhance the Raman signals for trace detection of low (and high) abundant species. All in all, the developed protocol provides an accurate

and routine analysis of Raman spectra for uranyl species identification and relative abundance elucidation. These advances are expected to provide a straight-forward approach for uranium species identified using LSPR spectroscopy, Raman spectroscopy, and SERS.

(434) **Structure and Plasmons of Single Bimetallic Nanorods during Reaction**; Jing Zhao[1], Sravan Thota[1], Shutang Chen[1], Yadong Zhou[2], Shengli Zou[2]; [1]University of Connecticut; [2]University of Central Florida

Bimetallic nanoparticles are promising materials in many applications because of their unique functionalities compared to their monometallic constituents. In this work, we synthesized Au-Cu bimetallic nanorods and used single particle spectroscopy to monitor the localized surface plasmon resonance (LSPR) of Au-Cu alloy nanorods during growth reaction. We found that the spectral features of the single particle scattering spectra of the nanorods are very different from the corresponding ensemble spectra. Specifically, multiple LSPR peaks were observed in the single nanorod scattering where the LSPR of the ensemble nanorod samples have only one resonance peak. With high resolution transmission microscopy and electrodynamics simulations, we found that atomic level geometric defects in the single nanorods split the scattering peaks, giving rise to higher order plasmon modes, which do not exist in perfect rods of similar geometry. The study shows that single particle scattering technique is as sensitive as high-resolution electron microscopy in revealing atomic level structural changes during the growth process of bimetallic Au-Cu nanoords.

(435) **Tunable 3D Plasmonic Cavity as as an Ultrasensitive SERS Platform**; François Lagugné-Labarthet[1], Mohammadali Tabatabaei[1], Mohamadreza Najiminaini[1], Jeffrey Carson[1]; [1]Western University

Metallic nanohole arrays (NHAs) with a high hole density have emerged with potential applications for surface-enhanced Raman spectroscopy (SERS) including the detection of analytes at ultra-low concentrations. However, these NHA structures generally yield weak localized surface plasmon resonance (LSPR) which is a prerequisite for SERS measurements. In this work, a compact three-dimensional (3D) tunable plasmonic cavity with extraordinary optical transmission properties serves as a molecular sensor with sub-femtomolar detection. The 3D nanosensor consists of a gold film containing a NHA with an underlying cavity and a gold nanocone array at the bottom of the cavity. These nanosensors provide remarkable surface plasmon polariton (SPP) and LSPR coupling resulting in a significantly improved detection performance. The plasmonic tunability is evaluated both experimentally and theoretically. A SERS limit of detection of 10^{-16} M for 4-Nitrothiophenol (4-NTP) is obtained along with distribution mapping of the molecule on the 3D plasmonic nanosensor. This results in an improved SERS enhancement factor (EF) of 10^7 obtained from a femtolitre plasmonic cavity volume. The tunability of these sensors can give rise to a potential opportunity for use in optical trapping while providing SERS sensing of a molecule of interest.

(436) **Scanning Angle Raman Spectroscopy Measurements of Thin Films and Buried Polymer Interfaces**; Emily Smith[1,2], Jonathan Bobbitt[1, 2], Craig Damin[1,2]; [1]Ames Laboratory; [2]Iowa State University

Scanning angle (SA) Raman spectroscopy is applied to study polymer films to simultaneously measure polymer thickness, chemical composition, structure and locate buried interfaces with tens of nanometer spatial resolution in the axial direction. The technique uses a prism on which the polymer is coated. Raman spectra are collected as the incident angle of the excitation laser is precisely varied from 30-70 degrees. SA Raman spectra of 10 nm to 2 micron polystyrene, polymethyl methacrylate and block copolymer poly(styrene-b-propylmethacrylate) films are collected to measure changes in thickness and domain formation. The results show the SA Raman data contain information to show how a variety of thermal and solvent annealing treatments affect film properties. SA Raman spectroscopy is a versatile method applicable whenever the chemical composition, structure and thickness of interfacial thin films need to be measured with high axial resolution. The technique will be useful in the field of polymer lithography and for work on thin film-containing photovoltaic devices. This research is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences through the Ames Laboratory. The Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under Contract No. DE-AC02-07CH11358.

(437) **Liquid Sampling-Atmospheric Pressure Glow Discharge Microplasmas: Evolving Towards Versatility, Practicality, and Transportability**; R. Kenneth Marcus[1]; [1]Clemson University

Miniaturization of chemical instrumentation has been a focus in analytical chemistry to increase the access to sophisticated methodologies as well as the potential for field deployment. In comparison to analytical instrumentation in the laboratory, field-deployable instruments must be smaller and more robust with respect to the introduced sample matrices (as sample preparation is difficult in the field). In case of atomic spectroscopy the development of miniaturized instrumentation has been almost nonexistent.

This laboratory developed the liquid sampling–atmospheric pressure glow discharge (LS-APGD) as a miniaturized excitation/ionization source. The plasma is initiated at the surface of an electrolytic solution flowing at rates of 10 – 100 μL min^{-1}, operating in a total consumption mode. The small volume (< 1 mm^3) microplasma yields high power densities (~ 10 W mm^{-3}) and electron number densities (~ 3 x 10^{15} cm^{-3}), which are comparable to those of an ICP. Their low power (< 100 W) requirements, small footprint and small sample amounts (~10 μL) make it attractive for field-based elemental/isotopic analysis. Importantly, the use of the microplasma as a secondary excitation/ionization source has been demonstrated for the case of laser ablation (LA) sample introduction. Fundamental studies have demonstrated a high level of robustness, with virtually no perturbation of excitation/ionization conditions across many “heavy” matrices.

While the LS-APGD has many of the target characteristics that one would desire for a miniaturized excitation/ionization source for elemental analysis, most surprising of recent developments has been the ability to operate the microplasma in modes which allow the identification of molecular species introduced via ambient desorption or flow injection mechanisms. For example, the identity and extent of uranyl ion ligation can be determined, while at the same time organic species ranging from dyes to proteins can be identified; all with the same device used for atomic spectroscopic analysis.

To be clear, there are always compromises that are realized as analytical devices are miniaturized. Therefore, there must be substantial upside in terms of versatility, practicality and transportability to allow such approaches to be accepted across diverse communities. It is believed that the LS-APGD is definitely a step in the right direction.

(438) **Mid-infrared Diffuse Reflection on Ultrafast Time Scales**; Eric Brauns[1]; [1]University of Idaho

Diffuse reflection (DR) is a fundamental optical phenomenon where light penetrates a translucent scattering solid and is re-emitted after undergoing numerous instances of reflection and refraction. As a result, photons can travel long distances through semitransparent samples. This

is routinely encountered in the spectral analysis of solids and turbid media. In some cases this is advantageous—e.g., in DR spectrometry where the long effective path lengths can increase the net absorption of a weakly absorbing analyte. In other cases it is undesirable—e.g., in imaging applications where DR degrades spatial resolution and contrast. Despite its significance, relatively little is understood about the underlying mechanism—particularly mid-infrared DR.

An important parameter in DR is the effective path lengths that photons travel. A direct determination of this requires a time-resolved approach. However, time-resolved techniques can be complicated and can pose unique challenges. This is especially true in the mid-IR spectral region on ultrafast time scales. I have developed an instrument that overcomes these hurdles. Briefly, femtosecond mid-IR pulses are generated by difference frequency mixing the output of an optical parametric amplifier that is pumped by a regeneratively amplified Ti:Sapphire laser. Time resolution is achieved by upconverting the diffusely reflected photons with pulses from the Ti:Sapphire oscillator. The result is an instrument capable of studying mid-IR DR with femtosecond time resolution.

In this talk, I will describe the instrument in detail and present experimental data on a series of powdered KBr samples containing varying amounts of carbon black. In addition to demonstrating the utility of the instrument, the results suggest that mid-IR photon transport on ultrafast time scales is actually not diffusive.

(439) **Electroosmotic Sampling and Microfluidic Determination of Extracellular Thiols in Brain Tissue Cultures**; Stephen Weber[1], Juanfang Wu[1], Jessie Jiang[1], James Landers[2], Erin Redman[3], J.P. Alarie[3],J. Michael Ramsey[3]; [1]University of Pittsburgh; [2]University of Virginia; [3]University of North Carolina

We have developed a platform for the determination of extracellular thiols in organotypic hippocampal cultures. The extracellular space is perfused with artificial cerebrospinal fluid (aCSF) using electroosmosis. Previous measurments in our lab showed that the zeta potential of the cultures is significant, about -22 mV. This fluid travels to a simple microfluidic chip for reaction with a Michael acceptor-based fluorogenic reagent and separation. We have determined cysteamine for the first time in the extracellular space and have used that to determine the likely degradation pathway of coenzyme A. We are also interested in the extracellular metabolism of glutathione and are currently working to establish conditions for the quantitative assessment of the rate of hydrolysis of this important peptide.

(440) **Fabrics as Platforms for Electrophoretic Separations**; Shashi Murthy[1], Tanya Narahari[1], Dhananjaya Dendukuri[2]; [1]Northeastern University; [2]Achira Labs

There is a rising need for low-cost and scalable platforms for sensitive medical diagnostic testing. Fabric weaving is a mature, scalable manufacturing technology and can be used as a platform to manufacture microfluidic diagnostic tests with controlled, tunable flow. Given its scalability, low manufacturing cost (<$0.25 per device) and potential for patterning multiplexed channel geometries, fabrics represent viable platforms for the development of analytical devices. This presentation will describe a fabric-based electrophoretic platform for protein separation. Appropriate yarns were selected for each region of the device and weaved into straight channel electrophoretic chips in a single step. A wide dynamic range of analyte molecules ranging from small molecule dyes (<1 kDa) to macromolecule proteins (67-150 kDa) were separated in the device. Individual yarns behave as a chromatographic medium for electrophoresis. We therefore explored the effect of yarn and fabric parameters on separation resolution. Separation speed and resolution were enhanced by increasing the number of yarns per unit area of fabric and decreasing yarn hydrophilicity. However, for protein analytes that often require hydrophilic, passivated surfaces, these effects need to be properly tuned to achieve well-resolved separations. An example of a device designed using these considerations will be presented to demonstrate estimation of electrophoretic mobility of napthol-blue-black bovine albumin. Lastly, the ability to tune separation may be used to predefine regions in the fabric for successive pre-concentrations and separations. The device may then be applied for the multiplexed detection of rare (low-abundance) proteins from complex biological samples such as serum and cell lysate.

(441) **Nanogels for Reversibly Patterned Electrophoretic Separations**; Lisa Holland[1], Brandon Durney[1], Tyler Davis[1], Srikanth Gattu[1]; [1]Chemistry Department, West Virginia University

Smart nanomaterials are an innovative alternative to support flexible and reprogrammable sample processing and tunable microscale bioseparations.[1-3] The morphology of phospholipid nanogels is temperature dependent, rendering the viscosity thermally responsive. The thermally responsive viscosity is utilized to steer fluids and pattern enzymes in microscale channels to support catalytic processing using nanoliter volumes of enzymes. These material features are leveraged to integrate sample processing and complex separations in a single platform. By harnessing a smart material, the on-board fluid is programmed to concentrate, process, steer, and then separate targeted analyte. This is significant because generic microscale channels generate a sophisticated multifunctional device for separation and selection. The reversible nanogel can be directed to different microfluidic channels using thermoelectric modules to spatially control the temperature in a microfluidic chip with a 2-second switching time. Phospholipid nanomaterials provide efficient separations of biopolymers based on hydrodynamic volume as well as chemical sieving. Glycans separated based on hydrodynamic size have theoretical plate counts of 600,000.[3] Glycan separations relevant to next generation pharmaceuticals and disease detection are modified to probe the glycan for structural information or to simplify a complex electropherogram with a plug of phospholipid gel containing lectin or enzyme. DNA separated based on chemical sieving has theoretical plate counts from 0.9 to 2 million and are applied to human identification, pathogen recognition, and clinical biomarkers.[4,5]

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(442) **Pressure-Actuated Microfluidic Devices for Pre-Term Birth Biomarker Analysis**; Vishal Sahore[1], Suresh Kumar[1], Adam Woolley[1]; [1]Brigham Young University

We are developing pressure-actuated microfluidic devices for pre-term birth (PTB) biomarker analysis, integrating immunoaffinity extraction, sample labeling/enrichment, and electrophoretic separation modules on a single platform. This approach utilizes on-chip pumps and valves to facilitate sample preparation and separation, enabling quantitation of PTB biomarkers. The electrophoresis module has been developed in a three-layer poly(dimethylsiloxane) (PDMS) microfluidic system. Each device has pneumatic valves that surround the injection intersection to capture a sample plug containing PTB biomarkers, which are subsequently separated using microchip electrophoresis. Moreover, an on-chip labeling and enrichment module has been developed with a reversed-phase porous polymer monolith in a cyclic olefin copolymer (COC) thermoplastic layer. Sample has been loaded on and eluted from this monolith using an on-chip peristaltic pump. Additionally, the labeling module has been integrated with an electrophoresis unit that allows quantitation of the separated PTB biomarkers. Currently we are developing an immunoaffinity extraction module with a porous polymer monolith to allow selective capture of PTB biomarkers from blood samples; this capability will also be integrated with the electrophoresis module. Our long-term goal is to combine all three modules and construct a fully automated integrated microfluidic device for pre-term birth diagnosis.

(443) **DNA Separation by Sequence**; Linda McGown[1], Jia Zhao[1], Steven Cramer[1], Cecily Wilbanks[1], Shekhar Garde[1], Xueru Tepke[1]; [1]Rensselaer Polytechnic Institute

DNA analysis has widespread applicability in biology, medicine, ecology, agriculture, biodefense, biotechnology and forensics. Most of these applications involve DNA separation by length, which is readily achieved using sieving gels in electrophoresis. Separation by sequence is a more complicated problem, generally requiring sufficient differences in native or induced conformation or differences in thermal or chemical stability of the strands that are hybridized prior to measurement. In many cases, applicability is limited detection of known target sequences. New approaches are needed that are analogous to separation by length in their simplicity and general applicability to any mixture of DNA. We have discovered such a technique that allows ssDNA to be separated by sequence using high concentrations of salts in simple buffer solutions. The approach does not require prior knowledge of the sequences or persistent conformational differences among the strands. This talk will discuss investigations of the basis of the separation, its implementation in capillary electrophoresis, and progress toward a two-dimensional microfluidic platform that combines conventional sieving gels to separate ssDNA based on length with separation of DNA by sequence. Applications of particular interest that could derive important benefits from this simple, rapid separation include point-of-care genetic analysis for patient care and metagenomic analysis of complex microbial communities and biofilms.

(444) **Time Regimes in Pulsed RF-GD-TOFMS: Properties and Effects on the In-Depth Profile Analysis of Thin Layers**; Nerea Bordel[1], Jorge Pisonero[1], Cristina González-Gago[1], Alfredo Sanz-Medel[1]; [1]University of Oviedo

Direct surface and depth profiling chemical analysis of multilayer materials demands a “multi-dimensional” knowledge, including simultaneous elemental and molecular information to characterize new materials. Radiofrequency glow discharges allow obtaining direct chemical information with high depth resolution from a great variety of materials in a fast and easy way.

In addition, when radiofrequency glow discharges are powered in pulsed mode (rf-PGD) the technique gains new and interesting properties. It has been demonstrated that a rf-PGD coupled to a time-of-flight mass spectrometer (TOFMS) is a suitable instrument to carry out depth profiling of very different types of coated materials with high depth resolution (range of nms).

In pulse mode, the discharge evolves according to the pulse frequency giving rise to three main regimes (prepeak, plateau and afterglow) with different ionization mechanisms.

Most of the previous studies have used the ion signals detected in the afterglow region to produce the qualitative depth profile of the samples under analysis. This region has always been considered the most interesting one since it is the most sensitive and the detection of molecular ions is also possible. Nevertheless the other pulse regions also contain the information about the samples being analysed and they could be used.

In this work the in-depth profiles of different samples (conductors, polymers, etc.) obtained with the ion signals detected in different parts of the GD pulse are investigated. The results show that the in-depth profiles of some elements exhibit noticeable differences depending on the pulse region being used while others are not affected. This work tries to gain understanding about the influence of the ionization mechanism on the resulting qualitative depth profiles and investigates the pulse region which provide better qualitative profiles.

(445) **Quantitative Reconstruction of the GDOES Sputter Depth Profile of a Monomolecular Layer Structure of Thiourea on Copper**; JiangYong Wang[1], Yi Liu[1], Wei Jian[1], Siegfried Hofmann[2], Ken Shimizu[3]; [1]Department of Physics, Shantou University; [2]Max Planck Institute for Intelligent Systems; [3]University Chemical Laboratory, Keio University

High-resolution depth profiles of a thiourea (CH4N2S) molecular monolayer on a copper substrate obtained by radio frequency glow discharge optical emission spectroscopy (rf-GDOES) are quantified by the Mixing-Roughness-Information depth (MRI) model. Based on the molecular structure of the self-assembled thiourea layer, the measured intensity-sputtering time profiles of N, S, C and Cu are fitted to the MRI model results with an appropriate depth resolution function. The sputtering rate is deter-mined accordingly, using two different approaches based on constant sputtering rate and on composition dependent sputtering rate. While the first approach requires an additional background for a fairly accept-able solution, the approach using a newly developed multielement dependent sputtering rate results in a complete and consistent reconstruction of all the measured elemental depth profiles. It is demonstrated that for depth profiling of the first few monolayers with rf-GDOES, the depth resolution can be as low as 0.5 nm, which is of the order of the theoretical limit.

(446) **Application of RF Glow Discharge Optical Emission Spectroscopy for Quantitative Depth Profile Analysis of Chemically Strengthened Glass**; Anna Nached[1], Georgiy Guryanov[1], Jamie Vargeson[1]; [1]Science and Technology Division, Corning Incorporated, Corning

Glass has a combination of desirable properties which constitute a unique asset for many modern-day needs when compared with other available materials such as metals, plastics, and most crystalline conglomerates. Among these might be listed transparency, hardness, good durability, low cost, ease of forming, and non-deformability. But glass also has an undesirable property which has severely limited its use throughout the ages, and this is its vulnerability to breakage, or lack of strength.

Over last 100 years, a great deal of study has gone into the understanding and improvement of glass strength. One of the methods to strengthen the glass is to make the chemical composition of the surface different from that of the interior. This can be accomplished via ion exchange, which is a chemical strengthening process where large ions are "stuffed" into the glass surface, creating a layer of compressive stress on the glass surface and making it more resistant to damage. Corning® Gorilla® glass is specially designed to maximize this behavior.

Fundamental understanding of the stress profile in the glass is closely connected to the compositional depth profile. RF GD-OES is an analytical technique that can be successfully utilized to provide accurate depth profiling from the sample surface down to 200 microns. With its high sputtering rate, excellent depth resolution, ability to analyze dielectric materials directly without any special sample preparation, and linearity of concentration calibration curves over quite a large dynamic range, RF GD-OES can close the gap between the surface sensitive techniques (1-5µm), such as, SIMS, and techniques that can be used for thicker layers by analyzing cross sections, such as, Electron Probe Micro Analysis (EPMA) that is inaccurate on first 1-2 µm.

In this talk, we will present results indicating the significant progress that has been made in RF GD-OES method development for the analysis of ion-exchanged glasses, including optimization of the depth calibration with varying sputtering rate during the profiling, method development for raw data quantification, and application of the plasma cleaning concept to improve accuracy of the results near the glass surface. Comparison with SIMS and EPMA profiles will also be presented.

(447) **Advances in Glow Discharge Mass Spectrometry for Elemental Analysis for low level detection**; Ekbal Patel[1]; [1]Mass Spectrometry Instruments Ltd

The glow Discharge is a well proven technique used for bulk material analysis. The technique requires minimal sample preparation whilst attaining a broad Elemental coverage to low level detection in a single Experiment. The principle, operations and advantages of the GD90 GDMS with specific application are included.

The latest development in RF technology for direct analysis of non conducting materials to low detection limits will be introduced . The advances in design approach and simplicity of use in interface and software will be presented.

With modern Instruments, is automation a reality to further enhance the quality issues, improve data, minimize any cross contamination and improve throughput.

(448) **Consequences of Heterogeneous Surface Composition in Depth-Resolved Glow Discharge Spectrometry**; Andrew P. Storey[1], Steven Ray[1], Maxim Voronov[2], Volker Hoffmann[2], Wolfgang Buscher[3], Carsten Engelhard[4],Gary Hieftje[1]; [1]Indiana University; [2]IFW Dresden; [3]University of Muenster; [4]University of Siegen

Over the last decade, several approaches have been developed to map the two-dimensional surface elemental composition of samples by means of glow-discharge (GD) imaging. Such methods are much faster than those that require a rastering probe. In principle, the combination of such an approach with time-resolved GD depth profiling would enable the rapid three-dimensional characterization of solids. However, successful implementation of such a technique has encountered several challenges, not the least of which is the question of how sputtering behavior is affected by surface heterogeneity in GD samples and how such irregularities propagate through a sample as sputtering proceeds. This presentation will cover insights derived from our studies of how heterogeneities are problematic for glow-discharge emission spectrometry and places these observations into broader context.

Computer simulations of heterogeneous materials eroded by glow-discharge sputtering, physical three-dimensional characterization of heterogeneous sputtered surfaces, earlier literature, and studies of minimized GD sputtering domains are brought together to elucidate these challenges. In short, slight differences in surface composition, inclusions, or imperfections at the interface of two layers or other causes of inconsistent sputtering can compromise GD depth profiling, which ordinarily assumes perfect surface homogeneity. Conventional GD depth profiling generally lacks effective means of flagging these error sources, a problem with which imaging GD could assist.

(449) **Shine Little Glow-Ken, Glimmer, Glimmer**; Joseph Caruso[1]; [1]University of Cincinnati

Ken Marcus has been a consistent achiever in the area of glow discharge spectrometry since he began his academic career. His success can be measured in many ways: his many publications, his impressive lectures and student presentations, his grant funding and his top-level reputation in the areas of atomic spectrometry and liquid chromatography. But there is one more measure; that of the influence and esteem he has on his colleagues and peers, plus the studies he has inspired. This talk reflects that inspiration as it pertains to this speaker.

We began our glow-discharge work by utilizing the glow-discharge cube sent to us from the Marcus Labs at Clemson. Influencing our initial studies was the major attractive feature of the rf glow-discharge; namely that it would allow analysis of both conducting and non-conducting cathode materials. After initial uses though, we became impressed with the stability and reproducibility of the discharge itself. After working with various microwave plasmas through the years, using the rf-GD was a pleasure and an excellent scientific advance. Furthermore, while it did not have the robustness of the ICP, it was more readily manageable plus far less expensive to operate, especially for gaseous sample work.

Given these pluses, we thought that instead of analyzing cathode materials, we might find excellent applications for vapor samples directly into the discharge. In fact, the performance with gaseous sample introduction was exceptional for gas chromatography and other methods of vapor introduction. This lead to looking further into this excellent plasma (discharge), even to the point of seeing that it could readily give both electron ionization type mass spectra as well as quantitative analysis. This talk will focus on sample vapor introduction to the rf-Glow-Discharge as a source for mass spectrometry.

(450) **Ferritin: A Clinical Biomarker and a Protein Cage for Nanoparticles**; Maria Montes-Bayon[1], Tobias Konz[1], F. Javier Alonso[1], Alfredo Sanz-Medel[1]; [1]University of Oviedo

Iron balance is tightly regulated in our organism and such regulation occurs exclusively at the site of absorption, as there is no physiological process to excrete the iron excess. The majority of iron absorption occurs via enterocytes in the proximal small intestine and is then transported to other sites within the body by transferrin, the main Fe transporter in human serum. Ferroportin is a newly identified iron efflux pump that mediates the export of iron from the enterocyte into the blood stream for transferrin binding.

Tranferrin carries Fe to, among others, the liver where the metal is stored in Ferritin. Ferritin makes iron available for critical cellular processes while protecting lipids, DNA, and proteins from the potentially toxic effects of iron. Ferritin is a 440 kDa protein composed by 24 subunits that form a hollow protein shell holding inside an internal cavity of about 8 nm internal diameter that can contain a variable amount of iron atoms (up to 4500) by forming a so-called biological nanoparticles. The understanding of the chemical structure of the ferritin core including the iron uptake, storage, and release mechanisms in detail would provide information to understand the etiologic origin of some neurological pathologies, for instance, such as Parkinson (PD) or Alzheimer's (AD) diseases. In PD patients a marked reduction of the ferritin expression in the brain as well as the alteration of neuromelanin structure seems to compromise the iron sequestration capabilities leading to the accumulation of free metal ion.

For the aim of studying Fe-ferritin related parameters an interesting possibility is the use of 57Fe isotopically enriched ferritin. Using a non-toxic isotopic label is especially favour able to investigate, among others, whether serum ferritin losses most of its Fe during or after effluxing from the cells in which it originates. In this work we evaluate the synthesis, characterization and analytical application of isotopically labelled ferritin as nanotracer to study ferritin in different disorders.

(451) **The Liquid Sampling – Atmospheric Pressure Glow Discharge: A Miniaturized Plasma for Giant Problems in Nuclear Forensics**; Benjamin T. Manard[1], Ning Xu[1], Alonso Castro[1], R. Kenneth Marcus[2]; [1]Los Alamos National Laboratory; [2]Clemson University

There is a need to perform rapid chemical analysis in the field, particularly in nuclear forensics. Currently samples are collected in the field and transported to fixed-base laboratories for analysis. For elemental analysis, inductively coupled plasma mass spectrometry (ICP-MS), secondary ionization mass spectrometry (SIMS), or thermal ionization mass spectrometry (TIMS) are commonly employed pending the results warranted. It is envisioned that rapid on-site screening of materials would greatly decrease the overall time as the sample load of fixed based laboratories would be greatly reduced. Additionally, preliminary results could provide vital information thus allowing rapid decisions to be made or prompt further sampling.

Here, the liquid sampling – atmospheric pressure glow discharge (LS-APGD) will be discussed as a potential plasma source for exciting/ionizing species for OES and MS detection. Characteristics including small footprint (<100 in2), low power requirements (<100 W), and minimal operating consumables (e.g. He gas) make this an ideal source for in field analyses. At Los Alamos National Laboratory, our main focus is to detect the elemental and/or isotopic composition of actinides such as plutonium and uranium in various nuclear or safeguards events (i.e. swipes or post-detonation debris). We have proposed to NNSA to explore the option of coupling the LS-APGD to a portable mass spectrometer so that isotopic ratios to a certain extent can be determined.

(452) **Ken Marcus, Champion of the Glow Discharge or Glow Discharge and Distance-of-Flight Mass Spectrometry: A Match Made in Heaven**; Steven Ray[1], Elise Dennis[1, 2], Christie Enke[2,3], Gary Hieftje[2], David Koppenaal[4]; [1]State University of New York at Buffalo; [2]Indiana University; [3]University of New Mexico; [4]PNNL

The success enjoyed by any analytical methodology is influenced by several factors. First, and foremost, is the degree to which the analytical technique holds scientific advantages over competing measurements or other means of obtaining the same information. Second, the impact of the method reflects how it informs critical chemical questions, and its relative range of application. Lastly, and most often overlooked, the successful development of any analytical method requires a group of dedicated champions that push the technological and theoretical development of the method, and push their analytical colleagues to adopt the technique. In the long and august history of the glow discharge a number of prominent individuals have acted in this role of 'champion', and among these figures Ken Marcus is certainly a shining example. In this presentation, the contributions of the award winner to the area of glow discharge spectroscopy will be examined. In addition to investigating past developments and their roots, the future of the technique will also be prognosticated upon. Special emphasis placed on aspects of an emerging new type of mass spectrometry known as Distance-of-Flight Mass Spectrometry (DOFMS), and the advantages of DOFMS when applied to glow discharge mass spectrometry.

(453) **Ken Marcus and the Glow on the Horizon**; Gary M. Hieftje[1], Andrew J. Schwartz[1], Steven J. Ray[1]; [1]Indiana University

Ken Marcus, deserving recipient of the 2015 Lester W. Strock Award, is best known for his seminal contributions to the development, characterization, and application of glow discharges to chemical analysis. Although conventional glow discharges, operated at modest (~60W) power levels and vacuum (~1 torr) settings, have long been used for bulk and depth-resolved analysis of metal samples, Marcus's championing of radiofrequency-powered discharges fostered a new wave of applications to non-conductive samples. More recently, Marcus and a host of others have turned to atmospheric-pressure glows, which differ in physical properties from reduced-pressure discharges and which again open new avenues in spectroscopic analysis. In this presentation, one of the newer atmospheric-pressure glows will be described and evaluated. Termed the Solution-Cathode Glow Discharge (SCGD), the source provides solution-based detection limits that rival or improve upon those available from ICP emission spectrometry; yet the SCGD requires no gas (argon or other) flow, needs no nebulizer or spray chamber, provides surprisingly uncluttered spectra, and operates at power levels typical of those for a conventional glow discharge (~65W).

(454) **Super-Resolution Imaging Using Multi-photon and Multi-photon-like Fluorescence Microscopy Techniques**; George Patterson[1], Maria Ingaramo[1], Andrew York[1]; [1]National Institutes of Health

Multi-photon microscopy provides deeper tissue imaging capacity than most linear fluorescence imaging techniques but often suffers from decreased resolution. Implementation of Multifocal Structured Illumination Microscopy (MSIM) with multi-photon excitation takes advantage of specimen penetration afforded by longer wavelengths but provides resolution doubled images with better sectioning and contrast in thick scattering samples. However, one disadvantage with multi-photon microscopy is the relatively high illumination intensities required for nonlinear excitation. We will discuss "two-step" fluorescence microscopy, a new approach to nonlinear imaging requiring orders of magnitude less light than conventional multi-photon excitation. This technique is based on positive reversible photoswitchable fluorescent probes, such as Padron, which display behaviors necessary for the "two-step" approach. These require that the fluorescent molecule photoswitch or ideally

rapidly equilibrate to a non-fluorescent state, that the probe photoswitch to an active fluorescent state, and that the same wavelength used to turn on the probe also excite the active state to produce fluoresce. Since both activation and excitation are linear processes requiring sequential absorption processes, the total fluorescent signal is proportional to the square of the illumination dose. Thus, two-step microscopy is similar in principle to two-photon microscopy but with orders-of-magnitude better cross-section. We will show that the quadratic nonlinearity from two-step Padron fluorescence using blue light excitation improves resolution, reduces out-of-focus signal, and is compatible with structured illumination microscopy. While no ideal two-step probe is known, improvements in the characteristics discussed earlier could lead to increased resolution and optical sectioning for many forms of conventional and super-resolution microscopy techniques.

(455) **Super-Resolution through Minimalist Representation of Chemical Imaging in Infected Single Red Blood Cell Components using Multiplex Hyperspectral Confocal Raman Imaging**; Nicolas Spegazzini[1], Rishikesh Pandey[1], Ishan Barman[2], Ramachandra Rao Dasari[2]; [1]Massachusttes Institute of Technology; [2]Johns Hopkins University

The malaria disease in early detection during asexual blood stage-erythrocytic phase has been a long-standing obstacle. In this report high speed and high resolution hyperspectral confocal spontaneous Raman imaging coupled with independent target restoration microscopy, can provide exhaustive multiplex information in spatial, and multichannel spectral resolution. Moreover decompose the chemical fingerprint information for each species considered in the study. We report the quantitat ive determination of chemical species and their multicomponent system. More than five major components in the red blood cell are observed: hemoglobin, parasite's food vacuole, hemozoin, and structural information membrane phospholipids and actin-spectrin from the protein component of the cytoskeleton.

(456) **Absorption Spectroscopy and Imaging from the Visible through Mid-IR with 20 nm Resolution Using AFM Probes**; Andrea Centrone[1]; [1]NIST, center for nanoscale Science and Technology

Correlated nanoscale composition and optical property maps are important to engineer nanomaterials in applications ranging from photovoltaics to sensing and therapeutics. Light absorption in the visible and near-IR measures electronic transitions in materials, providing information related to the band gap and defects. Light absorption in mid-IR probes vibrational transitions and provides information on chemical composition. However, light diffraction limits the lateral resolution of conventional micro-spectroscopic techniques to approximately half the wavelength of light, which is insufficient for characterizing materials at the nanoscale (especially in the mid-IR). Additionally, the wavelength-dependent resolution impedes direct comparison of spectral maps from different spectral ranges.

Photo Thermal Induced Resonance (PTIR) is a novel, label-free technique that circumvents light diffraction by employing an AFM tip as a local detector to measure light absorption with a wavelength-independent resolution. The function of the passive AFM tip is to locally transduce the thermal expansion of the sample induced by light absorption into large cantilever oscillations. Our PTIR setup combines an AFM microscope with three lasers providing wavelength-tunability from 500 nm to 16000 nm continuously. Local absorption spectra (electronic or vibrational) and maps are obtained recording the amplitude of the tip deflection as a function of wavelength and position, respectively.

The working principles of the PTIR technique, its lateral resolution, and linearity will be discussed first. Results show that the PTIR signal intensity is proportional to the local absorbed energy suggesting applicability of this technique for quantitative chemical analysis at nanoscale, at least for thin (< 1000 nm) samples. Additionally, a wavelength-independent resolution as high as 20 nm is demonstrated across the whole spectral range.

The recent development of plasmonic nanostructures with resonances in the mid-IR has generated considerable interest in surface-enhanced infrared absorption (SEIRA) spectroscopy due to chemical detection limits in the zeptomolar range; however, the poor resolution of conventional IR microscopy has prevented direct quantification of SEIRA enhancements at the nanoscale. In the second part, the PTIR technique will be used to quantify and map SEIRA hot-spots in the near-field and to extract important information for engineering plasmonic resonators arrays.

(457) **Fiber Bundle Arrays for Wide-Field, Dynamic SERS Nanoscopy**; Eric Languirand[1], Brian Cullum[1]; [1]University of Maryland, Baltimore County

Super-resolution chemical imaging via Raman scattering offers chemical-specific information. Using an imaging fiber bundle in conjunction with Raman scattering allows for vibrational information to be visualized. However, spontaneous Raman scattering is inherently weak making super-resolution imaging nearly impossible. To circumvent this - and increase sensitivity - surface enhanced Raman scattering (SERS) is employed with the use of imaging bundles. These imaging bundles consist of an array of 30,000 individual fiber elements that are 4μm in diameter with a total imaging diameter of approximately 1mm. When tapered, the fiber elements can be as small as 50nm in diameter with a total imaging diameter of approximately 20μm providing 100nm spatial resolution and resulting in sub-diffraction limited, wide-field SERS imaging. An acousto-optic tunable filter is used for rapid selection of the vibrational frequencies of interest for chemical specific and/or hyperspectral imaging.

Characterization of the fiber bundle on the nanoscale is necessary for understanding the sub-diffraction limited images obtained. While a scanning electron microscope (SEM) can provide high spatial resolution images for visualization of the surface of the probe, characterization of the cross-talk is essential to ensure the high resolution images are being obtained as expected. In this talk, the use of drop-coated quantum dots (QDs) will be explored for the evaluation of the cross-talk on the nano-scale. In addition, applications of these probes to biological and forensic imaging will also be discussed.

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(458) ***In situ* ATR-FTIR Spectroscopy and Imaging to Monitor the Purification Process of Antibodies**; Maxime Boulet-Audet[1], Bernadette Byrne[1], Sergei Kazarian[1]; [1]Imperial College London

In the next ten years, the pharmaceutical industry anticipates that revenue from biotherapeutics will surpass those from small drug molecules. Despite effectively treating a range of chronic and life-threatening diseases, high production cost severely limits the availability of biotherapeutic treatments. Using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, we investigated two critical issues associated with the production of antibodies: protein aggregation during storage and the fouling of affinity resin used for chromatographic purification.

Protein aggregation reduces yields, drug potency and can cause highly undesirable immunological effects. To identify processing conditions that maximize recovery while avoiding aggregation, we use heat resistance as a proxy for long-term aggregation propensity. We evaluated the potential of attenuated total reflection (ATR) FTIR spectroscopic imaging as a novel method for the high-throughput thermal stability assay of antibodies. Combined with focal plane array detection, ATR mode is very well suited to selectively probe precipitating aggregates *in situ*. Our FTIR spectroscopic approach tested 12 different buffer conditions simultaneously to assess the thermal stability over a wide range of buffer conditions.

Another important issue associated with antibody production is the prohibitive cost of the affinity chromatographic resin (>$16 000/kg). Resin cleaning-in-place (CIP) aims to slow the decay of the binding capacity. Assays can determine the resin's binding capacity from the mobile phase, but do not reveal the underlying causes of protein ligand degradation. The focus needed to be on the stationary phase to examine the effect of CIP on the ligand of the resin. To directly relate the binding capacity, local protein ligand concentration, and conformation, we developed a method based on ATR-FTIR spectroscopy. Applying a carefully controlled load to the chromatographic column produces an even and reproducible contact for probing resin beads with ATR mode. This approach revealed that ligand proteolysis does occur under typical CIP conditions, below 1M NaOH. However, concentrations of NaOH above 0.1 M cause significant changes in protein conformation. The addition of >0.4 M trehalose during CIP significantly reduced NaOH-induced ligand unfolding observed for one of the two Protein A resins tested. Such insights could help to optimize purification protocols in order to reduce antibody production costs.

(459) **Advanced Pattern Recognition Applied to Forensic Evidence**; Nicholas Petraco[1]; [1]John Jay College, City University of New York

Forensic practitioners are interested in numerical measures of quality for algorithmically generated associations between items of evidence under comparison, weather it be spectra, dust, soil, tool, hair, etc. There is no consensus in the general scientific community as to how to do this, such that practical applications will withstand long run scrutiny in the cauldrons of U.S. courtrooms.

Our approach is to cover both "statistical philosophies" and exploit frequentist and Bayesian methodologies. As a "frequentist" based approach, we will describe how conformal prediction theory can be used to put orthodox confidence levels onto any computer made "match" between a known and an unknown (Vovk 2005). For a "Bayesian" based approach we are pursuing an empirical Bayes' methodology. Modern machine learning methods output a voluminous amount of information when executing a discrimination task. This massive amount of output allows one to leverage Efron's empirical Bayes' model for an estimate of the posterior error probability of an unknown's source identity in a scientifically testable way (Efron 2006). Technically, the posterior error probability (PEP) gives an estimate that the source truly did not generate the unknown pattern. Once the PEP function is determined, a weight of evidence function or "likelihood ratio" is easily found.

(460) **A Bayesian Approach to Forensic Evidence Interpretation; Converting Analytical Data to Significance Using a Continuous Verbal Scale**; Jose Almirall[1], James Curran[2]; [1]Florida International University, [2]University of Auckland

Forensic scientists use the Bayesian approach in the interpretation of evidence for the assessment of a "likelihood ratio" to consider the probability of the evidence with respect to at least two possible explanations or hypotheses. A simple example of alternative hypothesis in the case of finding glass fragments on the clothing of a suspect might be "The suspect was present at the crime scene when the window was broken" and "The suspect was not present at the crime scene when the window was broken." The evaluation of these probabilities takes into account information such as the strength of the match between the crime scene source and fragments recovered from the suspect, and the relative rarity (or frequency) of glass with similar characteristics in the population. LA-ICP-MS data is considered the "gold standard" for comparing glass fragments suspected of having a coming origin. Quantitative analysis of glass is possible with excellent precision and accuracy using this technique and forensic scientists can better understand the "strength of the match", when a match is found, based on many studies conducted by various research groups around the world. Forensic scientists now also have a better understanding of "relative rarity" or how frequently we would expect glass fragments to have the same elemental profile even though the glass originated from different manufacturing plants. This presentation aims to improve on the current limited verbal scale used by forensic scientists to describe the significance of a match, when one is found by instead using a likelihood ratio that incorporates a numerical scale with higher granularity and better communicates the scientific data.

(461) **Statistical Method for Comparison of Mass Spectra: Applications for the Identification of Controlled Substances**; Ruth Waddell Smith[1], Melissa A. Bodnar-Willard[2], Victoria L. McGuffin[2]; [1]Forensic Science Program, Michigan State University; [2]Department of Chemistry, Michigan State University

Controlled substance samples submitted to a forensic laboratory are typically analyzed by gas chromatography-mass spectrometry to identify any controlled substances present. This identification is based on visual assessment of the resulting mass spectrum to a reference spectrum, which may be obtained from a suitable standard or from a database search. However, there is no statistical assessment to establish if the tentative identification based on this visual assessment of spectra is objectively correct.

In our laboratory, a method that can be used to assess the statistical equivalence of two mass spectra has been developed. The method uses an unequal variance t-test (also known as Welch's t-test) to compare every m/z in the scan range, testing the null hypothesis that the abundances at the given m/z values are not statistically different. If the null hypothesis is accepted at all m/z values, then the two spectra are considered to be statistically indistinguishable. A random-match probability can then be calculated to indicate the probability that the mass spectral fragmentation pattern in question occurs by random chance alone. This provides a statistical assessment of the veracity of the identification and is similar to methods currently used in DNA analysis. In contrast, if the null hypothesis is rejected at any m/z value, the two spectra are statistically distinguishable and the ions responsible for discrimination can be identified.

This presentation will first describe the development and validation of the statistical method. Specific applications for the identification of controlled substances in submitted samples will then be presented and discussed, highlighting problems in identification based on visual comparison of mass spectra and the potential of the statistical method to overcome such limitations.

(462) **Fusion of UV-visible Absorbance and Fluorescence Data for Forensic Discrimination of Dyed Textile Fibers**; Nathan C. Fuenffinger[1], Stephen L. Morgan[1]; [1]University of South Carolina, [2]University of South Carolina

UV-visible absorbance microspectrophotometry (MSP) provides a direct and relatively inexpensive means of analyzing forensic trace evidence such as textile fibers. Fluorescence MSP is often used as a follow-up method to absorbance MSP to further investigate the differences or

similarities of questioned and known fibers. In this study, fusion of absorbance and fluorescence MSP (collected using two excitation wavelengths) data is evaluated. Multivariate classifications in the form of naïve Bayes classification and quadratic discriminate analysis were applied to second derivative and mean centered spectra either before or after feature extraction by principal component analysis. Low-, intermediate-, and high-level data fusion strategies were employed in discrimination of textile fibers resulting in correct classification rates of 97.8%, 94.6%, and 93.8%, respectively. As a comparison, classification rates of 89.5%, 87.7% and 87.6% resulted from QDA models built from isolated absorbance, fluorescence with 405 nm excitation, and fluorescence with 546 nm excitation. The results suggest that data fusion is useful for providing additional discriminatory information on textile fibers when compared to single technique data evaluations.

(463) **Infrared Imaging and Multivariate Curve Resolution Applied to the Forensic Examination of Automotive Paints**; Barry Lavine[1], Matthew Allen[1], Koichi Nishikida[2], Mark Sandercock[3]; [1]Department of Chemistry, Oklahoma State University; [2]Materials Science Center, University of Wisconsin; [3]Forensic Laboratory, Royal Canadian Mounted Police, Canada

In the forensic examination of automotive paint, each layer of paint is analyzed individually by FTIR. The more unique are the paint layers, the more information is contained in the sample, and the stronger are the forensic conclusions that can be drawn. In North America, laboratories hand-section each layer of the paint and present each separated layer to the spectrometer for analysis which is time consuming. Sampling too close to the boundary between adjacent layers can also occur which produces an IR spectrum that is a mixture of two layers. Not having a "pure" spectrum of each layer prevents a meaningful comparison between each paint layer or, in the situation of searching an automotive paint database, will prevent the scientist from developing an accurate hit list of potential automotive suspects. These problems have been addressed by collecting concatenated IR data from all paint layers in a single analysis by scanning across the cross-sectioned layers of a microtomed paint sample using an FTIR imaging microscope equipped with a linear array detector. Decatenation of the concatenated IR data is achieved using multivariate curve resolution to obtain a "pure" IR spectrum of each automotive paint layer. This approach saves time, eliminates the need to analyze each layer separately and ensures that the final spectrum of each layer is "pure" and not a mixture. By integrating the imaging experiment including the use of multivariate curve resolution to improve spatial resolution with a prototype pattern recognition IR library searching system to identify the make and model within a limited production year range, we will show that forensic examination of automotive paints can be facilitated in terms of both the speed and accuracy of the analysis.

(464) **High Sensitivity Gas and Liquid Analysis Using Tunable Mid-Infrared Lasers**; Don Kuehl[1], Richard Sharp[1], Eugene Ma[1], Jinhong Kim[1], Charles Marshall[1]; [1]RedShift Systems Corp.

Mid-Infrared Quantum Cascade and Interband Cascade Lasers (QCL, ICL) have been used for some time now in commercial instrumentation for gas phase analysis. However, the high brightness of mid-IR lasers also holds promise for higher sensitivity measurements of optically dark samples such as analytes in water1,2. Prior results have been somewhat disappointing, which may be attributed in part to the immaturity of the first generation of commercial tunable QC lasers and to low frequency instabilities in the lasers and optical systems. To date, the main advantage of QCL's is shown in the ability to use longer pathlength transmission cells when measuring aqueous systems.

In this presentation, we will describe a new system for the measurement of aqueous solutions using low noise, continuous wave (CW) mid-IR lasers coupled with novel continuous flow microfluidic sampling techniques. The system not only provides the advantage of longer pathlength transmission cells, it exhibits a one to two order of magnitude improvement in sensitivity compared to an FTIR . By utilizing a multiplexed sampling method, the system is highly immune to short and long-term system noise, which also improves the accuracy for both quantitative and qualitative analysis. Some example measurements have been made on different types of samples and compared to FTIR for a number of promising applications, including organics in water.

[1] M. Brandstetter, T. Sumalowitsch, A. Genner, A E. Posch, C. Herwig, A. Drolzc, V. Fuhrmann, T. Perkmann and B. Lendl "Reagent-free monitoring of multiple clinically relevant parameters in human blood plasma using a mid-infrared quantum cascade laser based sensor system" Analyst 138, 4022-4028 (2013) .

[2] M. Brandstetter, L. Volgger, A. Genner, C. Jungbauer and B. Lendl "Direct determination of glucose, lactate and triglycerides in blood serum by a tunable quantum cascade laser-based mid-IR sensor" Appl. Phys. B: Laser and Optics. 110 233-239 (2013).

(465) **Toward Monolithic Integration of a Quantum Cascade Laser Array and an Echelle Grating Multiplexer for Widely-Tunable mid-IR Sources**; Mathieu Carras[1], Clément Gilles[1, 2], Luis Orbe[3], Guillermo Caprintero[3], Gregory Maisons[1]; [1]mirSense, France; [2]III-V Lab, France; [3]Universidad Carlos III de Madrid, Spain

In the mid-infrared (Mid-IR), arrays of Distributed Feedback Quantum Cascade Lasers (DFB-QCL) have been developed to extend the wavelength operation range of laser-based gas sensing systems improving the sensibility and selectivity. To go even further toward multi-species detection in a portable system, narrow-linewidth, single mode operation and wide tunability will then be gathered together on a single chip with high mechanical stability and compactness. In order to benefit from this extended wavelength range in a single output beam, integrated vertical adiabatic coupler and wavelength multiplexer have to be demonstrated separately.

We present the design, fabrication and performances of all the elements to realize widely tuneable Mid-IR monolithic source. We detailed photonic circuit consisting of an array of DFB-QCLs, taper couplers and passive multiplexers (MUX) on indium phosphide platform, which combines light generation, guiding and multiplexing functions together. The advantages of our monolithic approach are design flexibility, relatively simple processing and the need for only a single epitaxial growth for the entire structure. First, last results on gain medium of the laser array based on three distinct cascade designs, with respective gain at 7.3, 8.5 and 9.4 µm wavelengths will be shown. Then, evanescent coupler will be designed to transfer all light adiabatically from the active region to a low loss passive waveguide, while taking advantage of the high gain available in the quantum wells. Finally, the multiplexer is based on an etched diffraction grating, covering the whole range of the 30 lasers of the array while keeping a very compact size. These results are the first step to the realization of a monolithic widely tunable source in the Mid-IR and would therefore benefit to the development of fully integrated spectroscopic sensor systems.

(466) **Integrated Ring Laser Systems for Spectroscopy based on Quantum Cascade Structures**; Schrenk Werner[1], Rolf Szedlak[1, 2], Daniela Ristanic[1], Benedikt Schwarz[1], Peter Reininger[1], Andreas Harrer[1], Hermann Detz[1], Donald C. MacFarland[1], Aaron M. Andrews[1], Gottfried Strasser[1]; [1]Technische Universität Wien, Center for Micro- and Nanostructures and Institute for Solid State Electronics

Quantum Cascade Lasers are versatile light sources operating in the molecular fingerprint region of the electromagnetic spectrum. Recently, our group presented Ring Cavity Quantum Cascade Laser (Ring-QCL) emitting via the surface or substrate. A second order grating is used to select the emission wavelength and provide surface or substrate emission. The Ring-QCL geometry together with the surface or substrate emission allows the realization of dense 2-dimensional arrays of lasers with slightly different emission wavelengths to cover a larger spectral range. Each of this Ring-QCL does have a second order grating with slightly different grating period. In the case of substrate emission on chip focusing has been demonstrated by the use of a Metasubstrate. This increased the maximum intensity by a factor of six. Modified DFB gratings like abrupt and continuous Pi-Shifts or off-centered gratings can provide far fields with a central intensity maximum. We also demonstrate the possibility to integrate laser and detector on one chip by the use of bi-functional quantum cascade structures, allowing for further integration.

(467) **Monolithic Quantum Cascade Lasers And Their Applications**; Christian Pfluegl[1]; [1]Eos Photonics, Inc.

This presentation presents the spectroscopic concepts and results enabled by arrays of Distributed Feedback (DFB) QCLs, with each element at a slightly different wavelength than its neighbor. In optical systems, such as standoff detectors and in situ gas analyzers, this increases analyte sensitivity and selectivity by broadening spectral source coverage and by allowing for extremely fast all-electronic wavelength tuning with no moving parts. The QCL array is also an increasingly essential solution to the power scaling of QCLs. This talk will present the QCL array concept and our packaging systems before moving to discuss molecular spectroscopy results for a) multigas sensing, b) standoff explosives detection, and c) power scaling for directed energy.

(468) **Broadly-tunable Monolithic THz Quantum Cascade Laser Sources**; Mikhail Belkin[1]; [1]The University of Texas at Austin

Widely-tunable room-temperature, electrically-pumped semiconductor sources in 1-5 THz spectral range are highly desired for spectroscopy and imaging application. THz sources based on intra-cavity difference-frequency generation (DFG) in dual wavelength mid-infrared quantum cascade lasers (QCLs) with Cherenkov phase-matching are currently the only monolithic room-temperature THz devices that comparable in size, mass-producibility potential, and operation simplicity to infrared/visible diode lasers or mid-infrared QCLs. We will present our latest results on the development of broadly-tunable THz systems based on this technology. Both monolithic and external cavity configurations are explored. External-cavity THz DFG-QCL systems demonstrate continuous tuning at room temperature from 1.2 THz to 5.9 THz with THz peak power output varying between 5 and 90 μW, depending on the operating frequency. Beam steering of terahertz Cherenkov emission with frequency is suppressed by bonding devices onto high-resistivity silicon substrates that have virtually no refractive index dispersion and vanishingly-small optical loss in 1-6 THz range. To reduce the size and avoid mechanical complexity of external cavity tuners, we also demonstrate monolithic room-temperature THz DFG-QCL tuners with a tuning range from 3.44 THz to 4.02 THz, which corresponds to 15% of their center frequency. We have also fabricated an array of 10 of such monolithic THz DFG-QCL tuners that provide continuous tunability between 2 and 4 THz at room temperature with up to 105 μW of peak THz power output.

This work was supported NSF grant numbers ECCS-1150449 (CAREER) and ECCS-1408511. Walter Schottky Institute group acknowledges financial support from the excellence cluster “Nano Initiative Munich (NIM)”. Sample fabrication was carried out in the Microelectronics Research Center at the University of Texas at Austin, which is a member of the National Nanotechnology Infrastructure Network.

(469) **Standoff LIBS. Concepts and Scenes**; Javier Laserna[1]; [1]Universidad de Malaga

Analysis of distant objects using LIBS has experienced a spectacular growth in the past years. Although this application was demonstrated in the early years of LIBS, the use of lasers with improved beam properties, detectors with gating capabilities and tailored optical designs, has resulted in a new generation of LIBS systems of unprecedented performance. LIBS with transmission of the excitation and emission light beams through the atmosphere -also known as standoff LIBS- deserves special attention. This method has attracted considerable interest since it provides the solution to chemical measurements unapproachable by preceding technologies. The analysis of trace explosives at 50 m from the instrument, the inspection of liquid steel in ton-size converters, or the detection of pollutants in the façade of historical buildings, are just three examples of the exclusive capabilities of standoff LIBS. In this talk the fundamentals of this approach will be presented and a number of experimental variables governing the LIBS response will be discussed.

(470) **Application of Distance Correction to ChemCam LIBS Measurements**; Alissa Mezzacappa[1], Noureddine Melikechi[1], Agnes Cousin[2], Roger Wiens[3], Jeremie Lasue[2], Samuel Clegg[3],Robert Tokar[4], Steven Bender[4], Nina Lanza[3], Sylvestre Maurice[2]; [1]Optical Science Center for Applied Research, Delaware State University; [2]Institut de Recherche en Astophysique et Planetologie (IRAP), Universite’ Paul Sabatier, France; [3]Los Alamos National Laboratory; [4]Planetary Science Institute

Laser-induced breakdown spectroscopy (LIBS) provides chemical information from atomic, ionic, and molecular emissions from which geochemistry can be deciphered. Emission-specific distance response is a complicating factor to stand-off LIBS (ST-LIBS) spectral interpretation. To determine the behavior of a LIBS emission line with distance, it is necessary to separate the effects of many parameters such as laser energy, laser spot size, target homogeneity, and optical collection efficiency. ST-LIBS often requires variation in measurement distance between the laser and its targets from one measurement to the next, which presents an analytical challenge when comparing spectral results gleaned from slightly different experimental configurations. A preliminary spectral distance correction based on a proxy spectroscopic standard created from first-shot dust observations of Mars targets is applied to 16 lines (Mg, Si, Al, Ca, Fe, Na, and K) of pre-launch LIBS calibration data and ameliorates the distance bias in multivariate-based elemental-composition predictions[1]. We develop an expanded set of neutral and ionic spectral emissions that constrain distance bias in the ChemCam Mars LIBS data set. There are many emission lines identified that do not behave consistently with distance, as evidenced from laboratory analogue measurements and ChemCam data. By testing multiple proxy standards, we find a correction that minimizes the difference due to distance for spectral lines and increases spectral reproducibility for ChemCam data. However, there are many emission lines identified that do not behave consistently with distance, as evidenced from laboratory analogue measurements and ChemCam data. When the quantitative performance of the expanded distance correction on ChemCam data is assessed, there is improvement for SiO2, Al2O3, CaO, FeOT, Na2O, and K2O, which represent a majority of the major rock forming elements.

1. N. Melikechi, A. Mezzacappa, A. Cousin, N.L. Lanza, J. Lasue, S.M. Clegg, G. Berger, R.C. Wiens, S. Maurice, R.L. Tokar, S. Bender, O. Forni, E.A. Breves, M.D. Dyar, J. Frydenvang, D. Delapp, O. Gasnault, H. Newsom, A.M. Ollila, E. Lewin, B.C. Clark, B.L. Ehlmann, D. Blaney, C. Fabre: Correcting for variable laser-target distances of LIBS measurements with ChemCam using emission lines of Martian dust spectra. Spectrochimica Acta Part B Atomic Spectroscopy, 96, 51-60, 2014

(471) **Stand-off LIBS using Laser Filamentation: Fundamental Characterization for Quantitative Analysis**; Matthieu Baudelet[1,2], Matthew Weidman[1], Mark Ramme[1], Khan Lim[1], Magali Durand[1], Martin Richardson[1]; [1]Townes Laser Institute, University of Central Florida; [2]National Center for Forensic Science, University of Central Florida

The need to interrogate possible threats at distance over several hundreds of meters leads to the use of powerful lasers as sampling and excitation sources. To avoid the use of large focusing optics, femtosecond laser filamentation has been studied. Filaments enable the long distance projection of intense laser radiation, consequently enabling applications not otherwise possible using conventional laser focusing. Filamentation results in a clamped irradiance on the order of 10^{13} W/c^{m2}, and for laser-ablation based spectroscopy techniques, such as Laser Induced Breakdown Spectroscopy (LIBS), can be applied as the atomization and excitation source. Combined with LIBS, sensing at distances over hundreds of meters is possible and is commonly termed Filament-Induced Breakdown Spectroscopy (FIBS).

Because of the unique spatial-temporal properties associated with filamentation, the interaction with solid materials is different from traditional focused laser radiation. This talk discusses the quantitative characterization of filament-material interaction for applications such as stand-off spectroscopy. Characterization of the mass removal, the plasma composition as well as the directionality of the plasma emission enable reliable estimates of the stand-off ranges possible with single filament-induced LIBS materials detection. Analytical applications of filament-induced LIBS will be shown on organic samples.

This work was funded by the Army Research Office, the HEL/JTO, the Air Force Office of Scientific Research and the State of Florida.

(472) **Femtosecond Filament-Laser Ablation Molecular Isotopic Spectrometry**; George Chan[1], Huaming Hou[1], Xianglei Mao[1], Vassilia Zorba[1], Richard Russo[1]; [1]Lawrence Berkeley National Laboratory

Conventional laser-ablation based optical-emission methods (e.g., laser induced breakdown spectroscopy (LIBS) and laser-ablation molecular isotopic spectrometry (LAMIS)) are versatile tools for direct elemental and isotopic analyses in solid samples, and have been successfully demonstrated for stand-off analyses. However, for remote analysis over long distance, one challenge for the conventional approach is the classical optical diffraction limit of the laser-focusing optics, in which the minimum spot size that a laser beam can be focused increases linearly with its focusing distance. One solution to overcome this diffraction-limit constraint is to perform the ablation with a femtosecond-laser induced filament.

Femtosecond filamentation is a result of dynamic equilibrium of two opposing nonlinear effects – laser beam self-focusing and air ionization. Once launched in air, an intense (a few mJ) femtosecond laser beam automatically undergoes self-focusing as a consequence of the nonlinear refractive index of air (the Kerr effect). Self-focusing is similar to focusing of a laser beam by a lens except that this lens is created by the laser itself. This unconventional focusing effect is ultimately limited by very weak ionization of the air, sufficient to counter the change in refractive index, preventing the collapse of the beam that would have occurred in the absence of any saturating mechanism. As a consequence, the femtosecond laser beam is stabilized as a ~100 μm diameter filament, packing a laser pulse intensity of ~10^{14} W/cm2. High laser pulse intensity inside the core of the filament can therefore lead to ablation of a solid-state target over a long range.

In this proof-of-concept work, femtosecond filament (F^2)-LAMIS was performed inside a laboratory for a remote distance about 10 m. In this presentation, the underlying principles of F^2-LAMIS for remote isotopic analysis will be overviewed, and its analytical characteristics and performance will be discussed and compared with conventional LAMIS.

(473) **Mass Spectrometry of Airborne Nanoparticles**; Murray Johnston[1]; [1]University of Delaware

Airborne nanoparticles affect both climate and human health. Understanding these impacts requires knowledge of how nanoparticles form and grow, which in turn requires the development of novel analytical methods that can detect very small amounts of material and can be used to monitor fast changes in chemical composition. Our group has developed a nano aerosol mass spectrometer (NAMS) for characterization of ambient particles less than 20 nm in diameter. This instrument performs quantitative elemental analysis on a particle-by-particle basis and has been used to study time-dependent changes in nanoparticle composition in both urban and remote locations. These measurements show that the composition of particles in the air we breathe is dynamic and these changes give insight into the chemical mechanisms of nanoparticle formation and growth.

(474) **Rapid Measurement of Nanoparticle and Microparticle Size Distribution and Number Concentration by Inductively Coupled Plasma Mass Spectrometry**; Austin Wilson[1], Chuanqiang Sun[1], John W. Olesik[1]; [1]Ohio State University

Interest in nanoparticles, either natural or engineered, has risen in recent years due their wide range of uses and incompletely understand fate in the environment, plants, animals and humans. Engineered nanoparticles and microparticles can have a range of particle size distributions, dependent on the manufacturing process and control. As a result, a rapid, widely available technique to accurately measure nano- and micro-particle concentrations, chemical composition and size distribution is needed. ICP-MS could be used to rapidly measure the number of nano- or micro-particles, their particular composition and the particle size distribution. ICP-MS could be used in a sub-ms to ms time resolved mode to measure individual nano- or micro-particles (single particle (sp)-ICP-ms). We will briefly discuss the concepts, capabilities and limitations of sp-ICP-MS. We will discuss the attainable particle size distribution resolution and accuracy and potential instrumental sources of broadening of the measured particle size distribution. Among the instrumental sources of measured particle size distribution are: polydisperse aerosol sample introduction resulting in variable extents of particle vaporization and ion diffusion prior to the MS sampling orifice and ion detection efficiency variations dependent on the radial position of the particle in the plasma. ICP-MS based measurements of nano- and micro- particle size distributions of certified standard reference materials and scanning electron microscopy measurements will be compared. Experiments to assess potential sources of broadening of measured nano- and micro- particle sizes will be described.

(475) **A New Approach for Halogen Isotope Measurements with Focus of Compound-Specific Isotope Ratio Analysis**; Matthias Gehre[1], Julian Renpenning[1], Kristina Hitzfeld[1], Tetyana Gilevska[1]; [1]Helmholtz Centre for Environmental Research -UFZ

The feasibility of compound-specific chlorine isotope analysis of chlorine containing compounds via high-temperature conversion (HTC) was already demonstrated by Hitzfeld et al. (2011). However, analysis of chlorine isotopes after conversion to hydrochloric acid [H37Cl/H35Cl] revealed serious instabilities during stable isotope measurements. The instabilities could only be related to the conversion process and interference of the background matrix. Therefore, the HTC process was investigated in detail, in order to understand and optimize the conversion.

Characterization of conversion and simultaneous measurements of stable isotopes were established using a GC-HTC-MS/IRMS, equipped with a dual-detection system of ion trap MS and IRMS. While the conversion process could be monitored on-line using the ion trap MS, isotopes analysis was done simultaneously via IRMS. Remarkably, analysis of chlorinated and non-chlorinated hydrocarbons conversion revealed severe by-product formation at inappropriate HTC conditions. Detected by-product could be assigned to acetylene, ethylene, benzene and other interfering hydrocarbons in the mass range of the H37Cl as target ion. Therefore, previous instabilities of chorine isotope analysis could be assigned to overlapping ion signals.

By-product formation could be suppressed by modifying the HTC parameters (temperature, reactant gas, reactor load and residence time in reactor). Subsequent analysis of reference material (chloromethane, chloroethene, hexachlorocyclohexane and chloroacetic acid) was able to demonstrate very good precision ($1\sigma \sim 0.3$ ‰) and accurate (max. deviation ≤ 0.8 ‰) chlorine isotope measurements via GC-HTC-IRMS.

Reference

Renpenning, J.; Hitzfeld, K. L.; Gilevska, T.; Nijenhuis, I.; Gehre, M.; Richnow, H. H:

Development and Validation of an Universal Interface for Compound-Specific Stable Isotope Analysis of Chlorine ((37)Cl/(35)Cl) by GC-High-Temperature Conversion (HTC)-MS/IRMS.

Anal Chem 2015, 87, (5), 2832-2839.

(476) **Exploring Charge-Transfer Ionization Pathways with the Flowing Atmospheric-Pressure Afterglow (FAPA) Ambient Ionization Source to Expand the Range of Detectable Analytes**; Sunil Badal[1], Shawn Michalak[2], George Chan[3], Jacob Shelley[1]; [1]Department of Chemistry and Biochemistry, Kent State University; [2]Stark State College; [3]Lawrence Berkeley National Laboratory

Plasma-based ambient desorption/ionization techniques, which employ a low-energy plasma as a means to desorb and/or ionize molecular species, have attracted considerable attention in the last few years. These plasma sources are versatile in that they enable direct desorption and ionization of analytes from gaseous, liquid, and solid samples. However, ionization matrix effects caused by competitive ionization processes can worsen sensitivity and inhibit quantitative capabilities. Additionally, much remains to be learned about the fundamental mechanisms which govern desorption and ionization processes.

Here, we aim to expand the analytical capability of the flowing atmospheric pressure afterglow (FAPA) ambient ionization source by exploring additional types of ionization chemistry. We found that the abundance of certain reagent ions produced by FAPA source, and the corresponding ionization pathways of analytes, can be altered by changing the source working conditions. Reagent ion signals were monitored while changing the discharge current and gas flow rate. High abundance of charge transfer reagent ions, such as NO^+ and O_2^+, were preferentially formed at low helium flow rates and high discharge currents whereas high flow rates and low discharge currents produced more proton-transfer reagent species, e.g., protonated water clusters. To characterize ionization pathways under different source conditions, model analytes with well-known ionization properties were analyzed. In one example, biphenyl, which can undergo either charge transfer or proton transfer, was shown to significantly change ionization pathway based on source operating parameters, which demonstrated that different analyte ions can be formed under a unique set of operating condition showing two different operating regimes. More efficient ionization, produced by different modes of operation, made it possible to detect compounds, which are not capable of efficient proton-transfer ionization, such as 2,4,6-trichloroanisole and 2,2' dichloroquaterphenyl. Furthermore, we will demonstrate the ability to detect non polar analytes in the presence of easily ionized analytes in the charge-transfer mode, which would not be possible with conventional plasma-based ambient MS approaches. This added dimensionality of ambient MS analyses through alternative gas-phase chemistry increases the range and number of detectable analytes. In addition, potential mechanisms of reagent-ion formation and reasons for their variation with discharge current and gas flow rates will also be discussed.

(477) **High-Sensitivity Organohalogen Detection and Quantification by PARCI-MS**; Kaveh Jorabchi[1]; [1]Georgetown University

Halogenated compounds constitute a large fraction of persistent environmental pollutants and over 30% of the drugs. The halogen atoms can be used as intrinsic labels for detection of these compounds by chromatography coupled to elemental mass spectrometry (MS). Moreover, compound-independent calibration in elemental MS offers quantification without compound-specific standards, a highly desirable attribute for characterizing transformation products of organohalogens, e.g. in drug metabolism studies. Unfortunately, the conventional elemental ion sources lack the sensitivity for trace detection of halogens, particularly for Cl and F. Plasma assisted reaction chemical ionization (PARCI) addresses this shortcoming by utilizing a two-step ionization strategy. In the first step, a reactive plasma is used to quantitatively extract halogens form the analytes in the form of individual atoms and small molecules such as HBr. Ionization of these reaction products occurs in the plasma afterglow in negative mode, resulting in formation of F^-, Cl^-, and Br^- ions. In this presentation, we will discuss our fundamental ionization studies as well as the analytical performance of GC-PARCI-MS, demonstrating compound-independent response factors for halogens with detection limits of 750 fg F, 400 fg Cl, and 20 fg Br on column. The challenges and progress in development of LC-PARCI-MS will also be discussed.

(478) **Development of Raman Spectroscopy as a Rapid Identification Method for Raw Materials**; Tony Wang[1], David Meriage[1]; [1]Amgen
Advances in Raman spectroscopy have decreased the size of the spectrometer and created a number of commercially available handheld Raman analyzers. These handheld devices have allowed Biopharma to gradually adopt a new raw material receiving philosophy. Critical considerations for raw material method development using Raman spectroscopy will be discussed. Case studies will also be used to illustrate the importance of these considerations.

(479) **Micro-Raman Spectroscopy used as a PAT Tool and for Real Time Monitoring of Protein Stability during Freeze Drying**; Tatiana Starciuc[1,2], Laurent Paccou[1, 2], Yannick Guinet[1,2], Alain Hedoux[1,2]; [1]University of Lille 1 Sciences en Technology; [2]University Lille 1, UMET - UMR CNRS 8207
Freeze drying is widely used in Pharmaceutical industry in order to improve a long-term stability to the protein drug. An important drawback of this process is the proteins denaturation (loss of native three-dimensional structure), due to a series of physic-chemicals stresses at which proteins are exposed during process development (changes in temperature, pressure, dehydration, pH and concentration). In this context, different excipients (sugars, polyols, etc) are added in drug formulation for the protection of the protein structure during freeze drying cycles having also the role to stabilize the powder at a long-term storage. All of these formulations are used empirically without precise knowledge of the denaturation's origin and nature. Moreover, there is a lack of information regarding the protein-ice-excipients interactions and the correlation of structural changes in proteins with the presence or absence of various excipients.

Recently it was shown that micro-Raman spectroscopy offers the possibility to analyze the protein denaturation process in each freeze drying step (freezing, ice sublimation, water desorption) following spectral changes in the amide I band region (1650cm-1) (Hédoux et al., 2012, J. Pharm Sci 101(7) : 2316-2326). In the same time, this method can be applied to describe the distribution and mapping of different components in the lyophilized drug product (protein, ice, excipients, etc) and then to characterize the influence of these components on the protein stability during lyophilization (Hédoux et al., 2013, J. Pharm Sci., Wiley Online Library, 102 : 2484-2494).

In this presentation we will show the contribution of micro-Raman spectroscopy which can be used both as a process analytical technology tool and as a structural probe for real time monitoring the protein stability during the freeze drying process as well as long-term storage. The use of multivariate data analysis in order to detect subtle structural changes and to describe protein-excipient interactions will be presented. Deciphering the physical mechanism of protein stabilization will be discussed from the analysis of various protein formulations, and perspectives for future investigations will be presented.

(480) ***In situ*** **Raman Spectroscopic Monitoring of Multiple Biochemical Species During Microbial Fermentation Process Development**; Karin Balss[1], Sean Gilliam[3], Angelica Spinelli[1], Wojciech Czaja[2]; [1]Janssen Pharmaceuticals, [2]Depuy Synthes, [3]Kaiser Optical Systems
Recently, DePuy Synthes introduced SYNTHECEL® Dura Repair, a nanocellulose material for dura mater repair in adults. Continuous process improvements along with scale-up activities are important in order to meet future demands. Raman spectroscopy is an ideal process analytical technology (PAT) tool to assist with real-time characterization and product development understanding during the fermentation process, via the in situ monitoring of multiple media components and metabolites. In this study, we present the utilization of in situ Raman to quantitatively monitor multiple components, over the course of a 25L scale microbial fermentation process. Multivariate Partial Least Squares (PLS) regression models were then developed to correlate real-time Raman spectra to off-line reference values. To increase process understanding during fermentation, a critical process parameter was then varied between batches to understand the effects on the process. The real-time Raman spectra acquired throughout each batch were able to differentiate the batches based on the process parameter variability.

(481) **PAT Raman Data Acquisition in Biopharmaceutical Development and Manufacturing Environments Using Siemens SIPAT Framework**; Stefani Takahashi[1], John Paul Smelko[1], Brandon Berry[1], Robert Song[1]; [1]Biogen
Biogen has created an infrastructure for acquiring, storing, and transforming online Raman spectral data to enable real-time monitoring and control of key metabolites such as glucose in a large scale GMP environment. A calibration model was constructed from spectra and offline glucose sample data collected in both development and manufacturing environments. Predicted glucose concentrations were output to the control system and data historian where they can be accessed by our online multivariate monitoring software.

Future use of this infrastructure will allow for maintaining and controlling the production bioreactor glucose concentration level automatically without offline sampling, allowing for tighter control of metabolic profiles and more consistent batch to batch processing.

(482) **CQA focused Process Analytical Technology for Biologics Manufacturing**; Douglas Richardson[1], Zi Chen[1], Maria Khouzam[1], Daisy Richardson[1], John Higgins[1], David Pollard[1]; [1]Merck
In 2004 the FDA published an industry guidance for Process Analytical Technology (PAT) summarizing the principles for innovative pharmaceutical development. Evolving from the FDA Guidance numerous PAT applications have been studied for the manufacture of both small and large molecule pharmaceuticals. Quality by design (QbD) is a central part of biotherapeutic development, laying the framework for building quality into products through detailed process design and control. Implementation of PAT tools provides a means to improve the process and ensure final product quality through real-time monitoring of critical process and quality attributes (CPPs and CQAs) enabling rapid process decisions and potential real time release testing (RTRT). The majority of PAT applications to date have focused on monitoring process steps through spectroscopic techniques such as NIR, Raman, FTIR or UV-Vis. These techniques function very well for the monitoring of small molecule functional groups or chemical reactions including key nutrient components for biologics manufacturing. Limited applications exists applying spectroscopic PAT tools for monitoring therapeutic protein CQA's due to a lack of specificity and resolution for these complex attributes. This gap provides an opportunity for application of more selective online liquid chromatography PAT tools for direct monitoring of therapeutic protein CQA's.

This work highlights the expanding biologics PAT toolkit at Merck. Applications of the online ultra-performance liquid chromatography with UV and MS detection for biologics development will be discussed. Successful demonstration of both at-line and on-line sampling followed by reversed phase UPLC for titer and metabolites (RP-UPLC) , size exclusion for aggregates (UP-SEC) and ion exchange chromatography charge heterogeneity (IEX) will be presented. Cases studies for application of these PAT tools for online monitoring of continuous processing, forced degradation, and clinical compatibility studies will be reviewed.

(483) **Gold Nanostars: A Multi-Modality Nanoplatform for Diagnostic and Therapeutic Applications**; Tuan Vo-Dinh[1], Hsin-Neng Wang[1], Yang Liu[1], Andrew Fales[1]; [1]Duke University

This lecture presents the development and application of plasmonics-active gold nanostars for use in medical cancer diagnostics and therapy. This presentation describes several areas of research at the nexus of engineering, biology, medicine and nanotechnology. A new generation of nanoprobes using plasmonic nanomaterials including gold nanostars combining bio-recognition and nanotechnology has been developed for in vivo monitoring of biochemical processes in animal and plant cells. These studies demonstrate the applications of a new type of SERS plasmonics "molecular sentinel" nanoprobes for detection of microRNA biomarkers for disease. Also, new multi-modality nanoprobes provide a unique nanoplatform with capabilities to perform pre-operative tumor scanning with magnetic resonance imaging (MRI) and X-ray computed tomography (CT), intra-operative tumor detection with surface-enhanced Raman scattering (SERS) and two-photon luminescence (TPL) as well as photothermal therapy (PTT). Such nanoprobes open new horizons to a wide variety of applications ranging from in vivo sensors and in vitro diagnostics at the point of care to molecular imaging and sensing of biomarkers in plant cells for biofuel development as well as therapeutic applications.

(484) **Gold Superstructures for SERS-based Bioimaging**; Srikanth Singamaneni[1]; [1]Washington University in St. Louis

Plasmonics involves the control of light at the nanoscale using surface plasmons. One particular area where plasmonics is expected to make an enoromous impact is the field of life sciences, with applications in bioimaging, biosensing, and targted and externally-triggered therapy. We present a novel class of ultrabright surface enhanced Raman scattering (SERS) probes based on core-satellite and core-shell plasmonic nanostructures. We call the core-shell nanostructures as bi-layered Raman-intense gold nanostructures with hidden tags (BRIGHTs), which are nearly 20 times brighter compared to the exisiting SERS probes. Such BRIGHT probes can be incorporated into drug carrying vehicles (gold nanocages) to form multifunctional nanorattles that facilitate the monitoring of drug release non-invasively. We also demonstrate bio-enabled synthesis of a novel class of functional SERS probes with built-in and accessible electromagnetic hotspots formed by densely packed satellites grown on a plasmonic core. The accessible electromagnetic hotspots of the Au superstructures enable facile sampling of the surrounding complex biological milieu. The core-satellite superstructures serve as nanoscale sensors to spatiotemporally map intravesicular pH changes along endocytic pathways inside a living cell.

(485) **SERS Imaging of Gold Nanoparticles in Biological, Paper, and Granular Matricies**; Peter Vikesland[1], Rebecca Lahr[1], Matthew Chan[1]; [1]Virginia Polytechnic Institute and State University

This presentation will highlight our application of surface enhanced Raman spectroscopy (SERS) based imaging for the detection of gold nanoparticles and nanoparticle surface associated biomolecules in a variety of matrices. We will illustrate the broad utility of SERS imaging for 1) detection of intracellularly grown gold nanoparticles in the aquatic algae Pseudokirchneriella subcapitata and the identification of surface-associated biomolecules, 2) quantification of nanoparticle transit distances within paper-based microfluidic devices, and 3) evaluation of nanoparticle aggregation rates within granular media. In each case we utilize the exquisite sensitivity of SERS to detect both individual as well as aggregated gold and silver nanoparticles.

(486) **Selective Detection of 100 B. Anthracis Ames Spores in 20 Minutes using a Portable SERS Assay**; Stuart Farquharson[1], Chetan Shende[1], Wayne Smith[1], Carl Brouillette[1], Jay Sperry[3], Todd Sickler[2],Amber Prugh[2], Jason Guicheteau[2]; [1]Real-Time Analyzers, Inc.; [2]US Army; [3]University of Rhode Island

The May 2013 mailing of Ricin to President Barack Obama once again demonstrated the need for a bioagent analyzer to protect US citizens at home and the military abroad. Despite the substantial effort to develop bioagent analyzers, current analyzers 1) are too slow (e.g. PCR, 1 measurement/hour), 2) have high false-alarm rates (e.g. immunoassays), 3) lack sensitivity (e.g. Raman), 4) are not field-usable (e.g. GC-MS), or 5) cannot be multiplexed to identify multiple species (e.g. PCR). In an effort to overcome all of these limitations, we have developed a sample system that selectively binds specific bioagents and produces surface-enhanced Raman spectra. Measurements at the US Army's Edgewood Chemical Biological Center of *B. anthracis* Ames spores using this sample system coupled with a compact, portable Raman spectrometer will be presented.

(487) **Raman Spectroscopy and SERS Investigations of Rhizosphere and Medically Relevant Bacterial Communities**; Sneha Polisetti[1], Nameera Baig[3], Jennifer Morrell-Falvey[2], Joshua Shrout[4], Mitchel Doktycz[2], Paul Bohn[1,3]; [1]Department of Chemical & Biomolecular Engineering, University of Notre Dame; [2]BioSciences Division, Oak Ridge National Laboratory; [3]Department of Chemistry, University of Notre Dame; [4]Department of Civil and Environmental Engineering & Earth Sciences, University of Notre Dame

Biological samples are challenging to study due to their inherent complexity and heterogeneity. Raman studies of bacterial samples in their natural unaltered state, face issues like autofluorescence and complex spectra from known and unknown components of the sample. We are employing a nano-enabled approach to specifically target various components of bacterial communities and obtain chemical information by varying the enhancing mechanism, excitation wavelength, experimental conditions and multivariate statistical approaches. Resonant Raman has been utilized to characterize the presence of carotenoids in the cell membrane of wild type rhizospere bacteria YR343 and the absence of carotenoids in the mutant strain .Colloidal metal nano coatings have been used to obtain flavin adenine dinucleotide (FAD) signatures from the bacterial cells. We have also performed SERS detection of metabolites in communities of *Pseudomonas aeruginosa*. The work outlines the potential of Raman spectroscopy as a tool to non-invasively study bacterial systems while preserving the molecular integrity of these complex and fragile systems.

(488) **Plasmon-enhanced Spectroelectrochemistry – An Advanced Tool for Biosensing**; Christa Brosseau[1], Lili Zhao[1], Reem Karaballi[1], Jonathan Blackburn[2]; [1]Saint Mary, [2]University of Cape Town, Cape Town, South Africa

Recently our research has focused on combining nanoscale plasmonics and spectroelectrochemistry to allow for rapid, portable analysis of a multitude of target analytes. Specifically, we have developed an electrochemical surface-enhanced Raman spectroscopy (EC-SERS) system which we have used in our work to better understand and detect human disease. For example, we have recently demonstrated work which uses screen printed electrodes modified with multilayers of uniform Au and Ag nanoparticles to quantitatively detect a urine biomarker for preeclampsia in synthetic urine at clinically relevant concentrations. In addition, the design of an EC-SERS aptasensor capable of detecting DNA hybridization directly, without the need for costly labels is discussed. Our work towards the development of fabric-based sensing platforms is also discussed in this presentation.

(489) **Direct Detection of MicroRNA Based on Plasmon Hybridization of Nanoparticle Dimers**; Jennifer Chen[1]; [1]York University

MicroRNAs (miRNA) are short, non-coding oligonucleotides that are important for regulating a range of biochemical pathways. Abnormal levels of miRNA in cells or secreted into biological fluids have been identified in diseases. MiRNA can therefore be potential biomarkers for early disease diagnosis; however their detection and quantification are challenging. Herein we apply the sensing platform of discrete actuatable dimers for the detection of human miR-210 (hsa-miR-210-3p). The detection signal is a spectral blue shift in the hybridized plasmon mode as monitored by single-nanostructure spectroscopy. We investigate the specificity and detection limit of the platform and quantify miR-210 levels in RNA extracts of cells cultured under different oxygen tensions. In addition we demonstrate the feasibility of detection in complex media by examining miR-210 secreted in cell media. This sensing platform may be developed as a bioanalytical tool for validating miRNA profiles of biological fluids.

(490) **Filtration of Antigen-Assembled Gold Nanoparticles for SERS Detection**; Jeremy Driskell[1], Arielle Lopez[1], Francis Lovato[1]; [1]Illinois State University

Diagnostic tests are essential for disease detection and confirmation in order to effectively treat patients, control outbreaks, and monitor efficacy of therapeutics. Diagnostic tests are most beneficial when they provide high sensitivity detection and multiplexing capabilities. Recently, surface-enhanced Raman scattering (SERS) immunoassays have been developed for diagnostic applications to meet these demands. However, many emerging SERS-based assays are limited by diffusion kinetics to result in long assay times and are labor intensive, requiring many incubation and washing cylces. Herein we describe our effort to develop a simple, rapid homogeneous SERS immunoassay for proteins and viruses to address the time and labor challenges, yet retain the attributes of SERS-based detection.Our lab has developed SERS-active modified gold nanoparticles that undergo specific aggregation in the presence of antigen. The antigen-assembled aggregates are then separated from excess nanoparticle reagent by size selective filtration for quantitative analysis. In this presentation we will discuss the nanoparticle modification and characterization, antigen-mediated aggregation kinetics, and optimization of the filter for efficient capture and concentration of the nanoparticle aggregates. Moreover, the role of plasmonic coupling between the assembled nanoparticles will be discussed.

(491) **Integration of Electrophoretic Capture and Surface Plasmon Resonance Sensing in a Microfluidic Channel**; Karl Booksh[1], Ornella Sathoud[1], Joe Smith[1], Casey Kneale[1], Missy Postelwaite[1], Kimberly Hibsman[1]; [1]University of Delaware

Presented here will be our most recent results in combining surface plasmon resonance sensors with lab on a chip electrophoretic capture. Challenges include integration of multiple analytical methodology in a single platform. Selective electrografting of diazonium salts to functionalize an analytical and reference detector within a nanofluidic channel will be presented. We will demonstrate how the two sensors, place in close proximity, can compensate for temperature fluctuations during analysis.

(492) **Real-time Monitoring Bacterial Growth under Different Flow Rates with Surface Plasmon Resonance Imaging**; Pegah N. Abadian[1], Edgar Goluch[1]; [1]Northeastern University

Bacteria exist everywhere and when they find appropriate spot they will bind to the surface and form irreversible attachment. The irreversible attached bacteria will attract other bacteria to the spot and will form colonies, when the concentration of bacteria reaches to certain threshold they will start producing an exopolysaccharide (EPS) metrics which is known as biofilm and shield them from the outside environment. Bacteria inside biofilm are up to 1000 times more resistant to different kind of antibiotics. So it is very important to prevent bacteria from forming biofilm.

The fluid flow rate in the environment containing bacteria will generate a shear stress on the surface which will consequently decrease the initial attachment of bacteria on the surface; which will reduce biofilm formation as a result. It is also known that higher flow rates increase the rate of available fresh food for bacteria; this helps bacteria grow faster which will increase biofilm formation.

In this study we used surface plasmon resonance imaging sensor to real-time monitor Staphylococcus aureus growth and biofilm formation under continues fluidic flow over the surface. To study the effect of flow rate and consequent shear stress on bacterial growth, 15µl of S. aureus media were grown on the sensor surface under 10ul/min(slow flow rate) and 120ul/min flow rates (fast flow rate) in two different experiment sets. Each experiment was run over night.

The difference images generated by SPRi showed that bacterial growth is higher under the slower flow rate indicating that shear stress has a larger impact on preventing initial bacterial attachment and further biofilm formation. To confirm the results the sensor surface was removed from SPRi instrument after the experiment and stained with crystal violet to visually study the concentration of bacteria on the surface. In this experiment bacterial cells were stained violet. The staining results showed higher concentration of cells under slow flow rate which was consistent with the SPRi experiment.

(493) **Surface Plasmon Resonance and Fluorescence: A Novel Approach for Characterization of Biomolecules Interactions**; Jérémie Labrecque-Carbonneau[1], Jean-François Masson[1]; [1]University of Montreal

Biological systems characterization is of an utmost importance in the fields of medicine, pharmacology and environment. In order to accurately characterize biological systems like protein-ligands interactions, it is necessary to use sensors that conserve the biological activity of the targeted biomolecule. Surface Plasmon Resonance (SPR) emerged as a promising tool for the characterization of binding events between a ligand and a protein happening at the interface of a metal film and a dielectric medium. In the last decades, several surface modifications have been developed and confer to metallic films biocompatibility and anchoring properties for biomolecule attachment. Since SPR is a label-free technique, it also permits direct analysis of a receptor without further derivatization. Also, fluorescence measurements have been thoroughly used in the bioanalytical sciences and can grant valuable information about biological systems. Techniques like FRET and FRAP largely contributed to the field of cellular biology. Since fluorescence can be exalted (metal-enhanced fluorescence) in the vicinity of metal structures supporting a surface plasmon, it is an interesting alternative for the characterization of biological systems on a SPR sensor. This presentation will focus on the investigation of the amplification of the fluorescence for different fluorophores on gold and silver metal films. Then, the binding of different synthetic peptides binding on a protein (CD36) monitored in real-time by SPR and fluorescence in a single assay will be demonstrated. Both methods bring complementary information about the modification induced to the receptor following the binding event. Finally, a surface modification implying vesicle fusion on a metal-coated prism has been investigated in order to characterize the affinity of a transmembrane receptor (GM1) for various analytes by SPR.

(494)) **Histopathological Characterization of Biological Tissues using High-Resolution Infrared Spectroscopic Imaging**; Jayakrupakar Nallala[1], Gavin Lloyd[1, 2], Neil Shepherd[3], Nicholas Stone[4]; [1]Bio-physics, School of Physics, University of Exeter; [2]Biophotonics Research Unit, Gloucestershire Royal Hospitals; [3]Department of Pathology, Gloucestershire Hospitals; [4]Bio-physics, School of Physics, University of Exeter

Infrared (IR) spectroscopic imaging is a label-free method that provides bio-molecular fingerprint of cells and tissues in a non-destructive manner. It has shown the potential as a promising cancer diagnostic tool paving the way for automated spectral histopathology. The current new generation IR spectroscopy systems in conjunction with focal plane array (FPA) detectors have enabled faster imaging of large sample areas. More recently, with the development of novel optical systems it has also become possible to perform IR imaging at higher spatial resolutions. In this regard, a novel optical system that consists of a high-magnification optics retro-fitted onto a conventional FPA based FTIR imaging system was tested. Measurements were carried out on different pathologies of formalin fixed paraffin embedded colon tissues (normal, intermediate and tumoral) using the high-magnification system and also using the conventional imaging. In comparison to the conventional imaging, the high-magnification FTIR imaging enabled characterization of histo-pathological/cellular features of colon tissues in greater detail. This was achieved due to the significant reduction in the image pixel size, from 5.5x5.5 µm2 of the conventional technology to 1.1x1.1 µm2 in the high-magnification modality. Further analysis based on multivariate statistical methods is underway to observe the discriminatory capabilities of these two modalities between the three pathology groups considered. Furthermore, with the aim to bring IR spectral histopathology closer to clinical applications, efforts are underway to test a novel Supercontinuum based mid-IR light source, that could be tunable to discrete frequencies, in conjunction with the high-magnification optical system.

(495) **Predicting Vascularized Composite Allograft Outcome during Modulated Immunosuppression using Multimodal Imaging**; Nicole Crane[1,2,3], Rajiv Luthra[1, 3], Georg Furtmuller[4], Eric Elster[2], Gerald Brandacher[4], W. P. Andrew Lee[4]; [1]Naval Medical Research Center; [2]Uniformed Services University of Health Sciences; [3]Henry M. Jackson Foundation for the Advancement of Military Medicine; [4]Johns Hopkins University

Close to 40% of combat injuries sustained in OEF and OIF involve severe extremity and craniofacial trauma. Vascularized composite allograft transplantation is an ever increasing alternative when conventional techniques prove challenging. However, this technique is limited by the risks related to long-term, high-dose immunosuppression treatment. A key factor in the success of the composite tissue allograft is careful maintenance of immunosuppression. While over-immunosuppression can result in acute or systemic infections, insufficient immunosuppression can be equally threatening. Insufficient immunosuppression can lead to acute or chronic rejection episodes, the loss of graft function, or loss of the graft itself. The ability to precisely monitor the condition of the graft will allow for the reduction of immunosuppressive medication, while mitigating the risk of rejection. Integrating infrared (IR) and 3-charge coupled device (3-CCD) imaging techniques allows us to visually and quantitatively monitor the condition of the graft while modulating immunosuppression.

Heterotopic hind-limb transplantation is performed in a mini-pig model. Composite allografts are harvested from both hind limbs of a single donor animal and placed in two recipients. The skin tissue of the VCA grafts is oriented so as to be visible from the exterior of the hind leg, allowing for non-invasive monitoring. Recipient animals are assigned to one of three immunosuppression regimens. The first group receives no immunosuppression, leading to full-rejection the VCA graft within one week. The second group receives standard immunosuppression, as a control. The final group receives pulsed immunosuppression, leading to chronic rejection. Images of the graft and recipient were taken with 3CCD and IR cameras on the day of transplantation as well as on postoperative days.

As expected, in the animals receiving no immunosuppression, the graft was fully rejected within 5 days of transplantation. Animals receiving standard immunosuppression maintained their allograft without any clinical signs of rejection. Imaging results indicate little difference between the two groups up until 2 days after transplantation. Initial data from this study shows that adapting our previously established non-invasive imaging techniques are effective in detecting changes in the oxygenation and perfusion of the skin tissue element of the VCA graft.

(496) **From Fiber Spectrometers to Fiber Sensors**; Viacheslav Artyushenko[1]; [1]art photonics GmbH

There is a variety of fiber spectroscopy methods used for process control: transmission, diffuse reflection, fluorescence, absorption, Raman scattering. They can be used alone or in combinations for more sensitive and precise process control on-line or even in-line. Review of fiber spectrometers will be done to compare research spectroscopy systems with target specification of promising fiber sensors designed for customized applications. Portable and robust fiber sensors may accelerate development of various process-control solutions because of their low price, while their industrial application will result in a better yield of products with enhanced quality.

Fiber spectroscopy used for reaction monitoring in labs and for process control in industry provides well known advantages:

1) eliminates the need to take samples for lab analysis from a running process as fiber probes can monitor media composition in-line – i.e. in real time and from the critical points of reactor;

2) remote process-control can be done at long distance – up to 200-300m, - in UV-Vis-NIR range where silica fibers possess by high transmission;

3) robust design of industrial spectral probes enables to analyze toxic, aggressive or radioactive media and to monitor reactions at high pressure, high or low temperature, vibrations, etc.

While silica fibers are limited in transmission till 2.2µm, the new Mid IR-fibers enable analysis in 2-17µm finger-print range - the most informative for molecular vibration analysis.

Good knowledge of spectral changes specific for distinct chemical process can be extracted with the spectral fiber systems and used for process-control with correspondent chemometric model, but total cost of such a solution can be too high for volume applications. Substantial cost reduction of customized fiber sensors may avoid this trouble for a broader and faster PAT methods expansion into industrial applications. This possibility will be presented in review of promising fiber sensors based on LED or Fabry-Perot MEMS filters with the analysis of their key parameters, incorporated chemometric models and results of their experimental tests.

(497) **Spectroscopic Investigation of the Effects of Bioavailable Ions on Apatite Mineral Composition and Kinetics**; Mary Tecklenburg[1], Md. Shah Alam[1], Honey Madupalli[1], Andrew Derry[1], James Lamblin[1], Megan Ling[1]; [1]Central Michigan University

Biomineralization of bone and teeth is a complex process in which apatite precipitates in regular patterns within a collagen template. The dominant mineral phase within skeletal and dental tissue of vertebrates is similar to calcium hydroxylapatite, Ca10(PO4)6(OH)2 (HAp). Biomineral properties such as kinetics of crystallinity and solubility are significantly affected by substitutions of bioavailable ions and incorporated water. Organic ions and proteins have a role in controlling and enhancing biomineralization. In vitro studies have shown that crystals begin as a highly-disordered amorphous calcium phosphate (ACP) which eventually matures into biological apatite (Ap) containing partial substitutions and deficiencies in the HAp structural formula, most notably from carbonate. The goal of our work is to model biomineralization processes and assess the effects of constituent ions, temperature, and external force on kinetics and crystallinity of apatite formation.

We synthesized apatite from aqueous solutions in moderate temperatures (20 – 80 C) in a variety of conditions including ion availability, pH, and centrifugal force. Availability of aqueous carbonate produces substitution for phosphate (B-type carbonated apatite). We also heated some samples to 950 C under flowing CO2 to produce apatite with carbonate substitution for hydroxide (A-type and AB-type carbonated apatite). Spectral methods (Raman, IR, and XRD) were used for characterization of mineral phases, crystallinity, and interaction between ions. Chemical composition was measured by ICP-OES for inorganics and IR or Raman spectroscopy for organics. The kinetics of the amorphous ACP to apatite phase transition was strongly dependent on the type of additional ions available. We found that of among carbonate, citrate, and magnesium ions, carbonate is the least inhibiting of the phase transition while magnesium is the greatest. A combination of IR and Raman spectroscopy was used to evaluate the levels of A-type and B-type carbonate substitution in AB-type carbonated apatite.

(498) **Purification and Biochemical Characterization of Highly Active Manganese Peroxidase from Mutant Trametes Versicolor IBL-04 under Solid State Culture**; Muhammad Ramzan[1,2], Muhammad Asgher[1], Raymond Legge[3], Yan Feng[2]; [1]Department of Chemistry & Biochemistry, University of Agriculture Faisalabad, Pakistan; [2]Key State Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, China; [3]Department of Chemical Engineering, University of Waterloo, Canada

In this study, a novel heme containing extracellular manganese peroxidase (MnP) was purified from culture filtrate of mutant Trametes versicolor IBL-04, grown in SSF under static conditions using sugarcane bagasse as a substrate. The enzyme was purified (1.4-5.5 fold) to a homogenous form through ammonium sulphate precipitation, ion exchange chromatography, and gel filtration. The purified enzyme showed a single band on SDS polyacrylamide gel electrophoresis (SDS-PAGE) with an apparent molecular weight and molecular mass of 43 kDa. MnP showed optimum activity at 35°C in pH 5.0. The enzyme from mutant T. versicolor IBL-04 was studied in terms of their pH, temperature optima and kinetic characteristics. The enzyme activity was activated by Mn2+ and Co2+, and was inhibited by Ag+ and Cu2+. MnP from mutant T. versicolor IBL-04 retained 80% stability in the pH and temperature range of 4.5-6.5 and 30-45°C, respectively. The lower KM and higher Vmax values (2.31 mM; 782 µmol/mg/min), calculated from Lineweaver-Burk plots, suggested that the MnP from selected strain was more efficient and stable. The present work reports, for the first time, the purification and characterization of MnP from a mutant microbial community, were new findings in this study, which improved our knowledge. The results of this work demostrated that the higher activity and thermostability of enzyme suggest its role in operational stability and potential for commercial scale MnP production for diverse effective industrial applications.

(499) **Evaluation of Lipophilic versus Hydrophilic Delivery of Flufenamic Acid in *ex vivo* Human Skin by Confocal Raman microscopy**; Yelena Pyatski[1], Carol Flach[1], Qihong Zhang[1], Richard Mendelsohn[1]; [1]Rutgers University

For topical delivery to be effective, a drug or active agent must cross the stratum corneum (SC) barrier into viable tissue. As such, barrier modification has been the target of many approaches for epidermal, dermal and transdermal delivery. The use of permeation enhancers in formulations is certainly one of the most widespread approaches. In the current study, flufenamic acid (FluA), a non-steroidal anti-inflammatory drug, is used as a model active agent to investigate the influence of hydrophobic versus hydrophilic permeation enhancers on its penetration and spatial distribution in ex vivo human skin using confocal Raman microscopy. In separate experiments, FluA in octanol or propylene glycol/ethanol (75/25) is applied to the SC surface for varying time periods followed by confocal Raman mapping. Deuterated versions of the enhancers permit us to spectroscopically distinguish the exogenous chemicals from the endogenous SC lipids without affecting penetration parameters. The FluA pathway is tracked by the C=C stretching mode at ~1618 cm-1. The spatial distribution and relative concentration of exogenous materials along with perturbation to the skin's molecular and ultrastructure are imaged using both univariate and multivariate analysis. This, in turn, provides insight into different mechanisms of delivery for the permeation enhancers used herein.

(500) **New Routes for Tissue Pathology using Quantum Cascade Laser Based Imaging Microscopes**; Vishal Varma[1], Hari Sreedhar[1], Peter Nguyen[1], Andrew Graham[2], Francesca Gambacorta[2], Kyle Meinke[1],Oluwatobi Adelaja[1], Aliya Husain[1, 3], Grace Guzman[1], Michael Walsh[1]; [1]University of Illinois at Chicago; [2]University of Illinois at Urbana-Champaign; [3]University of Chicago

New Quantum Cascade Lasers (QCL) as sources of mid-infrared (IR) light represents the next advance in the technology of IR imaging microscopes. QCLs offer the advantage of being a highly bright source of IR, coupled with the ability to collect discrete frequencies and image in real-time. IR imaging has been demonstrated to be a potentially powerful approach to aid in tissue diagnosis in the field of pathology, due to its ability to derive rich biochemical images from tissue biopsies in an entirely label-free format. We have recently demonstrated the use of the Daylight Solution's Spero Microscope to collect tissue images from a wide number of organs with data of similar quality to a conventional FT-IR imaging microscope, but with the added benefits that a QCL microscope system brings; speed, discrete frequency collection and live imaging capabilities. Data will be presented from a number of projects including heart transplant rejection diagnosis, stromal changes in response to disease in the colon and diabetes associated changes in the kidney and liver. Finally, feedback from practicing pathologists using the QCL microscope in the hospital will be presented.

(501) **Metabolomic Characterization of Leishmania Major and Leishmania Donovani by 1H and 1H-13C HSQC NMR.**; Paulo Falco Cobra[1,2,3], John Markley[3], Otavio Thiemann[4], Luiz Colnago[2]; [1]Instituto de Quimica de Sao Carlos - Universidade de Sao Paulo; [2]EMBRAPA Instrumentacao; [3]Biochemistry Department - University of Wisconsin - Madison; [4]Instituto de Fisica de Sao Carlos - Universidade de Sao Paulo

Neglected tropical diseases (NTDs) are a group of 17 diseases that thrive mainly on developing countries. According to the World Health Organization (WHO), they are endemic in 149 countries and affect more than 1.4 billion people. Leishmaniasis is among these diseases, and its agent of infection, *Leishmania sp* protozoan, is transmitted through the bites of infected *Phlebotomus* sandflies. The commonest form of

leishmaniasis is the cutaneous, which usually causes ulcers on the face, arms and legs and has 1 million cases reported in the last 5 years. The most disfiguring form is the mucocutaneous as it destroys the soft tissues of the nose, mouth and throat. Another form of the disease, visceral leishmaniasis, attacks the internal organs, usually is fatal within 2 years and causes 20 thousand deaths annually.

We aim, in the current paper, to ascertain the metabolic profile of *Leishmania major* Friedling and *Leishmania donovani* DD8 by nuclear magnetic resonance (NMR) spectroscopy. A Bruker 600 Ascend (600 MHz for ^{1}H) was used to obtain 1D and 2D spectra. Excitation sculpting was used to suppress the water signal. ChenoMX® and rNMR software were used to assign the 1D and 2D data.

The ^{1}H spectra obtained presented around 400 peaks, and de ^{1}H-^{13}C HSQC presented around 150 peaks. Assigning these peaks to correspondent metabolite is an important step in the study of the organism and will allow a better characterization of the different species. To date, more than thirty metabolites were assigned to both species, including adenine, alanine, choline, leucine, methionine, threonine, tyrosine and uracil.

Comparison of the metabolites present in different *Leishmania* species will help us understand why some species are visceral while other are cutaneous. Ultimately, metabolomics studies of these organisms under normal growth conditions and under drug stress conditions should reveal why some species are resistant to some medicines and others are not.

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(502) **A pH Reporter Molecule for Measurements and 3D Imaging in Turbid Media**; Kevin Davies[1]; [1]Florida Gulf Coast University

The photoacoustic effect stems from a molecule's conversion of absorbed light into heat in a short (<ns) timeframe. The sudden heating of the adjacent solvent molecules results in a sudden volume expansion which can be recorded as an acoustic event. This effect may be used to perform noninvasive/remote measurements of pH and other chemical properties in turbid media, and with depth resolution. We present our recent work in identifying well-behaved photoacoustic reporter molecules, potential applications of this research, and an interesting result that should permit better signal-to-noise in these measurements.

(503) **Imaging and Feature Selection using GA-FDA Algorithm for the Classification of HSI Biomedical Images**; Rupali Mankar[1], Vishal Verma[1, 2], Michael Walsh[2], Bueso-Ramos Carlos[3], David Mayerich[1]; [1]University of Houston; [2]Department of Pathology, University of Illinois at Chicago; [3]Division of Pathology/Lab Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX

Vibrational spectroscopic imaging for the classification of cells types in tissue biopsies is a very promising technique for cancer diagnosis. The use of FTIR imaging provides quantitative information for classification, thereby reducing errors and variations that occur across laboratories using conventional chemical stains. However, supervised classification of FTIR images is computationally expensive, relying on dimension reduction and therefore prone to potential loss of important information. In addition, the acquisition of FTIR spectroscopic images is time consuming. Recent advances in QCL-based imaging techniques provide a means of improving performance, but require prior knowledge of the spectral features necessary for classification.

In this paper, authors propose use of genetic algorithms (GA) to reduce HSI image dimensionality. Genetic algorithms are very robust iterative searching algorithms which mimic the natural gene evolution process to get the optimal solution of problem. FTIR images are collected and pre-processed to remove scattering artifacts. These preprocessed images are used as supervised training and testing datasets for the GA-FDA feature selection algorithm and random forest classification. Training and validation data sets are prepared by labeling hyperspectral images with prior knowledge of class label from adjacent chemically stained biopsies. Initialization of the GA is a matrix of potential sets of randomly chosen features. Every generation of GA ranks each individual set of features based on a cost calculated using fitness function. GA uses Fisher's Discriminant (FDA) ratio as a fitness function, which maximizes the between-class and within-class scatter ratio. During evolution, the best individual subsets are reproduced to next generation, the next-best fit individuals go through crossover, and remaining vectors through the mutation process to produce individuals for the next generation (iteration). Validation of these optimal features selected by GA algorithm for classification of HSI data with a random forest gives promising results in both in accuracy, as calculated using the area under the ROC curve, and computation speed. This suggests that FTIR images can be used as a basis for QCL imaging, whereas FTIR data is used to identify important features for classification and QCL-based approaches are used in practice for diagnosis.

(504) **Deconstruction of Inclusion Bodies and Refolding of Bioactive Protein Using Archaeal Chaperones**; Maruda Shanmugasundaram[1], Nadya Pavlova[2, 3], Andrey Pavlov[4], Jin Y. Wang[5], James E. Galen[5], Alexei Slesarev[4], Antonio del Castillo-Olivares[5], Frank T. Robb[2, 3], Igor K. Lednev[1]; [1]Department of Chemistry, University at Albany, State University of New York, Albany, NY; [2]Department of Microbiology and Immunology, University of Maryland, MD; [3]Institute of Marine and Environmental Technology, University of Maryland, MD, USA; [4]Fidelity Systems, Inc., Gaithersburg, MD, [5]Center for Vaccine Development, University of Maryland, Department of Biology, Montgomery College

Inclusion bodies are aggregates of misfolded protein that pose a major problem in recombinant protein production. They are also implicated in several neurodegenerative disorders. For example, diseases such as Parkinson's and Huntington's are characterized by the occurrence of inclusions such as Lewy bodies. However, it has also been suggested that inclusions play a protective role by harboring much more harmful, toxic mutant proteins and oligomers from being released into the environment. Hence, it becomes desirable to not only deconstruct inclusion bodies but also refold them into native, bioactive form.

While conventional methods are available to disrupt extremely stable protein aggregates like inclusion bodies, they normally require harsh treatments such as the use of strong chaotropic agents. It is desirable to find milder conditions under which protein folding can be improved both in vitro and in vivo. To this end, we have utilized Archaeal chaperones, which we have recently shown to be capable of deconstructing amyloid fibrils without achieving refolding. Archaeal chaperones have several advantages over bacterial chaperones. The chaperone complement of Archaea is reduced in complexity, making recombinant expression simpler. In addition, Archaea have evolved to survive in the extremes of biology and their chaperones are much more stable across broader range of conditions.

In this work, we report the ability of an extremely thermostable Archaeal chaperone, a mutant HSP60 from Pyrococcus furiosus (Pf cpn MA), to improve protein refolding from inclusion bodies. The inclusion bodies are deconstructed in terms of morphology as shown by techniques such as Atomic Force Microscopy and Light Scattering. In addition, deep UV resonance Raman spectroscopy, which is highly sensitive for detecting

secondary structural changes, shows melting of β-sheet and α-helix conformations. We also show that the deconstruction of inclusion bodies leads to the refolding of native, bioactive protein in vitro. Additional studies carried out in vivo by co-expression of the green fluorescent protein with Archaeal chaperones show improved folding of GFP in bioactive form. Hence, this work has shown that Archaeal chaperones can be a viable solution for application in problems related to protein misfolding such as recombinant protein production and neurodegenerative disorders.

(505) **Analysis and Evaluation of the UV Radiation as a Disinfectant**; José Gabriel Aguilar Soto[1], Jorge Castro Ramos[1, 2], Humberto Miguel Sansebastián Aguilar[3], Diana Antonieta Sen Salinas[4]; [1]National Institute of Astrophysics, Optics and Electronics (INAOE); [2]National Institute of Astrophysics, Optics and Electronics (INAOE); [3]H&M Biomedical Technology International; [4]Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV)

This paper makes a documentary research on the effectiveness of ultraviolet radiation as a disinfectant for hospital areas. We evaluate the use of ultraviolet radiation as an alternative or complement to the disinfection of areas and surfaces, in order to reduce the microbial population in critical places in health centers. Therefore, bacteriological tests are performed, using an UV-C lamp to disinfect areas in hospitals, which can be displayed in quantitative and qualitative results of this research on the effectiveness of disinfection in question.

Finally, we describe these results and performed a thorough analysis of them in order to reach specific conclusions about the subject that help to the health center managers make decisions about the use of this disinfection method.

(506) **Diffuse Reflectance Spectroscopy and Automatic ABCDE Law Applied in Melanocytic Naevi**; Jorge Castro-Ramos[1], Adriana May-Salazar[2], Gabriel Aguilar-Soto[1], Diana Sen-Salazar[1], Francisco Gutierrez-Gonzalez[3], Reimer Romero-Hernandez[1],Karen Esmonde-White[4]; [1]Instituto Nacional de Astrofisica Optica y Electronica; [2]Instituto Mexicano del Seguro Social; [3]Centro para la prevención del cancer; [4]University of Michigan

We start a study to automate the ABCDE law in cancer detection for melanocytic naevi; for Asymmetry we use digital image processing, for Border we use image segmentation and splines, RGB parameters correlated with diffuse reflectance spectroscopy for Color was used

and for Diameter we use geometrical moments, finally time and diffuse reflectance spectroscopy for evolution was used. 50 patients with different kinds of moles were analyzed in this study.

(507) **Underwater Standoff Fluorescence Instrument for the Detection of Oil in the Seabed, Water Column and Under Ice**; Job Bello[1], Christina Gasbarro[1], Anton Smirnov[1]; [1]EIC Laboratories, Inc.

Even with strict rules and regulation of oil transportation, accidents leading to oil spills still occur frequently. Numerous accidents occur annually, with thousands of tons of oil being spilled into seas, rivers and lakes resulting in the contamination of the environment and endangering the ecology. It is evident that oil spills in coastal waters, harbors, and oil terminals are especially dangerous and necessitate a fast response in order to prevent contamination and long-lasting damage of marine habitats. Therefore, reliable oil spill detection systems are needed. The most effective detection tool for oil monitoring in water has been fluorescence spectroscopy. Because the main constituents of oils are aromatic compounds with absorption bands in the ultraviolet and visible regions of the spectrum, illumination of oil samples with light in this frequency range causes them to emit fluorescence. Fluorescence based methods have several advantages: they are non-contact, highly sensitive to the presence of aromatic hydrocarbons and easily miniaturized. We have developed a fluorescence based sensing instrument capable of detecting fluorescence from oil at a standoff distance. The instrument employs a laser projected telescopically outwards from the instrument body and then the fluorescence from the focused laser source is collected in 180° backscatter by the same telescopic optics of the instrument and then detected in real time. The instrument is capable of detecting oil from several meters away from the instrument. Results in the development and underwater testing of the fluorescence instrument will be presented.

(508) **Effect of CO2-laden Brine Temperature, Pressure and Salinity on the Temperature, Electron Density and Morphology of Laser-Induced Underwater Plasma, and Implications for Groundwater Monitoring in Geological CO2 Sequestration**; Christian Goueguel[1], Dustin McIntyre[1], Jinesh Jain[2], Cantwell Carson[1], Herve Sanghapi[3]; [1]USDOE, National Energy Technology Laboratory; [2]AECOM; [3]Institute for Clean Energy Technology

Geologic sequestration of CO_2 in brine-bearing formations has been proposed as a means of mitigating the impacts of CO_2 emissions from anthropogenic sources. For this procedure to have any meaningful impact on the global carbon cycle, vast quantities of CO_2 must be compressed to a supercritical state and injected into the subsurface formations and isolated from the biosphere for hundreds or thousands of years. However, establishing the environmental safety and efficacy of this procedure will require widespread monitoring of the process in the deep subsurface. If leakage was to occur, CO_2 will escape from the containment formations as a combination of supercritical CO_2 ($ScCO_2$) and native reservoir brine, with dissolved CO_2. In particular, $ScCO_2$ and/or CO2-saturated brine can potentially enter and contaminate underground sources of drinking water (USDWs), and subsequently resulting in locally high concentrations of CO2–brine in near-surface ecosystems. Therefore, the detection of CO_2–brine flow migration into shallow groundwater aquifers is important both to assess storage permanence and to evaluate impacts on USDWs. Laser-induced breakdown spectroscopy (LIBS) is one of the most promising technologies to be applied to CO_2 sequestration monitoring. The flexibility of probe design and use of fiber optics makes LIBS especially suitable for noninvasive standoff measurements under extreme environmental conditions. In order to ascertain the feasibility of using underwater LIBS for measuring CO_2–brine composition in high temperature, pressure, and salinity (T-P-X) environments, a LIBS measurement system was designed and set up which can be used for conducting experiments across the range of T-P-X conditions likely to be encountered in carbon sequestration applications. The system is comprised of a 5-ns Q-switched Nd:YAG laser, a Czerny-Turner spectrometer coupled with an ICCD camera, and a 458-mililiter stainless steel high-pressure vessel for supplying the high T-P-X environments. The pressurized CO_2 environment was achieved and maintained by a metering pump, and a temperature controller was used for heating the brine samples. Four cartridge heaters were attached to the vessel reactor, and pressure transducer and thermocouple were integrated to the vessel using Swagelok tube fitting connections for monitoring fluid pressure and temperature. The pressure of the system can reach 400 bar without heating, while the heating temperature could be about 150 °C. Utilizing this system, underwater LIBS experiments were conducted using standard brine samples in different salinity (in terms of NaCl equivalent), temperature and CO_2 pressure conditions. Results of the characterization of the excitation temperature, electron density, and morphology of the plasma are presented. The implications for carbon sequestration monitoring are discussed with respect to these results.

(509) **Ocean Floor Exploration using Multi-Sensor Active Spectroscopy: A Payload Concept**; Pablo Sobron[1,2]; [1]SETI Institute, [2]MalaUva Labs

This paper presents a new concept for a robotic payload for seafloor exploration. To keep up with the growing commercial and environmental interest and investment in exploration of the seafloor, new instruments and adaptive exploration concepts that are conducive to analyzing a variety of settings are needed. This paper describes the technology and operational aspects of the new payload concept. From these two standpoints, the concept puts forward three innovations for the in-situ exploration of the seafloor. First, it takes advantage of the high resolution 3D mapping enabled by LIDAR and Imaging and the synergistic data return of laser Raman, laser-induced breakdown spectroscopy, and laser-induced native fluorescence (LRS+LIBS+LINF), to perform integrated, context-preserving, stand-off, in-situ characterizations of fluids and precipitates. Second, it leverages the capability of a coring tool & robotic manipulator to acquire cores and reveal them to the LRS/LIBS/LINF sensor, thus enabling in-situ spectroscopic depth profiling of mineral precipitates and sediments. Third, it implements adaptive multi-sensor data processing and automated interpretation routines for real-time integrated data management and extraction of scientific information. The unique data-collection and processing abilities of this concept have the potential to enable new capabilities in seafloor sensing, such us rapid, in-situ, detailed geochemical and mineralogical imaging/mapping in the environmental assessment and analysis and natural resources exploration and valuation industries.

(510) **Infrared Spectroscopic Assessment of Biomass for Bioethanol Generation**; Ramyasri Ailavajhala[1], Mugdha Padalkar[1], Uday Palukuru[1], Arash Hanifi[1], Rashid Kaveh[1], Benoit Van Aken[1], Nancy Pleshko[1]; [1]Temple University

Lignocellulosic bioethanol research has been on the rise recently, with the goal of production of environmentally safer fuels. In particular, Switchgrass (Panicum virgatum), a perennial feedstock that is local to North America, can be used to produce bioethanol. Plants can also be used for cleaning up the environment through the process of phytoremediation, which can increase the competitiveness of second generation bioethanol. This work focuses on development of Fourier transform infrared (FTIR) spectroscopy techniques for non-destructive evaluation of the composition of Switchgrass in response to exposure to soil contaminants. We hypothesized that FTIR analysis was sensitive to changes in Switchgrass composition that resulted from exposure to the phytotoxic chemical 2,4,6-trinoitrotoluene (TNT). Methods: Switchgrass plants were grown and harvested at eight weeks in the presence of varying TNT concentrations up to 60mg/kg. Gravimetric measurements of the harvested plant shoots were made prior to spectral data collection. FTIR attenuated total reflectance data were acquired using a Perkin Elmer Spectrum 100 FTIR Spectrometer at 8cm-1 spectral resolution and 64 co-added scans in the range of 800 – 4000cm^{-1}. Partial least squares (PLS) regression models were built using the spectral data from 800-1810 cm^{-1} where TNT concentrations were used as the responders. To understand how individual components changed with concentration, the area under absorbances unique to lignin (1463 cm^{-1}) or cellulose (1322cm^{-1}) peaks were calculated. Results and Discussion: One way-ANOVA showed that shoot weight of the 30mg/kg group was significantly greater compared to the control group ($p<0.005$). Using cross validation, the PLS model predicted the concentration of TNT the switchgrass was exposed to with an R-squared value of .73 and RMSEP value of 5.93 (~ 10% of the concentration range). In addition, a linear trend existed between TNT concentration and the area under the cellulose peak ($R=0.96$ and $p=0.04$) up to a concentration of 30 mg/kg. Together, these data demonstrate that exposure to TNT increases cellulose in Switchgrass, findings that help further understanding of the impact of environmental contaminants on biomass productivity and digestibility in bioenergy feedstock plants.

(511) **Demonstration of Scalable Analytical Methods for the Screening of Algae Bloom Contaminated Surface Waters by UHPLC-TOFMS Equipped with a Novel and Automated Analyte Search Algorithm**; Stephen White[1], Nicole Lenca[2], Frank Kero[1], Jason Weisenseel[1], Benjamin Southwell[3], Bogdan Bogdanov[1],Craig Young[1], Judy Westrick[2]; [1]PerkinElmer, Oak Brook Technology Center; [2]Wayne State University; [3]Lake Superior State University

The advantages for mass spectrometry (MS) testing platforms have previously been reported for both small molecule screening and peptide applications versus traditional ELISA methods at ppb levels. Of particular interest to this study is the emergence of MS as a means to monitor chemical markers of algae bloom contamination in surface waters. Time-of-flight (TOF) MS is ideal for this application, since researchers may not know the exact nature of contaminants for geographically isolated samples. Fast scanning TOFMS instruments offer identification by exact mass measurements and when paired with chromatographic separation tools (e.g. LC, UHPLC) will provide data that is more informing versus many orthogonal qualitative techniques. Preliminary method development was completed at the University of Central Florida (3 analytes) prior to technology transfer to the PerkinElmer Technical Center (Oak Brook, IL). The analyte panel was extended to 6 analytes, consistent with current trends in the field. Additional method optimization was required. Authentic samples of contaminated surface waters were obtained and prepared by Wayne State University (Detroit, MI) and shipped to PerkinElmer (Oak Brook, IL) for analysis. To maximize the utility of this platform, a novel software package has been developed to allow for further interrogation of this sample set data without the need for further analytical method development or re-injection of the sample. The current template allows for >30 analytes and will continue to grow as new markers are identified. It is anticipated this data analysis template will prove a valuable tool in related environmental surveillance applications.

(512) **The Photoacoustic Effect from Moving Sources**; Wenyu Bai[1], Gerald Diebold[1]; [1]Department of Chemistry, Brown University

It has been shown some time ago that when a laser source moves with the sound speed in a one-dimensional geometry, the amplitude of the acoustic wave grows linearly in time. This phenomenon sheds light on the possibility of higher sensitivity trace gas detection than what is currently possible using modulated, continuous optical sources. To explore the possibility of this technique, we consider several different moving patterns of the laser source, e.g. acceleration, oscillation, and rotation, and extend the calculation geometry to two and three dimensions. Analytical and numerical solutions to the wave equation for pressure are provided. Especially, the idea of regarding the photoacoustic pressure as the mapping of optical energy deposition in space makes possible the calculation of almost any kind of moving pattern.

(513) **Polyethylene: A Novel Approach for Passively Sampling Fluorotelomer Alcohols**; Erik Dixon-Anderson[1], Rainer Lohmann[1]; [1]University of Rhode Island Graduate School of Oceanography

Fluorotelomer alcohols (FTOHs) and other poly-fluorinated alkyl substances (PFASs) are common and ubiquitous by-products of various industrial telomerization processes. This class of volatile and semi-volatile compounds has been shown to degrade into a wide variety of perfluorinated carboxylic acids (PFCAs) including perfluorooctanoic acid (PFOA), a persistent organic pollutant. Recent atmospheric studies have shown the presence of fluorotelomer alcohols and their degradation products present in high concentrations spreading out from point sources in North America, Europe, and Asia. This study developed a method for the widespread monitoring of fluorotelomer alcohols through the use of polyethylene passive samplers and gas chromatography-mass spectrometry (GC/MS). We assessed the uptake of these PFASs into the

polyethylene samplers as well as optimizing the extraction process from said samplers. In addition, we expect to generate the partitioning coefficients of water to polyethylene, KPEW, for an array of PFASs.

(514) **Oligomerizational Behaviour of Nitrophenol under Simulated Atmospheric Conditions**; Hafiz Muhammad Danish Sultan[2], Farhat Yasmeen[1], Muzafar Abbas[1]; [1]University of Engineering and Technology, Lahore; [2]University College of Pharmacy, University of Punjab, Lahore
Biomass burning is a major source of organic pollutants in atmosphere. Oligomerization potential of two major organic pollutants i.e;ortho-nitrophenols and para-nitrophenol was investigated under atmospheric conditions. Experiments were performed under simulated cloud conditions at day time and night time on ortho-nitrophenol and para-nitrophenol. uv-visible spectroscopy was used to investigation preliminary data about oligomerization potential . UV-Vis study was made at various acidic conditions (pH= 3, 4 & 5) and effect of time and nature of salt on oligomerization potential was studied during night and day time simulated conditions. Oligomerization was observed under simulated cloud conditions in night time conditions seemed more progressive as compared to day time conditions. It could be a major breakthrough in understanding and explaining the missing gaps of secondary organic aerosols formation and atmospheric pollution.

(515) **Investigation of Fluorescence Yield Variability in Emiliania huxleyi**; Stefan Faulkner[1], Cameron Rekully[1], Shawna Tazik[1], Joe Swanstrom[1], Timothy Shaw[1], Tammi Richardson[2],Michael Myrick[1]; [1]University of South Carolina Department of Chemistry and Biochemistry; [2]University of South Carolina Department of Biological Sciences
Phytoplankton are ubiquitous, microscopic, unicellular, and photosynthetic organisms that play a vital role in the biogeochemical performance of both marine and freshwater ecosystems. Fluorescence spectroscopy is a commonly employed method of phytoplankton detection. The Shipboard Streak Imaging Multivariate Optical Computing (SSIMOC) instrument was designed by our lab to quantify and classify marine phytoplankton optically. As part of our continuing development of the SSIMOC instrument, we have turned our attention to investigating sources of noise, both optical and physiological, and strategies for improving our overall signal to noise ratio. Several improvements to the SSIMOC have since been made which eliminate some larger sources of instrumental “noise” and increase overall signal. Recent work by this laboratory determined that fluorescence measurements on individual E. huxleyi cells varied rapidly (on the order of milliseconds) in intensity in a manner that could not be accounted for by experimental contributions to noise or by any of several other possible mechanisms. We present work, which will utilize improvements made to the SSIMOC allowing us to more precisely explore this fluorescence variability again, and compare it to standard fluorescent beads.

(516) **Design of Optical Interference Filters for Taxonomic Classification of Phytoplankton**; Cameron Rekully[1], Shawna Tazik[1], Stefan Faulkner[1], Timothy Shaw[1], Tammi Richardson[1], Michael Myrick[1]; [1]University of South Carolina
The development of methods for real-time characterization of phytoplankton community structure has important implications for environmental monitoring and the prediction of harmful algal blooms. Our research group has leveraged the power of multivariate optical computing to develop an instrument, the Imaging Multivariate Optical Computing (IMOC) photometer, which is capable of rapid classification of phytoplankton via imaging single-cell chlorophyll a fluorescence onto a CCD array. Collected images contain multiple streaks of emitted fluorescence produced by passing excitation light through a filter wheel containing a set of optical interference filters with different transmission profiles. The designs of these interference filters are optimized to yield transmission profiles that maximize differences in chlorophyll a emission intensity between classes. In the past, filter design was accomplished via a process involving linear discriminant analysis of single-cell fluorescence excitation spectra. Recently, however, our group has developed a native program for the generation of optical interference filter designs that offers many advantages over previously employed methods. This new process, in which filter designs are generated sequentially and work in sets, along with more sophisticated modeling of experimental noise, has allowed us to design filters that offer significantly more efficient predicted separations than previously observed. Here we will detail the development and associated performance improvements of this native interference filter design process.

(517) **Decrease in Cadmium Levels in Canadian Western Amber Durum from 1995 to 2013**; Anja Richter[1]; [1]Canadian Grain Commission
The Grain Research Laboratory, a division of the Canadian Grain Commission has programs in place to monitor cadmium (Cd) levels in Canadian Western Amber Durum (CWAD). One program in place since 1995 uses samples from selected cargo shipments of Canadian grain to monitor the safety of Canadian grains, including Cd in CWAD. Each year from the 1995 to 2013 these samples were analysed for Cd by atomic absorption spectroscopy and inductively coupled plasma-mass spectrometry after microwave digestion. The number of selected samples analysed per crop year varied and ranged from 9 to 273 samples per crop year. The yearly Cd means (in mg/kg) ranged from 0.072 to 0.156. Looking at the overall trend of samples analysed per crop year shows a decrease in Cd content found in CWAD grown in Canada. This reduction in Cd levels is due to the success of plant breeding programs which have produced low cadmium accumulating varieties of CWAD, which are now the most prevalent varieties grown in Canada.

(518) **Step-scan, Rapid Scan, and Interleaved Time-Resolved FTIR Spectroscopy: Signal-to-Noise Comparison**; Sergey Shilov[1], Michael Joerger[1], Thomas Tague[1]; [1]Bruker
Step-scan and rapid-scan spectroscopy are traditional ways to collect time-resolved spectra. Scanning mirror of interferometer moves with a constant speed and data are collected „on-fly“ if rapid-scan technique is used. Mirror speed and spectral resolution (defined by the mirror travel distance) limit time resolution to 10 ms per spectrum in this technique. Mirror moves stepwise in case of step-scan technique. Experiment is repeated and time-resolved data are acquired at each mirror position. Time-resolution in the step-scan experiment is limited by the response time of the detector (few ns for the current MIR detectors). Unfortunately, noise in the step-scan data is higher compare to the rapid-scan.

Interleaved spectroscopy is available for the Bruker Vertex series FTIR staring 2015. Instrument sends multiple triggers to the external setup while interferometer mirror moves continuously. After completion of single scan, data are resorted according to time and time-resolved spectra are calculated. Interleaved TRS method allows to record spectra with ns time resolution and with much less noise in compare to the step-scan technique.

Details, limitations, and applications of interleaved TRS method will be discussed in this presentation. Time-resolved data on emission of fast MIR laser recorded using different techniques will be presented. Signal-to-noise obtained by step-scan, rapid-scan, and interleaved TRS methods will be compared.

(519) **Non-destructive Analysis of Surface Coatings using the Agilent 4300 Handheld FTIR Analyzer**; Dipak Mainali[1], Leung Tang[1]; [1]Agilent Technologies

Protective coatings are commonly applied to automobiles, aircrafts, ships, railways, furniture, bridges, concretes, architectural constituents, industrial installations, and many other products that we encounter on a daily basis. A wide variety of coating formulations exist in the market and their use depends on the performance requirement of the final product. In order to ensure the desired characteristics of the coating on the substrate, it is important that the coatings with desired specifications and formulations are used. Similarly, for many of these coatings that are packaged and supplied by the manufacturers as two-part (2K) systems, it is necessary to maintain the proper mixing ratios of coating system components, either by volume or weight, before application to the product. The Agilent 4300 Handheld FTIR system is highly effective for positive material identification of coatings and determining the mix ratio of 2K coating systems. The spectrometer is particularly useful because of its portability and available sample interfaces, which enable the analysis and identification of specific formulations on a coated article regardless of location, size, and shape. The mid-IR technique is ideal for positive material identification and monitoring the mix ratio because the components involved in each coating have their own distinctive spectra related to their chemical makeup and the degree of final cure. Partial least squares based chemometrics algorithm, also known PLS-DA, was implemented for the discrimination and identification of similarly formulated acrylic-based coatings. A series of PLS-DA calibration models were combined into one method using the Agilent 4300 MicroLab PC Component Reporting capability, and this method quickly and successfully differentiated and identified these very similar coatings. In order to identify the mix ratio of two popular two-part coating systems: an epoxy primer and a polyurethane top coat, PLS quantitative model was built. In addition, a simple two-coat system with epoxy as primer and PU as a top coat was also modeled. The 4300 Handheld FTIR enables virtually instantaneous determination of the mixing ratio, helping to ensure that coatings meet their performance specifications and longevity requirements.

(520) **Near Infrared Spectral Evaluation of Tissue Engineered Cartilage Correlates to Gene Expression of constructs**; Farzad Yousefi[1], Ramyasri Ailavajhala[1], Uday Palukuru[1], Syeda Yusra Nahri[1], Nancy Pleshko[1]; [1]Department of Bioengineering, Temple University, PA, [2]Department of Bioengineering, Temple University, PA, [3]Department of Bioengineering, Temple University, PA, [4]Department of Bioengineering, Temple University, PA

Clinical need for tissue and cell replacements has created a considerable expansion in the field of tissue engineering. Currently available analytical techniques for monitoring tissue growth in cell culture studies enable us to probe factors such as RNA expression and intra/extracellular protein expression. However, these methods are invasive and destructive. The objective of the current study was to develop a non-destructive method to monitor cartilage tissue growth via near infrared (NIR) fiber optic spectroscopy. Methods: Polyglycolic acid (PGA) scaffolds were seeded dynamically with freshly harvested bovine chondrocytes in spinner flasks overnight. The seeded scaffolds were then statically cultured for six weeks in an incubator. NIR spectra were collected in reflectance mode using a 10 mm diameter quartz NIR fiber optic (Remspec Corp, Charlton, MA) coupled to a Matrix-F infrared spectrometer (Bruker, MA), three times a week after media changes. 256 and 128 co-added scans were used for background and sample spectra respectively with spectral resolution of 16 cm-1. Spectra were pre-processed using Savitzky-Golay smoothing (41 points) and second derivatives, and partial least squares (PLS) models were built using spectral data to predict gene expression data (Unscrambler, Camo software, Norway). At several time points (first, second, third and sixth weeks) engineered cartilage tissues were harvested, and scaffold thickness measured. RNA polymerase chain reaction (PCR) gene expression assays was conducted on scaffolds, with RNA isolated from the PGA scaffolds and then transcribed into cDNA from which the Collagen (I, II, IV) aggrecan and GAPDH expression levels were measured. Results and Conclusions: Scaffold thicknesses were not found to be significantly different and stayed at ~3-4 mm. The gene expression results suggested that aggregan and collagen amounts increased from week 1 to week 6 harvests. Root mean squared error of calibration (RMSEC) and prediction (RMSEP) for PLS models to predict aggrecan gene expression were 3.6 and 4 percent, respectively. Typically, gene expression correlates with compositional data, and thus it is likely that the NIR spectral data will also predict protein expression. Together, these data will help establish a non-invasive real time method to monitor cartilage development for tissue regeneration applications.

(521) **Differential Excitation Spectroscopy: A New Technique**; Boyd Hunter[1], Jason Cox[1], Paul Harrison[1], Bill Walters[1], Michael Miller[2]; [1]Kestrel Corporation; [2]Southwest Research Institute

Reliable detection of chemical, biological and explosive threats is the first step in a successful interdiction and control operation. In a laboratory environment, there are many techniques that can be used to identify unknown materials. In a non-laboratory environment, the detection process is compromised by the presence of various of various backgrounds and interferents.

Kestrel Corporation has developed a new spectroscopic technique called Differential Excitation Spectroscopy (DES) (patent pending). DES is a new pump-probe detection technique which characterizes molecules based the ground-state vs. rovibrational excited state differential using a multi-dimensional parameter set: pump and probe interrogation frequencies as well as the excited state lifetimes. Under appropriate excitation conditions, significant modulation of the ground state can result, providing a robust signal-to-noise ratio and a very material-specific response which has demonstrated high levels of immunity from common interferents and backgrounds. The immunity of the technique may be understood on the basis of the several parameters that must be correct to force the molecule into the excited state; the greater the number of factors that must be correct, the harder it is to generate a false positive signal.

DES results for various “threat” materials will be discussed: common explosive materials such as AN, UN, RDX and TNT(1) ; chemical warfare simulants such as DMMP and thiodiglycol (2) ; vapors emitted by benzene and various chlorinated solvents; and toxic gases such as sulfur dioxide, ethylene oxide, and boron trifluoride (3) .

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(522) **Vibrational Spectroscopy of an Imidazolium Ionic Liquid Confined in a Metal Organic Framework**; Johannes Kiefer[1], Manish Singh[2], James Anderson[2], Nilesh Dhumal[3], Hyung Kim[3]; [1]Universitaet Bremen, [2]University of Aberdeen, [3]Carnegie Mellon University Pittsburgh, [4]

Hybrid materials consisting of metal-organic frameworks (MOF) and room-temperature ionic liquids (IL) are promising candidates for advanced applications in energy and environmental technology. The properties of such a composite are determined by the structures of the MOF and IL, and the molecular interactions between the two. Hence, for the rational design, the interaction mechanisms need to be understood in order to develop a tailored material for a given application. In this work, Raman and FTIR are used to study the behavior of an ionic liquid (1-ethyl-3-methyl imidazolium ethyl sulfate, [EMIM][EtSO4]) confined in a micro-porous Cu-based MOF at the molecular level. The combination of Raman and IR spectroscopy provides a variety of new insights into a fascinating material. For example, the spectra reveal that the structure of the MOF is perturbed by interactions between Cu(II)-ions of the MOF and oxygen atoms of the IL's anion. In addition, a weakening of the intermolecular bonds of the linker within Cu-BTC can be observed. In turn, the structure of the ionic liquid inside the pores of MOF is significantly altered compared to the bulk liquid case. These modifications in the interactions are not uniform for all ion pairs of the ionic liquid. The ion pairs inside the pores experience an unexpected strengthening of the hydrogen bonding between the cation and the anion. The observed changes in the molecular interactions between the MOF and the IL can eventually be explained in terms of charge transfer phenomena, which are particularly interesting when the MOF/IL hybrid material is considered for use in energy devices.

(523) ***In-situ* Spectroscopic Study on Small Molecules Diffusion in Anion Exchange Membranes**; Ying Jin[1], Xiaohui Liu[1], Wenxu Zhang[1], E. Bryan Coughlin[1]; [1]Polymer Science and Engineering, University of Massachusetts Amherst

Processes of water, alcohols and salts diffusion in an anion-conducting polymer network are investigated by time-resolved attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. The anion-conducting polymer network can be used in alkaline anion exchange membrane fuel cells (AEMFCs). The fundamental understanding on the transport property of these AEMs is crucial for obtaining better performance of fuel cells. The transport property-membrane structure relationship is discussed in detail. The effects of membrane structures including ion exchange capacity (IEC), type of counter ions and the influences of diffusants on the diffusion coefficients were determined. The anion transport mechanism in AEMs was further evaluated.

(525) **Removal of Bone Marrow Contributions for Evaluation of Bone Water by Near Infrared (NIR) Spectroscopy**; Hee Jin Yang[1], Mugdha Padalkar[1], Michael Ispiryan[2], Chamith Rajapakse[2], Nancy Pleshko[1]; [1]Temple University; [2]University of Pennsylvania

Bone fracture risk increases with age and disease state. Recent studies have pointed to factors other than bone mineral density that contribute to fragility, including bone water. Near infrared spectroscopic (NIRS) methods are being proposed for evaluation of water in bone at microscopic resolution. However, there are significant interfering absorbances from bone marrow that complicate the NIRS analysis. The current study investigates methodology to reduce the bone marrow contribution to NIR spectra. Methods: Bovine tibias were harvested from slaughtered 2-4 month old animals (Research 87, Boylston, MA) and stored in phosphate buffered saline (pH 7.4, Invitrogen, Carlsbad, CA) at -20°C until processing. Thawed tibias were cut into 500 μm thick slices using a diamond wafering saw (Buehler Isomat 1000, Lake bluff, IL). NIR spectral images of the bone slices (~ 20 x 20 mm) were obtained using a Perkin Elmer Spotlight 400 imaging spectrometer (Shelton, CT). NIRS images were collected in transmission mode from 4000 to 7800 cm^{-1} at 64 cm^{-1} spectral resolution and 50 μm pixel resolution with 2 co-added scans. Each image took approximately an hour. The bone slices were placed between two glass slides to avoid water loss during imaging. After NIRS imaging, the slices were washed in an ultrasonicator (Emerson Industrial Automation, Danbury, CT) for 30 minutes at 45°C with 1% tergazyme solution, followed by gentle washing of the slices in DI water, and then NIRS data collection. NIRS images were analyzed using ISYS 5.0 software (Malvern Instruments, Columbia, MD). The mean intensities of NIR absorbance peaks at 5792 cm^{-1} (arising from the fat component of bone marrow) and 4608 cm^{-1} (from the bone collagen matrix) were analyzed in second derivative spectra (Savitzky Golay, 3rd order polymer and 7 points of smoothing) images before and after sonication. Results: It was found that the overall fat absorbance was reduced by 35% after ultrasonication, and in the trabecular region of bone, it was reduced by 97%. There were no significant changes to the collagen absorbances. These results indicate that the ultrasonication methodology can be used to remove bone marrow from bone prior to NIRS imaging.

(526) **Design and Performance of a New Diamond Attenuated Total Reflection-Video Microscopy Accessory**; David Schiering[1]; [1]Czitek

Type IIA diamond is a near perfect material for infrared (IR) spectroscopy applications. The combination diamond's IR transmission, hardness, and chemical resistivity are unsurpassed by alternative IR materials. Diamond offers another advantage – the optical transmission in the visible range – that allows the combination of IR spectroscopy with visible imaging of the specimen. This presentation will concern the design and performance of a new diamond attenuated total reflectance (ATR) – video microscopy accessory for Fourier transform infrared (FT-IR) instruments. The accessory employs a single reflection diamond internal reflection element (IRE) in a "roof top" prism configuration. The diamond is synthetic type IIA, single crystal. Microscopical observation is accomplished by imaging the specimen plane through the diamond "roof top" onto a megapixel camera. Live images are observed on a computer monitor via USB interface and video microscopy software. Images can be captured, analyzed, and documented using this software. In addition to the design attributes, the spectroscopic and microscopical performance will be presented with example applications.

(527) **Application of Near-Infrared Transflection and Transmission, and Hand-Held Raman Spectroscopy in Analysis of Ketoprofen in Pharmaceutical Gel**; Keith Freel[1], Ahmed Shawky[2, 4], Ahmed Ibrahim[2,4], Eman Elzanfaly[3], Maissa Salem[2], Ahmed El Gindy[2],Stephen Hoag[4]; [1]Metrohm USA, Inc.; [2]Analytical Chemistry Department, Faculty of Pharmacy, Misr International University, Egypt; [3]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Egypt; [4]School of Pharmacy, University of Maryland, MD

The feasibility of quantitative analysis of ketoprofen, the active ingredient in a gel drug delivery matrix, was explored utilizing both transmission and transflection methods of Near-Infrared (NIR) Spectroscopy and by hand-held Raman spectroscopy. Spectroscopic methods can offer several advantages when compared to traditional methods including rapid analysis (results in seconds), no sample preparation, high precision, and ease of use for routine analysis. Both are non-destructive methods, and will often match the accuracy of the primary method used for calibration. 27 samples were prepared with varied concentrations of ketoprofen ranging from 12.4 mg/g to 38.1 mg/g. 19 samples were used for calibration and 7 were used to validate the NIR and Raman methods. The samples were first analyzed by spectroscopy, then by HPLC. PLS regression models were developed based on the calibration set. Various pre-processing treatments were tested to optimize the model by evaluating various regression optimization parameters (RMSEC, RMSECV, RMESP, R2, etc.). The corresponding values for the root mean square error of prediction (RMSEP) were found to be 0.530 mg/g, 0.766 mg/g and 1.01 mg/g for NIR transflection, NIR transmission and hand-held Raman spectroscopy, respectively. These results showed that both NIR spectroscopy and Raman spectroscopy can be utilized for rapid quantitative analysis of ketoprofen. A comparison of method performance for NIR spectroscopy by transflectance, NIR by transmission, and hand-held Raman spectroscopy is reported.

(528) **Dynamics of an Internal Protonated Water Cluster: An Isotope Exchange Study of Photosynthetic Oxygen Evolution**; Udita Brahmachari[1], Bridgette Barry[1]; [1]Georgia Institute of Technology

Photosystem II generates oxygen from substrate water during oxygenic photosynthesis. The reaction occurs at the oxygen-evolving complex (OEC) and is initiated by photoinduced charge separation between redox-active cofactors. Photoexcitation leads to the stepwise oxidation of a Mn_4CaO_5 cluster that constitutes the OEC. The cluster stores these oxidizing equivalents and returns to its most reduced state by oxidizing water and generating molecular oxygen. The oxidation of the OEC proceeds in a stepwise fashion and these increasingly oxidized states are called the S_n states. The S_1 state is the resting or dark-adapted state. Oxygen evolution accompanies the S_3 to S_0 transition. In the present study, laser flashes are used to step the OEC through the entire S-state cycle. The effect of isotope exchange using $D_2{}^{16}O$ and $H_2{}^{18}O$ at pL 7.5 is investigated. A positive broad infrared band at 2850 cm^{-1}, associated with the S_1 to S_2 transition, is downshifted by $D_2{}^{16}O$ and $H_2{}^{18}O$ exchange. The sensitivity of the positive band to $D_2{}^{16}O$ and ^{18}O-isotope exchange supports the assignment of this band to an internal protonated water cluster. One possible explanation for the formation of this cluster is the deprotonation of substrate water during the S_1 to S_2 transition. This cluster stores this proton and then releases it to the lumen during the later S-state transitions.

(529) **Cryogenic Vibrational Spectroscopy Traps an Internal Protonated Water Cluster in Photosystem II**; Zhanjun Guo[1], Bridgette Barry[1]; [1]Georgia Institute of Technology

Many studies have been focused on proton movement in aqueous solution. Proteins require more organized structures to transfer proton quickly and to conduct biological reaction efficiently. The light-driven reactions of photosynthetic oxygen evolution occur in a chlorophyll-containing reaction center called photosystem II. During the photosynthetic oxygen-evolving reaction, water is oxidized at a Mn_4CaO_5 center, which cycles through five oxidation states, named the Sn states. In this work, experiments utilize reaction induced FT-IR spectroscopy at 190K and a plant photosystem II preparation. An absorptive, broad infrared band, centered at 2850 cm-[1], appears during S_1-to-S_2 transition. The band is eliminated by calcium depletion and ammonia substitution; both of these treatments inhibit oxygen evolution. $H_2{}^{18}O$ buffer exchange produces a frequency downshift, supporting the assignment of the band to a stretching vibration of cationic water. Taken together, the data support the conclusion that an internal water cluster acts as an intermediate proton acceptor during the oxygen-evolving reaction.

(530) **Monitoring the Disruption and Reformation of Stratum Corneum Lipids Packing Order *in vivo* with ATR IR**; Guangru Mao[1], Catherine Mack[1], Hao Ouyang[1]; [1]Johnson & Johnson Consumer Inc.

Stratum corneum (SC), the top layer of skin, provides most of skin's barrier function. SC lipids form highly ordered structure in the intercellular space and affects skin's barrier function. In this study, we used ATR IR to monitor the SC lipids packing order following local heat disruption on human volar forearm skin in vivo. Transepidermal water loss (TEWL) was also measured as an indicator of skin barrier function during the process. The high temperature greatly reduced the content of SC lipids packed in orthorhombic phase and it took ~80 minutes for the lipids to regain their initial packing order. Significant higher TEWL was also observed after heating. The TEWL was reversed back to its initial value in ~60 minutes. These results indicates that the exposure to high temperature disrupts the packing order of SC lipids and the prolonged recovery posts threats to skin's barrier function.

(531) **Infrared Imaging for Identification of Tissue Damage in Pathology**; Bennett Davidson[1,3], Michael Walsh[3], William Ennis[2], Timothy Koh[3], Andre Kajdacsy-Balla[3], Sagar Nadimpali[1]; [1]Department of Bioengineering, College of Engineering and Medicine, University of Illinois at Chicago; [2]Department of Surgery and Section of Wound Healing and Tissue Repair, College of Medicine, University of Illinois at Chicago; [3]Department of Pathology and Spectral Pathology Lab, College of Medicine, University of Illinois at Chicago

Chronic wounds are a prevalent issue in patients with diabetes and those that are bedridden. The current standard for diagnosing a non-healing wound relies upon the judgments from a trained pathologist and care provider. This method for tracking the progression of a wound's healing process is widely used, however it is qualitative, leaving the patient open to errors in judgment and extended hospital stays and expenses. Here, we discuss the applications of Fourier-Transform Infrared Spectroscopic imaging to gain insight into the underlying biochemical processes involved in the wound progression of chronic, non-healing, wounds. The University of Illinois at Chicago provided Mouse tissue samples, and the human samples were provided by the University of Illinois at Chicago Hospital's Center for Wound Healing, from 5 patients. We used FT-IR and QCL to scan, then Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to understand the biochemical differences in the collagen of progressive and non-progressive wounds for mouse wounds and a Bayesian Classifier to differentiate the main components that make up a chronic wound. From this, FT-IR and QCL offers promise in improving wound diagnosis and prognosis, leading to decreased hospital stays and costs.

(532) **Probing Triplet-Triplet Energy Transfer Efficiency in Artificial Photosynthetic Pigments using Resonance Raman Spectroscopy**; Elizabeth Kish[1], Katherine WongCarter[2], Smitha Pillai[2], Gerdenis Kodis[2], Dalvin D. Mendez-Hernandez, Junming Ho, Ana L. Moore[2,3], Thomas Moore[2], Devens Gust[2], Bruno Robert[1]; [1]Department of Life Sciences, CEA Saclay, France; [2]Department of Chemistry and Biochemistry, Arizona State University; [3]Department of Chemistry, Yale University

Photosynthesis is the process by which certain organisms, including most plants and many bacteria, obtain their energy for survival. During photosynthesis, photons are "captured" by chromophores in light harvesting complexes and start an electron transfer chain that ultimately results in the formation of energy. When there is light, there will ultimately be harmful species like singlets and triplets formed; it has been found that carotenoids are efficient quenchers of these harmful species in photosynthetic apparatuses.

Energy transfer mechanisms originating from photosynthetic machinery have gained much interest because biological systems represent possibilities for next generation energy sources. In our work, we have synthesized molecules (carotene-porphyrins) to mimic certain photosynthetic pigment networks and used them to study energy transfer mechanisms. In light harvesting complexes, a carotenoid molecule can quench a triplet coming from a chlorophyll molecule in its vicinity. Here, we have used porphyrins as a model for chlorophyll and covalently linked them to carotenoids in different orientations and studied them spectroscopically.

Resonance Raman spectroscopy (RRS) is a scattering technique that has been widely exploited to study carotenoids. It gives information on molecular vibrational states; when a molecule's electronic transition coincides with the incident wavelength of light, the technique becomes "resonant". This amplifies the signal by up to a factor of 10^6.

We have used RRS to study the carotene-porphyrins, specifically in their triplet forms. Depending on the relative orientation of the carotenoid to the porphyrin, we have seen that the triplet-triplet energy transfer triggers changes in nearly all bands of the RRS spectra (carotenoids have four characteristic bands; they are denoted ν_1 to ν_4). Specifically, the ν_1 band (the stretching frequency of the conjugated C=C moiety) noticeably downshifts when associated with a more delocalized triplet (and consequently more efficient triplet-triplet energy transfer). The band shifts represent the importance of molecular proximity to energy transfer mechanisms and give some insight into how this process may truly physically occur. We have done extensive modelling to corroborate our experimental results with calculations.

(533) **Effects of Molecular Absorption Cross-Section on Raman System Throughput**; Justin Cooper[1], Adam Hopkins[2]; [1]Alakai Defense Systems, [2]Alakai Defense Systems

Fundamental physics, and many published reports, indicate that Raman scattering cross-sections increase with decreasing wavelength. As electronic resonances are approached, the scattering cross-section increases further. This has led researchers to suggest that ever-decreasing wavelengths are required for Raman to be used for standoff and trace detection. We have evaluated the effects of wavelength on overall Raman throughput for several bulk liquids, and find that projected performance is dependent on, although more subject to absorbance, density, and molecular structure than previously established.

(534) **In-line Quality Control of Cross-linking for Photovoltaic Encapsulants via Raman Spectroscopy**; Mark Kemper[1], Christina Hirschl[2], Martin Kraft[2], Bradford Behr[1]; [1]Tornado Spectral Systems; [2]CTR Carinthian Tech Research AG

The long term operational viability of photovoltaic modules is strongly dependent on the encapsulant of choice. Currently, the preference is to use elastomeric ethylene vinyl acetate (EVA) which must be cured (crosslinked) on the module itself during manufacturing. Much work has been done for offline characterization of the degree of cross-linking and of particular interest has been the relationship between Raman microscope results and gel constant reference values obtained by Soxhlet-type analysis (ASTM D2765). In this paper we present the development of an in-line industrial high-throughput virtual slit (HTVS) Raman spectroscopy system for the real time analysis of polymer cross-linking. Initial investigatory work was conducted showing excellent correlation between Raman microscopy results and the industrial Raman system in off-line qualitative measurements. Next, a test system was designed by optimizing the probing system to ensure full characterization of the polymer layer and was integrated with an in-line demonstrator. By observing changes in CH-stretching in the vibrational region inferences on the degree of cross-linking were obtained. The method was extremely fast (sub 100 ms analysis times), robust (excellent correlation to Soxhlet values), and easy to use.

(535) **Transmission Raman Spectroscopy an Alternate Tool to Traditional HPLC to Determine the Content Uniformity of Solid Dosage Forms**; Michelle Raikes[1], Reggie Saraceno[1], Prince Korah[1], Julia Griffen[2]; [1]Boehringer Ingelheim Pharmaceuticals, [2]Cobalt Light Systems Ltd

Transmission Raman spectroscopy is an alternate tool to traditional HPLC to determine the content uniformity of solid dosage forms. This application is in high demand in the pharmaceutical industry and is in step with Quality by Design (QbD) and Process Analytical Technology (PAT) initiatives. A comprehensive study was conducted with a Transmission Raman Spectrometer analyzer (TRS100) from Cobalt Light Systems, Inc., to quantify the API content in pharmaceutical formulations such as powder blends, tablets and capsules. Calibration reference samples of powder blend and tablets were selected to build the calibration model utilizing an orthogonal design of experiment (DoE) approach. A correlation was established between the Raman signal and the corresponding reference HPLC assay values to build robust powder and tablet chemometric calibration models using partial least squares (PLS). This study successfully demonstrated the ability of TRS100 system to accurately predict API concentration in powder and tablet formulations. The study also demonstrated that powder or tablet calibration models can be used interchangeably for accurate assay prediction of tablet test samples. The specificity, accuracy, repeatability and linearity of the TRS method were demonstrated to be comparable with traditional HPLC analysis. The high chemical specificity of TRS and the non-invasive and non-destructive nature of the measurement allow this technique can be used as an alternate tool to the traditional HPLC for routine rapid quantitative analysis.

(536) **Multi-wavelength Dispersive Raman Spectrometer and Microscope for Non-destructive Pharmaceutical Ingredient Analysis**; Jack Qian[1]; [1]BaySpec Inc.

The characterization of the active pharmaceutical ingredient (API) and its distribution and physical properties in commercial medicine is necessary in drug research and development process in the pharmaceutical industry. Among various analytical techniques are employed for this purpose, Raman spectroscopy is gaining more popularity due to its advantages as non-destructive, non-invasive, fast spectrum acquisition in seconds, high reproducibility and so on. Having multiple wavelength excitations in one unit offers extreme flexibility and convenience to identify the best laser wavelength and investigate a great variety of real-world samples. In real-world Raman measurements, fluorescence is the biggest obstacle which significantly reduces the quality of the Raman spectra. Besides, resonance effect and difference cross sections make the multiple excitation wavelengths more important in Raman measurement. This paper will introduce a new class of Multi-wavelength Raman spectrometers and microscopes, realized by highly efficient, patented VPG gratings, fast optics, and TE-cooled CCD and InGaAs detectors. The confocal Raman microscopes feature with multiple laser excitations (532, 785, and 1064 nm), fully automated operation and high spatial/spectral resolutions. This work will focus on the experimental results obtained pharmaceutical samples. These data demonstrate that the multi-wavelength Raman is ultimately the solution for the analysis of the most complex fluorescence samples that traditional Raman systems are unattainable.

(537) **Raman Monitoring of the Carbonization Process of Metal–Organic Frameworks**; Szetsen Lee[1], Yu-Ting Gong[1], Bing-Han Li[1], Chia-Her Lin[1]; [1]Chung Yuan Christian University

The carbonization process of a series of aluminum-containing metal-organic frameworks (MOFs) was studied by Raman spectroscopy. Under inert gas, the formation rates of carbonized MOFs (CMOFs) were monitored by the variation of the Raman D band to G band intensity ratio with heat treatment time. XRD, BET, SEM, and EDS techniques have also been applied to confirm the quality of CMOFs. By varying treatment temperature, activation energies can be extracted from the temperature-dependent rate constants using the Arrhenius equation. The activation energies of the formation of CMOFs were correlated with the structural properties of MOF precursors such as the number of carbon atoms per ligand, pore size, and surface area. A reaction mechanism is proposed and discussed for the formation process of CMOFs.

(538) **Characterization of Graphene and Other Two-Dimensional Materials by Raman Spectroscopy**; Pierre Negri[1], Tim Prusnick[1], Ian Haywood, Olga Milikofu[3], Tim Batten[2]; [1]Renishaw Inc.; [2]Renishaw Plc.; [3]Renishaw K.K.

We demonstrate Raman spectroscopy as a valuable technique in the study/evaluation of graphene and its precursors. The analysis of the uniformity of quality, strain/stress and defects of these materials over very large areas (centimeter scale) demonstrates the variations in material quality on large production scales and guides process improvements in growth techniques. The large area analysis is essential as the need for large-scale production and the requirement for high quality graphene in industrial applications has grown significantly. Rapid characterization of materials being produced on an industrial scale, in a cost-effective manner with a high level of reproducibility, remains challenging. We demonstrate the utility and use of 3D Raman mapping for the examination of large h-BN crystals that were produced by a temperature gradient method. These results show that the magnitude and distribution of the internal local strain field can be visualized in three dimensions. This important information is being used to better understand growth mechanisms, improve growth techniques, and generate better single layer h-BN.

(539) **Coherent Raman Microscopic Study during the Stretching of a Homologous PE Blend**; Ying Jin[1], Ian Ryu[1], Chad Snyder[1], Young Jong Lee[1]; [1]National Institute of Standards and Technology

In this study, the broadband coherent anti-Stokes Raman scattering (CARS) microscopy method was applied to investigate a homologous PE blend under strain. Broadband CARS microscopy is a noninvasive and stain-free microscopic technique and can acquire compositional and orientation images simultaneously. As the physical orientation process of polymer materials has been an essential research topic. The microscopic structure and local molecular deformation of multicomponent semi-crystalline polymer blends during stretching were discussed. We demonstrated critical issues for performance of PE products during mechanical failure and the deformation mechanisms for semi-crystalline polymer materials.

(540) **Assessing Composition and Morphometry Properties of Ostrich Cartilage as Possible Tissue Engineering Scaffolds**; C. Erika Ramírez[1], Jorge L. Flores[1], Verónica M. Rodríaguez Betancourtt[1], JI Delgado-Saucedo[1], Héctor Pérez Ladrón de Guevara[3], Miguel Guzmán[2],Adán T. Paíno[1], Karen Esmonde-White[4]; [1]Universidad de Guadalajara - CUCEI; [2]Higher Technological Institute of Irapuato - ITESI; [3]Universidad de Guadalajara - CULAGOS; [4]University of Michigan - Medical School

The challenge of building artificial tissues lies with combining harvested cells and suitable scaffolds to provide a biomimetic tissue. We are interested in using natural products as artificial tissue scaffolds because they effectively model the hierarchical structure and function of native tissue. Ostrich is a sustainable meat source, and the cartilage represents otherwise to be discarded tissue, which can be potentially used as an engineered cartilage tissue scaffold. The goal of this project is to characterize composition and morphometry properties of cartilage under native, crosslinking or denaturing conditions. Ostrich tissue was obtained from a local abattoir and cartilage removed from the hock joint by blunt dissection. Tissue composition was measured using Raman spectroscopy. We examined ostrich cartilage under four denaturing conditions: 1) exposure to ribose or glucose solution to induce nonenzymatic crosslinking in collagen, 2) lyophilization/rehydration 3) exposure to acidic solution or 4) exposure to alkali solution. A Thermo Scientific DXR Raman Microscope instrument operating at 532 nm was used to collect Raman spectra of cartilage specimens. The Nanosurf easyScan 2 Atomic force microscopy was used to examine collagen morphometry in cartilage after lyophilization and rehydration. Chicken and turkey cartilage, native and stressed, were examined as controls. Raman spectroscopy bands corresponding to the amide I and amide III envelopes, chondroitin-6-sulfate of aggregan and collagen hydroxyproline proline were used to monitor matrix molecules during stress conditions. Raman spectroscopy results suggested that ostrich cartilage is more resistant to nonenzymatic crosslinking or pH-related denaturation than other bird cartilage specimens. AFM images demonstrate that lyophilization results in complete loss of collagen morphometry that cannot be rescued by rehydration. Compositional and morphometry characterization of cartilage in crosslinking and stress conditions provides insight denaturing mechanisms of cartilage. These data also provide a framework which will allow us to address future biomechanical and cell culture experiments, rapidly identify promising cartilage tissue scaffolds and optimize storage conditions.

Key words: Tissue engineering, ostrich cartilage, Raman spectroscopy, atomic force microscopy, nonenzymatic crosslink, collagen, glycation.

(541) **Automated Chemical ID of Particles using Raman Spectroscopy**; Vincent Larat[1], Eunah Lee[1], Bernd Bleisteiner[1], David Tuschel[1], Simon Fitzgerald[1]; [1]HORIBA Scientific

Raman spectroscopy has become one of the most powerful techniques for sample identification and characterization. Combined with a microscope and with automated sample stages, it is nowadays providing chemical images of heterogeneous materials with spatial resolution below 1 micrometer. Sample heterogeneity often means a mixture of materials within a matrix but it may also mean a dispersion of particles of various sizes, shapes and composition on a substrate. In that case, both particle morphological characterization and chemical identification are necessary to fully analyze the sample.

Using Raman micro-spectroscopy combined with optical analysis of particles dispersed on a substrate, it is possible to automatically detect particles and provide their complete characterization: size, shape, number and chemical identification. HORIBA Scientific's ParticleFinder™ systems provide a complete solution for particle characterization and identification.

This new methodology is already being embraced in various fields to answer real world problems which traditional techniques could not address. These include:

- Identification of contaminants in the pharmaceutical industry, and the application to USP788/789.
- Characterization of harmful respirable silica species in perlite powders used in building materials.
- Composition analysis of rocks through micro-particle characterization for mining exploration.
- Characterization of micro-plastic pollutants particles in natural water bodies.
- Rapid identification and selection of 2D crystals (phosphorene, MoS2) to characterize the crystal orientation.

(542) **Classification of High and Low Glycated Hemoglobin in Diabetic Patients with Raman Spectroscopy and PCA-SVM**; Villa Manriquez José Fabián[1], Castro Ramos Jorge[2], Gutierrez Delgado Francisco[3]; [1]Instituto Nacional de Astrofisica Optica y Electronica; [2]Instituto Nacional de Astrofisica Optica y Electronica; [3]Centro de Estudios y Prevención del Cancer a.c.

Glycated hemoglobin (HbA1c) has been increasingly accepted as the gold standard for diabetes monitoring, due to the HbA1c provides an alternate measure of total glycemic. In this work we show the result obtained after analysis of glycated hemoglobin in 71 diabetic patients volunteers using Raman spectroscopy in vivo. These measurements were made in three regions of the body (finger tip, ear lobe and forehead). The assignments of the measured Raman bands were performed to compare the difference between two groups diabetics controlled (HbA1c <

6.5%) (22 volunteers) and diabetics with risk (HbA1c ≥ 6.5%) (49 volunteers). Using, multivariable methods such as principal components analysis (PCA) combined with support vector machine (SVM) were used to develop effective diagnostic algorithms for classification among these two class. Results have shown which the best region is forehead with sensitivity and specificity of 78.37% and 90%, respectively. Efficacy of the diagnostic algorithm by receiver operating characteristic (ROC) curve was confirmed. The area under the ROC curve was 0.85. The preliminary results demonstrate that noninvasive detection Raman spectroscopy can provide high levels of glucose.

(543) **Comparing Raman Mapping of Colon Tissue with Immunohistochemistry for Tissue Classification**; Aaran Lewis[1], Riana Gaifulina[1], Jennifer Dorney[2], Martin Isabelle[3], Manuel Rodriguez-Justo[1], Naomi Guppy[1],Nick Stone[2], Catherine Kendall[1, 3], Katherine Lau[1, 4], Geraint Thomas[1]; [1]University College London; [2]University of Exeter; [3]Gloucestershire Hospitals NHS Foundation Trust; [4]Renishaw PLC

The use of Raman spectroscopy as a tool to differentiate between abnormal and healthy tissue types in a range of organs is well documented as Raman spectra provide detailed chemical information on the tissue under investigation. Due to its diagnostic potential, Raman spectroscopy is being evaluated for its applications in clinical pathology as it possesses the ability to provide rapid, label-free biochemical information in comparison to older immunohistochemical methods. Fundamentally, instrumental advances now permit Raman mapping with high enough spatial resolution to provide morphological information on tissue samples. This work aims to compare Raman mapping with traditional immunohistochemical techniques.

Normal and cancerous tissue specimens were obtained from patients undergoing colonoscopy or colon surgery. Samples were paraffin embedded and 8 µm tissue sections were prepared for Raman spectroscopy on stainless steel substrate. Contiguous tissue sections were cut at a thickness of 3 µm onto glass slides for haematoxylin and eosin staining and antibody staining with anti-desmin, anti-LGR549, anti-MUC2 anti-Collagen III and anti-Ki67. All samples were dewaxed with xylene prior to downstream analysis. Raman maps were collected across tissue regions at an acquisition time of 20 seconds per pixel, at 1.1 µm spatial resolution.

Discrete tissue signals were identified in Raman maps using principal components analysis and the spatial distributions of these components were compared with H&E stained tissue sections and immunohistochemistry data. The spatial distributions of components identified in Raman spectra could be mapped to cell nuclei, mucin, muscle and collagen and were deemed to be tissue-specific when compared with H&E and antibody data.

Results indicate that Raman mapping could offer a means of disease diagnosis by mapping the biochemical distribution of tissue sub-types without the need for complex laboratory processing and expensive antibodies. Essentially, Raman spectroscopy has the potential to provide clinicians with a means of improved diagnostic accuracy for the benefit of both pathologist and patient.

(544) **Identification of Polymeric Microfibers in Fish Stomach and Great Lakes Waters using Raman Spectroscopy**; Karen Esmonde-White[1], Rachel Cable[1, 2], Melissa Duhaime[2]; [1]University of Michigan Medical School; [2]University of Michigan

The accumulation of plastic debris in nature is one of the most pervasive environmental concerns of our time. Recently, plastic has been documented in the Great Lakes at the highest concentrations seen anywhere on the planet. Yet, the fate of these plastics and their role in ecosystem dynamics is poorly understood. We are particularly interested in the fate of polymeric microfibers, known as microplastics, after ingestion by fish and their ubiquity in Great Lakes waters. Microplastics were extracted from the stomach of fish and isolated onto stainless steel well plates for examination. Raman microspectroscopy (λ=785 nm) was used to examine microfibers isolated from fish stomach and reference organic and polymeric materials. Field samples of water were collected from St. Clair Lake and digested to reduce the amount of organic material present, resulting in a slurry-like liquid containing particles up to 1mm in diameter. Fiber-optic Raman spectroscopy (λ=785 nm) was used to examine bulk slurry-like liquids. Microliter volumes were extracted for drop deposition/Raman spectroscopy to enable slurry particle analysis at higher spatial resolution. We describe the applicability of Raman microspectroscopy and fiber-optic techniques for non-destructive, rapid analysis of microplastics. Preliminary results suggest that Raman may be useful for distinguishing microplastics from organic materials such as wood or feathers.

(545) **Comparison of Machine Learning Methods to Identify Bacteria using Raman Spectroscopy**; Cynthia Hanson, Elizabeth Vargis[1]; [1]Utah State University

Traditional bacterial identification methods take one to two days to complete, relying on large bacteria colonies for visual identification. This time-consuming analysis is unacceptable during time sensitive situations such as life threatening illnesses and bioterrorism. In order to decrease analysis time, laser spectroscopy methods have been employed in conjunction with machine learning techniques to identify bacteria spectrally. In this research, three similar mycobacteria were used to build spectral training and test datasets and determine which classification method was most accurate. In addition, two sets of data were evaluated to determine if analysis time could be saved using a shorter acquisition time and wavenumber range. The first dataset ranged from 400-1800cm-1 with an acquisition time of 30 seconds. The second dataset ranged from 900-1800cm-1 with an acquisition time of 10 seconds. Classification methods tested include linear discriminant analysis (LDA), classification trees, random forest, support vector machines, gradient boosting, tuned gradient boosting, and k-nearest neighbor. Linear discriminant analysis was found to be the most accurate for both acquisition times based on percent correctly classified values for cross validation and prediction onto the test dataset. Accuracy for LDA dropped from 87% to 71% as time and spectral range decreased from the first to the second dataset. Results indicate that spectra intended for LDA classification should be collected at longer acquisition times with a wavenumber range of 900-1800cm-1.

(546) **Measuring Copolymer Chemical Heterogeneity by Combining SEC with Offline Raman Spectroscopy**; Aaron Urbas[1], Andre Striegel[1], Leena Pitkanen[1]; [1]National Institute of Standards and Technology

The use of Raman spectroscopy was explored as an offline detection method for size-exclusion chromatography (SEC) for the purposes of determining copolymer chemical heterogeneity. Chemical heterogeneity, defined here as the change in average chemical composition as a function of copolymer molar mass, affects processing and end-use properties of copolymers such as toughness, brittleness, and biodegradability. In one-dimensional size-based macromolecular separations (e.g., size-exclusion or hydrodynamic chromatography, or flow field-flow fractionation), chemical heterogeneity is usually determined via multiple detection, employing a series of detectors with different sensitivities (e.g., refractometry combined with UV spectroscopy), or using chemically-sensitive detection methods such as infrared (IR) or, less commonly, nuclear magnetic resonance spectroscopy. In this work, heart-cutting fractions from the SEC peak of a gradient random copolymer of styrene and methyl methacrylate were collected, at concentrations similar to those of eluting slices in an analytical SEC experiment, and subsequently analyzed off-line by Raman spectroscopy. The amount (weight or mole percent) of styrene in each fraction was quantified by Raman spectra

using a band ratio based calibration model constructed from the analysis of several well-characterized block, alternating, and random S-MMA copolymers. The weight percentages of styrene obtained from SEC with off-line Raman analysis of the gradient copolymer were compared to results obtained using SEC with on-line multi-angle static light scattering, differential refractometry, and ultraviolet absorption detection.

(547) **A Robust Method for Capillary Isoelectric Focusing Coupled with Mass Spectrometry**; David Chen[1], Shuai Sherry Zhao[1]; [1]University of British Columbia

Here is the abstract

(548) **High Peak Capacity Separations of Proteins and Peptides Using Two Dimensional Micro Free Flow Electrophoresis**; Michael Bowser[1], Matthew Geiger[1], Alexander Johnson[1], Nicholas Frost[1]; [1]University of Minnesota, Department of Chemistry

Separations play a critical role in almost every aspect of chemical analysis. Traditional chromatography and electrophoresis separations provide excellent selectivity for samples containing up to several dozen analytes. While impressive, this is often insufficient for complex mixtures encountered in modern research, which may contain hundreds or even thousands of analytes.

Two dimensional (2D) separations offer the potential for assays with dramatically improved peak capacities. 2D approaches separate mixtures sequentially according to two independent mechanisms. The ideal peak capacity of a 2D separation approaches the product of the peak capacities for each individual separation. Existing hyphenated 2D separations rarely reach this ideal peak capacity due to under sampling of peaks as they elute off of the first dimension column. Instead current 2D separations are characterized by long separation times and peak capacities that fall well short of those predicted by theory.

Micro free flow electrophoresis (μFFE) is the ideal second stage for 2D separations. In FFE a thin stream of sample is continuously introduced into a planar flow chamber. An electric field is applied perpendicularly to the flow through the separation chamber. Analytes are deflected laterally in the electric field according to their electrophoretic mobility giving rise to individual stream paths. FFE has recently been miniaturized into a microfluidic format (μFFE), requiring less sample and reagents, a simplified flow profile and better heat dissipation.

The continuous analysis provided by μFFE further separates analyte peaks as they elute off the first separation column removing under sampling limitations encountered in previous 2D separations. Coupling with nano liquid chromatography (nLC) or capillary electrophoresis (CE) is as straightforward as inserting the column outlet directly into the μFFE separation chamber. No complicated valving or timing is required. We have demonstrated that coupling with μFFE increases peak capacity 20 to 30-fold with no sacrifice of efficiency or separation time in the first dimension separation. We have performed initial nLC×μFFE and CE×μFFE experiments using peptide digests that achieve peak capacities >2,300 in less than 10 minutes.

(549) **Multiplexed Separations for Biomarker Discovery in Metabolomics: Urinary Markers of Smoke-Exposure in Firefighters**; Philip Britz-McKibbin[1]; [1]Department of Chemistry & Chemical Biology, McMaster University, Hamilton, ON, Canada

Among first responders, firefighters suffer a higher incidence of work-related chronic disorders with reduced average life span, including cardiovascular disease and various cancers. In this work, dynamic changes in the urine metabolome of firefighters were characterized by multi-segment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS) as a multiplexed separation platform to identify wood smoke markers of exposure in urine. 15 adult male firefighters from 3 municipalities across Ontario provided 24-hour pre-exposure urine samples and personal questionnaires detailing work history and activities of daily living. Each group was exposed to controlled burns of wood-based materials for approximately 30 minutes during standardized training trials in a burn house while wearing full regulatory equipment. 24-hour post-challenge urine was obtained across three time intervals: 0 – 6 hours, 6 – 12 hours, and 12 – 24 hours. Despite wearing bunker gear and self-contained breathing apparatus, a significant increase in post-exposure urinary excretion of methoxyphenols, resin acids, and polyaromatic hydrocarbons was determined by targeted GC-MS analysis. Also, untargeted characterization of the urine metabolome was performed by MSI-CE-MS in order to identify dynamic changes after smoke exposure among over two hundred polar metabolites and their ionic conjugates. Due to the experimental design, multilevel paired and time-dependent statistical data analysis methods were implemented to increase statistical power as a result of the heterogeneity in apparent exposures, metabolism and/or excretion patterns of firefighters in different study sites. This work provides new insights into the occupational hazards of firefighting that is needed to develop effective mitigation steps to reduce chronic disease risk.

(550) **Coupling CE with MALDI Imaging MS and ESI MS for Enhanced Analysis of Signaling Molecules**; Lingjun Li[1], Xuefei Zhong[1], Shan Jiang[1], Zichuan Zhang[1]; [1]University of Wisconsin

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has been extensively used for neuropeptide analysis due to its high sensitivity, fast speed and in situ tissue imaging capability. However, it is often difficult to be coupled with separation techniques without compromising resolution in off-line coupling mode. With micrometer-scale spatial resolution, MALDI imaging MS (IMS) offers unique advantage in coupling with capillary electrophoresis (CE). IMS enables “continuous” collection of CE flow which increases electrophoretic resolution. Moreover, CE provides separation and quantitation ability for IMS.

In this presentation, I will describe our recent progress on the use of coupling of pressure-assisted CE with IMS (PACE-IMS) platform and the quantitation of complex biological samples using CE-IMS. By imaging of homogenously deposited CE trace on the sample plate, individual peaks (m/z) can be visualized as discrete image regions and extracted from the IMS data, thus eliminating issues with peak overlapping for complex samples. This new platform produced enhanced MS signals and significantly increased numbers of peptides being detected in both tryptic peptide mixtures and neural tissue extracts from crustacean model organisms. Furthermore, quantitation with high accuracy was obtained in combination with isotopic formaldehyde labeling.

In addition to coupling of CE to MALDI IMS on a TOF/TOF or an Orbitrap platform, we also explored the use of a hybrid analytical platform combining CE and electrospray ionization mass spectrometry (CE-ESI-MS) for profiling of small molecule neurotransmitters and metabolites extracted from the hemolymph and neuronal tissues, which enabled more comprehensive characterization of the signaling molecules. A ‘junction-at-the-tip’ type CE-ESI MS interface was used for online coupling of CE and Synapt G2 Q-ToF MS. Complementary mass features were identified by these hybrid platforms. Both data analysis and biological applications will be presented.

(551) **Analysis of Proteins, Protein Complexes and Proteomes under Native and Denaturing Conditions using Sheathless Capillary Electrophoresis Coupled with Mass Spectrometry**; Alexander R. Ivanov[1], Rosa Viner[2], Marcia R. Santos[2], Arseniy M. Belov[1], David R. Bush[1], Chitra K. Ratnayake[3], Barry L. Karger[1]; [1]Northeastern University, Barnett Institute of Chemical and Biological Analysis, Boston, MA; [2]Thermo Fisher Scientific, San Jose, CA; [3]Sciex, Brea, CA

Proteins, including biopharmaceuticals and building blocks of multimeric protein complexes, typically consist of heterogeneous collections of proteoforms, containing covalent and non-covalent modifications. Accurate knowledge of proteoform profiles is critical for assessing biological functions of proteins and protein assemblies as well as the safety and stability of biopharmaceuticals. Current methods of analyzing proteins and post-translational modifications (PTMs) require proteolysis or removal of PTM moieties (glycan profiling) that can result in loss of information on correlated abundances of PTMs. Minimal sample preparation, as used in native and top-down mass spectrometry (MS), is also desirable to minimize introduction of artifacts and to study labile PTMs.

To date, most MS studies of intact proteins and protein complexes have been based on direct infusion. Both native and top-down MS could greatly benefit from the direct coupling with high resolution separations. Here, we demonstrate the power of high performance capillary electrophoresis (CE) coupled to high-resolution MS using a sheathless interface (Sciex CESI) for both native and top-down MS, while enabling high sensitivity characterization of mixtures of native proteins, proteoforms, complexes and even proteome-level samples.

Experimental conditions for separation, in-source ion desolvation, fragmentation, and CESI were optimized, and then efficient separation and high sensitivity analysis (e.g., low fmole) was achieved. Additional proteoforms were detected using the native CE-ESI-MS approach in comparison to experiments performed using direct infusion native MS or CE-ESI-MS under denaturing conditions. Protein identities were confirmed using MS/MS. By optimizing sample handling, separation and injection conditions, we were able to resolve glycan isomers of interferon-β1 and determine the correlation in abundance of certain modifications. We have been also able to profile more than 100 PSA glycoforms under native or denaturing conditions. The performance of the native CE-MS approach for higher complexity samples was assessed in the analysis of the E. coli ribosomal proteome. Ribosomal proteins and their complexes were identified using both accurate mass and fragmentation experiments. In summary, sheathless CE-ESI-MS in combination with advanced MS and bioinformatics techniques has demonstrated to provide excellent minimally destructive capabilities for identification and quantitative characterization of proteins, protein complexes and even proteomes in their native and intact states.

(552) **From the VG 9000 to Nu Astrum – How Magnetic Sector GDMS was Made a Commercial Reality**; John Cantle[1]; [1]Nu Instruments
John Cantle, Glyn Churchill, DeAnn Barnhart, Christopher Page, Sergey Ryabov, Andrew Burrows.

The ability to accurately and precisely quantify elemental composition from matrix to sub-ppb (parts per billion, ng/g) levels is virtually unique to GD-MS.

The Nu Astrum high resolution glow discharge mass spectrometer uses a low flow, low power, cryo-cooled discharge cell which allows a large range of solid materials to be analysed. It is the successor to the highly accomplished VG9000 which first appeared in the 1980s and for which around 80 units were supplied world-wide. This paper will discuss the trials and tribulations of the commercial introduction.

The first Astrum from Nu Instruments was commissioned in 2010, and there are now over 12 installed worldwide including the US, UK, Japan and China. The Astrum has a diverse customer base ranging from refineries for high purity materials including copper, indium and gallium to manufacturers of metal alloys such as nickel and cobalt based superalloys. The Astrum is also being utilised in research and contract laboratories, both environments requiring the analysis of a wide range of materials on the same instrument.

The continuing development of the Astrum has resulted in numerous improvements, including a new liquid nitrogen cooling system. The new cooling system enables the use of non-pressurised dewars, increases cooling efficiency and reduces liquid nitrogen consumption. With the new cooling system cell alignment is also improved, and the use of boron nitride is completely eliminated from the instrument, thus eliminating the potential for accidental boron contamination of samples. Additional improvements include the cell exit slits, which are now laser cut to aperture sizes which have been individually optimised for flat and pin samples. The new cell exit slits improve sensitivity whilst (in tandem with the new cooling system) ensuring greater consistency between cells without causing a significant change to abundance sensitivity or molecular and multiple charged interferences.

Examples of these and other improvements to the Astrum will be given in this talk as well as data obtained during the development processes. All of this will be set in the context of the first appearance of commercial GDMS from VG Instruments.

(553) **Pushing the Boundaries in Glow-Discharge Spectrometry— a Tribute to Edward Steers**; Gary M. Hieftje[1], Andrew P. Storey[1], Jacob T. Shelley[2], Steven J. Ray[1]; [1]Indiana University, [2]Kent State University, [3], [4]

The name of Edward Steers is synonymous with fundamental investigation and application of glow discharges. Active in the field for many decades, Steers has been responsible for a host of innovations and explanations into glow discharge behavior. He has been a steadfast promoter not only of this versatile source but also of others working in similar areas. This symposium is a most fitting tribute to a pioneer in the truest sense of the word, in his first attendance of a FACSS (SciX) meeting.

Recent work into glow discharges has extended the range of pressures and power levels at which glows operate. Here, one unusual sort of glow will be described and evaluated. Termed the Flowing Atmospheric-Pressure Afterglow (FAPA), the source is operated at atmospheric pressure in helium. When the discharge products flow into the ambient atmosphere, they generate a host of products, such as protonated water clusters and charged nitrogen-oxygen ions, which together are capable of ionizing a broad range of analyte molecules. Moreover, the effluent from the discharge cell is heated by the plasma, making it capable of desorbing molecules from the surface of a sample. In concert, these features make the FAPA useful for the “soft” ionization of many substances, to yield mass spectra that are both simple and sensitive. In this presentation, fundamental features and new applications of the FAPA source will be featured, with a view toward future versions of the source that enhance is capabilities further.

SciX 2015 ABSTRACTS

(554) **Measurement of Oxygen in Solid Samples using Analytical Glow Discharges with Optical or Mass Spectrometric Detection**; Volker Hoffmann[1], Edward Steers [2], Sohail Mushtaq[2], Juliet Pickering[3], Cristina Gonzalez Gago[4], Petr Smid[4],Thomas Hofmann[4], Cornel Venzago[4], Wolfgang Gruner[1], [2]; [1]IFW Dresden; [2]London Metropolitan University; [3]Imperial College, London; [4]AQura GmbH

The analytical determination of the light elements hydrogen, carbon, nitrogen and oxygen is usually applied in practice with the so-called non-metal analysis by carrier gas hot extraction or combustion methods for individual elements - seldom pairwise like for oxygen plus nitrogen. Analytical glow discharges with optical (GD-OES) and mass spectrometric (GD-MS) detection are able to obtain even depth resolved information about these light elements in solid samples, where most of the other analytical techniques fail. However, the interpretation or even quantification of the measured signals is very difficult. Effects of a physical nature (plasma processes such as the 'Hydrogen effect' and the Doppler effect or diffusion of hydrogen in the sample during sputtering) and a chemical nature (e.g. formation of compounds with argon or the matrix, poisoning of the sample or gettering) go along with instrumental problems (e.g. of the sensitivity and vacuum quality) and problems at calibration (such as lack of calibration material or matrix dependence).

Therefore, fundamental investigations have been made about the influence of O in analytical glow discharges [1] and the effect of Ar-O mixtures compared with that of O released from the sample [2]. This comparison proved that O in a concentration, which is generated by sputtering of an oxide, has no major effect on the emission of spectra in GD-OES.

Investigations about matrix independent calibration of O became possible with the preparation of sintered samples consisting of a metallic powder like Mg, Al or Cu representing the matrix and a defined addition of powderous oxides of interest. We will show that in GD-OES the emission yield of line O I 130 nm depends on the matrix, but using O I 777 nm, matrix independence is obtained. GD-MS also produced promising results for the calibration of O with these sintered samples, when the sputtering rate was included in the evaluation.

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[3] C. Gonzalez-Gago, P. Smid, T. Hofmann, C. Venzago, V. Hoffmann, W. Gruner, The use of matrix-specific calibrations for oxygen in analytical glow discharge spectrometry, Anal Bioanal Chem 406 (2014) 7473–7482.

(555) **Investigations towards Matrix Independent Calibrations in Glow Discharge Mass Spectrometry**; Petr Smid[1], Cristina Gonzalez-Gago[2], Volker Hoffmann[3], Cornel Venzago[1], Thomas Hofmann[1]; [1]AQura GmbH; [2]University of Oviedo; [3]IFW Dresden

Mass spectrometry (MS) utilizing analytical glow discharges (GD) as an atomization and ionization source has proved to be a very sensitive, fast and reliable analytical technique for direct solid state trace and ultra-trace elemental analysis with small requirements on sample preparation. Over a few decades, the analytical technique of GD-MS has been successfully applied in various fields of analytical applications such as high purity metals for electronic industry, semi-conductors and layered materials for micro-electronics and photovoltaics, special alloys for aerospace industry, etc. The quantification approach applied traditionally in GD-MS is based on the concept of standard relative sensitivity factors (StdRSF). This approach compensates the lack of matrix-specific reference materials for various elements in trace concentration range and provides fairly accurate analytical results; the uncertainty associated with this quantification concept amounts up to a factor of 2 depending on the element analyzed. Matrix and plasma parameter dependence of the StdRSF when using the so called 'static flow' cryo-cooled GD source being used in the GD-MS instruments since 80s was found to be relatively small in comparison to the 'fast-flow' GD sources implemented in the commercial GD-MS systems later on. The main advantage of the fast-flow concept is the high sample throughput compared to the earlier GD-MS concept based on the 'static-flow' GD.

The lack of knowledge of matrix and plasma parameter dependence of the StdRSF was the main motivation for the recent investigations carried out within the European Metrology Research Project (EMRP) SIB09 ELEMENTS with the objective to improve the overall measurement uncertainty of GD-MS with the fast-flow GD sources. Relevant reference materials with Al, Cu and Zn matrices containing defined traces of metallic elements were investigated. A special attention was paid to the effect of voltage, current and gas flow. Suitable working parameters were selected for calibration and extensive experimental work including sputter rate measurements was undertaken. A suitable model for matrix independent calibration was achieved when normalizing the measured signals to Fe.

This work was conducted within the EMRP project SIB09 ELEMENTS. The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.

(556) **One Thing Leads to Another: Sixty Years of Spectroscopy Research**; Edward Steers[1]; [1]London Metropolitan University

I first encountered experimental spectroscopy as a 2nd year physics undergraduate at Imperial College. We all had three weeks, 6 hours/week in the spectroscopy lab – a very tough time but I was hooked on spectroscopy, and went on to a PhD on intensity measurements on the nitrogen electronic spectra. A three year fellowship at AERE, Harwell, followed, recording the emission spectra of rare earths and transuranics in the 1-2.5 μm region to provide data for term analysis.

In 1959, I became a lecturer at Northern Polytechnic, a forerunner of London Metropolitan University, where there was a small spectroscopy research group. An important local firm was Hilger & Watts, then the major UK manufacturer of spectrographic equipment; I went there to discuss running relevant short courses. This led to a 3-day course "The Principles of Atomic Spectroscopy", which attracted about 35 attendees. The main reaction – "Why didn't you include applications?" This led to a series of short courses, from 3 days to 2 weeks long, covering basic theory and analytical applications, mainly on AAS, then under steady development, but also on ICP, DCP etc. All the major manufacturers provided instruments and demonstrators for practical sessions, and this built up a number of important industrial contacts. e.g. with Pye Unicam, then owned by Philips, Eindhoven and Cathodeon, a hollow cathode manufacturer, and joint PhD projects. As a result, a colleague spent a year with Prof. Boumans in Eindhoven, and while he was there, we both visited ISAS, Dortmund. In 1984, I spent eight months at ISAS, working under Prof Laqua on GD-OES, followed by a number of further short visits. Since then, I have studied excitation processes in GD for OES and MS, and developed more and more contacts with those working with analytical GD.

EW-GDS was founded in 1992 to collaborate on GD-OES compositional depth profiling; in 1993, its scope was extended to bulk analysis, fundamentals as well as applications and both OES and MS. The field was then very fragmented, and, apart from my research activity, much of my time since has been devoted to fostering collaboration between groups.

(557) **To Subtract or Not to Subtract, that is the Question… In Interpretation of Soil Organic Matter Spectra**; Francisco Calderon[1], Andrew Margenot[1, 2], Sanjai Parikh[2]; [1]USDA-ARS, Akron, Colorado; [2]Department of Land, Air and Water Resources, University of California Davis

There is currently no gold standard method for the study of soil organic matter (SOM) molecular structure as of yet. NMR, DRIFTS, analytical pyrolysis, thermogravimetry, and NEXAFS all have advantages and disadvantages. However, DRIFTS is gaining popularity because it is quick, relatively inexpensive, and yields information-rich data sets. Soils are complex mixture of a variety of minerals, plant residues, and the hard to characterize SOM. Spectral subtraction has been used for enhancing SOM bands in an otherwise mostly mineral spectrum. There are instances where subtraction is not advisable, but there are other situations where subtractions clearly have potential to unravel important details in the spectral data. Soil scientist should strive to arrive at a convention about what is the appropriate way to use and report spectral subtractions. In this presentation, we will address SOM removal techniques such as ashing or wet chemical oxidations with permanganate or hypochlorite, and also clay removal by hydrofluoric acid as basis for subtraction from intact soil spectra. We will also present subtractions using soil spectra from permafrost affected Arctic soils rich in SOM, and soils from the sandhills of Nebraska, low in clay content. We postulate that with judiciuos criteria, spectral subtraction of soil DRIFTS data can be performed and used as a valuable tool to measure changes in SOM due to agricultural management or other disturbances.

(558) **Modeling the Time-Resolved Diffuse Reflectance**; Arnold Kim[1]; [1]University of California, Merced

We give a systematic derivation of the Kubelka-Munk equations from the equation of radiative transfer. In doing so, we derive an intermediate approximation, which we call the generalized Kubelka-Munk equations. The generalized Kubelka-Munk equations are better, especially for strong absorbing and anisotropic scattering media, and only slightly more difficult to solve than the Kubelka-Munk equations. Therefore, we introduce their use in modeling the diffuse reflectance. We then extend this theory to model the time-resolved diffuse reflectance. We propose the use of this model for interpreting measurements of the mid-infrared diffuse reflection on ultrafast time scales recently obtained by E. Brauns (Appl. Spectrosc. 2014. 68(1): 1-4).

(559) **QCL-Standoff MIR Reflectance Spectroscopy Measurements of Hazardous Chemicals and Biological Threats**; Samuel P. Hernández-Rivera[1], Leonardo C. Pacheco-Londoño[1], Amira Padilla-Jimenez[1], Nataly J. Galan-Freylr[1], Carlos Rios[1,2], John R. Castro-Suarez[1]; [1]Department of Chemistry, University of Puerto Rico-Mayaguez

Standoff (SO) mid-infrared (MIR) reflectance spectroscopy using quantum cascade lasers (QCL) sources has been used to tackle various analytical problems involving detection of hazardous threat chemicals, biological threats agents simulants, landmine explosives in soils; distribution of content of active pharmaceutical ingredients in formulations; and even detection of oil residues in soils. The high collimation and spectral radiance (brightness) of the QC lasers used made possible the researches in up to recent elusive problem. In other cases, results were as good as those that can be obtained using attenuated total reflection (ATR) modality for FT-IR based systems with the added capability of sensing at a distance.

Highly energetic materials (HEM), homemade explosives (HME), chemical and biological agents are classified as imminent threats which provide terrorists means to cause damage to civilians, troops or first responders and to private and public property. Research with SO-QCL spectroscopic systems has allowed detecting, discriminating and quantifying HEM/HME present as trace residues on metal substrates (neat or painted) and on non-reflective substrates in a fast and reliable manner. Substrates such as aluminum, travel bags, cardboard and wood were used. HEMs investigated included 2,4,6-trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), and 1,3,5-trinitroperhydro-1,3,5-triazine (RDX). Three chemometrics algorithms were applied to analyze the spectral characteristics. Partial least squares (PLS) regression analysis was used to find the best correlation between the MIR signals and the surface concentrations of the samples. Principal component analysis (PCA) and PLS combined with discriminant analysis (PLS-DA) were used to discriminate, classify and identity similarities in the spectral data sets. The results demonstrate that the MIR vibrational method used in these studies can be useful for a rapid analysis of HEMs on any substrate type.

In another application, thin layer chromatography was coupled to QCL reflectance spectroscopy (TLC-QCLS) and used as a portable hyphenated technique for fieldable applications of trace analysis of HEM.

(560) **Quantitative Infrared Directional/Hemispherical Reflectance Measurements**; Thomas Blake[1], Carolyn Brauer[1], Yin-Fong Su[1], Russell Tonkyn[1], Tanya Myers[1], Brenda Kunkel[1], Bruce Bernacki[1], Timothy Johnson[1]; [1]Pacific Northwest National Laboratory

Past laboratory and field work by researchers performing thermal infrared sensing of geological surfaces, for example, has shown that for most materials a laboratory measurement of the total directional/hemispherical reflectance, R_T, of a target surface and the use of Kirchhoff's law gives a good approximation to the emissivity, ε, of that surface: $\varepsilon = 1 - R_T$. Using a Fourier transform spectrometer (FTS) and a commercial-off-the-shelf integrating sphere with a matte gold coating we have begun to construct a quantitative database of infrared directional/hemispherical (specular + diffuse and diffuse-only) reflectance spectra covering the wavelength range 1.3 to 16.7 μm for materials relevant to longwave infrared field sensing. Protocols for measuring specular + diffuse and diffuse-only reflectance spectra of surfaces using the FTS and sphere are provided and estimates for systematic and random error in the measurements are given. Results for measurements of reflectance standard materials as well as data from several materials of interest will also be presented. A complementary reflectance database for the same materials is also being developed in the visible and near infrared (VNIR). The VNIR data, which is measured using a dispersive spectrometer and Spectralon coated sphere, overlaps with the infrared data in the shortwave infrared region. Reflectance data comparisons from the two instruments in the overlap region will also be given.

(561) **Detection Limits for Blood on Fabrics via IR Diffuse Reflection**; Michael Myrick[1], Stephanie DeJong[1], Ray Belliveau[1], Stephen Morgan[1], Brianna Cassidy[1], Zhenyu Lu[1]; [1]University of South Carolina

Detection limits in forensic situations are often not well understood, and some of the best-known methods for detecting blood have literature values for detection limits that vary by many orders of magnitude. Further, the units in which detection limits are cast have been mainly units of

convenience that are not readily converted to the kind of units that an analytical chemist would recognize. Detection limits using IR or NIR spectroscopy are at best crude estimates to date.

As time allows, we will explore a case study to determine the detection limits for blood on fabrics via infrared diffuse reflection (DR) under the simplest conditions of no additional interferences and with well-controlled laboratory samples.

Working in reflectance mode, the system is pretty nonlinear – but models are not appreciably better in log(1/R) mode. We find that high order derivative processing works well and holds a number of surprises. A few of our observations about derivative processing are: high order numerical gap derivatives can have signal to noise ratios greater than the undifferentiated original spectra; the numerical gap derivative, when optimized, works as well or better than traditional Savitzky-Golay derivative processing in every case we studied; the high-order gap derivative can behave like a matched filter for improving recognition of the analyte; and a gap derivative can be designed to be invertible for ease of analyzing component spectra.

The detection limits we found for different spectral windows on 4 different fabrics will be given, with the best we found being in the range of 0.01 %w/w. Generally we found that the mid-IR spectral window gave better results than any of the near IR spectral windows. We were also curious what factors restrict the detection limit for blood in the different spectral windows, and our work to answer this question will be described.

(562) **Evaluation of Phage Susceptibility in Acinetobacter baumannii**; Meron Ghebremedhin[1,4], Nicole Crane[1, 2, 4], James Regeimbal[1,2], Anna Jacobs[3], Brendan Corey[3]; [1]Naval Medical Research Center; [2]Uniformed Services University of Health Sciences; [3]Walter Reed Army Institute of Research; [4]Henry M. Jackson Foundation for the Advancement of Military Medicine

The emergence of Multi Drug Resistance (MDR) infections has become a global problem for civilian and military populations alike. In combat wounded soldiers, these infections are particularly problematic when associated with traumatic blast wounds sometimes resulting in adverse wound outcomes such as limb amputation. Consequently, there has been a great deal of interest in developing phage based therapies against MDR pathogens such as Acinetobacter baumannii, which are often responsible for hospital acquired infections. Due to the wide variety of phages, the development of a custom therapeutic using an entire phage library will require a rapid method of identification like Raman spectroscopy to inform phage selection.

This study aims to evaluate the potential of Raman spectroscopy to predict phage susceptibility in A. baumannii strains. We collected Raman spectra of a set of A. baumannii strains that are susceptible to AB5075 Phage (AB5075Φ) which included AB5075 and AB4490, and of those not susceptible to AB5075Φ which included AB967, AB4857 and AB5711. In order to monitor spectral changes, spectra of the strains were collected pre and post AB5075Φ treatment.

Analysis of Raman spectra revealed inherent spectral markers, predominantly the presence of Raman spectral band at 979cm-1 in all strains that are AB5075Φ susceptible but absent in those that are not AB5075 Φ susceptible. Other spectral markers were also observed at 1422 cm-1, 1379 cm-1, 641cm-1 and 620 cm-1 Raman spectral bands only in the AB5075Φ susceptible strains. Furthermore, when AB5075 Φ susceptible strains were passaged to select for AB5075 Φ resistant mutants, these mutants no longer displayed these markers, including the 979 cm-1 band.

Based on these results, it can be postulated that Raman spectra for A. baumannii strains contain markers which can be used to predict AB5075Φ susceptibility. While this is a preliminary study involving a subset of A. baumannii diversity set and AB5075Φ, current work is ongoing to expand the library of relevant diverse phage and A. baumannii strains along with Raman spectroscopic data.

(563) **Direct Bacterial Analysis by Ambient Ionization Mass Spectrometry**; Pu Wei[1], Christopher Pulliam[1], Soumabha Bag[1], Alan K. Jamusch[1], Saerom Kim[1], Rafal M. Pielak[2], R. Graham Cooks[1]; [1]Purdue University; [2]L'Oréal California Research Center, San Francisco, CA

Discrimination of microorganisms plays an important role in numerous fields, including clinical medicine, public health, biotechnology, and food safety. Genetic approaches, such as whole-genome shotgun metagenomics sequencing, are commonly used to identify bacteria and fungi down to the strain level. However, these methods are often labor-intensive, time-consuming, and expensive. Ambient ionization mass spectrometry allows for rapid method to differentiate bacteria and fungi at the species level based on characteristic lipid profiles. In this work, three different ambient ionization methods, paper spray (PS), zero volt PS, and swab touch spray (STS) were investigated for discrimination of several prevalent microorganisms, including *Staphylococcus, Streptococcus, Propionibacterium, Pseudomonas, Acinetobacter, Corynebacterium, Candida,* and *Malassezia* species. Microorganism (bacteria and fungi) isolates were cultured from frozen samples, and sub-cultured samples from an isolated colony were analyzed using a linear ion trap mass spectrometer. Full scan spectra were recorded for 1-2 min/sample in both positive and negative ion mode, and structures of the major lipids were confirmed with MS/MS analysis. Multivariate data analysis, which is based on principal component analysis (PCA), followed by linear discriminant analysis (LDA), was performed to demonstrate the *in vitro* discrimination of microorganisms, and the average successful prediction rate was over 90%. Three ionization methods are evaluated based on their sensitivity, reproducibility, average successful prediction rates, etc. Using the most appropriate method selected from previous study, microorganisms from human fingerprint are cultured and analyzed. Metabolisms of selected microorganisms under different culturing conditions are studied by examining changes of their lipids. In addition, selected species were analyzed by PS coupled with a portable mass spectrometer, the Mini 12, which shows great potential for *in situ* analysis of microorganisms in future. This is also the first demonstration of analyzing large molecules like lipids using a miniature mass spectrometer.

(564) **An Analytical Approach to Designing Clinical MALDI-TOF Mass Spectrometer Instrumentation**; James VanGordon[1]; [1]bioMerieux, Inc.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometers are increasingly used in a clinical setting for the identification of unknown microorganisms. These systems use a laser to ionize a sample via the MALDI process and electrostatically accelerate the resulting ionized proteins and peptides in vacuum. The times of flight are subsequently measured to determine the mass composition of the sample. However, the corresponding subsystems within the mass spectrometer must be operating correctly and in a unified manner to achieve clinically significant results. The resulting instrument can be described as a cross-coupled, multivariable design problem. Mathematical time-of-flight theory can be used as a preliminary tool to ensure that the multivariable problem includes and addresses many of the practical limitations for a MALDI-TOF instrument. Typical operation of a MALDI-TOF mass spectrometer as it relates to microbial identification will be discussed.

Additionally, this paper will pose design considerations for linear MALDI-TOF mass spectrometers to be used in a clinical setting. Analytical techniques will be used to describe the effects of design parameters such as vacuum pressure, delayed extraction times, and timing jitter on the mass spectra.

(565) **Surface Enhanced Raman Spectroscopy of Bacteria-Infected Wound Effluent of Combat Related Injuries**; Nicole Crane[1,2,3], Shubha Yesupriya[1], Meron Ghebremedhin[1,3]; [1]Naval Medical Research Center; [2]Uniformed Services University of Health Sciences; [3]Henry M. Jackson Foundation for the Advancement of Military Medicine

In military medicine, one of the challenges in dealing with combat-related injuries is the prevalence of bacterial infection. Current methods of identifying bacterial infection rely on culturing microbes from patient material and performing biochemical tests, which together can take 2-3 days to complete. Surface Enhanced Raman Spectroscopy (SERS) is a powerful vibrational spectroscopy technique that allows for highly sensitive detection of analytes adsorbed onto specially prepared metal surfaces. In the past, we have been able to discriminate between bacterial isolates grown on solid culture media using standard Raman spectroscopic methods. Here, we use SERS to assess the presence of bacteria in wound effluent samples taken directly from patients. Citrate reduced silver colloid solution was prepared and pipetted onto gold slides. Wound effluent was pipetted and dried onto each nanoparticle spot and SERS spectra were collected. The infected samples have a diverse population of gram positive and gram negative bacteria of 9 different species including *Acinetobacter haemolyticus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterobacter cloacae*. In order to quantify the magnitude of signal enhancement, SERS spectra were compared to the Raman spectra of effluent samples. Little to no Raman signal was detected in any of the samples when effluent was spotted directly onto the gold slides without nanoparticles. SERS signal was clearly evident when layered on top of the nanoparticle spots, with at least 2 fold enhancement compared to standard Raman spectra of the samples. This study demonstrates an important step towards developing Surface Enhanced Raman Spectroscopy (SERS) as a powerful and rapid tool for detecting bacterial infections in complex clinical samples. Future studies will include fine-tuning silver nanoparticle morphology to fully exploit SERS signal enhancement and development of classification models for bacterial identification using SERS effluent spectra.

(566) **Monitoring Bacterial Biofilm Growth and Removal using a Quartz Crystal Microbalance**; Hunter Sismaet[1], Pegah Abadian[1], Edgar Goluch[1]; [1]Northeastern University

Bacteria form complex intercellular communication networks known as biofilms that allow them to adapt to a wide-range of environmental stimuli, making them highly resistant to extreme conditions and antimicrobial agents. As a result, highly-persistent bacterial species have emerged in the hospital setting. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were recently classified as serious threats to modern healthcare by the Centers for Disease Control and Prevention due to their antibiotic-resistant character and prevalence in hospital-acquired infections. Development and application of a novel platform for studying these bacteria at the onset of infection as well as strategies for bacterial growth and removal can provide useful insight into how bacteria grow and adapt to their environment. To address these needs, a quartz crystal microbalance (QCM) was used to investigate bacterial biofilm growth and removal.

QCMs measure the precise oscillation of a quartz sensor at their resonant frequency when an alternating voltage is applied. Mass deposition and removal on the surface of the quartz causes a frequency shift response that is measured by the QCM in real time. These highly sensitive mass measurements can be used to monitor how bacteria adhere, grow, and form biofilms on surfaces.

Bacterial species *Pseudomonas aeruginosa* and *Staphylococcus aureus* were grown overnight in lysogeny broth (LB) growth media at 37 °C. Cells were centrifuged to remove the supernatant and reconstituted in fresh LB. Bacterial species were diluted to approximately 10,000 cells/µL and loaded at 50 µL/min for 30 minutes. The bacteria were allowed to adhere and grow on the gold-coated quartz surface over the course of several hours. Changes in both frequency and damping were recorded to monitor bacterial accumulation on the surface. Once the bacterial cells were stably attached to the surface, a 1% SDS detergent was loaded into the system to study the effect of biofilm removal. Biofilm removal was determined by the sensor's response as it returned to the original baseline and confirmed by visual inspection using the device's optical window. This study validates the use of a QCM for studying bacterial adhesion and removal from surfaces as well as investigating the effectiveness of biofilm removal agents.

(567) **Resonance Enhanced AFM-IR Induced by Quantum Cascade Laser**; Alexandre Dazzi[1], Jérémie Mathurin[1], Johanna Saunier[2], Najet Yagoubi[2], Ariane Deniset-Besseau[1], Kevin Kjoller[3]; [1]Laboratoire de Chimie Physique - Université Paris-Sud; [2]Matériaux pour la santé, Faculté de Pharmacie, Chatenay-Malabry; [3]Anasys Instruments

We present results obtained with Resonance Enhanced AFM-IR, a new generation of AFM-IR technique[1] allowing analysis of much thinner samples by making use of a Quantum Cascade Laser (QCL) as the IR source. With this new technique, it is possible to increase significantly the sensitivity and the spatial resolution of the AFM-IR technique. Imaging of self-assembled monolayers was realized [2] proving the high sensitivity and resolution of this new technique which is achieved by measuring molecular expansion using force detection.

The first demonstration will be illustrated by a direct application in pharmaceutics domain [3]. The technique is used to detect some traces of organics materials (lubricant) on polymer (polyurethane) used in hospital to make implantable catheters. Surface state is one of the most important parameter on the biocompatibility of an implantable medical device. By using the resonance enhanced mode, it is possible to identify with high resolution topographic and chemical maps the chemical nature of very thin film observed on the surface.

The second illustration will deal with proteins spectra obtained on biomaterials. Streptomyces is a filament bacteria that growths naturally into the earth. Usually the mycelium evolves from basal to aerial state with an autophagy process. The result of such a process is to find ghosts inside the cell culture which are "empty filaments" composed only of cell walls and membranes. The resonance enhanced technique will allow us to detect the proteins by their infrared spectra directly on the ghost filament.

(568) **Linear and Nonlinear Vibrational Mid-Infrared Photothermal Spectroscopy with a Compact Fiber Laser Probe**; Atcha Totachawattana[1,3], Shyamsunder Erramilli[1, 2, 3, 4], Michelle Sander[1,3,4]; [1]Electrical and Computer Engineering, Boston University; [2]Physics and Biomedical Engineering, Boston University; [3]BU Photonics Center; [4]Materials Science and Engineering, Boston University

We present a mid-infrared photothermal spectroscopy system with a quantum cascade pump laser and a near-infrared fiber probe laser for label-free characterization of mid-infrared normal vibrational modes in molecular systems. Photothermal mid-infrared spectroscopy offers the potential

for ultra-high sensitivity with enhanced signal-to-background contrast, compared to conventional Fourier Transform Infrared Spectroscopy: High spectral brightness quantum cascade lasers are combined with mature detector technology in the near-infrared, without the need for temperature cooling. The fiber laser, operating at wavelengths far removed from the absorption features in the mid-infrared, provides a low noise, robust and turn-key laser configuration that is highly customizable.

Here, we demonstrate linear and nonlinear photothermal studies of the C-H scissoring band around 1607cm-1 in a 6µm-thick liquid crystal 4-Octyl-4'-Cyanobiphenyl (8CB) at room temperature in the smectic-A phase. A pulsed QCL pump that is tunable over the wavenumber range of 1570-1740cm-1 and a fiber probe beam are co-aligned and coaxially focused onto the sample. The photothermal signal is collected in a heterodyne detection scheme by a commercial photodetector combined with a lock-in amplifier. At low mid-infrared pump powers (~1mW), system optimization and the use of higher probe laser power leads to improvements in the signal-to-background contrast by one order of magnitude, from ~600 to ~6500.

An interesting spectroscopic regime is observed for higher mid-infrared pump power values where nonlinear photothermal studies are conducted: The main absorption band around 1607cm-1 undergoes a series of characteristic pitchfork bifurcations with spectral narrowing. High reproducibility for different probe laser parameters underlines the possibility of utilizing these nonlinear spectral signatures for unique sample-specific identification. In addition, this regime offers the potential for higher spatial resolution and simultaneous characterization of vibrational spectroscopic signatures with thermal diffusion parameters. In summary, we present mid-infrared photothermal spectroscopy in the linear and nonlinear regime as a novel and sensitive method for bond-specific spectral characterization.

(569) **Laser Direct IR Imaging - A New Paradigm for Mid-IR Spectroscopic Imaging**; Charles Hoke[1], Yuri Beregovski[1], Andrew Ghetler[1], Yang Han[1], Christopher Moon[1], Richard Tella[1]; [1]Agilent Technologies, Inc.

The performance of broadly tunable external cavity quantum cascade lasers has improved dramatically over the past several years. These spectrally bright light sources have clear benefits for traditional spectroscopic applications and profound advantages in areas like trace gas detection. However, the coherent nature of these sources creates numerous challenges in microscopy and imaging applications. Traditional image sensor based approaches, either linear or 2D array, give rise to numerous coherence artifacts that significantly degrade image quality and impede quantitative interpretation. Laser Direct IR (LDIR) imaging is a fundamentally new Mid-IR imaging architecture that has been developed to overcome these limitations. LDIR retains the speed advantages inherent in a QCL imaging system and additionally enables the user, for the first time, to generate metrology grade images and spectra. An overview of LDIR will be presented along with quantitative measures of instrument performance along with numerous application examples.

(570) **Fast Time-resolved IR Measurements using EC-QCL**; Michael George[1]; [1]University of Nottingham

Fast Time-resolved IR spectroscopy has proved a powerful tool for following chemical process on timescales from femtoseconds to seconds providing key information for identifying reaction intermediates and elucidating reaction mechanisms. This lecture will focus on using EC-QCLs as the iR source for fast time-resolved IR measurements and will show very fast sensitive measurements can be obtained with relative ease and provide important information on the nature and reactivity of excited states and reaction intermediates.

(571) **EC-QC Laser Spectroscopy for mid-IR Transmission Measure-Ments of Proteins in Aqueous Solution**; Bernhard Lendl[1], Andreas Schwaighofer[1], Mirta R. Alcaraz[1]; [1]Technische Universität Wien

Mid-IR transmission measurements of the protein amide I band in aqueous solution at large optical paths is presented. A room-temperature operated tunable external-cavity quantum cascade laser (EC-QCL) operated in pulsed mode allowed applying path length of up to 38 µm. To minimize temperature-induced variations caused by background absorption of the bending vibration of water overlapping with the amide I region, a highly-stable temperature control unit with relative temperature stability within 0.005 °C was developed. An advanced data processing protocol (COW) was established to overcome fluctuations in the fine structure of the emission curve that are inherent to the employed EC-QCL due to its mechanical instabilities. Employing this setup, characteristic spectral features of five well-studied proteins exhibiting different secondary structures could be elucidated at concentrations as low as 2.5 mg ml–1. In addition recent results on protein folding studied by EC-QCL spectroscopy will be presented, too.

(572) **Contribution and Impact of Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) and Laser Induced Breakdown Spectroscopy (LIBS) Tandem system to Forensic Evidence Analysis**; Jhanis J. Gonzalez[1,2], C. Derrick Quarles Jr.[2], Dayana D. Oropeza[1], Charles Sisson[2], Xianglei Mao[1], Vassilia Zorba[1], Rick Russo[1,2]; [1]Lawrence Berkeley National Laboratory; [2]Applied Spectra, Inc.

Improvements in Laser Ablation for material sampling over the past few decades have led to the emergence of several applications of this technique (in its two modalities LIBS and LA-ICP-MS) to some important forensic measurements. These Laser Ablation modalities are commonly used for both elemental and isotopic analyses, and has multiple advantages compared to dissolution techniques, notably higher spatial resolution, easier and faster sample preparation, and for many applications it is a non-destructive method. However, LA-ICP-MS suffers of a significant limitation related to its elemental coverage. For example, non-metals such as H, N, O and halogens such as F, are difficult or impossible to analyze by conventional ICP-MS systems. On the other hand, one of the major benefits for using Laser Induced Breakdown Spectroscopy (LIBS) is its elemental coverage, and of course its ability to detect elements that are difficult or impossible to analyze by most analytical techniques and in particular those mentioned above. The combination of the two Laser Ablation modalities provides complementary measurements for elements that are separately unattainable due to low sensitivity and/or strong interferences. In this presentation we will discuss some of the recent advances and applications using the J200-Tandem system from Applied Spectra, Inc.

(573) **The Discrimination of Printing Inks Using Laser Induced Breakdown Spectroscopy for Forensic Applications**; Ruthmara Corzo[1], Jose Almirall[1]; [1]Florida International University

The advent of improved printing technology has facilitated the production of fraudulent documents that closely resemble the authentic document. Elemental analysis is a valuable tool for the chemical characterization of printing inks, which can aid in the forensic discrimination of samples originating from different sources or in the association of samples originating from the same source. In this study, 45 inkjet and 57 toner inks, the two most commonly encountered printing inks in forensic document examinations, were analyzed directly from the paper substrate using Laser Induced Breakdown Spectroscopy (LIBS). The sample set included the four primary colors used in the printing process (black, cyan, magenta, and yellow). Previous studies in our group have shown Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry to be useful for the

analysis of printing inks, resulting in greater than 99% discrimination between different samples within each ink type as well as nearly 100% correct association of samples known to originate from the same source. On the other hand, Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) provided good discrimination for toners (>97%) with 100% correct association, but was limited for the analysis of inkjets due to the technique's lower sensitivity. Nevertheless, SEM-EDS offered some complementary information to LA-ICP-MS and was able to discriminate some samples that were indistinguishable by LA-ICP-MS due to the presence of polyatomic interferences for several elements (S, Si, Ca, Cl, K, and Fe). LIBS offers shorter analysis time as well as greater sensitivity compared to SEM-EDS and is capable of detecting the elements that are problematic for LA-ICP-MS, thus potentially providing complementary information to LA-ICP-MS. The discrimination and association results of LIBS analysis of printing inks, as well as a comparison to previous results reported using LA-ICP-MS and SEM-EDS, is presently reported.

(574) **Quantitative Evaluation of Spectral Interference in LIBS**; Jessica Chappell[1,3], Brandon Seesahai[2], Martin Richardson[2], Michael Sigman[1,3], Matthieu Baudelet[1,3]; [1]National Center for Forensic Science, University of Central Florida; [2]Townes Laser Institute, CREOL-The College of Optics and Photonics, University of Central Florida; [3]Chemistry Department, University of Central Florida

Quantitative analysis in any Optical Emission Spectroscopy starts with a high level of confidence in the spectral line assignment from databases. Nevertheless, the resolution of the spectra is usually degraded by the instrument and/or the line broadening. This low spectral resolution makes it possible that spectral interferences occur for the majority of the lines. Such interference makes the elemental profile uncertain.

This presentation shows the development of a factor to quantify the level of confidence attributed to line assignment in LIBS as an example and its extension to a complete quantitative atomic profiling. This factor is combining a physical understanding of the plasma emission with a statistical analysis of the spectrum.

The possible applications and outcomes of using such a quantitative factor to evaluate line assignment in plasma spectroscopy will be discussed in the context of forensic science.

The work presented is funded by the US National Institute of Justice (2012-DN-BX-K027: "Level of Confidence in Elemental Analysis by LIBS") and the State of Florida.

(575) **Evaluation of a Handheld LIBS Instrument for First Responder and Forensic Applications**; Richard R. Hark[1,2,3], David Day[1, 2, 3], Anthony H. Downey[2,3], Adam Miller[2], John Plumer[2]; [1]SciAps, Inc.; [2]Synergos Global Security, LLC; [3]Juniata College, Department of Chemistry

First Responders are routinely challenged with the task of rapidly, safely, and reliably identifying unknown and potentially hazardous substances in field conditions. Currently, multiple instruments and wet chemical tests are employed to distinguish hazardous materials, including chemical, biological, nuclear, radiological, or explosive (CBRNE) threats, from benign substances (e.g., harmless white powders) commonly encountered during First Responder operations. Forensic scientists are similarly tasked with analyzing trace evidence to determine if there is a match between material found at a crime scene and that collected from a potential suspect.

Laser-induced breakdown spectroscopy (LIBS) is an atomic emission spectroscopic technique that allows for rapid elemental analysis of a wide variety of samples. LIBS has been shown to be an effective method for the analysis of CBRNE materials and items of forensic interest because it allows for simultaneous identification and quantification of all elements by comparison against a spectral library. Much of this work has been performed using laboratory-based LIBS instruments but stand-off and portable systems have been employed as well. The recent introduction of handheld LIBS instrumentation and the application of sophisticated chemometric analysis algorithms provides a valuable new tool for First Responders and forensic investigators. The capabilities of a commercially available handheld LIBS unit (Z500, SciAps, Inc.), were evaluated under field conditions. Gunshot residue (GSR) and a suite of non-pathogenic biological agent simulants and other materials commonly encountered in white powder scares were tested. The goal was to determine if the handheld LIBS unit can be used to more efficiently, effectively, and economically mitigate the risks and consequences of hazardous materials incidents and provide useful information of presumptive or confirmatory value in forensic investigations.

(576) **Quantitative Elemental Composition Measurements of Plant Materials using LIBS**; Amy Bauer[1], Markus Gaelli[1], Steven Buckley[1], Robert Robinsky[1]; [1]TSI, Incorporated

Because of matrix heterogeneity and complexity and highly variable water content, quantitative LIBS analyses on plant material has generally been proven to be difficult. This paper will document an investigation to determine whether emission features that originate in the moisture in fresh plant materials can be used to correct for the plasma quenching that this water introduces.

Previous researchers have successfully performed quantitative analysis on a variety of plants, largely with the strategy of drying, grinding and pelletizing the samples.1 In one case, the material is extracted with acid and then pelletized. This sample manipulation makes the data analysis simpler and removes problems related to heterogeneity, but it makes a fieldable application much less convenient. Prior research has also been performed on fresh materials, but these studies tend to be qualitative, rather than quantitative.

Based on work performed by Jin and coworkers in which plasmas formed on potato samples were characterized for both plasma temperature and electron temperature, we will begin an investigation of LIBS analysis of fresh plant materials using fresh potato skins. A wide variety of samples will be split for comparative LIBS and ICP analysis. Using the Hα line at 565 nm and the OH (A→X) vibrational features at 318 nm, we will explore normalization and other forms of correction to enable quantitative LIBS analysis of a variety of elements in potato skins. We will report on the results, and the likelihood of overall technique being applicable to measurements outside the laboratory.

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(577) **Enhanced Analysis of Adherent Human Cells and Molecular Imaging of Microbial Growth by Laser Ablation Electrospray Ionization Mass Spectrometry**; Akos Vertes[1], Hang Li[1], Pranav Balan[1], Linwen Zhang[1]; [1]George Washington University

Adherent human cells exhibit significant metabolic differences from their circulating counterparts. Due to the difficulties associated with sampling individual adherent cells, very little is known about their compositional heterogeneity. We introduced capillary microsampling in combination with electrospray ionization (ESI) mass spectrometry (MS) and ion mobility separation (IMS) for the analysis of metabolic changes in single human hepatocytes. Based on these measurements, more than 70 chemical species were identified. To assess the cellular response to metabolic modulators, the adenylate energy charge (AEC) levels for untreated and rotenone treated cells were evaluated. A significant shift in the AEC distribution was observed for rotenone treated cells. Cellular heterogeneity for the GSH/GSSG redox pair was found essentially unchanged in oxidative stress but the distribution of the [UDP-HexNAc]/[UDP-hexose] ratio exhibited dramatic broadening. Lipid turnover rates were studied by stabile isotope labeling pulse-chase experiments at the single cell level. These techniques can reveal cellular energy and redox state distributions and their changes in response to environmental stimuli or drug treatment. Determining turnover rates for small subpopulations can highlight phenotypic differences in cell dynamics. The ability to measure metabolic noise for multiple chemical species in a cell can illuminate the role of the corresponding species in the metabolic network. Another ambient ionization method, laser ablation electrospray ionization (LAESI), relies on accurate sampling of tissues and cells by mid-IR laser pulses. The deposited energy is used to eject small volumes of, e.g., a microbial culture. Using LAESI, two and three-dimensional MS imaging (MSI) were achieved on colonies of microorganisms. Spatiotemporal changes in metabolite and lipid levels were followed by LAESI-IMS-MSI during the interactions of these systems with their environment, including antibiotics and other microbial strains. This approach provided a new method to rapidly determine the susceptibility of different bacteria to specific antibiotics.

(578) **Applications of Chemometrics to Ambient Ionization MS Analysis**; Valentina Pirro[1], Alan K. Jarmusch[1], Christina R. Ferreira[1], R. Graham Cooks[1]; [1]Chemistry Department, Purdue University

Ambient ionization refers to ion generation under ambient conditions, requiring minimal-to-no sample preparation prior to MS analysis. Ambient techniques recently emerged and have since expanded into a variety of methods. The ability to rapidly examine the sample in its native state is the primary advantage of ambient MS, as well as the acquisition of chemical images. Ambient MS techniques usually require minute amounts of sample and can be non-destructive, hence amenable to multi-way/-level/-block experiments using the same specimen, which provide a vast amount of chemical information requiring a chemometric approach to be comprehensively explored and interpreted. Such approaches may precede the actual characterization of the molecular signature, as in early genome sequencing projects. Parallel development of analysis strategies for multivariate data are needed to assess the complexity of experimental data as a whole, as relevant insights might be revealed through the identification of similar/dissimilar patterns, outliers intended as uncommon cases, and present/absence of interrelationships among samples and/or variables. This approach contrasts that of biomarker discovery, following a single “channel” of information (e.g. molecule), but rather follows organism function and the dynamic intertwined relationships between molecules and their biological role. Data mining is one approach to explore large amounts of data in which biological relationships exist, and some methods are inspired by biology itself, e.g. artificial neural networks. In this presentation, biomedical applications of various ambient MS methods, with focus on desorption electrospray ionization (DESI), will illustrate chemometrics features and strategies, particularly for pattern recognition adapted to the MS data. Examples of applications include end-point cellular metabolism assessment of small metabolites (< 200 Da) and lipids in intact mammalian gametes, stem cells, and tissues. Chemometric strategies applied to the molecular signatures are used to understand cellular metabolism, differentiation, stemness, and tumorigenesis. Our results show that the molecular mosaic recovered by MS can indicate physiological or aberrant cellular activity, guide further analysis in upstream metabolism, and provide fundamental understanding, diagnostic information, and translation. We envisage that chemometrics applied on complex chemical profiles generated by ambient MS will provide better understanding of divergent pathways and cancer diagnostics, embryo development and stem cell differentiation.

(579) **Mass Spectrometry Imaging of Secondary Metabolites Directly on Fungal Cultures**; Nicholas Oberlies[1]; [1]University of North Carolina at Greensboro

Of the over 250,000 compounds that have been described from Nature, only about 15,000 of these have been from fungi. This is somewhat surprising, since fungi are ubiquitous and found throughout the world, able to thrive in almost any environment. Moreover, fungi were the source of penicillin, perhaps the most important drug of the 20th century. It has been estimated there are between 1.5 and 5 million species, and likely more. Fungi are prodigious chemists, and research supports the notion that each fungus can produce unique metabolites. My team’s goal is to discover these compounds, and do so in an efficient, and where possible, creative manner.

Ambient ionization mass spectrometry techniques have recently become very prevalent in natural product research due to their ability to examine an organism in situ. Profiling a sample directly on an organism without the need for lengthy regrowth and extraction procedures is extremely attractive, especially if one wants to profile many different fungi as rapidly as possible. However, direct ambient ionization techniques, such as DESI and nanoDESI, lose the valuable capability of chromatographic separation, thus limiting the ability to differentiate isomers and confirm metabolite identities. To overcome this limitation, a droplet-liquid microjunction-surface sampling probe (droplet-LMJ-SSP) can be coupled with UPLC-PDA-HRM-MS/MS, thus gaining the separation, retention times, and UV data. By capturing these mutually supportive data, the identity of secondary metabolites can be assigned confidently and rapidly. Using the droplet-LMJ-SSP, a protocol was constructed to dereplicate fungal cultures directly from the Petri dish.

(580) **The Bioaccumulation of a Toxic Ionic Liquid in Zebrafish (Danio rerio) Analyzed by DESI-MS Imaging**; Consuelo Perez[1], Alessandra Tata[1], Michel L. DeCampos[3], Chun Peng[2], Demian R. Ifa[1]; [1]Department of Chemistry, York University, Toronto, Ontario, Canada; [2]Department of Biology, York University, Toronto, Ontario, Canada; [3]UNICAMP, Campinas, São Paulo, Brazil

Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) has become a powerful tool for surface analysis with applications in chemistry, biology, and medical diagnostics. It is particularly useful in the distribution of endogenous biomarkers and metabolites in animal organs and whole body tissues. In this work, we introduce DESI-MSI as a rapid bioaccumulation screening method for the localization of a toxic ionic liquid (IL) in adult male zebrafish (Danio rerio). AMMOENG 130™ is a toxic quaternary ammonium IL, [C38H80N][Cl], with a MW of 586.5g/mol. Acute zebrafish toxicity studies have reported a LC50 of 5.2 mg/L; causing extensive damage to gill secondary lamellae, increasing membrane permeability, physically altering lipid bilayers and affecting the fish gas-exchange process. In this study, AMMOENG 130™ was characterized for the first time by ESI-MS and ESI-MS/MS. Zebrafish were exposed for 96hrs to induce IL bioaccumulation. In the presence of

5.0 and 2.5mg/L, zebrafish were deceased within 24hrs, whereas fish exposed to 1.25mg/L survived the study. Zebrafish gill tissue sections were analyzed by DESI-MS in search of IL bioaccumulation. In the lowest concentration, the intensity of IL ions changed considerably. The molecular ion of m/z 551 decreased in intensity, whereas the IL ion of m/z 313 significantly increased. Also, the degradation of the IL in water was investigated; however it was clear that water did not distinctly promote IL degradation into m/z 313. Further investigations are underway to establish whether IL metabolism is actively occurring in the zebrafish. DESI-MS images of whole body zebrafish were mapped for IL distribution. In higher concentrations, the IL was mainly localized in the respiratory system, while in the lowest concentration it was found in the respiratory, nervous system and other regions. Interestingly, the IL was able to diffuse across the blood brain barrier and accumulate in the brain. These preliminary results demonstrate that the distribution of exogenous, toxic or persistent compounds is feasible by DESI-MSI. DESI-MSI shows great promise in becoming a rapid ambient mass spectrometry technique for compound localization in small aquatic organisms from environmental exposure of chemical pollutants, pesticides and drugs from the aquatic environment.

(581) **Direct Analysis of Copper and Molybdenum in water by Microwave Plasma Torch Coupled with the Linear Ion Trap Mass Spectrometry**; Tao Jiang[1], Xiaohong Xiong[1], Wenhao Qi[1], Meilin Yang[1], Qiuju Liu[1], Lanfang Yi[1], Zhiqiang Zhu[1], Huanwen Chen[1]; [1]Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology

Copper and molybdenum are important transition metal elements. Both they are the indispensable trace elements in living body and play key roles in the biochemical cycles and metabolism. Moreover, there are apparent antagonistic and synergistic actions between copper and molybdenum in the process of metabolism, i.e. the excess intake of molybdenum element would hold-up the absorption and utilization of copper, and vice versa. However, the redundance of molybdenum or copper is toxic to human body, and can lead to a variety of diseases. Therefore, developing the direct analytical method of water samples to measure the content of copper and molybdenum ions are significant for the health protection and the research on metabolism of metal elements. In addition, the fast analysis of copper and molybdenum will be also potential in the application of many mine-related fields. Here, we have developed a direct and sensitive method based on the microwave plasma torch coupled with a linear ion trap mass spectrometer for the sub- synchronous detection of trace levels of copper and molybdenum ions in aqueous liquids. Sampling solution was nebulized and dried and then flowed across the plasma flame through the central cube of the MPT, without any other pretreatments. The generated complex ions contained molybdenum or copper in plasma were detected in negative and positive ion modes, respectively, and were characterized further in collision induced dissociated (CID) experiments. Under the optimized conditions, the limited of detection (LOD) using MS2 procedure, were estimated to be at the level 10-9g/ml (1ppb) for copper and molybdenum. The linear dynamics rages cover at least 2 orders of magnitude. The analysis of a single aqueous sample can be completed in 5~6 minutes to obtain simultaneously the contents of copper and molybdenum with a reasonable semi-quantitative sense. Some practical aqueous samples and milk were analyzed qualitatively with reasonable recovery rates. These experimental data demonstrated the MPT MS is promising and hopeful in scene analysis of copper and molybdenum in water, and has potential applications involving several fields, such as environment controlling, exploration of hydrogeology, water quality inspection, and can be also used as the supplement of ICP-MS.

(582) **Product Development Challenges in Specialty Chemicals**; Steven Scheifers[1]; [1]Stepan Company

It is the nature of batch reactions that they are operated with one or more variables changing value over the course of the reaction. This includes reagent and product concentrations, temperature, reactor mass loading, and pressure. In addition, multiple phases may be present at one or more stages of the reaction profile. The plethora of changing variables presents many challenges to acquiring and analyzing the data from batch reactions. Examples of Raman spectra that were acquired and analyzed are presented for reactions where more than the concentration of reactants and products are changing. Emphasis is placed on methods to process the data in order to mitigate the impact of the changes on the data analysis.

(583) **Optimally Dividing Available Samples into Calibration and Validation Sets for**; Bryan Bowie[1]; [1]ExxonMobil Research & Engineering

ASTM Standard Practice E1655 on Infrared Multivariate Quantitative Analysis requires that calibration models be validated via the analysis of a set of independent samples which were not used in the construction of the model. While the practice provides guidance on the appropriate number of calibration and validation samples based on the complexity of the model, and on the appropriate distribution of the samples in the multivariate space, it does not provide guidance on how to split available samples into a calibration and validation set.

This paper will review several algorithms that have been proposed to accomplish this split, and compare their performance on selected datasets. The comparison will include the Kennard-Stone algorithm, the reduced nearest neighbor algorithm of Shenk and Westerhaus, an “Onion” algorithm developed by N. Gallegher et. al., and a genetic algorithm method (Optsplit) developed by J. Brown. Evaluating each of these algorithms will show the criticality of creating a properly balanced calibration and validation set. Criteria for judging the optimality of the split in terms of the requirements of E1655 will be presented and used to tailor the performance.

(584) **Raman Spectroscopy for Lab Scale *in situ* Applications**; Xianghuai Wang[1], John Roberts[1], Kevin Wier[1], Dimitris Katsoulis[1]; [1]Dow Corning Corporation

In situ reaction monitoring using Raman spectroscopy is a novel, unobtrusive technique for monitoring reaction kinetics and it has become an important tool for industrial applications. In this talk, two *in situ* Raman spectroscopy applications on reactions in sealed reactors are discussed. One application is to utilize the in situ Raman technique for monitoring the chlorination of $Si(OMe)_4$ with a chloride source. With the help of frequency prediction by density functional theory (DFT) calculations, the Raman shift peaks of all the reaction intermediates were identified, normalized, and plotted versus time. The other application is to investigate the compatibility of acetoxysilanes with various materials, such as would be encountered during processing or packaging. The in situ Raman technique is employed to monitor the stability of Si-O-C(=O) bond which is sensitive to moisture.

(585) **Moving Vibrational Spectroscopy from Chemical Process Monitoring to Bio-Process Monitoring**; Jim Cronin[1]; [1]Jim Cronin

DuPont has a long history of developing and implementing Process Measurements centered around vibrational spectroscopy. Recent growth in and focus on bio-processing has created opportunities to evaluate commercial scale fermentations and post processing unit operations for potential process control applications. This presentation will provide examples of recent successes in applying vibrational spectroscopic measurements in bio-processing applications.

(586) **PAT Application in Lubricant Industry**; Henry Xiao[1]; [1]Infineum

This paper reviews and discusses the application of process analytical technologies (PAT) in lubricant additive manufacturing processes. The impact and potential effects of PAT on the additive production is assessed. Many PAT initiatives have carried out to achieve the fundamental understanding of different batch chemical processes and new product development. In addition, PAT has been implemented to help running process optimization studies on current products to improve product quality and cycle times.

The process excellence has demanded better and faster analytical tools for monitoring of chemical process. With recent advances in spectroscopic techniques, such as NIR, Raman and FTIR, coupled with multivariate analysis techniques, PAT provides us many options to understand and control the process in a new and different way from traditional practice. However, PAT development and final implementation to an industrial setting process are rather complex and challenging. Due to the nature of additive industry, each application has to deal with different chemistry, process condition and unique equipment. The performance of analyzers needs to endure harsh industrial environment to provide a reliable and long term analytical results with minimal intervention. The integration and transfer of the models built from multivariate analysis based on lab data is difficult and can result in uncertainty with respect to validation and process variation. The knowledge gained from lab experiments and pilot plant trials is the key to the final implementation of PAT in plant.

A few cases, both success and failure, will be discussed to demonstrate the benefits resulting from the adoption of PAT within the lubricant additive industry.

(587) **Biomedical Applications of SESORS: Through Bone and Blood Vessels**; Bhavya Sharma[1], Richard Van Duyne[1]; [1]Northwestern University

We are combining the power of two Raman spectroscopies, surface-enhanced Raman spectroscopy (SERS) and spatially offset Raman spectroscopy (SORS) for biomedical applications. SERS is a powerful vibrational spectroscopy that allows for label-free, highly sensitive and selective detection of low concentration analytes through amplification of the localized plasmon resonances of small noble metal nanoparticles. SORS allows Raman measurements to be made through distinctly different layers within a diffusely scattering medium. This combined surface-enhanced SORS is designated as SESORS.

Here we present progress we have made on developing SESORS for the measurement of analytes, such as neurotransmitters including dopamine and serotonin, in the brain through the skull. Little is known about the local concentrations of neurotransmitters, except from a few experiments where holes are drilled through the skull to insert electrodes into the parts of the brain that release dopamine. We are working to develop a non-invasive methodology to measure concentrations of key neurochemicals in vivo. Additionally, we will present preliminary results on using SESORS and SORS for detection of calcifications in arteries, as a through the skin method for detection of atherosclerosis.

(588) **Development of SORS for Nonchemical Subsurface Analysis**; Nick Stone[1], Ben Gardner[1], Pavel Matousek[2]; [1]University of Exeter, UK, [2]Central Laser Facility, STFC Rutherford Appleton Laboratory

Spatially offset Raman spectroscopy (SORS) has shown great promise for measuring the chemical composition of materials beneath the surface of turbid media. Here we explore the novel development of SORS for more advanced subsurface analysis.

Temperature-SORS or T-SORS, a novel methodology combining the approaches of Stokes/Anti-Stokes peak ratios and SORS is proposed here. T-SORS enables temperature measurements of chemically specific targets buried in turbid media, with numerous potential applications.

The results of demonstration experiments will be outlined and discussed.

(589) **Development of Micro-SORS for Subsurface Analysis of Thin Layers in Art and other Areas**; Claudia Conti[1], Chiara Colombo[1], Marco Realini[1], Pavel Matousek[2]; [1]Consiglio Nazionale delle Ricerche, Istituto per la Conservazione e la Valorizzazione dei Beni Culturali (ICVBC), Via Cozzi 53, 20125, Milano, Italy, [2]Central Laser Facility, STFC Rutherford Appleton Laboratory

Here we present the development of micro-SORS, the extension of Spatially Offset Raman Spectroscopy to thin (tens of micrometres thick) highly turbid stratified media [1,2] to the non-destructive interrogation of painted layers of real objects of art where conventional Raman microscopy is not applicable. The concept is demonstrated by recovering the pure Raman spectra of paint sublayers that are obscured by the surface layer(s) of paint. The application of micro-SORS to the analysis of real painted objects of art has a great relevance to the conservation and valorization of cultural heritage avoiding the cross sectional analysis. Other application examples outside cultural heritage will also be presented.

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(590) ***In vivo* Raman Spectroscopy for Assessing Tissues in Chronic Wounds**; Karen Esmonde-White[1], Crystal Holmes[1], Michael Morris[2]; [1]University of Michigan Medical School, [2]University of Michigan, [3], [4]

Chronic wounds, primarily manifest as sacral pressure wounds, digital ulcers or diabetic foot ulcers, are serious complications which can result in patient morbidity and mortality. Recent studies have used Raman spectroscopy or diffuse reflectance spectroscopy to show alterations in soft tissue composition including reduced tissue oxygenation, decreased skin lubricity and collagen defibrillation. Our previous work focused on the examination of bone in osteomyelitis (bone infection) of diabetic foot wounds. Raman microspectroscopy and scanning electron microscopy studies suggested pathological crystal deposition on bone in osteomyelitis, and Raman detection of these pathological crystals may serve as a molecular risk marker for osteomyelitis. We describe the translation of fiber-optic Raman spectroscopy tools for assessing mineralized tissues or deposits. Using a unique research workflow that integrated Raman measurements into a patient's clinical care visit, we longitudinally monitored three patients with diabetic foot ulcers. *In vivo* patient measurements quickly informed on probe performance, ergonomics, safety and tolerability and provided critical feedback for next-generation Raman probes, instrumentation and patient foot holders. Technological challenges for point-of-care Raman spectroscopy and tomography will be discussed, including measurement times, motion artifacts and ambient light conditions. Infrastructure support is equally critical to successful translation and we discuss how clinical and research infrastructures have affected our *in vivo* studies.

(591) **Implementation of SORS for Three-dimensional Breast Margin Assessment**; Anita Mahadevan-Jansen[1], T Quyen Nguyen[2], Jennifer Giltnane[1], Brittany Caldwell[1], Melinda Sanders[1], Mark Kelley[1]; [1]Vanderbilt University, [2]Northwestern University

The risk of local recurrence for breast cancers is strongly correlated with the presence of tumor within 1-2 mm of the surgical margin on the excised specimen. Spatially offset Raman spectroscopy (SORS) holds much promise for intraoperative margin analysis. We have demonstrated the ability of SORS to distinguish between two soft tissue layers and have designed and tested a SORS probe with multiple source-detector offsets. This probe was tested in breast lumpectomy specimens intra-operatively. Spectra from each S-D separation were averaged to create a composite spectrum with biochemical information covering the entire range from the tissue surface to ~2 mm below the surface, and a probabilistic approach was used to classify these composite spectra as having "negative" or "positive" margins. This discrimination was performed with 95% sensitivity and 100% specificity. More recently we have designed and developed a three-dimensional scanner that can assess the margin of an entire lump (~3 cm) in a clinical feasible time frame while the patient is in the operating room. Results of a pilot study validating the ability of this scanner will also be presented

(592) **Investigating the Technology and Provenance of Bloomery Iron Using Slag Chemistry: Methodological Challenges and Archaeological Potential**; Michael Charlton[1]; [1]UCL Institute of Archaeology

The production of iron and its alloys created a unique set of technological opportunities that has shaped the evolution of human societies for the past three millennia. Indeed, iron finds its way into almost every aspect of life from agriculture and architecture to transportation and warfare. Most early pre-industrial iron was produced in the solid state via bloomery, or direct process, smelting. First evidenced in the Near East around 1500 BC, bloomery smelting spread to most areas of the Old World by 500 BC and to the Americas in the 16th century AD. Explaining this technology and its diversity can shed significant light on cultural, economic and social processes.

Though iron is the material of interest, investigating smelting technology is often better served by analysis of its essential by-product, slag. The fluid slag is created from the non-reduced components of the ore, but also serves as the medium through which reduced iron particles sinter into a usable mass of metal, or bloom. Slag chemistry is also influenced by temperature, redox conditions, and dissolved furnace wall and fuel ash. Its chemistry is thus a reflection of individual smelting recipes that can be analysed to reveal pathways of technological change. Similarly, because some slag is inevitably included in the bloom and artefacts smithed from it, slag chemistry is also a gateway to iron provenance analysis and the exploration of economic exchange networks.

This paper explores many of the archaeological potentials that chemical characterization of slag promises, but tempers it with an assessment of practical problems these analyses present. Major challenges still exist for the creation of comparable data sets derived from multiple instruments housed in multiple labs. There also remains a lack of consensus on appropriate elements or isotope ratios to examine, let alone the best analytical strategies and statistical techniques to employ. These issues can be successfully addressed, but only through the collective action of analysts seeking to make their data relevant beyond the single case study.

(593) **Classification and Geographic Origin of Garnets using Laser-Induced Breakdown Spectroscopy (LIBS)**; Richard R. Hark[1], Peter A. Defnet[1], Michael Wise[2], Russell S. Harmon[3]; [1]Department of Chemistry, Juniata College; [2]Department of Mineral Sciences, National Museum of Natural History, Smithsonian Institution; [3]Department of Marine, Earth and Atmospheric Sciences, North Carolina State University

Garnet is a silicate mineral that conforms to the general compositional formula A_3B_2 $(SiO_4)_3$, where the 'A' site is occupied by rather large divalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, or Mn^{2+}) and the 'B' site hosts smaller trivalent cations (Al^{3+}, Fe^{3+}, or Cr^{3+}). There are six common species of garnet that comprise two groups on the basis of their ideal end-member chemical compositions. Previously, Alvey et al. (**2010**, *Appl. Opt.* 49, C168) analyzed 157 samples described as garnets from 92 locations worldwide using laser-induced breakdown spectroscopy (LIBS) and demonstrated that LIBS has the potential to discern garnet geographic origin using the concept of 'geochemical fingerprinting'. In this study, an expanded suite of over 203 garnets was examined using electron microprobe microanalysis (EPMA) to correctly classify the samples on the basis of their major element composition and then ascertain the efficacy of LIBS for classification and provenance determination. The LIBS spectral data were processed using multivariate statistical pattern recognition methods (e.g., PCA, PLSDA) and the resulting classification models then used to successfully classify unknown garnet samples of a specific compositional type according to their geographic origin. Various preprocessing techniques (normalization, spectral outlier removal) were implemented to optimize the LIBS classification results. This study demonstrates that LIBS offers a good alternative for identification of unknown garnet samples based on their geographic provenance.

(594) **Characterisation of Binders Used in Aboriginal and European Painted Artefacts Using Pyrolysis Gas Chromatography Mass Spectrometry**; Rachel Popelka-Filcoff[1], Tiffany Reeves[1], Fabien Pottier[2], Claire Lenehan[1]; [1]Flinders University, [2]Museum National D

Pigment binders are complex mixtures of several compounds from a variety of materials that are used to adhere pigments to each other and to the supporting material. Compounds used vary between cultures but generally are made of waxes, resins and protein-based materials. In the past, Aboriginal Australians traditionally used naturally sourced materials, however after European settlement in Australia, Aboriginal Australians began using European-style binders as well as those obtained from native flora and fauna, however when and how this transition occurred is unclear. There has been extensive characterisation of European binders, but much less of Aboriginal media. Such characterisation provides a basis for conservation, restoration, authentication, and dating of Indigenous artefacts from these time periods, as well as providing insight into Australian history.

This research has focussed on optimising a pyrolysis gas chromatography mass spectrometry (Py-GCMS) method to analyse a wide range of binding materials. This involves rapid heating of a sample to form small volatile compounds that are subsequently separated and detected to determine the chemical composition of the sample, and requires only very small samples (0.5 mg) and minimal sample preparation, making it highly advantageous for use in analysing valuable artefacts.

A method capable of distinguishing between binder types and materials from European and Aboriginal Australian cultures has been developed, and a library of known binders has been compiled for comparison to Indigenous objects for identification. In addition, method development was accomplished for identification of binders mixed with pigments. This work is the first comprehensive analysis of binder compounds used across the history of Australian pigments.

(595) **Archaeometry and Human Life Ways**; David George[1]; [1]Saint Anselm College

This paper will examine the uses that archaeometric data can (and cannot) have in reconstructing human life ways in archaeological research. It will consider first some of the ways in which archaeometric data has been used in shaping research design in excavations in advance of the data collection. While other examples will be considered, the paper will use examples from excavations that the author has directed. Next the paper will discuss some of the epistemological implications of shaping questions designed around archaeometric data collection prior to excavation. It will then discuss the consequences of asking questions archaeometric questions of material post excavation. Finally it will suggest methods for applying archaeometric data to enhancing our understanding of human life ways not just relating to economic activity but also cognitive and religious.

(596) **Understanding the Materials and Techniques of Early Photographs through Non-Invasive Analysis and Reconstructions**; Silvia A. Centeno[1], Anna Vila[, 2], Emmanuelle Marquis[3], Yimeng Chen[3], Julia Kohanek[3], Yan Dong[3],Alejandro Schrott[4], Lisa Barro[5], Nora W. Kennedy[5]; [1]The Metropolitan Museum of Art, Department of Scientific Research.; [2]Statens Museum for Kunst, Center for Art Technological Studies and Conservation,; [3]Department of Materials Science and Engineering, University of Michigan; [4]IBM Research, Thomas J. Watson Center,[5]The Metropolitan Museum of Art, Department of Photograph Conservation

The identification of photographic techniques traditionally has been based on stylistic considerations, knowledge of the artist's working methods, and observation of the photographs texture and deterioration characteristics. Because of the complex chemistry and physics of the different early photographic processes and of the interest many artists had in experimentation, differentiation by visual means alone may give only partial or inconclusive information.

The talk will present results of the identification of image materials and of deterioration processes in turn of the 20th century platinum-based prints and in daguerreotypes dating to the 1850s through Raman, XRF, FTIR, TEM, and SEM-EDS analyses, complemented by research into the technical literature and historic materials of the 19th and early 20th centuries, and reconstructions, with the ultimate goal of better understanding the artists' working methods. Identification of the methods used by artists not only enhances dating, attribution, and broader art historical knowledge but it also plays a key role in the preservation and conservation of the works. 1-6

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(597) **Plasmonic Sensing at the Single Particle Level**; Emilie Ringe[1]; [1]Rice University

Nanotechnology provides unprecedented means to miniaturize devices and tailor the physical behavior of materials. Most properties, including optical, catalytic, and electronic, can be fine tuned through choice of composition, size, and shape of nanoparticles, while ever-smaller sensors, down to the single particle and single molecule level, are now achievable. However, much of the understanding of structure-function relationships in optical sensors, specifically those based on localized surface plasmon resonances, remain to be unraveled as it is often blurred by the unavoidable heterogeneity in nanostructures produced either by top-down or bottom-up methods.

This talk will discuss how such difficulties can be overcome with, and what can be learned from, single particle studies of plasmonic nanostructures, with a specific focus on development and optimization of chemical and biological sensors. The single particle spectroscopic approaches we employ have identified structural factors most critical for high refractive index sensitivity and sensing figure of merit, as well as the effect of aggregation and substrate. We have correlated results from these studies with state-of-the-art structural and near-field characterization in high-resolution, monochromated scanning transmission electron microscopy. Such results show new predictive and quantitative understanding of plasmonic structures towards the development of nanoscale sensors.

(598) **Mapping Electric Fields Induced By Plasmons with Vibrational Stark Shifts**; Zachary Schultz[1], Daniel Kwasnieski[1], Hao Wang[1], James Marr[1]; [1]University of Notre Dame

The excitation of plasmon resonances in metal nanostructures results in confined electric fields that have been exploited in research areas ranging from chemical catalysis to trace detection. Plasmonic nanostructures increase the rate of reactions on their surfaces when illuminated at their plasmon resonance frequency. On nanostructure arrays, heterogeneous reactivity has been observed, implicating "hotspots", areas of intense electric fields, as important for catalysis. The excitation of plasmons has long been associated with nanostructure-enhanced spectroscopies, such as surface enhanced (SERS) and tip enhanced (TERS) Raman scattering. It remains challenging to experimentally assess the magnitude of the electric field induced by plasmons. Recent work in our lab indicates that vibrational Stark shifts observed from nitrile adlayers on plasmonic materials provide a direct measurement of the local electric field around nanostructures. The frequency of the CN moiety shifts in a correlated fashion with the SERS intensity. These shifts are attributed to a vibrational Stark shift arising from rectification of the optical field, which gives rise to a DC potential on the surface. Here we present a model where the Stark shift of the nitrile probe can be correlated to the optical field, providing an intensity independent measurement of the local electric field environment.

(599) **Reaching Beyond All-Metallic Plasmonics with Optoplasmonic Metallo-Dielectric Hybrid Materials**; Bjoern Reinhard[1]; [1]Boston University

Optoplasmonic hybrid materials that combine plasmonic and dielectric building blocks in discrete clusters and extended arrays of defined geometry are enabling materials for sensing and other application fields that seek to control the distribution of light in both spatial and frequency domain with sub-diffraction limit resolution. Optoplasmonic materials that encompass building blocks of different chemical composition also provide opportunities for engineering an intricate phase landscape. With the development of template-guided self-assembly strategies that facilitate the integration of plasmonic and dielectric nanoparticles into optoplasmonic hybrid structures at pre-defined locations, the experimental

realization of intricate optoplasmonic structures is now possible. In this presentation the fundamental design criteria underlying discrete and extended optoplasmonic structures will be discussed before two implementations are analyzed. The first class of materials discussed combines dielectric microspheres as whispering gallery mode resonators with plasmonic antennas at defined locations in close vicinity of (but not attached to) the dielectric microsphere. The interaction of WGMs with plasmonic resonators located in their evanescent field is analyzed. The second example describes two-dimensional interdigitated arrays of 250 nm diameter TiO2 NPs and clusters of electromagnetically strongly coupled 60 nm gold nanoparticles. It is demonstrated that delocalized photonic–plasmonic modes in the arrays achieve a cascaded E-field enhancement in the gap junctions of the Au NP clusters and that this boost is accompanied by a general increase of the E-field intensity throughout the entire array.

(600) **Tuning Surface Plasmon Resonances on Gold Nanostars**; Laura Fabris[1], Theodoros Tsoulos[1], Roney Thomas[1,3], Swarnapali Indrasekara[1,2]; [1]Rutgers University; [2]Rice University; [3]Wesleyan University

Near field techniques, such as surface enhanced Raman spectroscopy (SERS), rely on the ability of plasmonic nanoparticles to induce localized electromagnetic field enhancements in close proximity to the metallic surface. The possibility of achieving SERS signal enhancements high enough to enable sensitive identification of analytes down to the single molecule level is correlated to the presence of the so-called “hot spots”, that are locations of extremely intense electromagnetic field enhancement, which can be located at the vertices, edges, or crevices in isolated nanoparticles or at narrow junctions between assembled nanoparticles. The presence of finely tunable hot spots enables to apply SERS as a reliable spectroscopic technique in the analytical and biomedical fields. Gold nanostars have emerged recently as a powerful SERS substrate leading to SERS enhancement factors of 10^10 and are therefore promising for the fabrication of SERS sensing platforms and SERS tags.

In this talk I will present our recent efforts in combining simulations and experiments in a feedback loop aimed at understanding plasmonic resonances in model gold nanostars that closely resemble those produced via bottom-up seed-mediated synthetic approaches. Employing 3D finite element electromagnetic field simulations, and guided by TEM tomography, we were able to reproduce experimental optical extinction spectra taking into account asymmetry in the nanostar morphology, polydispersity, and variable angular interactions with the incoming radiation that are typical of ensemble measurements. I will also report on the application of gold nanostars in SERS-based sensing platforms with detection limits in the low femtomolar regime and on the regiospecific bottom-up assembly of gold nanostars and nanospheres that we optimized with the goal of further improving plasmonic near field enhancement for application of these systems as SERS tags.

(601) **One-Pot Synthesis of Silver Nanoparticles with an Ultra-Thin Silica Shell and Their Integration into SERS and LSPR Substrates**; Daniel Willett[1], George Chumanov[1]; [1]Clemson University

Ultra-thin silica shells (UTSS) are an effective way to improve the stability and compatibility of nanoparticles without significantly attenuating their intrinsic optical properties. This is of particular importance to plasmonic particles used in sensing or surface related studies such as localized surface plasmon resonance (LSPR) or surface enhanced Raman spectroscopy (SERS). For silver nanoparticles an UTSS can both hinder oxidation of the Ag in both air and water as well as establish a scaffold for functionalization through well-established silane chemistry.

UTSS on metallic nanoparticles such as gold or silver generally involve first using a silane coupling agent to render the surface vitreophilic followed by precipitation of silica by the Stober method or addition of sodium silicate species. Here we present a method to obtain silver nanoparticles with a silica shell of a thickness between 1-20 nm on silver nanoparticles ranging in size from 20-300 nm. It is obtained during the growth of the nanoparticles themselves by addition of a silica source to the well-established method of silver nanoparticle synthesis by hydrogen reduction of silver(I) oxide in water that both catalyzes the reaction as well as generates the shell. This allows for fairly monodisperse spherical silver nanoparticles to be obtained in concentrations of approximately 4.8E12 AgNP per litre (for 100 nm AgNP with 1 nm silica shell). This silica shell greatly improves the particle stability allowing for further concentration of the particles in water up to solutions containing 50% Ag by weight. In addition we demonstrated the ability of these particles to be easily integrated into substrates for both SERS and LSPR applications. Specifically for the detection of uranyl complexes and proteins by SERS using the method of shell isolated nanoparticle enhanced Raman spectroscopy (SHINERS). These particles were also integrated into a novel approach to measuring the LSPR shift using differential measurements achieving sensitivities for bulk of 4.8E-6 RIU.

(602) **Synchrotron Infrared Nano-Spectroscopy (SINS) of Fungal Cell Wall Composition**; Kathleen Gough[1], Catherine Findlay[1], Robert Johns[2], Hans Bechtel[2], Michael Martin[2], Susan Kaminskyj[3],Tanya Dahms[4]; [1]University of Manitoba; [2]Advanced Light Source Berkeley Lab; [3]University of Saskatchewan; [4]University of Regina

The spatial resolution of far-field FTIR spectrochemical imaging is limited to about 1 μm, under the best circumstances, by the relatively long-wavelengths of infrared light. For many applications, however, sub-micron resolution is not only desirable, but critical to fully characterize the sample. Synchrotron infrared nanospectroscopy (SINS) overcomes the spatial resolution limitation (Bechtel et al., PNAS 2014), enabling near-field broadband infrared spectroscopy with a tip-limited spatial resolution of <30 nm, in conjunction with AFM imaging. In this technique, synchrotron IR light is focused onto the oscillating tip of an AFM, located within an asymmetric Michelson interferometer, and the elastically scattered light is detected at harmonics of the tip oscillation frequency in order to discriminate between the near-field signal and the far-field background. The moving mirror inside the interferometer enables the weakly scattered signal to be frequency resolved and amplified by the stronger reference beam. Following transformation of the interferogram, we obtain a near-field spectrum of material located within less than 100 nm of the surface. Here, we examine the hyphal walls of wild type *Aspergillus nidulans* and several gene-deletion strains wherein single deletions responsible for biosynthesis of critical major and minor wall compounds have been shown to have significant effects on cell growth and morphology. In contrast to the whole cell information that is obtained by far-field FTIR imaging, SINS is ideally suited to probe the thin (30 - 200 nm) walls of fungi, providing spectrochemical data on the changes in cell wall composition. A better understanding of fungal wall composition and the gene-deletion induced changes in the cell wall is essential to understanding the wall’s role as the interface between this eukaryotic cell and its targets, whether in relation to controlling fungal pathogens or to advancing biotechnology.

(603) **Synchrotron Based Broadband IR Microspectroscopy and Spectromicrotomography**; Caol Hirschmugl[1]; [1]UW-Milwaukee

Synchrotron Based Infrared sources are broadband sources, with high signal to noise characteristics that can be beneficial for a wide variety of experiments. Two recent implementations will be discussed, with broad applications described. In particular, 2D Projection imaging at the diffraction limit[1] and 3D imaging at the diffraction limit[2]. In the latter, hyperspectral cubes of (x,y, z, Abs (λ)) are obtained employing spectromicrotomography2, a label free approach, it inherently evaluates a broad array of wide organic materials, with minimal sample

preparation and modification. Examples presented here (polymer composites, single cells and colonies of cells) demonstrate the broad applicability of this approach to detect complex chemical information of intact samples.

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(604) **Vibrational Spectroscopy: Disease Diagnostics and Beyond**; Hugh J. Byrne[1]; [1]Dublin Institute of Technology

The presentation will describe the efforts of the group in DIT to develop the applications of vibrational spectroscopy for disease diagnostics and beyond, into the realm of in vitro characterisation of the effects of external agents, including chemotherapeutic agents and nanomaterials.

The potential of vibrational spectroscopy, both Raman scattering and FTIR absorption, for label free screening and analysis of disease onset and progression is well established, and applications in cervical tissue biopsies and cytological samples will be described. In skin samples, the relative merits of the two techniques will be compared and contrasted, demonstrating that Raman spectroscopy provides significantly better spectral and spatial resolution. In skin tissue sections, Raman can be employed to profile the different layers of the epithelium, and to monitor the effects of solar radiation damage, demonstrating that the techniques can be used to analyse the early onset of tissue dysfunction at a molecular level.

In cervical cell lines, spectroscopy can discriminate between cells of different degrees of infection with the Human Papilloma Virus, and it is demonstrated that partial least squares regression can be employed to correlate the changes in the spectral profiles to both HPV copy number as well as the upregulation of the protein p16INK4A.

In a lung adenocarcinoma cell line, the action of the commercial chemotherapeutic agents cisplatin and vincristine can be characterised on a subcellular level using Raman spectroscopy. Characteristic spectroscopic signatures of groove binding and intercalation can be identified and differentiated, and the direct chemical effects of the action of the drug can be differentiated from the indirect physiological effects. Multivariate regression models can be established to compare the efficacies of the different chemotherapeutic agents.

In the field of nanotoxicology, Raman spectroscopy can be demonstrated to be an effective monitor of the cellular response, but also as an effective technology to localise and identify the nanoparticles subcellularly, as well as to explore the local environment of the nanoparticle and thus probe the subcellular trafficing and mechanisms of response.

Vibrational spectroscopy is thus demonstrated to represent a truly label free probe of cellular responses beyond the realm of disease diagnostics

(605) **Infrared Spectral Imaging of Live Cells though Water**; Peter Gardner[1], James Doherty[1], Michael Pilling[1], Zhe Zhang[1], Graeme Clemens[1], Alex Henderson[1], Gianfelice Cinque[2]; [1]Manchester Institute of Biotechnology, University of Manchester, [2]Diamond Light Source, Diamond House, [3], [4]

Infrared spectroscopy is now widely used in biomedical research, for example in drug-cell interaction studies. Ideally, measurements of live cells exposed to drugs, followed as a function of time, would give the greatest insight into the cellular cytotoxic response. Water, however, is a strong infrared absorber which results in several large peaks in the live cell spectrum. These peaks overlap with protein bands and the fingerprint region which contains most of the significant spectral information for cells. It is important therefore to find a reliable method of removing the water contribution from the recorded spectrum. In a previous study [1], infrared spectra of cells in a microfluidic device were measured and a water correction method used to reveal a normal looking cell spectrum in both the lipid and the fingerprint region. However, in order to allow the cells to flow, the microfluidic channel depth was such that the OH stretching band was saturated and therefore could not be used for fitting. This can potentially lead to an over correction of water. In order to address this water correction problem we have returned to using a static cell in which the path length is restricted to enable the intensity of OH stretching band to be measured and subsequently used in fitting [2,3]. The aim of this work was to develop a water correction method that does not over-fit the water and which can subsequently be used for live cell imaging. Using fixed cells initially, an evaluation of fitting methods results show that using the Amide A band in the fitting algorithm considerably improves the consistency of the corrected data. Data obtained at using the MIRIAM beamline at Diamond Light Source and laboratory based sources including QCLs are compared.

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(606) **Single Cell Analysis for Biological and Biomedical Applications Using Mid-IR Synchrotron Light and Laser Sources**; Ganesh D Sockalingum[1], Valérie Untereiner[1], Abigail V Rutter[2], Marie Guilbert[1], Nick R Forsyth[2], Christophe Sandt[4], Paul Dumas[4], Katia Wehbe[, 3], Gianfelice Cinque[, 3], Josep Sule-Suso[1]; [1]University of Reims Champagne -Ardenne, FR; [2]University of Keele, UK; [3]diamond Synchrotron, UK; [4]soleil Synchrotron, FR

This paper reports on the use of both laser Raman microspectroscopy (LRM) and synchrotron infrared microspectroscopy (SIRM) at the SOLEIL (France) and DIAMOND (UK) light sources to probe single cells. The aim is to develop spectral cytology for biological and biomedical applications in cell typing, cell/drug interaction monitoring, and cell-microenvironment interactions. SIRM and LRM were used to successfully differentiate between lung cancer cells sensitive and resistant to a chemotherapy drug. The onset of drug action could be identified via time- and drug concentration-dependence SIRM data. Cutting-edge research with SIRM can help to improve the understanding of single cancer cell/microenvironment interactions, i.e., here young and old type I collagen as a models for the extracellular matrix. Spectral images of single fibrosarcoma cells in interaction with the collagen matrices were acquired and clustering analysis with k-means delineated tumor cells from their surrounding collagen networks and the latter as a function of age, mainly due to specific changes in the sugar absorption region. The Fuzzy c-means analysis gave a better nuance of the spectral images. A progression of the biochemical information was observed upon going from the cellular compartments to the pericellular contact regions and to the intact parts of the different age collagens. Two spectral markers based on sugar and protein bands via the intensity ratio (I1032/I1655) and band area ratio (Asugar/Aamide II), showed an increase in advanced glycation endproducts (AGEs) with age. A clear separation of the three age groups was obtained for spectra originating from the peripheral contact areas mainly due to changes in protein band intensities. The above-described markers decreased to constant levels for the age conditions indicating a masking of the biochemical information. These results are important to better understand the impact of age on tumor progression processes while highlighting new markers of the tumor cell invasion front.

(607) **Single Molecule Detection with Nanometer-Scale Pores**; John Kasianowicz[1], Joseph Robertson[1], Arvind Balijepalli[1], Sina Bavari[2], Rekha Panchal[2], Jingyue Ju[3],Minchien Chen[3], Shiv Kumar[3], Carl Fuller[3], Joseph Reiner[4]; [1]NIST, Physical Measurement Laboratory, Gaithersburg, MD; [2]US Army Medical Research Institute for Infectious Diseases, Ft. Detrick, MD; [3]Dept. of Chemical Engineering, Columbia University, New York, NY; [4]Department of Physics, Virginia Commonwealth University, Richmond, VA

Proteinaceous nanometer-scale pores provide the molecular basis of action of nerve, muscle and other tissues. For the past 25 years, we have been developing nanopores for the detection, characterization, identification, and quantitation of single molecules. We will discuss the use of these methods for practical applications, including DNA sequencing, single molecule "mass spectrometry", therapeutics development, and the identification of proteins and other biomarkers.

(608) **Engineered Protein Nanopores for Challenging Tasks in Molecular Diagnosis**; Liviu Movileanu[1]; [1]Syracuse University

Protein nanopore-based sensing elements represent a pressing need in molecular biomedical diagnosis. However, the integration of protein nanopores with other solid-state nanofluidic devices is a challenging task. This is especially true if we consider that isolated single proteins are in general fragile and unstable under harsh conditions of detection. Here, I will present a strategy for improving the stability of a redesigned nanopore using ferric hydroxamate uptake component A (FhuA), a beta-barrel membrane protein channel of E. coli (Mohammad, Iyer, Howard, McPike, Borer & Movileanu, 2012). The primary function of FhuA is to facilitate the energy-driven, high-affinity Fe3+ uptake complexed by the siderophore ferrichrome. The key ingredient of this strategy was the coupling of direct genetic engineering of FhuA with a fast-dilution refolding approach to obtain an unusually stable protein nanopore under a broad range of experimentation. These advantageous characteristics were recently demonstrated by examining proteolytic activity of an enzyme at a highly acidic pH, a condition at which majority of beta-barrel protein nanopores are normally gated or unfolded. Future membrane protein design work will not only reveal a better understanding of the processes employed in membrane protein folding and stability, but will also serve as a platform for the integration of robust protein components into devices.

This research has been supported by NIH R01 GM088403.

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(609) **Molecular-Level Design of Nanoscale Tools for Enhanced Single-Molecule Sensing**; Julie Whelan[1], Nuwan Bandara[1], Buddini Karawdeniya[1], Jason Dwyer[1]; [1]University of Rhode Island

Our group uses thin-film silicon nitride membranes with well-defined micro- and nanoscale pores to make single-molecule chemical sensing devices. Nanopores are nanometer-scale channels fabricated through thin, free-standing membranes. A powerful single molecule sensor results when the nanopore membrane is used to divide two halves of an electrochemical cell. An applied voltage drives electrolyte ions and analyte species through the nanopore, and analytes can be detected and characterized through their effects on the nanopore ionic current. Tremendous challenges—and unique possibilities—emerge from even transiently constraining an analyte molecule within the zeptoliter volume of a typical nanopore: jurisdiction over—and correct treatment and consideration of—nanopore surface effects are vitally important in such a setting. To ensure modification of the hidden inner surfaces of the nanopore, we relied upon electroless plating, a method pulled from the broader suit of electrochemical surface modification techniques. Our development of electroless gold plating chemistry suitable for gold-plating silicon nitride has provided us with a straightforward, solution-based method to tune nanopore physical dimensions through time- and temperature-dependent plating rates, and to customize nanopore surface chemistry through thiol self-assembly methods. We have made progress in this area, but the length-scale of our pores presents severe challenges, especially when imaging the pores to confirm that a gold coating and subsequent monolayer assembly are present within their interior geometries. To overcome this obstacle, our group has developed a much simpler method of studying the nanopore interior based on measuring the flow of small monovalent ions through the pore. This technique measures the nanopore conductance, which depends in a direct way upon the nanopore size and shape so that pore narrowing due to gold or monolayer coatings can be directly and nondestructively measured. Our chemical control over the internal sensing region of the nanopore, where the influence of the surface chemistry cannot be ignored, allows for the exploration of a broad range of analyte molecules, some of which had previously presented analytical challenges in unpassivated nanopores.

(610) **Solid-State Single-Molecule Detection of DNA in Nanochannels**; John Oliver[1]; [1]Nabsys Inc

We have developed solid-state, electronic nanodetectors that generate long range mapping information from single molecules of DNA that are hundreds of kilobases in length. Long fragments of DNA are tagged at specific sequences. The molecules are translocated through nanochannels and the presence of attached tags is observed through changes in the resistance of the nanochannel. The locations of the tags on each fragment of

DNA are determined from the time stamps of the event after correcting for the effect of viscous drag. The events are assembled with high efficiency and used to generate accurate reference maps for genomes.

The detection and biophysics of long DNA strand translocation will be discussed in the context of creating genome scale physical maps that have higher resolution and accuracy than optically derived maps.

(611) **Deformation of Bacterial Morphology in Sub-Micrometer Constrictions under Applied Pressure**; Nil Tandogan[1], Edgar D. Goluch[1]; [1]Northeastern University

Filtration techniques use size-specific separation to eliminate bacteria from fluids. Slow sand filtration which is widely used in developing countries applies the same principle to obtain potable water. Recent studies, however, showed that bacterial cells deform their morphology to fit into structures that are narrower than their own diameter. It is not well known how fluid flow affects the bacterial behavior in porous structures, which could be critical at water filtration. Here, we studied the relation between bacterial species and pressure in pores by using microfluidic devices. Miniaturized systems are excellent tools to mimic porous structures, and observe bacterial cells at a single cell level. Our microfluidic devices consist of an inlet channel where the pressure is applied, and an outlet channel which is open to atmosphere. The channels are connected via 950 nm tall constrictions with widths ranging from 1.5-3 μm.

The microfluidic devices were fabricated using lithography techniques. Constrictions were patterned on a 3 inch silicon wafer by electron beam lithography, and their height was adjusted by sputtering Chromium metal. Microchannels were aligned onto the constrictions with photolithography. The completed master wafer was used to produce poly-dimethylsiloxane (PDMS) devices, which were then permanently bonded to glass cover slips. In this study, we used two model microorganisms: *Pseudomonas aeruginosa* and *Escherichia coli*. The cells were initially grown overnight in lysogeny broth at 37 °C. Before flowing cells through the constrictions, the cultures were diluted to avoid accumulation of bacterial cells at the entrance of the constrictions. The pressure was applied at increasing increments by using a microfluidics flow control system, which was connected to a house airline. The images were taken after each pressure application.

Our results showed that the size and motility of bacteria are very important in determining the pressure profiles of species in narrow structures. *P. aeruginosa*, which is smaller in diameter and more motile than *E. coli*, required less pressure values to enter the constrictions. As the constriction size became narrower than the diameter of both bacteria, the pressure applied significantly increased. Further analysis is crucial to determine the optimum parameters for separating pathogens to improve the filtration processes.

(612) **A Study of Strategic Calibrations and Analyte Calculations**; Kim Marshall[1]; [1]Leco Corporation

Glow Discharge Optical Emission Spectroscopy (GD-OES) is an important tool for elemental analysis. GD-OES is used for both quantitative bulk analysis and compositional depth profile analysis (CDP). And, like all such optical emission techniques, GD-OES utilizes the measured intensities of one or more atomic emission line for quantification of the measured elements. An important characteristic of OES is that there is a plurality of atomic emission lines to choose from for each and every analyte. Thus the selection of the specific analytical line or lines to be utilized for quantification is one of the more important tasks of the analyst during method development.

While this task is paramount perhaps it seems too obvious. Thus little work has been published on automating the selection and eventual use of this complementary information. Historically, when most spectrometers were based on polychromators, these choices were made when the spectrometer was built and were typically selected for the materials which that specific instrument was designed to analyze. During method development the analysts was limited by these manufacturing selections to only one or a very few emission lines. With the advent of array based detection, however, this choice could be made during method development or even post analysis. Examples of early work of this kind are referenced below.(1,2 However, even as array-based spectrometers have become more common, little has been published detailing the best strategies for making such choices.

The work presented here is an attempt to describe and evaluate some possible approaches to making these line selections. Not only does this involve line selection but also approaches to determining when and how to use or combine the apparent complementary information that is available with modern array based spectrometer systems. This presentation will outline the factors that influence line selection for a given analytical problem. Approaches to numerical evaluation and data convolution will be detailed and specific problem scenarios and possible automated solution strategies will be described.

1. D. F. Wirsz and M. W. Blades, J. Anal. At. Spectrom., 3 (1988), p363

2. D. A. Sadler and D. Littlejohn, J. Anal. At. Spectrom., 11 (1996) p1105

(613) **Applied Applications for GD OES in First Solar's Material Analysis Department**; Kristin Robison[1]; [1]First Solar

In a fast paced Research and Development/Manufacturing environment it is important to produce quick, accurate & meaningful results; radiofrequency glow discharge optical emission spectroscopy (rf-GD OES) is one of the tools used at First Solar, in the Materials Analysis Department, to produce such results. Each year thousands of samples are analyzed via rf-GD OES. The GD analysis' are used to look at elemental migration induced by various conditions, elemental concentration levels and specific profile shape for elements of interest.

(614) **Comparison of Two Analytical Techniques GD-MS and SIMS Applied in Depth Profile Analysis**; Piotr Konarski[1]; [1]Institute of Tele and Radio Technology

We compare two mass spectrometry techniques used in the depth profile analysis of layered structures: glow discharge mass spectrometry (GD-MS) and secondary ion mass spectrometry (SIMS).

The basic phenomenon used in both of these techniques is an ion sputtering, which enables the removal of successive atomic layers from the surface. In GD-MS, the sample is a fragment of the wall of the glow discharge cell, which is subjected to ion bombardment from the direction of the glow discharge.

The SIMS technique uses focused ion beam etching of the selected area of the sample.

The second basic phenomenon is an ionization process. In GD-MS we extract ions of the sputtered material from the glow discharge region. This extraction occurs usually a few milimeters above the analysed surface. By contrast, the SIMS technique analyses the secondary ions, which are formed directly at the etched surface, at the spot where primary ion beam hits the surface.

Basic analytical parameters of the two depth profile techniques, as well as their main advantages and major drawbacks will be discussed. For example, we will present some comparative analyses of chosen layer structures. The results of our studies were obtained using two quadrupole mass analyser based systems.

(615) **The More You Look, the More You Find! – The Role of Penning and Charge Transfer Processes in Analytical Glow Discharges**; Edward Steers[1], Sohail Mushtaq[1], Volker Hoffmann[2], Juliet Pickering[3], Petr Smid[1,4], Zdenek Weiss[4]; [1]London Metropolitan University; [2]Leibniz Institute for Solid State and Material Research Dresden; [3]Imperial College, London; [4]LECO

It is generally accepted that collisions of the second kind, Penning ionisation (PI) and Asymmetric Charge Transfer (ACT), play a major role in the production of ground state and excited ions in glow discharges for OES and MS; Penning excitation (PE) can lead to the production of excited atoms or ions. For PI, the metastable atom, normally argon, must have at least sufficient energy to produce ionisation of the sample, but an exact energy match is not required, as the third particle formed, an electron, can take away surplus energy. On the other hand, both PE and ACT require a close energy match to conserve energy and linear momentum in the collision, so affect spectral line intensities very selectively

The presence of molecular gases – hydrogen, oxygen or nitrogen – in the plasma gas, possibly arising from the sample, can have a major effect on the number densities of ions and metastable atoms, and so can affect both ion signals in GD-MS, and line intensities in GD-OES. The effect of hydrogen is the most significant. We have carried out a large amount of work on these effects, using e.g. hydrides and oxides, and also adding small amounts of molecular gas to the main plasma gas. Comparisons with different plasma gases, argon, helium, neon and krypton and mixtures, with different added gases and with different electrical parameters can help to identify the detailed processes involved. For example, if hydrogen is added to argon, Ar-ACT is greatly reduced, but H-ACT and H2-ACT can be observed. The concept of transition rate diagrams, recently introduced by Weiss [1], provides a valuable aid for identification of excitation processes for ionic spectra, and also illustrates the importance of collisions with metastable excited states of sample atoms. Recently observed examples of these processes will be discussed in detail.

[1] Zdeněk Weiss, Edward B.M. Steers and Juliet C. Pickering. Transition rates and transition rate diagrams in atomic emission spectroscopy: a review, 2015, Spectrochim Acta, Part B, *submitted*

(616) **Glow Discharge Optical Emission (GD-OES) Application for Thin Film Analysis Of Metal Powders**; Arne Bengtson[1], Mats Randelius[1]; [1]Swerea KIMAB AB

A Glow Discharge Optical Emission (GD-OES) application for thin film analysis of metal powders has been developed. The challenge is to get the metal powder to stick to a flat metal surface with sufficient adhesion to make normal GD-OES operation possible. Various techniques have been tried, but the one that has been successful is to press a minute amount of powder into the surface of a softer metal substrate. In this work, chromium steel and type 316 high alloy steel powders were pressed into aluminum substrates. Since this application is intended to study the very thin surface oxide on the powders, a measure to reduce the "startup" effects of the lamp was to sputter coat the samples with an approximately 50 - 100 nm gold layer. This layer is rapidly sputtered in 0,1 – 0,2 s, enough time to stabilize the plasma. When quantifying the data, the gold channel is simply deselected in order to just display the profile beginning with the sample surface. The results show that very thin surface layers of metallic powder can be analyzed by GD-OES using the sample preparation technique developed, with good reproducibility. This technique should work for bulk analysis of metal powders as well.

(617) **The Education of a Former Academic or Women Who Gave Me the Business**; Alexander Scheeline[1]; [1]SpectroClick

After 33 years as a faculty member and 7 years as co-founder of another enterprise (Anchor Science), the author "retired" to build a small business (SpectroClick). The origins of the business, dating to early undergraduate experiences, are reported. Converting a clever student's ideas into a product presented technical, financial, management, and personal challenges. Even technologically intensive businesses are primarily about people, their motivations, passions, and limitations. After a lifetime of wishing to be left alone to be productive, the author learned that he couldn't be productive alone. The wisdom of his mentors, students, colleagues, family, and even antagonists were all helpful in getting the business off the ground.

(618) **Ideas to Finance Your Spectroscopy Innovation**; Roshan Shettty[1]; [1]Anasys Instruments

Angels or licenses? Dilutive or non-dilutive? Loans or grants? Successfully navigating the myriad options for financing innovation is critical to the viability of a technology-based company. The business plan, investor pitches and market analysis depends on how the business will be funded. Do you have an idea that you'd like to create a company around? If finance is your constraint, this talk will discuss the various options available ranging from Friends/Family to Angel to Venture Capital to federal grant and how these sources can fit into each stage of a company's development.

(619) **Finding a Niche Market in Busy Spectroscopy Field**; Rina Dukor[1]; [1]BioTools, Inc.

Spectroscopy, and specifically Vibrational spectroscopy, is a very busy field filled with everything from small hand-held devices to sophisticated large and expensive spectrometers. How does one build a successful business based on spectroscopy? Understating the problem that needs to be solved, the gap in the existing products, the available market and customer requirements are the most important keys to success. But in my experience as entrepreneur, it takes much more than that. Differentiating oneself from the rest – even in a smaller niche market – could be the most important element. With an added undying passion and incredibly hard work – and always keeping the customer in mind – one can in fact build a disruptive new technology and successful business. In this presentation I will share my experience, discuss lessons learned and the need for constant innovation.

(620) **When a Great Medical Device Isn't Great for the Company**; Bradford Clay[1]; [1]bioMerieux, Inc.

Medical devices are regulated in most countries of the world The regulations address rigorous design methodology and good manufacturing practices, but it is still possible to "achieve defeat from the jaws of success".

A case study will be presented of a medical device which caused manufacturing, quality and regulatory issues when product sales expanded faster than anticipated.

(621) **Improving Multivariate Calibration Results for Optical Spectrometers**; Michael F Roberto[1], Randy Pell[1], L. Scott Ramos[1], Brian G. Rohrback[1]; [1]Infometrix, Inc.

A project has been undertaken to assess how to reduce the amount of effort devoted to maintaining and optimizing spectroscopic model performance in support of refinery and chemical plant labs. Over the last five years, a series of algorithmic approaches have been examined with the goal of streamlining the process of chemometric model construction to make the models significantly more robust when put into routine practice. This effort generated the following observations:

1.Even though there are published "Best Practices" for generating chemometric models, these practices are infrequently followed;
2.Recalibration of an optical spectrometer is perceived to be warranted due to changes in crude slates and blending component composition, but may not be required;
3.Even if a calibration was performed properly during initial installation, staffing changes and lack of training undermines subsequent recalibrations; and
4.It is of benefit to minimize software maintenance frequency to control product giveaway.

In order to use optical analyzers effectively, the analyst needs to consider the limitations that ultimately determine the eventual success and life-expectancy of any calibration. There are many areas that have an impact on the ultimate quality of a chemometrics calibration: outlier detection, selection of number of factors, and even choice of algorithm. This presentation reports on a multi-organization effort leading to an improvement in calibration procedures for on-line and laboratory multiwavelength spectrometers. Here the process of calibration is examined in detail and a path is outlined for building calibrations that are more reliable and less sensitive to process shifts. Much of this improvement can be attained without requiring replacement of either the hardware or software in place. Additional improvement in calibration quality is available through the use of well-referenced methods that constitute the best technologies available.

(622) **SERS Detection of Foodborne Pathogens at 10 cfu/g Food in Less than 5 Hours**; Chetan Shende[1], Katie Dana[1], Jay Sperry[2], Stuart Farquharson[1]; [1]Real-Time Analyzers, Inc.; [2]University of Rhode Island

Each year foodborne pathogen infections result in ~50 million illnesses, ~130,000 hospitalizations, and 3000 deaths in the USA alone. Preventing the distribution and consumption of contaminated foods is challenging because just 100 bacterial cells can rapidly multiply to millions, reaching infectious doses within a few days. Unfortunately, current methods used to detect these few cells rely on similar growth steps to multiply the cells to the point of detection, which also takes a few days. Consequently, there is a critical need for an analyzer that can rapidly detect foodborne pathogens at 10 colony forming units per gram (cfu/g) of food in a few hours (not days). In an effort to meet this need, we have been developing an assay that extracts such pathogens from food, selectively binds these pathogens, and produces surface-enhanced Raman spectra (SERS) when read by a Raman analyzer. Measurements of *Campylobacter jejuni* in chicken, *Escherichia coli* in ground beef, *Listeria monocytogenes* in cheese and *Salmonella typhimurium* in cantaloupe at 10 cfu/g using this assay will be presented.

(623) **Data Transfer between a FT-NIR Laboratory and a Miniaturized Hand-held NIR Spectrometer**; Heinz Siesler[1], Uwe Hoffmann[2], Frank Pfeifer[1]; [1]University of Duisburg-Essen, [2]nir-tools

The aim of this contribution is to demonstrate the transfer of spectra that have been measured on two different laboratory FT-NIR spectrometers to the format of a handheld instrument by measuring only a few samples with both spectrometer types. Thus, despite the extreme differences in spectral range and resolution, data sets which have been collected and calibrations which have been developed thereof, respectively, over a long period on a laboratory instrument can be conveniently transferred to the handheld system without the requirement for elaborate complete rescanning of spectra.

The chemometric procedure will be described for the data sets of a quantitative and a qualitative calibration case study. For this purpose the spectra measured on the FT-NIR laboratory spectrometers were used as "master" data and transferred to the "target" format of the handheld instrument.

(624) **Two-Dimensional Infrared Correlation Analysis of Time-Resolved Infrared Spectra to Probe the Structure Development of the Thermally Reversible Gel Made of a Bio-based, Biodegradable Polymer**; Isao Noda[1], Brian Sobieski[1], Liang Gong[1], C.J. McBrin[1], John Rabolt[1], Bruce Chase[1]; [1]Department of Materials Science and Engineering, University of Delaware

Time-dependent infrared spectra were used in this study to probe the thermally reversible gelation process of solutions of bio-based, biodegradable poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (Nodax™) to gain insight into the dynamic evolution of the gel micro-structure. In-depth analysis of the time-resolved infrared spectra was performed using two-dimensional correlation spectroscopy to determine the origin of the gel micro-structure development. Although the fundamental driving force of the gelation is a thermodynamic transition in nature, development of the gel micro-structure is kinetic, meaning that once the polymer solution is cooled, it takes a finite amount of time for the solution to become a gel. The growth of said structure occurs in a rapid, almost step-wise fashion that can only be observed through a fast spectral sampling, where rapid-scan Fourier transform infrared (FTIR) and planar array infrared (PAIR) spectroscopy techniques are well suited. 2D correlation spectroscopy allowed the subtle evolution of spectra during the gelation, which can be difficult to observe, to be analyzed in depth and easily compared.

(625) **Near-Infrared Compositional Analysis of Acid Components in Etchant Solution in Combination with a Feature Selection Method and Investigation of Inter-Component Interactions using Dimensional (2D) Correlation Analysis**; Kyeol Chang[1], Hoeil Chung[1]; [1]Department of Chemistry, College of Natural Sciences, Hanyang University

Near-infrared (NIR) spectroscopy has been used to determine the concentrations of major inorganic acids (H3PO4, H2SO4, HNO3 and HCl) in etchant solutions. The measurement is largely based on the recognition of the varied spectral feature of water absorption bands by their presence in the samples, rather than direct NIR absorption by themselves. As a result, the resulting spectral variations according the concentration change are relatively indistinct and unforeseen spectral features under the water bands are necessary to be recognized for multivariate quantitative analysis. First, we have prepared binary and ternary mixture samples contained the tested components as a combinatorial fashion and their inter-influences on the calibration accuracies were examined. In parallel, to probe their interactions with water, two-dimensional (2D) correlation

analysis was used to investigate the perturbed water bands by the components. Second, for more accurate determination of component concentrations using the sample spectra with indistinct analyte-related features, we have evaluated Markov blanket (MB) discovery algorithms as a feature selection method, which intends to find a set of minimal number of variables (wavelengths) to explain the target variables (concentrations) on the basis of conditional independence of all the variables to be connected in a Bayesian network. The resulting accuracies in the determination of component concentrations using MB were compared with those using partial least squares.

(626) **Characterization of Atomic Lifetimes and Linewidths in Laser Induced Plasmas using Tunable Laser Absorption Spectroscopy**; Mark Phillips[1]; [1]Pacific Northwest National Laboratory

Spectral linewidths and lifetimes of atomic species are key parameters for isotopic analysis in laser-induced plasmas. Tunable laser absorption spectroscopy provides information on species populations with high temporal and spectral resolution. In addition, absorption measurements allow probing of species during later times in the plasma evolution at which the lower temperature and electron density results in narrow spectral linewidths. We have recently characterized atomic absorption in laser-induced plasmas over wide ranges of parameters including time, temperature, and pressure. Time-resolved absorption from different elements, ionization states, and energy levels are measured to provide key information on plasma physics and chemistry and allow differentiation of various spectral line broadening mechanisms. In particular, we have measured absorption spectra of multiple neutral and ionic transitions in uranium and find spectral linewidths typically < 5 GHz (5 pm) at atmospheric pressure in air. The spectral linewidths are smaller than the isotope splitting for many transitions, opening up new possibilities for isotopic analysis in laser plasmas.

(627) **Laser Ablation Molecular Isotopic Spectrometry (LAMIS): Factors Influencing Analytical Precision and Accuracy**; Xianglei Mao[1], George Chan[1], Alexander Bol'shakov[2], Huaming Hou[1], Vassilia Zorba[1], Richard Russo[1]; [1]Lawrence Berkeley National Laboratory; [2]Applied Spectra, Inc.

Laser Induced Breakdown Spectroscopy (LIBS) is a powerful and versatile analytical tool for direct and fast elemental analysis at atmospheric pressure, for virtually any type of sample with minimal sample preparation. However, LIBS generally provides elemental information for a given sample, but not its isotopic composition because of small isotope shift in atomic or ionic lines. Our recent development of Laser Ablation Molecular Isotopic Spectrometry (LAMIS) enables real-time isotopic analysis at atmospheric pressure conditions. LAMIS uses radiative transitions from molecular species either directly vaporized from a sample or formed by associative mechanisms of atoms or ions in a laser ablation plume. The isotope shift in molecular bands is several orders of magnitude larger than that of atomic or ionic lines.

As is true with any analytical technique, the resulting analytical performance is only as good as the calibration model. In LAMIS, there are two different approaches to obtain isotopic information of the sample from the measured molecular spectra, namely spectral-pattern recognition and multi-variable non-linear spectral fitting. In the pattern recognition approach, spectral patterns are measured from a series of isotopic standards for calibration. In this approach, the analytical precision can readily reach per-mil level, as demonstrated in our previous work. The second approach – theoretical multi-variable non-linear spectral fitting requires no isotopic-enriched standard. However, the availability of an accurate set of molecular spectroscopic constants is essential in this case.

In this presentation, the underlying principles and calibration methods for LAMIS will be overviewed, and factors influencing the analytical precision and accuracy of this technique will be discussed.

(628) **Hybrid Interferometric/Dispersive Atomic Spectroscopy of Uranium**; Phyllis Ko[1], Jill Scott[2], Igor Jovanovic[1]; [1]Penn State University, [2]Idaho National Laboratory

An established optical emission technique, laser-induced breakdown spectroscopy (LIBS), is of significant interest for its rapid analysis capability and compatibility with field use. In LIBS, the optical emission spectrum of the laser-pulse generated plasma is usually analyzed to yield elemental and molecular information of the analyte. LIBS can also be used to measure isotopic composition, for example the enrichment of uranium. This capability can support important nuclear security objectives such as nuclear safeguards and nonproliferation. The measurement of uranium enrichment using LIBS is challenging, one reason being the high spectral resolution required to resolve the small isotope shift. For example, the isotope shift of the commonly studied 424-nm U(II) line is only 25 pm. High-resolution spectrometer systems used for such measurements to date required a large and expensive spectrometer. An alternative approach, consisting of a relatively inexpensive and compact Fabry-Perot etalon coupled to a grating spectrometer, can be used to perform high-resolution spectral measurements for LIBS. We describe a method to reconstruct the high-resolution spectra from the spectrally and angularly encoded interference pattern of a Fabry-Perot etalon. We demonstrate the use of our method to conduct LIBS measurements of uranium with isotopic sensitivity.

(629) **H and D Analysis Using Laser Induced Breakdown Spectroscopy in Helium Gas**; Koo Hendrik Kurniawan[1], Kiichiro Kagawa[1, 2]; [1]Maju Makmur Mandiri Research Center, [2]Fukui University

A crucial safety measure to be strictly observed in the operation of heavy water nuclear power plants is the mandatory regular inspection of the concentration of deuterium penetrated into the zircaloy fuel vessels. The existing standard method requires tedious, destructive and costly sample preparation process involving the removal of remaining fuel in the vessel and melting away part of the zircaloy pipe. An alternative method of laser-induced breakdown spectrometry (LIBS) is proposed by employing orthogonal double pulse laser in flowing atmospheric He gas. The special setup of ps and ns laser systems operated for the separate ablation of sample target and the generation of He gas plasma respectively, with properly controlled relative timing, has succeeded to produce the desired sharp D I 656.10 nm emission line with effective suppression of the interfering H I 656.28 nm emission by proper control of the ps laser ablation energy and time synchronization between ps and ns laser operation. Under optimal experimental condition, a linear calibration line is attained with practically zero intercept and 5 μg/g detection limit for D analysis of zircaloy sample while creating a crater of only 10 μm diameter. This method therefore promises its potential application for the practical, in-situ and virtually non-destructive quantitative micro-area analysis of D, thereby supporting the more efficient operation and maintenance of the heavy water nuclear power plants. Further, it will also meet the anticipated needs of future nuclear fusion power plants as well as other important fields of application in the foreseable future.

(630) **Detection of Isotopes in a Matrix with LIBS**; Alan Ford[1], Charlemagne Akpovo[2], Staci Brown[2], Jorge Martinez[2], Lewis Johnson[2]; [1]Alakai Defense Systems; [2]FAMU

The detection of isotopes, especially radioactive ones, has been a long-time concern that has elevated over the years with the increased possibility of rogue states and terror groups developing nuclear weapons or so-called "dirty' bombs. Though Geiger counters and scintillators can detect radioactive substances, it is desirable to find such hazardous materials without exposure. In addition, there may be occasions where detection of non-radioactive or weakly emitting sources need to be detected as a prelude to enrichment or as a material associated with nuclear bomb construction.

One tool that allows for standoff detection of isotopes is laser-induced breakdown spectroscopy (LIBS). Not only is it standoff, but it can be made man-portable. It offers real-time analyses of isotopes of most materials, especially with the technique of laser ablation molecular isotopic spectrometry (LAMIS). It can also be quantitative in both elemental detection and isotopic ratios, which is especially useful for nuclear forensics.

However, LIBS/LAMIS for isotope detection has a major drawback – matrix effects – though these matrix effects can be overcome in multiple ways. The first method utilizes improved chemometric techniques. Chemometrics can be a powerful tool, especially if the concern is simply interference from the matrix, as long as multiple features of the analyte of interest are present. A second method involves the use of additional hardware to selectively excite the material of interest. For example, extra lasers tuned to a transition of a given species might be used to enhance the emission of that species above the background.

In this presentation, techniques for quantifying isotopes/isotopolgues in the presence of a matrix are provided. Demonstrations of the power of the techniques are provided, and the advantages of each are discussed.

(631) **Distinguishing Isobaric Drugs using Online Derivatization and Direct Analysis in Real Time (DART)**; William D. Hoffmann[1], Glen P. Jackson[1, 2]; [1]West Virginia University, Department of Forensic and Investigative Science, [2]West Virginia University, C. Eugene Bennett Department of Chemistry

The time required to perform a chromatographic separation, typically 20-30 minutes for GC, is a major factor contributing the backlogs at crime labs across the country. One method of overcoming the throughput challenge posed by GC/MS analysis time has been the use of ambient ionization techniques that often bypass chromatographic separations. We therefore explore the possibility to quickly and easily differentiate the drug isobars in positive ion mode using online derivatization and Direct Analysis in Real Time (DART).

Isobaric interferences pose a major problem in the analyses of illicit substances.

For example, the major cannabinoids of marijuana are the main psychoactive component tetrahydrocannabinol (THC) and a non-psychoactive component, cannabidiol (CBD). CBD and THC are easily differentiated using GC/MS based on their distinct retention times and electron ionization mass spectra, in spite of their identical chemical formula and similar structure. Although CBD and THC can be distinguished using electron ionization, under soft ionization conditions, the full and tandem mass spectra of CBD and THC are virtually indistinguishable in the protonated form. However under HDX conditions the isotopic envelope shift suggests that CBD uptakes a greater number of deuterium atoms than THC, which is consistent with the greater number of exchangeable sites on CBD. Interestingly, the number of substitutions is greater than expected for both CBD (two exchangeable sites) and THC (one exchangeable site). Because CBD and THC are constitutional isomers, the protonated peak for CBD and THC should appear at *m/z* 315. However upon HDX the isotopic envelopes for CBD and THC shift to *m/z* 319 and *m/z* 317, respectively. The difference in isotopic distributions for CBD and THC prepared with deuterated solvent suggests that the known interconversion of cannabidiol and tetrahydrocannabinol is slow relative to the hydrogen/deuterium exchange process and therefore does not preclude the use of HDX. Tandem mass spectra of CBD and THC were analyzed using *m/z* 318 as the precursor peak, which was chosen because it was present in sufficient abundance to be isolated and fragmented from both species.

(632) **Rapid Detection of Rare Earth Elements by Microwave Plasma Torch Coupled with the Linear Ion Trap Mass Spectrometry**; Xiaohong Xiong[1], Tao Jiang[1], Meiling Yang[1], Wenhao Qi[1], Saijin Xiao[1], Zhiqiang Zhu[1], Huanwen Chen[1]; [1]Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology

Rare earth elements are important industry material and have really extensive applications in various fields. Along with large amounts of the exploited of rare earth ores, rare earth elements accessed inevitably environment, food chain and the human body. The demand of analytical method for rare earth elements with high sensitivity is increasing in current society. In this work, a sensitive mass spectrometric analysis method based on the microwave plasma technique is development for the fast detection of rare earth elements (REEs) in aqueous solution at the trace level. The plasma was produced from an Ar assisted microwave plasma torch (MPT) under atmospheric pressure, and was directly used as ambient ion source of a linear ion trap mass spectrometry (LTQ). Dry nebulized sampling solution flowed across the plasma flame through the central cube of the MPT, without any other pretreatments. For the REEs including La, Ce, Pr, Eu, Nd, Sm, Y, etc., the generated complex ions in plasma were detected in both positive and negative ion modes and further characterized in collision induced dissociated (CID) experiments. These mass spectra showed that these REEs could keep their original valence state as that in aqueous through the plasma excited, which is distinct from that of the ICP-MS. Under the optimized conditions, the limited of detection (LOD) of these REEs, using MS2 procedure, were estimated to be at the level 10-10g/ml with a linear dynamics rage larger than 2 orders of magnitude. The analysis of a single aqueous sample can be completed in 2~3 minutes with a reasonable semi-quantitative sense. Two practical aqueous samples and orange juice were analyzed qualitatively without extraction separation of REEs. These experimental data demonstrated the MPT-MS is hopeful to be applied in scene analysis involving several fields, such as environment controlling, food safety and living-body, and can be used as the supplement of ICP-MS.

(633) **Analysis of Vapor Samples with Interrupted Helium Flow for the Flowing Atmospheric-Pressure Afterglow Mass Spectrometry**; Andrew P. Storey[1], Offer Zeiri[1, 2], Steven Ray[1], Allen White[1,3], Gary Hieftje[1]; [1]Indiana University; [2]Nuclear Research Center Negev; [3]Rose-Hulman Institute of Technology

The flowing atmospheric pressure afterglow (FAPA) has been demonstrated to be one of the promising sources for ambient desorption-ionization mass spectrometry (ADI-MS). Like most discharge-based ADI-MS sources, the FAPA requires the use of helium, a nonrenewable resource that has begun to face price and supply pressures. Recently, our group has demonstrated that the pin-to-capillary version of the FAPA source can be maintained for extended periods with minimal helium flow, which conserves helium and without dramatic changes to the source or extended

warm-up periods prior to analysis. This previous work focused on the ability of the source to remain stable and to preserve performance characteristics with solid samples. Experiments involving the introduction of vapor species encouraged additional characterization.

Mass spectra and gas-flow patterns both show important differences between vapor and solid sampling when discontinuous helium flow is utilized. Schlieren photography revealed initial turbulence when helium flow is restored, and thermal and diffusion processes when helium flow is absent. This behavior can be correlated to time-resolved mass spectra and thermography data. A broad set of volatile organic compounds were examined and alternative sample-introduction arrangements employed to characterize the interaction of the afterglow from the FAPA source and the resulting ions that can be sampled into a mass spectrometer. In some cases, unusual product ions are observed and signal levels vary as the interval of helium flow evolves. From these data, advantages and challenges of the use of interrupted helium flow for the analysis of vapor compounds with FAPA-MS will be evaluated.

(634) **The Liquid Sampling-Atmospheric Pressure Glow Discharge: A Miniaturized Ambient Glow Discharge Ionization Source for Elemental and Molecular Mass Spectrometry**; Lynn X. Zhang[1], R. Kenneth Marcus[1]; [1]Clemson Univeristy

The liquid sampling-atmospheric pressure glow discharge (LS-APGD) was first developed as an excitation source for atomic emission spectroscopy by Marcus and co-workers. It has recently been improved and demonstrated for use as a low power, small footprint, and cost efficient ionization source for elemental mass spectrometry, molecular mass spectrometry, and ambient desorption mass spectrometry. The multiple functions in one package puts the LS-APGD in a unique position among other atmospheric pressure plasmas. It also has the capabilities of working with different sample forms including liquids, bulk solids, and solution residues. The high salt/matrix tolerance reduces sample prep work, thus provides convenience for its potential use as a field-based ionization source. These versatilities all come on a small platform.

The LS-APGD microplasma is sustained between the surface of an electrolytic solution (5-50 µL min^{-1}), and a solid stainless steel counter electrode mounted at a 90° angle, at discharge currents of <60 mA with d.c. power of <50 W. The electrolytic solution is introduced through a 0.28 mm (i.d.) capillary housed inside a 1 mm (i.d.) metal capillary, with the helium sheath gas (0.1-1.0 L min^{-1}) flowing in between. Presented here are the design and fundamental operation aspects of the LS-APGD operating in various mass spectrometry ionization modes. Sample analysis involving direct elemental analysis, organometallic speciation and organic compound determinations in solution, and direct surface analysis of solid samples and liquid residues will be presented. It is believed that the LS-APGD ionization source holds a unique position and has great potential to be utilized in many fields of chemical analysis.

(635) **Direct Analyte-Probed Nanoextraxtion (DAPNe) Coupled to Nanospray Ionization Mass Spectrometry Applied to Document Analysis**; Vivian Huynh[1], Kristina Williams[1], Phillip Mach[1], Zachary Sasiene[1], Teresa Golden[1], Guido Verbeck[1]; [1]University of North Texas

Microscopy with direct analyte-probed nanoextraction-coupled to nanospray ionization mass spectrometry (DAPNe-NSI-MS) has been proven to extract ink from documents with little to no physical or chemical footprint. The DAPNe technique opens the window for many forensic applications with its ability to be easily coupled with other instrumentation. Raman spectroscopy and fluorescence microscopy was used to determine if a document was modified (e.g., altering a four to a nine) and laser ablation was used to determine the ink chemistry of redacted text (i.e., the writing is concealed with other ink). Fluorescence microscopy and Raman spectroscopy were able to discern the original text from the altered text due to the different formulation of the pens. Raman spectroscopy with mapping capabilities distinguished between the two different pens used. Results indicated high intensities of cellulose from the paper at 1086cm-1 and 1121cm-1, C-O-C stretch from ink pen 1 at 1273cm-1 which is most likely due to one of the organic additives used as a stabilizer, and ink pen 2 indicated C-H bending at 1360cm-1 from a methyl or methylene.

If the modification were to occur with the same exact pen, the oxidation products of the new and old ink can be differentiated due to the different times the ink was written by quantitating the percent relative peak area. DAPNe-NSI-MS was able to track the process of oxidation, since inks start to age and oxidize as soon as they are deposited. The extractions are also dependent on the NSI solvent used. For example, a chelator such as EDTA added to the NSI solvent was able to chelate the inorganic components of iron gall ink (Fe and Mn); and a solvent such as Methanol:H2O:1%Acetic acid extracted organic components of the ink such as polyethylene glycol, ethoxy diglycol, and rhodamine dye. The solvent tip chemistry can selectively target specific components in the ink, significantly reducing potential matrix effects and ion suppression.

(636) **Microfluidic Gas Chromatography-Bringing the Analyzer to the Sample**; Joshua Whiting[1], Pierre Puget[2], Eric Colinet[2], Philippe Andreucci[2], David Faulkner[3], Philippe Coric[3]; [1]3 Degrees of Separation; [2]APIX Analytics; [3]EIF-Astute

There has been an increased focus on bringing the analyzer to the sample in process analysis, thus eliminating the need for long fast loop sample lines and delayed response to process changes. 15 years ago CPAC introduced the NeSSI standard with this goal in mind. Gas chromatography (GC) has long been one of the most versatile analytical instruments, with a long history in process analysis. Thru the use of low power microfabricated components to minimize size, power, and sample volume to reduce the explosion risk, these instruments have begun to move out of instrument sheds and onto the pipe to provide faster turnaround of process data reduced sample waste and near real time monitoring.

APIX Analytics in collaboration with EIF-Astute have introduced a new instrument dedicated to process analysis the MAX ONE based around the APIX modular concept. The heart of the system is the Multi-Physics detector package where the NEMS resonator detector developed in collaboration between Caltech and CEA-LETI is coupled with a microfabricated TCD to provide sensitive universal detection for analytes ranging from permanent gases to diesel range species. The MAX ONE is designed from the start to be placed anywhere in the process environment from shed to pipe with an anticipated ATEX Zone 1 certification and integrated NeSSI compatible design. An overview of the MAX ONE system with modular design - up to 4 parallel complete GC channels - and performance will be presented.

(637) **Fast GC Performance in the Real World: Multi Lab Studies for Repeatability & Reproducibility**; John Crandall[1], Steve Bostic[1], Ned Roques[1]; [1]Falcon Analytical

Repeatability, reproducibility, precision and accuracy are terms tossed around in both laboratory and process analysis communities. While very clear definitions of these terms exist, all are intended to help users understand the performance of their analytical systems. This is critical in single plants: support lab and process analyzer data must at least correlate one to the other. But what if data from multiple plants not only correlated but within a defined range were statistically “identical?” And what if GCs could run 24/7 for more than 2 years without retention time

drift, without maintenance and without downtime? This paper will discuss analytical consistency in the context of both process and lab system performance including the ASTM D7798 method showing results from 7 independent laboratories using this simulated distillation method.

(638) **FTIR & GC-FTIR Single Analyzer for Comprehensive Process Monitoring and Control**; Charles Phillips[1], Martin Spartz[1], Anthony Bonanno[1], Stacey Larson[1], Alice Delia[1]; [1]Prism Analytical Technologies, Inc.

An optimal detection system for process analysis should provide qualitative and quantitative capabilities for many classes of compounds over a large dynamic range from % to pptr levels. A single analyzer with these capabilities would allow for measurement of many chemical process parameters and simultaneous monitoring of process modifications/control, reducing the install and maintenance costs for multiple analyzers.

Prism has coupled a gas chromatograph (GC) with a Fourier Transform Infrared Spectrometer (FTIR) gas analyzer in an innovative way that enhances the compound separation dimension of the GC with the qualitative and quantitative dimension of FTIR. The FTIR gas analyzer utilizes a multiple path gas cell that collects the analytes into the gas cell for integration and signal averaging allowing for ppb to pptr detection limits. By using this gas cell concept the need for high resolution GC is removed since co-eluting compounds are easily resolved in the spectral domain. Thus, fast GC separations with minimal theoretical plates can easily be employed.

Additionally, through this unique coupling, the FTIR gas analyzer can also be configured to operate as a stand-alone analyzer for those situations where the analyte is not conducive to chromatography or is at higher concentration levels.

Prism's MAX™ technology can be setup in less than a day and is not damaged if too much sample or analyte were to reach the detector. Since the system is based upon analysis of infrared spectra, it requires a one-time calibration which is transferrable from instrument-to-instrument. This detection system is ideal for both real-time process analyzers and laboratories where productivity is improved by eliminating redundant and/or frequent calibration or where known or unknown samples can wreak havoc with large concentration swings potentially affecting results or operation of the analyzer.

Among the process applications where MAX™ capabilities have been demonstrated are: determination of both low and high levels of hazardous air pollutants emanating from a process which has the capability of destroying expensive catalysts, on-line measurement of oxygenates in fuel without the need for GC x GC methodology, and near-real time monitoring of siloxanes in purified biogas streams.

(639) **Application of Low Thermal Mass Column Technology to On-line Process Gas Chromatography**; Eric Schmidt[1], Anna Sandlin[1], Linda Heinicke[1], Bill Winniford[1], Wilco Hoogerwerf[2], Jasper Van Noyen[3],Dale Ashworth[4], Chris Bishop[4]; [1]Analytical Sciences, The Dow Chemical Company, Freeport, TX ; [2]Analytical Sciences, The Dow Chemical Company, Terneuzen, The Netherlands; [3]Hydrocarbons R&D, The Dow Chemical Company, Terneuzen, The Netherlands; [4]Valco Instruments Company, Inc. , Houston, TX

Understanding conversion and selectivity of gas phase hydrocarbon processes is often enhanced by the application of on-line analytical measurements to observe the chemistry in near real-time so that the end-user of the data can more easily link "cause and effect." The agility and power of liberating the technology affords end-users a means to determine the conditions for optimum yield, assure process control, understand process upsets, and characterize unknown streams with urgency not possible by conventional means.

On-line process gas chromatography (GC) applications are typically limited to packed column and isothermal oven technology. This presentation will describe the use of low thermal mass (LTM) column technology as applied to process GC to gather the analytical information needed to better understand, optimize, and probe chemical problems. The results illustrate simplifying a GC method by combining multiple isothermal column trains into a LTM unit thereby reducing method development time and improving peak resolution

(640) **Portable Plant Performance Analyzer System**; Matthew MacConnell[1]; [1]Air Products and Chemicals

Characterization of operating plant performance requires analytical measurements in points of the process that are often not sampled during normal operations. Plant design feedback for continous improvement, troubleshooting and plant optimization is possible given sufficient process measurements across and within unit operations such as in this case, cryogenic distillation and mole sieve adsorption.

To meet this objective, a portable analyzer system was designed to provide multiple sample points and sufficient analytical information to close material balances and optimize processes in a cryogenic air separation plant. This paper will explore the analysis and integration challenges that were met in building this multiple crate portable analyzer system and how it was deployed so far in the US and China.

The portable system is designed for shipment around the world and utilizes digital integrated systems to reduce wiring and improve measurement rangeability and precision. Analyzers incorporated in the system include mass spec, GC, gas filter correlation IR, electrochemical, and paramagnetic methods. The system also incorporates Pressure drop and moisture transmitters. A sample switching multiplexer is included for easy user customization of sample selection and timing on more than 20 sample points. The system incorporates a Siemens PCS7 distributed control system and utilizes Profibus DP communications along with OPC to the analyzers and sample systems.

(641) **An Infrared (IR) and Vibrational Circular Dichroism (VCD) Spectroscopic Study of Solvent Effects on Hydrogen Bonding by (S-(+)-2-(4-Isobutylphenyl)-Propionic Acid**; Douglas Minick[1], Randy Rutkowske[1], Mark Hemling[1]; [1]GlaxoSmithKline R&D

Vibrational circular dichroism (VCD) is now a well-established technique for elucidating the absolute configuration of chiral organic molecules. The degree of reliability for a VCD assignment is largely determined by the accuracy of the in silico simulation of a molecule's solution phase conformational state since results from this analysis are used to generate the conformationally averaged VCD spectrum upon which the assignment is based. As the structural complexity of a molecule increases, so does the complexity of its conformational state, requiring a deeper understanding of the experimental infrared (IR) spectrum in order to design model systems that better capture the molecule's actual solution state. Hydrogen bonding is perhaps the most problematic complication for VCD analysis. These interactions are often pervasive under experimental conditions and capable of producing a variety of different types of H-bonded structures. One of the ways to reduce H-bond interactions is to use an H-bond disrupting solvent. Dimethyl sulfoxide-d6 (DMSO-d6), a 'universal' solvent in VCD analysis, is generally considered to be strongly H-bond disrupting. In our experience, however, this solvent changes but does not simplify the intermolecular H-bonding interactions typically exhibited by carboxylic acid. As a case in point, we report herein findings from the VCD analysis of (S-(+)-2-(4-isobutylphenyl)-propionic acid ((S)-ibuprofen) in three solvents: CDCl3 (weak proton donor), DMSO-d6 (proton acceptor) and ACN-d3 (proton acceptor). Our results are consistent with the formation of a typical H-bonded acid dimer by (S)-ibuprofen in CDCl3, a complex H-bonded chain in DMSO-d6, and a

solvated monomer in ACN-d3. By providing a better understanding of self-association by carboxylic acids in these solvents and in particular, DMSO-d6, our findings should facilitate the selection of computational models in VCD studies of chiral molecules containing this functionality.

(642) **Quantitation of Enantiomers by Vibrational Circular Dichroism (VCD)**; Laila Kott[1]; [1]Takeda Pharmaceuticals International Company
Vibrational circular dichroism (VCD) is a technique that uses the infrared regions (IR) of the electromagnetic spectrum and similar to the Ultraviolet radiation based CD (ECD) spectra, can differentiate between enantiomers in solutions. This technique works well with molecules that contain IR active groups and some rigidity (i.e. ring structures). Similar to ECD, the VCD spectra of enantiomers are of equal intensity and opposite in sign and because, of this it, was possible to construct chemometric calibration models to detect the presence of low levels of compounds in the presence of its enantiomer. By normalizing the observed intensities of the VCD signals with the observed IR spectra or by concentration, a partial least squares (PLS) model can be constructed for chiral compounds in order to predict/determine the percent of the undesired enantiomer. Using the model compound of α-pinene (i.e. amount of R-α-pinene enantiomer in S-α- pinene), the feasibility of this approach was demonstrated resulting in a chemometric model that produced a root mean squared error of cross validation (RMSECV) of 0.69%. This indicates that the enantiomeric purity of S-α-pinene could be determined to an accuracy of ±0.69%. Building upon these feasibility studies, other models were developed using research compounds. This work demonstrates that VCD can be used for the low level detection of a compound in the presence of its enantiomer and thus eliminates the need for a UV chromophore and chromatographic separation of the two enantiomers.

(643) **Pharmaceutical Applications of Vibrational Circular Dichroism (VCD)**; Steven Wesolowski[1]; [1]AstraZeneca
The absolute stereochemistry of chiral drugs is usually established via X-ray crystallography. However, vibrational circular dichroism (VCD) spectroscopy coupled with quantum mechanics simulations offers a rapid alternative to crystallography and is readily applied to both crystalline and non-crystalline samples. VCD is an effective complement to X-ray analysis of drug candidates, and it can be used as a high-throughput means of assessing absolute stereochemistry at all phases of the discovery process (hundreds of assignments per year). The practical implementation (or fee-for-service outsourcing) of VCD and selected case studies are illustrated with an emphasis on providing utility and impact to pharmaceutical discovery programs.

(644) **Advancing Supercritical Fluid Chromatography (SFC) Technology and Its Applications in Drug Discovery**; Yingru Zhang[1], Chunlei Wang[1], Jun Dai[1]; [1]Bristol-Myers Squibb Co
Despite the overwhelming success of GC and HPLC in separation science, Supercritical Fluid Chromatography (SFC) has shown a great momentum to become a mainstream technique in recent years. The impetus was largely propelled by the dominant performance of SFC in resolving and isolating mg to kg of chiral drug molecules in the pharmaceutical industry. The successful industry adoption of packed column SFC not only led to the "killer application", but also demonstrated its undeniable advantages with respect to super- or sub-critical fluid's unique combination of diffusivity, viscosity and solvation. This presentation highlights a few technical innovation and development we have done in the Drug Discovery enironment to advance the capability and exploit the unique property of for chiral resolution. The highlighted techniques and research include parallel-column SFC for method development of chiral separation, Simulated-moving Column SFC technique for difficult chiral resolution on columns with Insufficient selectivity, and the tandem-column SFC using column back pressure as a unique parameter for chiral separation.

(645) **Development and Validation of a Normal Phase Chiral HPLC Method for verification of Afoxolaner as a Racemic Mixture Using a Chiralpak® AD-3 Column**; Jinyou Zhuang[1], Satish Kumar[1], Abu Rustum[1]; [1]Merial, A Sanofi Company
Afoxolaner is a new insecticide-acaricide molecule from the isoxazoline family that acts on the insect aminobutyric acid (GABA) and glutamate receptors. Isoxazoline family of compounds have been employed as active pharmaceutical ingredient in drug products prescribed for control of fleas and ticks in dogs. Afoxolaner, with a chiral center at isoxazoline ring, exists as a racemic mixture of two enantiomers. A chiral normal phase high performance liquid chromatography (HPLC) method has been developed to verify that Afoxolaner is a racemic mixture as demonstrated by specific rotation. A Chiralpak® AD-3 column (150 mm × 4.6 mm I.D.) maintained at 35 °C was used in the method. Analytes were analyzed with an isocratic elution using 89% Hexane/ 10% IPA/ 1% MeOH (v/v/v) as the mobile phase with a detection wavelength of 312 nm. Desired separation of the two enantiomers was achieved in less than 15 minutes. The resolution and selectivity factors of the two enantiomers was 5.3 and 1.6 respectively. The Limit of Quantitation (LOQ) of the method is 1.6 ug/mL of Afoxolaner. This method was appropriately validated according to ICH guidelines for its intended use. ® All marks are the property of their respective owners.

(646) **Validation of *in-vivo* Raman Spectroscopy for Bladder Cancer Diagnosis**; Christiaan van Swol[1], Michelle Agenant[1, 2], Trudy Jonges[2], Olivier Wegelin[1], Ruud Bosch[2], Harm van Melick[1],Matthijs Grimbergen[1]; [1]St. Antonius Hospital Nieuwegein; [2]University Medical Center Utrecht
Bladder cancer is a significant public health problem responsible for more than 130,000 deaths annually worldwide. It is a malignant neoplasm originating from the surface lining (mucosa) of the bladder. Early diagnosis and complete resection of tumor lesions is essential to bring a change in prognosis. Currently disease assessment in bladder cancer is based on visual cystoscopic evaluation of the bladder wall. Fluorescence imaging techniques are being used to enhance visual contrast. This results in improved sensitivity but lacks the specificity required for use in general urologic clinics. Raman spectroscopy is an inherently specific technique that may allow tissue discrimination to the "pathologist's conclusion" level in a single spot within seconds. The aim of our study is to clinically use and validate the optimized Raman probe in a patient database in order to validate the differentiation between benign and malignant bladder lesions in vivo and to explore the possibility to use this technique for tumor grading and staging.

Patients, who were diagnosed with a tumor at the out-patient clinic were scheduled for transurethral resection of bladder tumor (TURBT) to evaluate the origin of the tumor (grade and stage). During this procedure a fiber-optic probe was advanced through the cystoscope, to obtain high-quality spectra from suspicious and non-suspicious bladder locations with collection times of 1-5 s. A biopsy was taken at the same time for histopathological analysis. Mathematical modelling using principal component analysis followed by linear discriminant analysis was used to correlate Raman spectra with histopathological diagnoses.

Two hundred and thirty-six Raman spectra were measured from a total of 90 patients. The algorithm was able to distinguish bladder cancer from normal bladder locations with a sensitivity of 75% and a specificity of 89%.

The in-vivo Raman probe system is able to distinguish between benign and malignant bladder tissue, within clinically acceptable measuring times (< 5 seconds). Evidence exists that it may be possible to use this technique for assessment of stage and grade of the disease, but further study is needed.

(647) **Raman Spectroscopic Techniques for the Identification of Ionizing Radiation Induced Damage in Tumour Cells**; Andrew Jirasek[1], Quinn Matthews[2, 3], Samantha Harder[2], Martin Isabelle[4], Julian Lum[3], Alex Brolo[2]; [1]University of British Columbia; [2]University of Victoria; [3]BC Cancer Agency; [4]Gloucester Royal Hospital

In this talk we assess the potential of Raman spectroscopic techniques in detecting radiotherapy-based ionizing radiation damage in tumour cells. We show that, in conjunction with Principal Component Analysis, Raman techniques can identify unique, independent signatures of radiation damage in an array of cancer cell lines irradiated in vitro. Damage signature can be traced to biological processes initiated as a result of radiation exposure. Finally, preliminary in vivo murine model experiments show promise in extending the technique to in vivo systems. The results point to the possibility of utilizing Raman techniques for patient monitoring or radiation therapy progression.

(648) **Exploration of Wavelength Effects on Deep Tissue Detection in Transmission Raman spectroscopy**; Adrian Ghita[1], Pavel Matousek[2], Nick Stone[1]; [1]University of Exeter, UK, [2]STFC Rutherford Appleton

Transmission Raman spectroscopy is a simple and versatile analytical technique for detecting the chemical composition inside bulk turbid samples. This non-invasive approach represents a promising tool for deep tissue detection of breast calcification which could also be used as a complementary technique to standard mammographic screening. The proof of principle of this approach was demonstrated by Stone and Matousek when Raman signal from a hydroxyapatite vial was detected inside 26 mm thick porcine tissue. Although the results are promising, in order to establish full clinical relevance of the method, the Raman signal has to be retrieved from tissue samples as thick as 40-50 mm. An important step in this direction is to find the optimum laser wavelength for detecting the calcifications located inside the breast tissue.

For this reason several scenarios in regards to sample position within the tissue and laser wavelength were examined experimentally. The sample consisting of a vial filled with trans-stilbene, was placed in front, middle and back of the phantom tissue to account for representative possible locations of calcification inside the breast. The search for the optimum laser excitation wavelength was carried out in region between 770 nm and 830 nm. For each laser wavelength and sample position the Raman spectra were recorded by a CCD detector.

To assess the Raman detection efficiency at different excitation wavelengths, 'peak visibility' of the specific Raman band (~1000 cm-1) was calculated for each measurement. The peak visibility was calculated using the ratio between the Raman band intensity and the average adjacent background noise level. The results obtained evidence different detection efficiency in respect to sample location within the tissue and laser excitation wavelength. The lowest likelihood of detection on the detection axis was found for a situation when the sample ('lesion') is located in the middle of the tissue. In conclusion the optimum laser wavelength for the detection of breast calcification is around 790 nm. Furthermore the sample position, or location of the volume of interest, represents a crucial factor in deciding the laser wavelength of choice for deep tissue screening.

(649) **Multiplexed Raman Micro-Spectroscopy using Spatial Light Modulators**; Faris Sinjab[1], Graham Gibson[1, 2], Miles Padgett[2], Ioan Notingher[1]; [1]University of Nottingham; [2]The University of Glasgow

Raman spectroscopy is a powerful tool which is increasingly used for materials characterization and investigation across a wide range of fields. Spontaneous Raman scattering is observed when a suitable laser is used to excite a sample, and the scattered radiation is typically analysed with a dispersive Raman spectrometer. When used in a micro-spectroscopy setup, the laser is focused onto the sample through a microscope objective, allowing a single acquisition at a time. This is a major restriction on when multiple point measurements are desired, such as in Raman imaging.

Multiple sampling points can be generated by using techniques from holographic optical tweezing [1], which use a computer-controlled liquid-crystal spatial light modulator (SLM) to adjust the phase of a laser beam such that a desired pattern of spots can be created when focused. Using this setup, an arbitrary number of sampling points can be created to allow multiplexed readout of Raman spectra, limited only by the maximum power of the laser and Raman CCD detector size, which can, in principle, be scaled up.

We have built and tested a prototype instrument, characterizing the performance in terms of spectral resolution, and testing simultaneous acquisitions of up to 25 Raman spectra from arbitrary positions on the sample. Furthermore, we have also demonstrated the potential for this technique in speeding up the intra-operative diagnosis of skin cancer, which has previously been demonstrated using a selective point scanning method [2].

For this application, the multiplexed Raman readout provides a further 10-fold decrease in diagnosis time. We showed that for skin resections obtained during Mohs micrographic surgery, diagnosis cab be obtained in only 5-10 minutes.

This new method for Raman micro-spectroscopy has great potential for increasing measurement throughput, which will have a large impact in industrial material characterization and medical and pharmaceutical sciences. Additionally, this new approach is also of interest in academic research in areas such as Raman optical tweezers, where novel types of experiment could be performed.

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(650) **Rapid Fiber-Optic Raman Spectroscopy Enhances *in vivo* Diagnosis of Adenomatous Polyps at Colonoscopy**; Zhiwei Huang[1]; [1]National University of Singapore

Colorectal cancer can be prevented if detected early. Endoscopic differential diagnosis of hyperplastic polyps and adenomas remains an unambiguous clinical challenge. Raman spectroscopy is an optical vibrational technique capable of probing biomolecular changes of tissue associated with neoplastic transformation. This work aims to apply a rapid fiber-optic Raman spectroscopy technique for real-time in vivo assessment of adenomatous polyps during clinical colonoscopy. We have developed a fiber-optic Raman endoscopic technique capable of simultaneously acquiring both the FP (i.e., 800–1800 cm-1) and HW (i.e., 2800–3600 cm-1) Raman spectra from colorectal tissue subsurface (<200 μm) for real-time assessment of colorectal carcinogenesis. In vivo FP/HW Raman spectra were acquired from 50 patients with 17

colorectal polyps during clinical colonoscopy. Prominent Raman spectral differences ($p<0.001$) were found between hyperplastic (n=118 spectra), adenoma (n=184 spectra) that could be attributed to changes in inter- and intra-cellular proteins, lipids, DNA and water structures and conformations. Simultaneous FP/HW Raman endoscopy provides a diagnostic sensitivity of 90.9% and specificity of 83.3% for differentiating adenoma from hyperplastic polyps, which is superior to either the FP or HW Raman technique alone. This study demonstrates that simultaneous FP/HW Raman spectroscopy technique has the potential to be a clinically powerful tool for improving early diagnosis of adenomatous polyps in vivo during colonoscopic examination.

(651) **Pushing the Envelope in Raman Spectroscopy: Identification of Organic Media in Art**; Celine Daher[1], Ludovic Bellot-Gurlet[2], Francesca Casadio[1]; [1]Art Institute of Chicago, [2]MONARIS UMR 8233 UPMC/CNRS

Raman spectroscopy is widely used to characterize different kind of materials in the cultural heritage field[1]. Indeed, it is fast, has high spatial resolution (of the order of μm), does not require elaborate sample preparation steps, it is non-destructive—the collected sample can be re-used for further analyses—and finally, it allows the possibility of *in-situ* non-invasive analyses with miniaturized portable devices [2, 3].

For many years the identification of organic materials has remained a challenging task for Raman analysis because of the high fluorescence they might induce, covering the vibrational features. These natural organic substances such as resins, oils, waxes, glues or gums are used both to create art (as binders for paintings, varnishes, inks, adhesives…) and to treat it. The two main approaches to remove the fluorescence background and recover the vibrational features of organic compounds, some studies present new mathematical approaches [4, 5]; but the most common way is to use FT-Raman instrumentation, equipped with a 1064 nm near IR excitation wavelength[6-9].

Another challenge regarding organic media is that they are often present as mixtures. This is the case for binders, mixed with minerals like pigments and charges or with other organic compounds such as varnishes[10]. How to identify a binder if the pigments it is mixed with give the strongest signal? How to detect an additive in a varnish if the main constituent's features dominate the spectrum? In some cases, additional data treatment (such as chemometrics) are necessary to go further in the data interpretation than just observing vibrational bands[11, 12].

Finally, natural organic media can exhibit different states of preservation leading to different spectral features[13]. Nonetheless, vibrational databases are built up on modern reference samples. How can an aged material be identified if no ancient references are available?

This paper will address these and other challenges posed to FT-Raman spectroscopy by the analysis of organic materials. Selected case studies and methods for advanced data treatment will be presented.

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(653) **Identification of Copper Resinate in Artworks: In a Quest for the Optimal Raman Procedure**; Jana Striova[1]; [1]National Institute of Optics-National Research Council

The acquisition of good quality Raman spectra is determined by many factors such as excitation wavelength or the Raman cross-section of the analyzed compound. Since the Raman signal intensity is proportional to λ^{-4}, short wavelengths are preferred but in this spectral region the fluorescence phenomena may compete and hinder the Raman signal. In this talk, the advantages and drawbacks of six different visible and near infrared laser sources in the acquisition of copper resinate pigment Raman spectra will be discussed. Painters such as Vermeer - Böcklin were supposedly fond of this green glaze but did also Caravaggio use it? We will inspect together whether and how the Raman spectrocopy can provide answer to this question by analyzing the Madonna dei Pellegrini painting. A brief excursion into other application fields, such as monitoring of consolidating treatments or cleaning operations, will show the Raman spectroscopy as a key information providing tool in the cultural heritage related tasks.

(654) **Detection of Natural and Synthetic Organic Colorants in Historic Oil Paintings using Surface-Enhanced Raman Spectroscopy**; Kristin Wustholz[1], Kristen Frano[1], Shelley Svoboda[2]; [1]College of William and Mary; [2]Colonial Williamsburg Foundation

Although several analytical methods provide conservators with the ability to identify inorganic colorants in works of art, the ultrasensitive detection of organic materials, those often most prone to fading and decomposition, is challenging. Recent advances in the field of surface-enhanced Raman scattering (SERS) spectroscopy have enabled the identification of various organic dyestuffs in small disperse samples and cross-sections from oil paintings. Here, we apply several SERS-based approaches to study red and blue pigments in a series of oil paintings that represents a variety of materials, artists, and techniques from Britain to North America during the 18th century. In particular, we examine the use of carmine lake, vermilion, Prussian blue, and indigo in Portrait of Frances Parke Custis by the Jaquelin-Brodnax Limner (ca. 1722), Portrait of Evelyn Byrd by an unknown London-based artist (probably 1725-1726), Portrait of John Custis IV by Charles Bridges (ca. 1735), Portrait of Elizabeth Allen by Jeremiah Theus (1759), and Portrait of Isaac Barre by Sir Joshua Reynolds (1766). The results of these SERS-based analyses reveal the presence of organic pigments in 18th-century oil paintings, not only in broad-scale quantity-rich areas of the composition, but also the more challenging fleshtones and small, yet important, detail regions. Moreover, several modern 20th-century pigments were detected in three

18th-century paintings using SERS, likely a result of past conservation treatment. Collectively, these SERS findings provide a more accurate reflection of organic materials in 18th-century British-North American Transatlantic portraits and ultimately, inform the digital reconstruction of two portraits so that art historians and conservators can better understand the original intent of the artist.

(655) **Raman Spectroscopy for Cultural Heritage: A Powerful Technique in the Conservation Scientist's Toolbox**; Federica Pozzi[1]; [1]Solomon R. Guggenheim Museum

Since the first reported application to the analysis of artifacts in the late 1970s, the field of Raman spectroscopy for cultural heritage has grown enormously. Initially used for pigment identification, this technique has gradually found application in the study of a wide variety of materials - including minerals and gems, traditional and modern synthetic pigments, enamels and glazes, gums, waxes, and resins - from objects of artistic, historical and archaeological significance. The increasing body of publications that has appeared in the literature in the past decades has highlighted, among the main advantages, its high molecular specificity, sensitivity, spatial and spectral resolution, versatility in terms of experimental and instrumental set-up, non-destructivity, suitability for the study of whole artifacts and inhomogeneous surfaces, as well as equipment mobility and portability enabling analysis in the field. The need of overcoming everyday issues, e.g. the swamping of the intrinsically weak Raman signal by an intense fluorescence emission from the analyte or other fluorophores contained in the specimen under study, has prompted researchers to push the technique to its limits, exploring the application of alternative methods, such as subtracted shifted and surface-enhanced Raman spectroscopies (SSRS and SERS), for the characterization of natural dyes and other fluorescing organic molecules. Recent additional achievements in the field include the elucidation of materials' degradation processes, the use of chemometrics and mathematical approaches for quantitative Raman analysis, the spectral mapping of surfaces, and the application of confocal and micrometer-scale spatially offset Raman spectroscopy (SORS) for the analysis of paint layers.

In this talk, technological advances and object-based research that have turned Raman spectroscopy into a routine technique in academia, museums and other cultural heritage institutions will be discussed, along with recent examples of its application to the analysis of artworks from the Art Institute of Chicago, the Metropolitan Museum of Art and the Solomon R. Guggenheim Museum.

(656) **Mass Spectrometry Tools for Probing Cell to Cell Chemical Heterogeneity**; Jonathan Sweedler[1]; [1]University of Illlinois

Cell-to-cell chemical heterogeneity is a fundamental property of multicellular organisms. Subpopulations and rare cells can often be found even within presumably homogeneous cell populations. To study these subpopulations, high-throughput methods that can perform large numbers of single cell measurements are required. Several mass spectrometry (MS) approaches that can assay the small molecules and peptides from individual cells are highlighted including direct single cell MS and mass spectrometry imaging (MSI). We have developed several approaches to work with specific cells, and now have adapted these methods to work with larger populations of cells. As one approach, we use optical microscopy-guided single cell MSI to probe thousands of individual cells. The cells are deposited onto a microscope slide at low to medium density. Cell locations on the slide are then determined using microscopy, and these locations guide subsequent MS analysis specifically to each cell. The MS data sets are analyzed to using principal component analysis (PCA) and PCA-based outlier detection. When used together, these approaches reveal both major and minor data patterns to enable large subpopulations and rare cells to be found. Because MSI typically consumes a small portion of the available analytes from the sample, we can perform post analyses on selected cells after our MS characterization. We demonstrate this approach on several cellular samples including neurons and endocrine cells from rodents. These MS-based tools do not require analyte preselection and are well suited for looking at complex and poorly characterized cellular populations.

(657) **Microchip Electrophoresis: A Mid-Career Method?**; Adam Woolley[1]; [1]Brigham Young University

The field of microchip electrophoresis has seen extensive growth since its introduction in the early 1990's. With the passage of time, is microchip electrophoresis in the middle part of its life cycle, or even becoming a "mature" technique? From the vantage point of my research group, microchip electrophoresis continues to be an enabling technology for bioanalysis. We have been fabricating integrated microfluidic systems for the determination of biomarkers linked to pre-term birth. We are constructing porous polymer monoliths within microdevices for affinity extraction of targeted peptides and proteins, and for solid-phase enrichment and fluorescence labeling of the captured analytes. We then combine these on-chip sample handling steps with rapid microchip electrophoresis separation in an integrated microdevice, thus providing automated analysis. In previous work, we have shown that immunoaffinity extraction can be combined with microchip electrophoresis for the quantitation of cancer biomarkers [1]. Furthermore, we have demonstrated on-chip solid-phase extraction and fluorescence labeling on monolithic columns in microfluidic devices [2-3]. We are utilizing electric fields, as well as pressure-based systems having on-chip valves and pumps, to drive fluids in our devices. Electrically driven fluid handling provides a simpler experimental approach; on the other hand, the use of valves and pumps allows for better control of small volumes, improving reliability of loading and elution. Current efforts are directed toward performing affinity extraction of pre-term birth biomarkers, followed by preconcentration and fluorescent labeling, and then subsequent separation through microchip electrophoresis all on a single integrated microfluidic device. Our approach is well suited for the analysis of trace biomarkers in a sample-sparing format, with microchip electrophoresis playing a key role in these bioanalysis systems.

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(658) **AC-Electrokinetics at Nanoscales: from Complex Nanocolloids to Macromolecules**; Elaine Zhu[1]; [1]University of Notre Dame

Despite the emerging interest in empolying AC-electrokinetics for rapid and effective manipulation and assembly of advanced functional materials for broad applications, detailed AC-polarization mechanism at nanoscales remains little understood. Deciphered by fluorescence imaging and fluorescence correlation spectroscopy at a single molecule or particle level, this talk will emphasize our recent studies of the structural dynamics of drug-encapsulated micellar nanoparticles, synthetic polyelectrolytes, and amyloid proteins in response to uniform and non-uniform AC-electric fields of varied frequencies and amplitudes. For nanoparticles whose size is reduced to be comparable to or less than the Debye screening length, the dynamic double-layer charging effect becomes critical to the interfacial polarization of nanocolloids. As a result, distinct interior polarization from nanocolloidal surface polarization under AC-fields could cause the disintegration of complex nanocolloids and

the release of interior chemical encapsulate. For synthetic polyelectrolytes and proteins whose surface charge is not fixed, conformational structures can be controlled by AC-fields with a strong dependence of applied AC-frequency when the applied AC-field voltage exceeds a threshold to effectively migrate counter-ions at charged macromolecular interfaces. Such AC-field induced conformational transition can be further exploited for supramolecular assembly.

(659) **Dielectrophoretic Monitoring of Alterations in C.difficile Colonization Due to Inter-Strain Antagonism**; Nathan Swami[1], Yi-Hsuan Su[1], Ali Rohani[1], Cirle Warren[2]; [1]Electrical Engineering, University of Virginia; [2]Infectious Diseases, University of Virginia

Clostridium difficile (*C.difficile*) infection (CDI) is a global toxin-mediated intestinal disease that is commonly attributed to exposure to pathogenic *C.difficile* strains following the elimination of healthy microflora in the gut by broad-spectra antibiotics. An emerging body of scientific and clinical studies has demonstrated that CDI rates can be controlled through promoting antagonistic inter-strain interactions on pathogenic *C.difficile* strains by pre-colonization with non-toxigenic *C.difficile* and other probiotic microorganisms, due to ensuing competition for the same nutrient and colonization niche. The emerging interest in restoration of a healthy microbiota to treat CDI highlights the need to understand and optimize the complex and dynamic interaction between the commensal polymicrobial environment and pathogenic *C.difficile* strains. Current methods to monitor the alterations in *C.difficile* colonization following culture within environments including non-toxigenic *C.difficile* and other probiotic microorganisms utilize cumbersome adhesion assays on human epithelial cell lines (HCT8). These are time consuming, thereby limiting the permutations of interactions that can be studied and do not provide direct information on the causes underlying the alterations in colonization. S-layer glycoproteins are part of the cell wall envelope in gram positive and gram negative bacteria, and are integral to surface recognition, gut colonization, host-pathogen adhesion and virulence. Hence, alterations in colonization properties of pathogenic *C.difficile* strains due to dynamical interactions with non-toxigenic *C.difficile* and other probiotic microorganisms are likely to alter the S-layer on the cell wall envelope. We demonstrate here that these alterations in the S-layer on the cell wall envelope of pathogenic *C.difficile* strains due to interactions with non-toxigenic *C.difficile* cause systematic alterations in call wall capacitance, thereby enabling their facile monitoring within just as few minutes, based on the characteristic shifts in their dielectrophoretic frequency spectra. We compare the influence of static versus dynamic co-culture methods on alterations in *C.difficile* colonization and benchmark this dielectrophoretic method versus conventional adhesion assays. In this manner, we envision the application of this microfluidic platform towards optimization of polymicrobial therapies to arrest CDI.

(660) **XFEL Diffraction from Protein Nanocrystals Isolated using a Microfluidic Sorter**; Bahige Abdallah[1], Nadia Zatsepin[1], Shatabdi Roy-Chowdhury[1], Jesse Coe[1], Katerina Dorner[1], Raymond Sierra[2], Hilary Stevenson[3], Guillermo Calero[3], Petra Fromme[1], Alexandra Ros[1]; [1]Arizona State University; [2]Stanford PULSE Institute; [3]University of Pittsburgh

The advent and application of the X-ray free-electron laser (XFEL) has uncovered the structures of proteins that could not previously be solved using traditional crystallography. While this new technology is powerful, optimization of the process is still needed to improve data quality and analysis efficiency. One area is protein crystal size heterogeneity , where variations in crystal size (among other factors) lead to the requirement of large data sets (and thus 10-100 mg of protein) for determining accurate structures. To decrease sample dispersity, we developed a high-throughput microfluidic sorter operating on the principle of dielectrophoresis, whereby polydisperse particles can be transported into various fluid streams for size fractionation. Using this microsorter, we isolated several milliliters of photosystem I nanocrystal fractions ranging from 200 to 600 nm in size as characterized by dynamic light scattering, nanoparticle tracking, and electron microscopy. Sorted nanocrystals were delivered in a liquid jet via the gas dynamic virtual nozzle into the path of the XFEL at the Linac Coherent Light Source where we obtained diffraction to ~ 4 Å resolution, indicating that the small crystals were not damaged by the sorting process. Using simulations, we show that narrow crystal size distributions significantly improve merged data quality in serial crystallography. Methods to accomplish this have not yet been implemented at an XFEL, thus from this novel proof-of-concept work, we can expect that the automated size-sorting of protein crystals will become an important step for sample production by reducing the volume of data needed for a high quality final structure.

(661) **Capturing Viruses using Dielectrophoretic Microdevice**; Jie Ding[1], Robert Lawrence[2, 3], Brenda Hogue[2,3], Paul Jones[1], Mark Hayes[1,4]; [1]Department of Chemistry and Biochemistry, Arizona State University; [2]School of Life Sciences, Arizona State University; [3]The Center for Infectious Diseases and Vaccinology, The Biodesign Institute, Arizona State University; [4]Arizona State University

Biotechnology, separation science, and clinical research are impacted by separation and manipulation of bioparticles such as DNA, protein and viruses in microfluidic devices. Microfluidics systems provide many attractive features, including small sample volume, rapid detection, high sensitivity and short processing time. Dielectrophoresis (DEP) and electrophoresis are especially well suited to microscale bioparticle control and have been demonstrated in many formats.

Since the first discovery in 1892, viruses have been a focus of study to reduce its unpredictable threat to human lives. Most detection methods are time-consuming and expensive, which would lead to delayed diagnosis and further loss on time, money and lives. To reduce the risk caused by virus, a lot of work has been done on developing an accurate, quick-response and cheap virus detection method. Considering all the advantages of dielectrophoresis, it has broad prospects in achieving this goal.

In this work, an optimized gradient insulator-based DEP (g-iDEP) device was utilized for concentration of Sindbis virus (SINV), an arbovirus (arthoropod-borne virus) with a diameter of 70 nm. It is also the archetype of alphavirus in the Togaviridae family with a typical icosahedral shape. It is the first time that SINV is captured using an evolved g-iDEP device. The concentrating behaviour of SINV has been investigated using a simplified model. Preliminary result on bacteriophage indicated the possibility of capturing different viruses in this g-iDEP device. Compared with other common separation techniques such as membrane filtering, centrifugation, and chromatograph, DEP-based microfluidics shows high sensitivity and more significantly it allows for fast separation and concentration of viruses in a single step within a short time.

(662) **Investigation of Spatial and Temporal Dynamics of Electrophoretic Exclusion on a Microdevice**; Fanyi Zhu[1]; [1]Arizona State University

Numerous separation techniques have been developed with enhanced sensitivity and resolution. While capillary electrophoresis is an exemplary technique, it suffers from poor mass sensitivity and therefore preconcentration strategies, including counterflow gradient focusing, isotachophoresis, filed-amplified sample stacking and so forth have been developed to minimize this limitation. Here we show an enhanced electrophoretic exclusion, a novel counterflow gradient focusing technique, which exploits hydrodynamic flow and electrophoretic forces to exclude, enrich, and separate analytes. Unlike a continuous gradient generated along the entire channel by most of the counterflow techniques,

our technique creates discrete gradients about the area between reservoirs and channels, making exclusion occur near the entrance to the channel. As a result, separation is independent of the length of the channel and can be accomplished through a microdevice with parallel or serial format. Moreover, species are separated in bulk solution based upon their native properties without the need for pretreatment, and it can be easily coupled to various techniques for detection, such as mass spectrometry, making it a promising technique for future separation development.

In the current work, we adapted this technique to a microdevice, which was designed to have one large entrance reservoir and three parallel separation components, with a specific voltage configuration for each. Qualitative studies focused on channel area with fluorescent dye molecules, demonstrating exclusion phenomenon when electric field was applied. When voltage was released, a bright bolus passed through the channel under hydrodynamic flow, which further verified the exclusion. Moreover, for this microdevice, finite element analysis models using commercial COMSOL software were built to aid our understanding of this technique. Based on our previous work, it is believed that steeper gradient, smaller channel diameter and slower flow rate all contribute to a better resolution on benchtop devices. Similar factors were also manipulated in microdevice both by simulations and experiments to explore the temporal and spatial dynamics, the resolving power, the degree of concentration and so forth. The combination of information studied above can be beneficial for next generation design aiming at better resolution, higher capacity and easier manipulation.

(663) **MPT-MS, a Versatile Platform for Analytical Chemistry**; Huanwen Chen[1], Haidong Wang[1], Rui Su[2], Konstaintin Chingin[1]; [1]Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology; [2]Changchun University of Chinese Medicine

Ambient mass spectrometry (AMS) is of increasing interest for the direct molecular analysis of raw chemical samples owing to the high speed and simplicity of analysis. However, the majority of AMS methods favors soft ionization process, and thus lacks power to desorb and ionize sufficient amount of materials in condensed phases [1-3].

Microwave plasma torch (MPT) was originally introduced in 1985 as a light source for atomic emission spectroscopy (AES) by Prof. Q. Jin[4]. Unlike the traditional inductively coupled plasma (ICP), MPT utilizes microwave energy to sustain plasma, which allows much lower operation power and gas consumption. In our lab we are exploring the possibility of applying MPT for the direct molecular ionization in ambient environment. MPT offers considerably higher energy of excitation plasma compared to the available ambient ion sources. Owing to its compact size, MPT can be easily mounted and adjusted in front of MS instruments with atmospheric interface.

Our preliminary results indicate that MPT-MS can be considered as a highly versatile tool for the ambient ionization of various samples. Importantly, the amount of energy coupled to the sample during MPT ionization is directly controlled by the distance between the end of the torch and the sampling spot. At large distances (> 3 cm), the plasma temperature is relatively low, so that the intact molecular ions are preferentially produced. At shorter distances, where the plasma is substantially hotter, analyte ionization is accompanied by the notable in-source fragmentation. Thereby, the same MPT platform can be operated in the complementary modes of analysis covering broad range of ionization energies. Further, the ambient reactions of analyte molecules with MPT ions, e.g., water cations and nitrate anions, substantially enhance the chemical specificity of MPT-MS. In present report, these and other aspects of ambient MPT-MS analysis are discussed with regard to model applications.

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(664) **Interpreting Geological Mineral Mixtures with Combined Raman-LIBS Spectroscopy (RLS)**; Nina Lanza[1], Samuel Clegg[1], Roger Wiens[1], Rhonda McInroy[1]; [1]Los Alamos National Laboratory

Raman and laser-induced breakdown spectroscopy (LIBS) are highly synergistic analytical techniques that are each capable of detailed characterization of geological materials. Raman is sensitive to molecular structure while LIBS provides information about elemental composition; these complimentary measurements are highly useful for many geological applications. This is because stoichiometry alone cannot always provide a unique solution for mineralogy (crystal structure), and vice versa. For example, although the minerals calcite and aragonite have the same chemical formula (CaCO3), their mineral structures are different; calcite forms trigonal lattices while aragonite forms orthorhombic lattices. Similarly, two minerals may have the same crystal structure but different stochiometries; calcite (CaCO3) and siderite (FeCO3) are structurally identical but chemically distinct. Most natural rocks are composed of suites of minerals that may or may not be geochemically related, depending on the rock's origin. Although minerals in igneous rocks are geochemically related by a distinct crystallization sequence, sedimentary rocks commonly contain physical mixtures of unrelated minerals. In order to correctly identify and interpret sedimentary materials, it is vital to identify both their chemistry and mineralogy.

Both Raman and LIBS are laser-based analytical techniques that can gather information about samples at a standoff distance. Both have previously been separately applied to geological problems, most notably LIBS as part of the ChemCam instrument suite onboard the Curiosity Mars rover. Because of its unique capabilities, a combined Raman-LIBS instrument, SuperCam, has been selected to fly on the upcoming Mars 2020 rover mission. Here we will report the results of a laboratory study currently underway to understand the Raman-LIBS signatures of physical mixtures of geological materials. Of particular interest is determining the limits of detection for low abundance mineral species within such mixtures. Geological standard materials with a range of compositions and mineralogies were selected, as well as one basalt (mixed mineralogy). These standards were mixed in varying proportions with the basalt standard using a mortar and pestle and then pressed into pellets for LIBS and Raman analysis. Unmixed pellets of each standard were also analyzed to provide end-member spectra. Results are forthcoming.

(665) **Atmospheric Microwave & Micro Plasmas for Ambient Desorption/Ionization Mass Spectrometry**; Yixiang Duan[1]; [1]Research Center of Analytical Instrumentation, College of Life Science, Sichuan University

Ionization source is the most important part in mass spectrometer system. Since the desorption electrospray ionization (DESI) technology was first described in 2004, dozens of ambient desorption/ionization technologies have been developed with various capabilities for mass spectrometry. Ambient desorption/ionization technologies have been typically classified by desorption mechanisms as spray droplet assisted desorption, laser desorption/ablation techniques, and plasma-based techniques. Comparing with spray droplet assisted desorption, plasma-based sources employ various electrical discharges instead of charged droplets to generate radicals, excited atoms, and electrons. There have been several sources such as direct analysis in real time (DART) source, dielectric barrier discharge ionization (DBDI), atmospheric pressure glow discharge desorption/ionization (APGDI), flowing atmospheric-pressure afterglow (FAPA) source, microhollow cathode discharge microplasma source, low temperature plasma (LTP) source, plasma-assisted desorption/ionization (PADI) and so on. In our work, we have developed several atmospheric plasma desorption/ionization sources, mainly micro-fabricated glow discharge plasma (MFGDP) and microwave induced plasma desorption/ionization (MIPDI) source. These sources can be used for solid, liquid, gaseous and real sample analysis. MIPDI source was constructed in a Surfatron structure, where non-equilibrium microwave-induced argon or helium plasma can be generated. Distinguished from other plasma-based ion sources, the MIPDI source can be used for polymer (e.g., PEG 800) analysis with a molecular weight over 1,000 Da. The simple construction, reduced gas flow rate, and ability to sustain plasmas with alternative gases for efficiently desorb and ionize analytes directly from samples surface in the ambient conditions make the MFGDP and MIPDI satisfactory ionization sources for ambient mass spectrometry. MFGDP device has a very simple configuration with two platinum electrodes set face to face within an alumina ceramic-constructed discharge chamber. The micro-plasma can be generated and maintained by DC micro glow discharge with Ar/He as working gases. Detection limits of solution and dry spots were calculated to be in a level of ng to fg mm2. Applications on pesticide residue screening were conducted by analyzing multi-class compounds in spiked solutions, extracts of fruits and vegetables, and real samples. Limits of detection (LODs) at levels down to 0.13 ng g-1 mm-2 of the spiked extracts of tomato, green pepper, and orange were obtained, with RSDs of 3.48%-13.46%. Taking advantage of the low temperature of the micro-plasma, distribution of diphenylamine in apples was mapped through scanning cut apple piece-by-piece in depth directly.

(666) **Analytical Instrumentation for Future Planetary Exploration Missions**; Peter Edwards[1], Ian Hutchinson[1], Richard Ingley[1]; [1]University of Leicester

Planetary exploration presents unique challenges for analytical science; instruments must be low mass, have minimal moving parts, operate autonomously, robust and determine chemical or molecular information about a target.

Laser Induced Breakdown Spectroscopy (LIBS) provides remote chemical analysis of a target. ChemCam, an instrument on NASA's Mars Science Laboratory rover, is the first LIBS instrument to be utilised on a planetary mission. Around 228000 individual spectra have been acquired for more than 700 targets enabling the characterisation of many of the environments along Curiosity's route (the instrument is capable of determining the composition of a target area diameter ~ 500µm at around 7m).

However, the small spot size is not ideal for bulk analysis; a particular mineral grain may give an unrepresentative composition of a target rock. Sulphur peaks are also low in intensity compared to other elements, making quantitative analysis of sulphate rich environments challenging. With only elemental data available, molecular identification can be complicated (two distinct minerals can have the same composition); therefore it is advantageous to combine LIBS with other techniques to acquire information on material composition to answer the fundamental science questions of a particular mission.

A number of analytical methods could provide complementary data. For example, Raman spectroscopy is another laser based technique which provides molecular and mineralogical information, as is Laser Induced Fluorescence (LIF). X-ray florescence (XRF) provides information on the chemical content of a sample, whilst X-ray diffraction (XRD) gives molecular/structural information.

No single technique is ideal for all materials and mission science goals. Each one has particular strengths and weaknesses and the optimum combination of techniques depends on a number of different factors and trade-offs. In this work we compare the performance of particular analytical combinations using a combination of laboratory data obtained with portable and ExoMars prototype instruments, MSL data and radiometric simulations. We also comment on the challenges/limitations involved in building such combination instruments in order to recommend specific payload configurations.

(667) **Advanced Experimental Design for Simultaneous Acquisition of Laser Induced Plasma and Raman Signals**; Soo-Jin Choi[1], Jae-Jun Choi[1], Dae-Hyoung Kim[1], Dong-Woo Han[1], Jack J. Yoh[1]; [1]Seoul National University

LIBS (Laser-Induced Breakdown Spectroscopy) is a technique to analyze atomic composition and isotopes by detecting plasma light occurred by the laser ablation. Raman spectroscopy, on the other hand, uses the shifted emission light which is unique for each molecule for its composition analysis. The combined Raman-LIBS system requires two lasers due to difference of proper irradiance for each method while most of the optical components can be shared as a single unit. A method for obtaining both LIBS and Raman signals simultaneously using a single Gaussian laser beam was reported in [1], even though optimum gate width for their Raman detection was never used, followed by the plasma signal detection.

A beam expander is employed to extend the laser beam for obtaining the Raman signal without interference with the plasma. Also a focusing lens of small diameter was used to generate the laser induced plasma for LIBS. By the proposed experimental design, simultaneous acquisition of laser induced plasma and Raman signals is possible using the optimum gate width for Raman detection which is about hundred nano-seconds.

(668) **Comparative Peptidomic Analysis Towards Functional Discovery of Neuropeptides**; Lingjun Li[1]; [1]University of Wisconsin

All nervous systems employ many neuropeptides as chemical messengers. Using a sensitive mass spectrometry (MS)-based platform, more than 300 neuropeptides have been discovered in a well-defined crustacean nervous system, revealing that even a relatively simple neural network contains an unexpectedly-rich diversity of neuropeptides. To further explore the functional consequences of peptide multiplicity, MS imaging techniques and in vivo microdialysis sampling technique are employed to obtain spatial and temporal information of peptidergic signaling, respectively. Furthermore, novel isotopic and isobaric labeling techniques have been developed and employed to produce differential display of neuropeptidomes under different physiological conditions. Examples of neuropeptide regulation of feeding behavior and environmental stress will be highlighted. Collectively, these combined studies will help elucidate the functional roles that neuropeptides play in regulating neural plasticity.

(669) **Pushing the Limits of Vibrational Spectroscopic Imaging with New Technology**; Rohit Bhargava[1]; [1]University of Illinois at Urbana-Champaign

Vibrational spectroscopic imaging offers the convenient integration of microscopy and spectroscopy, yet offers significant challenges that go beyond either field. While microscopic data recording complicates interpretation of spectra compared to traditional approaches, large volumes of data complicate information extraction compared to conventional optical microscopy. We show that these complications can not only require careful understanding of the data acquisition process but can also lead to new opportunities for faster, higher performing and insightful spectroscopic analyses. We first describe recent advances in theory of infrared microscopy that allow an appreciation of the resolution question in chemical imaging. Next, we present several examples from IR and Raman microscopy to demonstrate how the interpretation of straightforward systems can be complicated by various confounding aspects. We present corrections and theory to demonstrate that these effects can be understood and corrected in some cases. Finally, we present results from instrumentation developed in the past few months to demonstrate that high-resolution and rapid imaging can lead to novel biomedical images that allow significantly improved information for the user.

(670) **Automating Epigenomics: Progress towards a Microfluidic Chromatin ImmunoCapture Device**; Ryan Bailey[1], Yi Xu[1], Steven Doonan[1], Richard Graybill[1], Tamas Ordog[2]; [1]University of Illinois at Urbana-Champaign; [2]Mayo Clinic

Epigenomic tests, and in particular assays that probe protein-DNA interactions, are an emerging tool in the development of personalized cancer treatment strategies. Chromatin immunoprecipitation (ChIP) is the gold standard for analyzing protein-DNA interactions; however, conventional ChIP is limited in its clinical utility as it is extremely laborious and requires large cellular input (1-10 million cells). These requirements significantly reduce the applicability of ChIP in a clinical setting—particularly when minimal sample is available, such as in the analysis of tumor biopsies, stem cells, or circulating tumor cells.

Microfluidic devices offer many attractive benefits over traditional macro-scale methods including reduced volume requirements, parallelization capability, and automated operation, which make them particularly well-suited to sample-constrained epigenomic analyses. We are developing a powerful and versatile, droplet microfluidics-based platform that will allow for automated ChIP analyses. Our platform incorporates every major step in the ChIP workflow into an automated device, including cell lysis, chromatin digestion, immunocapture, and DNA purification. Importantly, these processes will eventually be carried out at the single cell level, which promises to provide unique insights into epigenomic tumor heterogeneity. Beyond single cells, the unprecedented ability to handle samples of variable input will also facilitate robust validation against traditional ChIP assays to demonstrate broad genomic coverage. Taken together, we feel that the resulting platform will be a powerful new tool that helps enable the translation of epigenomic insight into individualized cancer treatment at the point of care.

(671) **Measuring Neurochemicals *in vivo* using LC-MS**; Robert Kennedy[1]; [1]University of Michigan

Measuring neurochemicals in vivo offers an unparalleled window on the brain. With such measurements it is possible to identify chemical signals associated with brain functions, distrubances from diseases, and pharmacological effects. Sensors and imaging techniques are powerful methods for such measurements; however, they lack ability to monitor multiple neurochemicals. Furthermore, it can be exceedingly difficult to develop new probes for each neurotransmitter. Sampling from the brain and then analyzing fractions by LC-MS can overcome these problems. We have developed comprehensive LC-MS methods that allow nearly all small molecule neurotransmitters to be measured. We have also developed methods to monitor neuropeptides in vivo. These molecules are challenging because of their low concentration and multiple adsorptive mechanisms. These methods have been used to phenotype animals, identify changes associated with learning, define novel circuits, and eluciate new drug mechanisms. These methods and their applications will be described.

(672) **Single-cell Mass Spectrometry Tells of Asymmetry in the Body Plan of the Early Developing Embryo**; Peter Nemes[1], Rosemary Onjiko[1], Sally Moody[1]; [1]The George Washington University

Characterization of biomolecular pathways underlying the patterning of the body axis is essential to understanding normal embryonic development and finding therapeutics for congenital anomalies. How small molecules, particularly metabolites, are implicated in the establishment of developmental asymmetry is little known because it has not been technologically feasible to measure a broad spectrum of small molecules in single embryonic cells (blastomeres). Here, we utilize a single-cell mass spectrometry technology and develop new measurement methodology to compare metabolite production between single blastomeres that occupy the left and right half of the embryo at the earliest stages of development when microinjection studies confirmed asymmetry. We micro-dissected D1 blastomeres from 8-cell and D11 blastomeres from 16-cell embryos that give rise to nervous tissues of the South African clawed frog (Xenopus laevis), a popular model in cell and developmental biology. After extracting the polar and apolar portion of their metabolomes in 5 µL using a two-pronged approach, metabolites from a 10 nanoliter volume of these extracts were separated using a custom-built single-cell capillary electrophoresis instrument. Metabolites were identified and relatively quantified by an electrospray ionization high-resolution mass spectrometer to test our hypothesis that the metabolome reveals left-right asymmetry.

Multivariate and statistical evaluation of the resulting mass spectrometric data revealed complex metabolic heterogeneity between blastomeres on the left and right sides. Of 130+ molecular features, 80+ distinct metabolites were consistently measured, 40 of which were identified with outstanding confidence. A total of 12 distinct metabolites were differentially enriched between the left and right D1 blastomeres (t-test's p value below 0.05 and fold change above 2), and these differences were also detectable for a subset of metabolites in the D11 blastomeres. Furthermore, we extended our measurements to ask whether developmental asymmetry correlates with molecular chirality. That a spectrum of metabolites tells of developmental asymmetry already in the 8-cell-stage of the embryo is surprising and contributes to a deeper understanding of body patterning in developmental biology. The results we describe here also underscore the central role that bioanalytical chemistry and instrument development holds to enable us to ask new types of questions in basic and translational research.

(673) **A Portable Clinical Grade Raman Device for Point-of-Care Diagnosis of Gout and Pseudogout**; Ozan Akkus[1], Bolan Li[1], Nora Singer[2], Donard Haggins[3], Yener Yeni[3]; [1]Case Western Reserve University; [2]Metro Health Hospital; [3]Henry Ford Hospital

Background: Monosodium urate monohydrate (MSU, causes gout) and calcium pyrophosphate dihydrate (CPPD, causes Pseudogout) are two main crystals leading to joint diseases. Raman spectroscopic analysis of synovial aspirates carries a diagnostic potential. In this work, Raman spectroscopy was translated to clinical applications by developing a cost-efficient and portable device that can identify synovial crystal species at the point of care. This compact clinical grade Raman device can speed the time of clinical diagnosis of gout and pseudogout while maintaining the accuracy comparable to that attained by microscopic analysis.

Materials and Method: Synovial aspirates were obtained from Metro Health Hospital (Cleveland, OH) and Henry Ford Hospital (Detroit, MI). The synovial fluids were briefly digested with chemicals (hyaluronidase, proteinase K, and SDS) to dissolve the organic debris and release the crystals. The digested synovial fluid was transferred to a regular syringe and filtered through a custom-made filter cartridge mounted at the syringe tip; the filter cartridge was designed to guide and constrict the flow of the fluid to a spot, retaining crystals over a ~1 mm diameter spot. Following the microfiltration, the filter cartridge was inserted into a cost-efficient automated Raman device (CARD) designed for identifying crystal species.

Results and Discussion: By using CARD, the diagnosis procedure only took ~1 hour, and the limits of detection of MSU and CPPD crystals were 0.1 μg/mL and 1 μg/mL respectively, far less than the reported clinical concentrations (10 ~ 100 μg/mL). Based on the preliminary clinical study (N = 174), CARD carries a sensitivity of ~90%, and a specificity of 100%. While detecting crystal species, CARD can also estimate the crystal concentrations, which would relate to the disease severity. CARD has potential to be a novel tool for gout/pseudogout diagnosis, and could potentially improve use of inpatient resources and the overall quality of patient care.

(674) **Bone Tissue Composition and Heterogeneity at the Micro and Nano-scale**; Adele Boskey[1], Eduardo Villareal[1], Lyudmila Spevak[1], Richard Mendelsohn[2]; [1]Hospital for Special Surgery; [2]Rutgers University

Bone is a heterogeneous tissue with structural variations at low (microscopic) and high (AFM) levels of resolution. We have previously used Fourier transform infrared microscopy (FTIRM) and imaging (FTIRI) to demonstrate variations in chemical composition in terms of bone mineral and matrix content in human, non-human vertebrate, ovine and rodent bones. In the FTIRI studies, heterogeneity was defined as the line-width at half-maximum of the pixel distribution for each calculated parameter (mineral-to-matrix ratio; carbonate-to-phosphate ratio; crystallinity; acid phosphate substitution; collagen maturity). At the micron-level, in the case of human bones, heterogeneity of FTIRI variables decreased with patient age; comparing age-matched pairs of patients with fragility fractures, the only variation in heterogeneity was an increase in crystallinity heterogeneity in the cortical regions of the bone. This was associated with an age-independent significant increase in the mean values for crystallinity. At the nano-level using AFM-IR (Analysis) a 4 um repeat distance was noted for each of these parameters in osteonal bone. We suggest these variations are significant contributors to the bone tissue heterogeneity which in turn effects bone mechanical properties.

(675) **Emerging Magnetic Resonance Imaging and Spectroscopy Methods for the Assessment of Osteoporosis**; Chamith Rajapakse[1]; [1]University of Pennsylvania

It is estimated that over 200 million people worldwide suffer from osteoporosis, predisposing them to bone fractures. Hip fracture, for example, is a devastating event—within a year of the injury, 20-30% of patients die and 50% lose the ability to walk. Bone mineral density derived from Dual energy X-ray absorptiometry, an approach which has many limitations, is still used as the clinical standard for diagnosing and managing osteoporosis. A reliable method is required to determine who might benefit from osteoporosis medication that can reduce fracture risk. Recent advances in high resolution magnetic resonance imaging (MRI) and spectroscopy (MRS) have paved the way for novel noninvasive techniques for three-dimensional quantification of bone quality. For example, it is now possible to obtain high-resolution images of the proximal femur depicting the trabecular and cortical microstructure in human subjects. These images can be analyzed to estimate the structural and mechanical competence of bone. More recently, bone water assessed by MRI has been proposed as a new biomarker for bone quality. MRS of the bone marrow has also been proposed as another measure of bone quality. The flexibility of magnetic resonance techniques to provide information from molecular to macro scale could improve the assessment of bone quality in the future.

(676) **Developing Raman Spectroscopy for Clinical Detection of Peripheral Nerve Injury**; Katherine E. Cilwa[1,2], Eric A. Elster[3,4], Benjamin K. Potter[1,3,4], Jonathan A. Forsberg[1,3,4], Nicole J. Crane[1,2,3]; [1]Regenerative Medicine, Naval Medical Research Center; [2]Henry M. Jackson Foundation for the Advancement of Military Medicine; [3]Uniformed Services University of Health Sciences; [4]Walter Reed National Military Medical Center

Peripheral nerve dysfunction is the second leading cause of long-term disability in injured service members and injury is present in over 30% of combat injuries. This is in striking contrast to an incidence of only 3% in civilian trauma. Innervation of organs and musculoskeletal tissue is necessary for wound repair and preservation of healthy tissue. Without repair, peripheral nerve injury may lead to sensory and motor dysfunction, neuroma formation, and atrophy of distal tissue. The ability to determine tissue viability during the surgical repair of extremity injuries is vital to future return to normal function. The purpose of this study is to develop Raman spectroscopy as a methodology for detection of nerve damage. Normal and injured intact human peripheral nerve specimens were obtained as part of a discarded tissue protocol with Walter Reed National Military Medical Center. Following excision specimens were immediately snap-frozen at -80 °C until Raman collection. Raman spectra were collected every 5 mm on both sides of each intact nerve using a Kaiser Rxn1 System with PhAT probe attachment, 785 nm excitation, and 3mm spot size (Kaiser Optical Systems, Inc., Ann Arbor, MI). Following Raman collection, specimens were fixed in neutral buffered formalin, paraffin embedded, sectioned for histological staining and evaluation. Preprocessing of spectra was conducted using in-house MATLAB® scripts, and Raman metrics were determined using custom peak fitting MATLAB®. Band area ratios (BARs) were calculated using curve fit derived band areas of peaks of interest. BARs calculated include protein lipid (1240/1270 cm^{-1}) and disordered protein/total protein (1240/1340 cm^{-1}). BARs reveal decreased lipid content and increased protein disorder within injured nerves. This surgically deployable tool may be used to assess nerve viability and combined with other biomarkers to guide surgical decision making to ultimately speed recovery and reduce disability.

(677) **Mid Infrared Fiber Optic Evaluation of Ligament and Tendon Composition**; Mugdha Padalkar[1], Cushla McGoverin[1], Arash Hanifi[1], Nicholas Caccese[1], Padraig Glenn[1], Scott Barbash[2],Eric Kropf[2], Nancy Pleshko[1]; [1]Dept. of Bioengineering, College of Engineering, Temple University, Philadelphia PA; [2]Dept. of Orthopaedic Surgery and Sports Medicine, Temple University, School of Medicine, Philadelphia, PA

More than 350,000 anterior cruciate ligament (ACL) injuries occur every year in the United States. A torn ACL is typically replaced with an allograft or autograft tendon (patellar, quadriceps or hamstring). However, the process of ligamentization, transformation of a tendon graft to a healthy functional ligament, is poorly understood. Currently, histology, which requires harvesting of the tissue, is the gold standard method to assess the remodeling of the graft in animal studies. Ideally, a non-destructive method for assessment of graft composition could be implemented in a clinical setting. Fourier transform infrared (FT-IR) spectroscopy exhibits a high degree of specificity to molecular structure and compositional changes in tissue. The mid infrared (MIR) spectral absorbances from connective tissues (primarily collagen and other proteins) are well understood and widely used to study connective tissues. Here, we investigated the feasibility of infrared spectroscopy fiber optic analysis to differentiate between ligament and tendon as a first step toward development of a spectroscopic tool for clinical assessment of ligamentization.

MIR fiber optic data were collected from freshly dissected bovine ACLs (n=12) and patellar tendons (PT) (n=12) using a Thermo Scientific Nicolet iS5 FT-IR spectrometer with a TGS detector fitted with a fiber optic coupler (Harrick Scientific Products, Inc., Pleasantville, New York), and a silver halide ATR-loop mid-infrared fiber optic probe with a 6 mm diameter (Art Photonics, Berlin, Germany). Spectra were collected in the frequency range of 1000-1800 cm^{-1} at $8cm^{-1}$ spectral resolution with 32 co-added scans. Spectra were multiplicative scatter corrected followed by a 13 point smooth second derivative processing. A partial least squares discriminant analysis (PLS-DA) model was developed to differentiate between ligament and tendon. The performance of the model was determined based on the percent correct classification of ACL and PT. It was found that PLS-DA based on mid-IR spectral data resulted in 95% correct classification, with the primary absorbances contributing to the model based in the protein amide II region. This study will lay a foundation to develop a minimally invasive method for evaluation of healing ligament injuries. Better understanding of ligamentization could lead toward improved therapeutic interventions and post-operative protocols.

(678) **Rapid Analysis of Breast and Prostate Tissue using Conventional FTIR and Tuneable Infrared Quantum Cascade Laser (QCL) Based Imaging**; Peter Gardner[1], Michael Pilling[1], Alex Henderson[1], Ben Bird[2]; [1]Manchester Institute of Biotechnology, University of Manchester; [2]Daylight Solutions

Infrared hyperspectral chemical imaging is proving to be a promising technique that can complement digital histopathology. Applications include screening of biopsy tissue via automated recognition of tissue/cell type and disease state based on the chemical information from the spectrum. Integration into clinical practice, however requires, a reduction in data acquisition times in order to be compatible with standard pathology laboratory workflows and timescales. Fourier Transform Infrared (FTIR) imaging with focal plane array (FPA) detectors is currently the state-of-the-art instrumentation for infrared absorption chemical imaging. Recent developments in broadly tunable mid-IR lasers, however, have transformed the IR spectroscopic landscape and opened up new possibilities for rapid large area IR imaging 1-4. Although the multiplex advantage of FTIR is ideal when it is necessary to exploit the full mid IR spectral range, it is also the case that when just a few frequencies are required, significant speed improvements can be realised using discrete frequency chemical imaging. In this paper we show how once spectral biomarkers have been obtained (using conventional FTIR) these can be used these can be exploited using a quantum cascade laser (QCL) based spectral imaging microscope. Examples will be given of rapid imaging of prostate and breast tissue TMAs demonstrating the potential utility of this methodology in the clinical environment.

We gratefully acknowledge Daylight solutions for the loan of the Spero QCL microscope which enabled this study to be carried out.

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(679) **The Role of High Resolution FTIR Spectrochemical Imaging in Resolving Clinical Issues**; Kathleen Gough[1]; [1]University of Manitoba

The limits to spatial resolution in FTIR spectrochemical imaging of tissues are being pushed to, and beyond, the diffraction limit. With the appropriate optics, nominal 1.1 micron pixel edge spatial resolution can be achieved with a thermal-source FTIR microscope (in our lab: Agilent Cary 620 microscope, 64x64 Focal Plane Array attached to an Agilent Cary 670 spectrometer). This enables rapid biodiagnostic ex vivo tissue imaging in-house, with images collected over larger areas, in less time (minutes) and with comparable quality and resolution to the highest current synchrotron source FTIR wide-field imaging capability. With the micro-ATR attachment, pixel dimensions of ~250 nm can be achieved (Findlay et. al. Analyst 2015,140:2493-2503). With our in-house designed tomo accessory, bench-top FTIR tomographic imaging of individual cells is now possible. The implications for clinical applications are numerous. Rapid, high resolution imaging of tissues offers the promise of rapid screening in a clinical setting, provided the appropriate protocols can be defined. The benefits and potential for this approach will be discussed in the context of two on-going research projects in our group: analysis of cells in neurodegenerative disease and analysis of soft tissue damage and repair in stressed tendon. The results from high resolution imaging can inform applications at any length scale, whether the long term goal is single cell analysis or rapid, large area analysis for spectropathological screening and classification.

(680) **Introducing Infrared Imaging Human of Blood Serum for High-throughput Biomedical Screening**; Caryn Hughes[1,2], Graeme Clemens, [2], Benjamin Bird[3], Matthew Barre[3], Jeremy Rowlette[3], Matthew Baker[2]; [1]University of Manchester; [2]University of Strathclyde; [3]Daylight Solutions Inc.

There are ever-increasing strains on biomedical services to deliver rapid, accurate and cost-effective diagnostic solutions to healthcare providers. A rapid screening blood serum assay could not only result in a fast turnaround from sample processing to outcome, but could also positively impact detection rates of early-stage disease, leading to a more favorable overall prognosis in the long term.

There is increasing evidence that blood serum droplets from patients with disease are distinguishable from control specimens by their distinctive spectral infrared signature. A large body of work that has demonstrated this for brain, bladder and ovarian cancers, for instance, using total attenuated reflection Fourier Transform Infrared (ATR-FITR) spectroscopy. ATR-FTIR is a promising clinical tool for point of care testing due to minimal sample preparation and data processing requirements.

The uses of microarrays are favored for high-throughput efficiency. Serum can be prepared via robotic dispensing and subsequent dehydration to create stable multi-patient microarrays with the option for storage. These microarrays can analyzed by acquiring spatially-resolved chemical maps using FTIR-imaging. Imaging creates the opportunity to perform robust quality control regimes for data processing in advance of searching for spectral signatures of disease. This is especially important when high reproducibility is required as sample preparation using sessile drop formation can result in heterogeneity in the morphological and chemical composition of the dried drop - this can heavily influence its infrared spectrum.

Discrete frequency infrared (DFIR) presents a new approach to IR imaging, whereby a broadly tunable mid-infrared quantum cascade laser (QCL) is applied to acquire key spectral ranges can be selected in the experimental design. The use of a QCLs coupled to a microscope with room temperature detectors provide practical opportunities for rapid IR data collection with enhanced efficiency and high pixel resolution.

With a focus on cancer, we present preliminary results that suggest it is possible to rapdily classify brain, breast, lung and skin cancer patient samples against non-cancerous control specimens using a classification model with DFIR data.

(681) **Infrared Spectroscopy in 3D and at Nano Scales**; Michael C. Martin[1]; [1]Advanced Light Source, Lawrence Berkeley National Laboratory
Synchrotron infrared beamlines use the diffraction-limited beam properties to enable a variety of cutting edge science at the micron length scale in both reflection and transmission. It has enabled a wide variety users to perform science across numerous fields. In this talk, I will explore how can we go further?

I will describe two new techniques we have recently developed and demonstrated. First, 3D FTIR tomography [1] provides spectrally rich, label-free, nondestructive visualizations of distinctive chemical compositions throughout intact biological or materials samples. The technique has combined Fourier Transform Infrared (FTIR) spectroscopy with computed tomography (CT) to create a non-destructive 3D imaging technique that provides molecular-level chemical information of unprecedented detail on biological and other specimens with no need to stain or alter the specimen.

Second, by combining scattering-scanning near-field optical microscopy (s-SNOM) with mid-infrared synchrotron radiation, Synchrotron Infrared Nano-Spectroscopy (SINS) enables molecular and phonon vibrational spectroscopic imaging, with rapid spectral acquisition, spanning the full mid-infrared (500-5000 cm-1) region with nanoscale spatial resolution [2]. This highly powerful combination provides access to a qualitatively new form of nano-chemometric analysis with the investigation of nanoscale, mesoscale, and surface phenomena that were previously impossible to study with IR techniques. We demonstrate the performance of synchrotron infrared nano-spectroscopy (SINS) on semiconductor, biomineral and protein nanostructures, providing vibrational chemical imaging with sub-zeptomole sensitivity.

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(682) ***In situ* Attenuated Total Reflection Fourier Transform Infrared Analysis of Live Cells**; K. L. Andrew Chan[1], Pedro L. Fale[1]; [1]King
Whilst it is important to understand the response of cells to drugs in the development of effective anti-cancer agents, current methods of assessment of cellular responses often are destructive and/or require external labelling which might lead to artefacts or high running cost. This presentation reports a new demonstration, validation and application of a non-destructive label-free method for characterising drug diffusion and cellular response to anti-cancer drugs based on live cells studies using FTIR spectroscopy.

The results of the quantification of 5-fluorouracil (5-FU) in living cells at a therapeutic-relevant concentration will be first described. The demonstrated method overcame the challenges of FTIR measurement of live cells, such as the low sensitivity, high water content and the fact that living cells are bathed in culture medium. We achieved this by the use of a longpass filter and a multi-bounce ATR to maximise the signal of the drug in cells and reduce the noise in the spectrum. We report that it is possible to quantify 5-FU, a low molecular weight drug, at at least 20 μM, an order of magnitude better than the expected limit of detection offered by FTIR.

We will also report on the result of the ATR FTIR measurement of a chemotherapy drug, doxorubicin, in three different live human cell lines at different drug concentrations *in situ*, with a focus on the biochemical alterations caused by the drug. The three different cell lines, HeLa, PC3 and Caco2, were chosen with different degrees of resistance to the drug to demonstrate that FTIR can be a fast and cost effective method to determine the sensitivity of cancer cells to the drug and at the same time providing information on the mechanism of drug resistance. We have validated the drug sensitivity results using standard MTT assays as well as complementary fluorescence measurements.

We have also analysed the difference spectra to reveal subtle changes inside living cells under treatment providing information about the mechanism of resistance such as the inhibition of DNA synthesis and the significant energy consumption of the resistive cells as a result of the efflux of the drug.

(683) **The Early History of LIBS at Los Alamos National Laboratory, 1979-1983**; Leon Radziemski[1]; [1]Piezo Energy Technologies
Shortly after the invention of the laser in the early 1960's, scientists around the world started looking for applications. Early spectrochemical techniques used the laser for ablation into a conventional spark. Much research was done on plasma properties such as temperature and electron density. But through the 1970's advances were hampered by the cost and complexity of lasers. Late in that decade much simpler Nd:YAG and tunable dye lasers spurred interest in laser based spectrochemistry.

In 1979 spectroscopists at Los Alamos National Laboratory (LANL) began investigating the laser-induced plasma for spectrochemical information bearing on environmental, processing, and safety issues. This led to the first two publications from LANL in 1981, in Plasma Chemistry and Plasma Processing. In the first paper, time integrated experiments were applied to detection of sodium and potassium in a coal gasifier stream, to beryllium particulate in air, and to detection of toxic organic compounds via signature atoms such as phosphorous, chlorine, and sulfur. The term LIBS was used for the first time for Laser-Induced Breakdown Spectroscopy. In the second paper, experiments were extended by adding a time-resolved capability. That technique was called time-resolved LIBS or TRELIBS. Although the latter term was never used again, time-resolution became an important part of many LIBS experiments.

Following those publications LIBS development at LANL was stimulated by the need for a real time monitor for beryllium dust during beryllium machining. An internal R&D grant funded work on LIBS as a possible solution. This led to the demonstration of LIBS' capability to perform spectrochemical analysis of gases, aerosols, solids, and in liquids with little or no sample preparation. Another strength of the technique was its

relative simplicity, at least for qualitative and semi-quantitative analysis. That led to many ideas for applications both at Los Alamos, and at other laboratories all over the world.

We will review the early publications from the LANL group, and ideas that surfaced and were developed later at various laboratories.

(684) **From the Calibration Curve to Machine Learning:**; Matthieu Baudelet[1,2]; [1]Townes Laser Institute, University of Central Florida; [2]National Center for Forensic Science, University of Central Florida

Laser-Induced Breakdown Spectroscopy was one of the first analytical technique that was developed using the laser with Raman spectroscopy. The conventional approach to quantitative analysis was using working curves and it was shown from the beginning for minor elements in a metallic alloy. Protocols not being largely developed in the community, matrix and plasma effects have been handled since then by deviating the calibration curve from the linear form and expanding to multi-variate analysis.

This review will show the evolution of quantitative analysis by LIBS. From the traditional calibration curve to the more complex machine learning techniques, the community has worked on ways to handle different excitation protocols and a variety of matrices for elemental analysis. But not only "unsupervised" calibration methods have shown interesting results, the integration of fundamentals of laser ablation as well as plasma physics has been studied in order to obtain more robust models for analysis.

A discussion on the different approaches will be opened to determine the direction(s) where quantitative analysis by LIBS should head to.

(685) **Calibration- and Calibration-Free LIBS: Past and Future**; Igor Gornushkin[1], Wolfram Bremser[1], Andrey Demidov[1], Ulrich Panne[1,2]; [1]Federal Institute for Materials Research and Testing (BAM); [2]Humboldt-Universität zu Berlin, Department of Chemistry

Laser induced breakdown spectroscopy (LIBS) is a widely used analytical method which yet lacks a well-established analytical protocol. No strict recommendations exist on which laser, which laser parameters, spectrometer, spectral resolution, experimental geometry, calibration standards and calibration procedures ought to be used in order to obtain reliable and reproducible results.

The current presentation will address these issues based on partly literature data, partly past inter-laboratory tests, and partly current analytical work at the BAM Federal Institute for Materials Research and Testing.

Several calibration-free, i.e. CF- and MC- (Monte Carlo) LIBS methods will also be discussed along with their advantages and shortcomings.

(686) **Calibration-Free LIBS, As It Was and As It Is**; Vincenzo Palleschi[1], Emanuela Grifoni[1], Stefano Legnaioli[1], Giulia Lorenzetti[1], Stefano Pagnotta[1]; [1]Applied and Laser Spectroscopy Laboratory, ICCOM-CNR, Pisa, Italy

The Calibration-Free method for the quantitative analysis of LIBS spectra was introduced in 1999 by the Pisa group, with the aim of overcoming the drawbacks associated to the classical approaches based on the build of calibration curves. Starting from the fundamental assumption of a plasma in Local Thermal Equilibrium, the CF-LIBS method has quickly gained interest for its capability of performing precise quantitative analysis without the need of reference samples and the intrinsic feature of overcoming the matrix effect, which is one of the most important limiting factor on the application of LIBS as an analytical technique. The CF-LIBS approach was widely used in the following years for the analysis of biological and geological samples, for the study of Cultural Heritage and for application in industrial environment. Several review papers have been since then published on the theoretical and practical aspect of CF-LIBS. With the intent of improving the accuracy of the CF-LIBS analysis, in recent years several groups have proposed the use of mixed or hybrid methods for overcoming the major drawback of the CF-LIBS technique, i.e. the complexity of the algorithm and its strong dependence on the precise knowledge of the spectral parameters of the emission lines in the LIBS spectrum.

In this communication, we will present and discuss the fundamental ideas at the basis of the CF-LIBS approach and its evolution from the original algorithms to the present formulation of the method.

(687) **Application of Laser Induced Breakdown Spectroscopy (LIBS) for On-Line Elemental Analysis**; Paul Coffey[1], Philip Martin[1], James Thomson[1]; [1]Manchester university

This work explores the application of laser induced breakdown spectroscopy (LIBS) for non-invasive, in-line elemental analysis in the process industries. In this technique a laser is used to ablate and ionise a small sample of material into a plasma and the optical emission lines are interrogated for the characteristic elemental emission lines. No sample preparation is required. Advantages and disadvantages of LIBS will be discussed. Examples will be given of results from the manufacture of advanced materials such carbon fibre reinforced polymers with the detection of impurity species. Examples from the nuclear industry will also be given. Finally we will discuss the potential for combining LIBS with Raman spectroscopy for combined elemental and molecular structural sampling.

(688) **Inline Analysis for Rapid Optimization of Continuous Flow Processes**; Richard Bourne[1], Nicholas Holmes[1]; [1]University of Leeds

Abstract: This talk will focus on the development of automated continuous flow systems to rapidly optimize processes. In particular, recent research on self-optimizing systems where the reactor and its process control instrumentation become an autonomous unit into which the reactants are pumped, and from which products emerge with optimized yield without requiring any intervention from the operator. This presentation will outline the use of different inline analytical techniques such as infrared spectroscopy, liquid chromatography and direct mass spectrometry to optimize several recent examples. The use of statistical optimization (design of experiments) will be compared to development of kinetic models and evolutionary feedback algorithms.

Biography: Richard A. Bourne is currently an independent Tenure Track Fellow at the University of Leeds. He completed a PhD under the supervision of Sir Prof. Martyn Poliakoff, CBE, FRS, followed by postdoctoral research with Sir Prof. Martyn Poliakoff and Prof. Michael W. George looking at reactions of singlet oxygen as part of the EU FP7 SYNFLOW project. He is now an independent academic and works at the Institute of Process Research and Development (IPRD) at Leeds, working on rapid process development and continuous flow chemistry. His research group is particularly focussed on rapid optimisation using automated systems. He is currently working as part of the H2020 EU ProPAT project developing and evaluating new sensor technologies.

SciX 2015 ABSTRACTS

(689) **Automated Stability Testing – How *in-situ* Measurements Deliver Rapid Product Development**; Andy Brookes[1], Faye Turner[1], Helen Williams[1]; [1]Astrazeneca

Throughout the development of a potential new pharmaceutical product gaining a rapid understanding of the stability of a material (e.g. drug substance intermediates, API, development formulations) can be critical to effectively delivering a viable new medicine.

This presentation describes a novel automated technology and associated methodology which enables investigative stability studies to be undertaken more efficiently whilst simultaneously generating more detailed data sets, giving us an insight into the effect of temperature and humidity.

In-situ analytical tools and advanced data evaluation approaches allow us to evaluate the physical stability of the sample (polymorphic form, hydratation state). The impact physical changes have on chemical stability will be highlighted.

Examples of applications in screening prototype formulations and predictive stability study designs will be shown to demonstrate the impact of this new approach.

(690) **Mid-infrared Spectroscopy Based on a Supercontinuum Source and a MOEMS-based Fabry-Perot Microspectrometer**; Markus Brandstetter[1], Jakob Kilgus[1], Petra Müller[1], Peter M. Moselund[2]; [1]RECENDT GmbH - Research Center for Non-Destructive Testing; [2]NKT Photonics A/S

We present a broadband spectroscopic setup suitable for process analytical purposes based on a novel mid IR supercontinuum (SC) source and a Fabry-Perot microspectrometer (FPMS).

SC sources feature high brightness combined with broadband spectral emission and collimated laser propagation. Their emission range is currently extending from the near-IR into the mid-IR spectral region1. Thus, SC sources become attractive as potential replacement for thermal emitters for dedicated spectroscopic applications. Due to their ability to be focused to diffraction limited spot sizes they may also serve as source for chemical mapping. However, while other emerging technologies, such as mid IR quantum cascade lasers (QCL), already find practical application in the field of optical sensing, SC sources have yet to prove their applicability.

SC sources obtain a number of unique features that may give them precedence over QCLs. SC emission does, for instance, not depend on inherent wavelength selection, such as QCLs in external cavity or distributed feedback configuration. The whole broadband spectrum is available in each single pulse at pulse durations in the low nanosecond regime. Furthermore, the fibre-based principle of SC sources could be advantageous in process analytical environments. On the downside, SC sources exhibit intensity fluctuations arising from the non-linear nature of the SC generation process2. We present results of the characterization of a mid-IR SC source (NKT Photonics, 1.75 µm – 4.2 µm, ~75 mW) and its application as broadband source for spectroscopy. We realized a dispersive set-up, using a MOEMS-based Fabry-Perot micro-spectrometer and compared its performance with that of a FTIR configuration. Absorption spectra were acquired from various samples (aqueous solutions, oils, polymers and metal surfaces) in various configurations, including transmission, Attenuated Total Reflection (ATR) and reflection geometries. Satisfactory results were obtained, even at low acquisition time, making this setup a promising concept for process analytical application.

1) Peterson C.R., Møller U., Kubat I., Zhou B., Dupont S., Ramsay J., Benson T., Sujecki S., Abdel-Moneim N., Tang Z., Furniss D., Seddon A., Bang O., (2014), Nature Photonics, 8, 830–834.

2) Corwin K.L., Newbury N.R., Dudley J.M., Coen S., Diddams S.A., Weber K., Windeler R.S., (2003), Phys. Rev. Lett. 90, 113904.

(691) **Strategy to Improve Raman Multivariate Calibration Life-Cycle Model Performance: A Pharmaceutical Tablet Assay Example**; Md. Nayeem Hossain[1], Md. Anik Alam[1], Benoît Igne[3], Carl Anderson[1,2,4], James Drennen[1,2]; [1]Graduate School of Pharmaceutical Sciences, Duquesne University; [2]Duquesne University Center for Pharmaceutical Technology, Duquesne University; [3]RD Platform Technology and Science, Glaxo SmithKline, King of Prussia; [4]Duquesne University

To develop a robust spectroscopic calibration model, unique sources of variability that are not directly related to the components of interest should be included in the calibration samples. In pharmaceutical formulations, multiple components (excipients) are required in addition to the active pharmaceutical ingredient (API) for the purpose of aiding manufacturing. Many excipients are hygroscopic in nature and will contribute variability in moisture content as a function of manufacturing lot, geographic origin, storage conditions, or seasonal variation. Additionally, some pharmaceutical operations (e.g. wet granulation) cause exposure of excipients and API to water. Although water is a weak Raman scatterer, moisture variability might have indirect effect on analytical model performance. Because excipients have intrinsic fluorescent characteristics (with broad spectral features), moisture variability may cause spectral artifacts in the form of baseline variation associated with fluorescence quenching. When the variability that is present during routine manufacturing is not included in the calibration, test and validation sets, the long-term performance will be limited. Long-term performance is becoming more critical as pharmaceutical companies implement multivariate PAT models more often. However limited work has been performed on model management specifically on testing and updating of models. This work will be discussed how moisture variation affected the predictive ability of a Raman calibration model for active ingredient in solid oral dosage forms and the direction of method management for better model performance. Novel efficient calibration model based on pure components will be proposed to develop robust Raman calibration model as an alternate method management, which will help to reduce time and expense for robust model development and long term model management.

(692) **Toward Understanding the Origin of VCD Intensity Enhancement in Protein Fibrils**; Laurence Nafie[1]; [1]Syracuse University

Amyloid fibrils are a form of protein structure having high stability and extremely low thermodynamic potential energy. Such fibrils have been linked to a variety neurological diseases, such as Huntington's Disease and Alzheimer's Disease. Although fibrils possess a high degree of structural rigidity, their extreme length prevents crystallization and study by X-ray crystallography. In 2007 we reported that vibrational circular dichroism (VCD) shows unusual sensitivity to the growth and development of protein fibrils in aqueous solution as the result of a previously unexplored supramolecular chiral order. [1] Subsequent publications since then have explored the pH control and reversal of the supramolecular chirality of protein fibrils and the correlation of this chirality with the morphology of fibrils in the film state as imaged by scanning electron microscopy (SEM) and atomic force microscopy (AFM). [2] We have found that individual fibril filaments can twist with either a left- or right-handed helical sense, and in addition, fibrils can further aggregate into multi-filament fibril braids. Fibril braiding and structural chiral evolution

has been correlated with increasing VCD intensity with no additional increase in the intensity of the underlying IR absorption. Despite these advances in exploring the VCD of fibrils a deeper understanding of the origin of the, in many cases enormous, intensity enhancement is still lacking. Possible origins of this enhancement will be described and approaches to calculating the VCD of fibrils will be described.

1. Shengli Ma, Xiaolin Cao, Mimi Mak, Adeola Sadik, Christoph Walkner, Teresa B. Freedman, Igor Lednev, Rina K. Dukor and Laurence A. Nafie, J. Am. Chem. Soc. 129, 12364-12365 (2007).

2. Dmitry Kurouski, Xuefang Lu, Ludmila Popova, William Wan, Maruda Shanmugasundaram, Gerald Stubbs, Rina K. Dukor, Igor K. Lednev and Laurence A. Nafie, J. Am. Chem. Soc. 136, 2302-2312 (2014).

(693) **Role of Side-Chains and Environmental Variation in Forming Peptide Aggregates and Fibrils. IR and VCD Spectroscopic Studies**;

Tim Keiderling[1], Fernando Tobias[1], Ge Zhang[1], Heng Chi[1]; [1]University of Illinois at Chicago

Formation of peptide aggregates and fibrils must ultimately depend on balancing interactions of side chains with conformational constraints of the polymeric structure, as modified by their relative stabilities in various environments (solvent, pH, ionic strength, etc.). Typically the lower solubility of hydrophobic sequences in aqueous media favors desolvation by formation of interchain contacts and sometimes ordered interdigitization, such as in poly-glutamic acid at low pH. The Glu side chain –COOH groups form bifurcated H-bonds with the amide C=O groups in what is termed a β2 sheet, evidenced by large shifts of the amide I and formation of stacked β-sheets. Vibrational spectroscopic methods have been used to establish β-sheet structures underlying many related structures. Our analyses of the of Glu10 based peptide IR and VCD spectra show they form β2 sheets, antiparallel but out of register, as determined using selective isotope labeling and theoretical modeling. Extending those studies to alternate (Glu-Aaa)n variants, where Aaa is Leu, Ile, Phe, Val, also shows this pattern of intersheet side-chain interdigitation. By contrast, small or polar residues (Aaa = Ala, Thr) show mostly disordered conformations, not fibrils. Peptides with β-carbon branched hydrophobic residue side-chains exhibited the β2 conformation while peptides with γ-carbon branched side-chains favored the classic β1 conformation. However, these distinctions were very dependent on preparation and incubation conditions. VCD signal enhancement correlated to fibril formation as confirmed by TEM images. In studies of alternate, larger systems, we have prepared fibrils of insulin using various protocols, pH, salt, heating, and concentration variations, and have been able to disaggregate the fibrils by addition of DMSO. As reported, the morphological character of these fibrils varies with preparation conditions. We have been able to interconvert them with DMSO addition in some cases and have differentiated the forms with VCD and induced CD of fibril-bound ThT supported with TEM images. Theoretical modeling of -sheet IR, VCD and Raman spectra have been used to clarify some of these spectral effects.

(694) **UV Resonance Raman (UVRR) Structural Studies of Polyglutamine (polyQ) Side Chains and Fibrils**; David Punihaole[1], Zhenmin Hong[1], Elizabeth Dahlburg[1], Ryan Jakubek[1], Riley Workman[2], Jeffry Madura[2],Sanford Asher[1]; [1]University of Pittsburgh; [2]Duquesne University

PolyQ expansions are linked to the formation of large plaques composed of amyloid-like fibril aggregates that are involved in Huntington's and other neurdegerative diseases. There is currently little that is known, however, about the structure of polyQ fibrils. Importantly, almost nothing is known about the role that the primary amides of the glutamine (Gln) side chains play in stabilizing the fibril cross-β structure. The lack of atomic resolution structures of polyQ-rich aggregates motivates the need to develop incisive, new biophysical methods that can interrogate amyloid-like fibrils.

New spectroscopic markers need to be found in order to obtain high-resolution structures of polyQ and other amyloid-like fibrils. Here, we discuss our recent discovery of a novel vibrational marker that reports on the structure of Gln side chains. Our DFT calculations show that the Amide III^P (AmIII^P) vibrational frequency depends sinusoidally on the OCCC dihedral angle of primary amides (the χ_3 angle of Gln). We use UVRR and visible Raman to experimentally correlate the AmIII^P frequency to the χ_3 angle dihedral angle of Gln. The structural sensitivity of the AmIII^P derives from the C_β-C_γ stretching component of the vibration since this bond length is inversely correlated with the frequency. The C_β-C_γ bond length dependence on the χ_3 dihedral angle results from hyperconjugation between the C_δ=O_ϵ π* and C_β-C_γ σ orbitals of the Gln side chain.

The discovery of this vibrational marker enables important new insights into the solution phase and fibril structures of polyQ-rich peptides. Combining our knowledge of the AmIII^P band with other primary amide bands such as the AmI^P and AmII^P, we determine, for the first time, the structure, hydrogen bonding, and local dielectric environments of the Gln side chains in *bona fide* polyQ fibrils. We also synergistically couple Molecular Dynamics (MD) simulations with UVRR to determine the peptide backbone structure of polyQ fibrils. *Most importantly, we believe that searching for conformationally dependent hyperconjugation interactions will aid in the discovery of new and incisive vibrational spectroscopic markers.*

(695) **Structure and Stability of Amyloid Fibrils Studied by Vibrational Spectroscopy and Surface Probe Microscopy**;

Maruda Shanmugasundaram[1], Dmitry Kurouski[1], Marketa Pazderkova[2], Tomas Pazderka[2], William Wan[5], Gerald Stubbs[5], Rina K. Dukor[3], Laurence A. Nafie[3, 4], Igor K. Lednev[1]; [1]Department of Chemistry, University at Albany, State University of New York; [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic; [3]BioTools Inc.; [4]Department of Chemistry, Syracuse University; [5]Department of Biological Sciences and Center for Structural Biology, Vanderbilt University

Amyloid fibrils, highly ordered protein aggregates implicated in neurodegenerative diseases, are known for their stability. However, the structure and morphology of fibrils can be controlled by changing the solution environment. The ability to control the polymorphic state is important as it enables switching of the biological activity and associated toxicity of amyloids. Our recent studies have shown that changing solution pH by less than one unit can result in spontaneous changes in fibril morphology and refolding from one polymorph to another. This refolding process is accompanied by the reversal of the Vibrational Circular Dichroism (VCD) signal and the change in twist handedness observed by surface probe microscopy. In this presentation, we will discuss whether the fibril refolding is a general phenomenon, what mechanism(s) is behind this process, and how well the VCD data correlate with surface probe microscopic results. The discussion will be based on our most recent studies of the refolding of fibrils formed from a prion forming peptide HET-s (218-289) and lysozyme. The prion fibrils show reversal of VCD signage and accompanying change in morphology with elevation of the solution pH, indicating that refolding is a general phenomenon. A further kinetic investigation revealed that change in the surface interaction between filaments plays a key role in the prion fibril refolding mechanism. However, a reversal in VCD signage is not accompanied by the appearance of a completely reversed twist handedness detectable by the surface probe microscopy. This discrepancy could indicate that the two techniques probe different levels of chirality in protein fibrils. The latter was further confirmed by studying lysozyme fibrils grown at a specific pH, where 'reversed' VCD signage was accompanied by 'normal' (left-twisted)

morphology. While this result itself was consistent with the seldom-observed process of handedness inversion, it demonstrates that the surface probe microscopy probes the chirality at the surface level and VCD probes the chirality at the filament level. Thus, this work proposes that the refolding of amyloid fibrils is a general phenomenon, and shows that VCD and surface probe microscopy provide complementary information, and both methods are required to understand the chirality of macromolecular assemblies.

(696) **Detailed Analysis of Protein Fibers by Vibrational Sum-Frequency Scattering and Second-Harmonic Generation Imaging**; Patrik Johansson[1], Patrick Koelsch[1, 2]; [1]University of Washington, [2]University of Washington, Department of Bioengineering

A detailed understanding of the structure, function, interaction, orientation, conformation, and dynamics of protein fibers is critical – not only for the development of treatments for progressive diseases involving protein fibers, but also for refining strategies in tissue engineering or optimizing structures for drug delivery. In this contribution, we demonstrate how sum-frequency scattering (SFS) can be utilized towards these important goals in bioengineering research. Vibrational SFS spectroscopy probes structures with inherent ordering, dispersed in a 3D-environment. This yields chemical information through vibrational spectra, from which abundance and relative orientations of chemical groups can be extracted.

In our first study, we applied SFS on collagen fibers as a key demonstration of this principle on protein fibers. We detected SFS signals in the NH, CH stretching and bending, and amide I regions. Our experiments indicate that signals are dependent on the scattering angle, making it possible to selectively probe various features of the collagen fibers. Data analysis routines including maximum entropy method calculations revealed opposite signs for the imaginary part of the nonlinear susceptibility in spectral regions that correspond to amide I and side-chain vibrations, respectively. This distinction allows spectral separation of both contributions and is linked to relative orientations of these groups. Information like this is pivotal for applications involving protein fibers - such as the engineering of scaffolds in regenerative medicine, or the development of treatments for fiber-related diseases like Alzheimer's disease.

Our second study was dedicated to the chemical and structural analysis of amyloid spherulites, another form of protein superstructures. These include fibrillar beta-sheets growing radially from an amorphous core into micron-sized spherulites having high regional ordering suitable for SFS characterization. These structures are interesting candidates for drug-delivery applications. They are also present in Alzheimer's disease although their role is still unclear, which calls for more detailed characterization of their structure and physico-chemical properties. Here we show the first second-harmonic generation (SHG) images and discuss SFS measurements of various amyloid spherulites in aqueous solution without needs for labeling approaches. Our results show that SFS analysis of protein superstructures can provide unique information and possibly break new ground in bioengineering research.

(697) **High Speed Raman Mapping for Pathology Classification in Esophageal Cancer.**; Catherine Kendall[1], Oliver Old[1], Martin Isabelle[1], Gavin Lloyd[1], Katherine Lau[2], Neil Shepherd[1],Hugh Barr[1], Nick Stone[3]; [1]Gloucestershire Hospitals NHS Trust; [2]Renishaw PLC; [3]University of Exeter, UK

Raman spectroscopy has been shown to accurately classify tissue pathology in a variety of organ and tissue types. Much of this work has been performed using Raman microspectrometers on ex-vivo tissue sections with long acquisition times resulting in total mapping acquisitions taking many hours. To enable translation of this technology to a clinical setting, either as an adjunct for pathologists or an in vivo probe for point of care testing, measurement times must be reduced. By using Renishaw's StreamlineTM technology, which combines a line focusing technique and pixel binning, it is possible to collect Raman spectral measurements much faster without compromising signal to noise. This study aims to assess the ability of this technique to accurately classify tissue pathology, using an esophageal model.

Tissue was collected from patients undergoing endoscopy or surgery on the esophagus. Specimens were collected from patients with Barrett's esophagus (BE), dysplasia and adenocarcinoma, and snap frozen in liquid nitrogen. 8µm tissue sections were prepared onto calcium fluoride slides, with contiguous sections stained with haematoxylin and eosin (H&E) for histological comparison. Raman spectra were collected across homogeneous regions of tissue pathology, using StreamlineTM acquisitions of 60 seconds per pixel, at 1.1µm spatial resolution.

Advanced multivariate statistic analysis tools were used to develop pathology classification models, which were then tested using leave-one-out cross-validation. The sensitivity and specificity of this pathology classification model using Raman Spectroscopy to discriminate dysplasia/adenocarcinoma from Barrett's esophagus produced sensitivity and specificities >77% and >80%, respectively.

By combining multivariate statistical analysis with StreamlineTM Raman acquisition of spectral data, we have demonstrated good sensitivities and specificities. This study illustrates the potential of non-invasive rapid Raman spectral mapping measurements and development of a robust and validated esophageal classification model that are able to classify tissue pathology both providing a clinical tool for pathologists and clinicians.

(698) **Good vibrations: Shining Light on Metabolism**; Roy Goodacre[1], Katherine Hollywood[1], Lorna Ashton[1], David Cowcher[1]; [1]University of Manchester, UK

There is increasing interest in being able to measure metabolic changes in biological samples as a function of space. In order to achieve this we and others have been developing a range of powerful mass spectrometry techniques for the analysis of small molecules that effect spatial analysis from cells and tissues. However, MS is inherently destructive to the sample and this limits serial analyses on cells and tissues. By contrast methods based on vibrational spectroscopy are non-invasive and can be collected readily through transparent media. This talk will highlight our recent research in both Fourier transform infrared (FT-IR) and Raman spectroscopies. These will include: using FT-IR spectroscopy as a high throughput phenotypic screening method for generating metabolic fingerprints prior to in depth GC-MS and LC-MS analyses; enhancing Raman spectroscopy for trace detection of multiple small molecules simultaneously; and how vibrational spectroscopy can be developed for the analysis of tissues as well as single algal, mammalian and bacterial cells.

(699) **Raman Spectroscopy for Immunological Research**; Alison Hobro[1], Nicolas Pavillon[1], Nicholas Smith[1]; [1]Biophotonics Laboratory, Immunology Frontier Research Center, Osaka University, Osaka, Japan

The initial stages of an immune response are dependent on the actions of immune cells in response to the presence of an infective agent. Failure to respond to 'foreign' agents results in infection and disease while, at the opposite end of the scale, autoimmune diseases are caused by the overaction of immune cells attacking 'self' cells and tissues. An effective immune response is a careful balance between these two extremes. This is achieved through the actions of individual cell types as well as the coordination of these responses between cell types.

Raman spectroscopy, with its applicability to measuring both live cells and tissues, can provide spectral signatures, reflecting the biomolecular content of a sample. In many cases the differences in Raman spectra are sufficient to distinguish between different cell types and states. Here we will show the extents to which Raman spectroscopy can be used to identify immune cells as well as their initial responses to selected immune stimulants, using lymphocytes and macrophages as examples

(700) **Utility of Short-Wave Infrared Raman for SERS and SORS**; Neil Shand[1]; [1]Defence Science and Technology Laboratory

A general trend in the development of portable Raman material idenitifcation is to utilise ever longer excitation wavelengths. This work explores the application of instrumentation using wavelengths beyond 1 micron to SERS nanotags, coventional Raman analysis and spatially off-set Raman. This work shows that wavelengths such as 1280 nm can reduce fluorescence but also have additonal benefits for penetration of barriers as a result of the trade-off between scattering and transmission.

(701) **Hyperspectral Raman Imaging of Lipid Rafts in Artificial Monolayer Membranes**; Jun Ando[1,2,3], Masanao Kinoshita[2, 4], Jin Cui[2,4], Hiroyuki Yamakoshi[3], Kosuke Dodo[1,3], Katsumasa Fujita[1,2],Michio Murata[2,4], Mikiko Sodeoka[1, 3]; [1]AMED-CREST, AMED; [2]Osaka University; [3]RIKEN; [4]Lipid Active Structure Project, JST, ERATO

Raman microscopy provides chemical information and distribution of molecules in a specimen without labeling or modification. Recently, we developed slit-scanning Raman microscopy, which improved imaging speed more than 100 times faster than that of conventional point-scanning Raman microscopy. Molecular dynamics of a living sample, such as cytokinesis of biological cells and cytochrome c release during apoptosis, have been observed by providing both high spatiotemporal resolution and chemical information.

Here we demonstrate hyperspectral Raman imaging of a lipid membrane monolayer. Due to the weak scattering signal from a limited number of molecules in a detection volume, spontaneous Raman imaging of a lipid membrane has been thought to be infeasible. Highly efficient optical system of our developed microscopy overcame this problem, with the information of full spectrum at each pixel. As a target component of lipid membrane, we focused on sphingomyelin (SM), which is known as an important component of lipid rafts. To specifically observe SM in a membrane, we synthesized SM analog tagged with diyne moiety (diyne-SM). Diyne-tag exhibits strong Raman peak at silent region of biomolecules, while maintains the original property of lipid molecule due to its small chemical structure. We observed a diyne-SM/DOPC/cholesterol ternary monolayer as a model of lipid raft, which was prepared on a quartz substrate. Raman intensity distribution, reconstructed by peak intensity of diyne-tag, visualized heterogeneous distribution of diyne-SM, forming micrometer-sized round domains in a membrane. High spatial resolution of our system further allows us to analyze fine structures inside of the domain. It was found out that the dinye-SM was enriched in the central area of the domain rather than the peripheral area. Furthermore, spatially-resolved Raman spectra at different phase in ternary monolayer discriminated the phase-dependent change in peak profile of intrinsic vibrational mode of lipid. Our proposed alkyne-tag Raman imaging, performed here with single lipid-layer sensitivity and hyperspectral imaging capability, will contribute to lipid membrane research, especially for lipid rafts.

(702) **Portable SERS Analysis of Industrial and Environmental Analysis**; Mark Peterman[1], Merwan Benhabib[1], Samuel Kleinman[1]; [1]OndaVia, Inc.

Fast, on-site chemical analysis increases process yields and decreases operational costs. OndaVia has developed a portable, Raman-spectroscopy-based analysis system that enables real-time, trace-level chemical analysis in complex industrial fluids. This analysis system consists of a small, transportable spectrometer, and a disposable, chemical-specific cartridge. The system enables analysis of ppth-level constituents in aqueous solutions down to ppb-level contaminant measurements in ground water. We demonstrate the ability to quantify analytes in complex mixtures over eight orders of magnitude in detection limit, while also achieving laboratory-quality accuracy. For example, triazine-based hydrogen sulfide scavengers are widely used to remove corrosive materials during natural gas and crude oil operations. These compounds are challenging to measure. The alternative laboratory methods require time-consuming derivitization steps due to temperature instability of the starting materials. We demonstrate direct detection and quantification of ppth-levels of MEA-triazine and its by-product, dithiazine, with 10% accuracy in complex spent scavenger solutions. Downstream, the use of these scavengers yields the tramp amine, monoethanolamine, in refinery process waters. Using surface-enhanced Raman spectroscopy, we achieve 0- to 100-ppm measurements and 10% accuracy for monoethanolamine in complex refinery waters with a two-minute, portable test. With surface-enhanced Raman spectroscopy and intelligent surface treatments, we also demonstrate ppb-level detection and quantification of perchlorate in ground water samples in a ten-minute field test. Our novel approach to Raman spectroscopy provides a fast, accurate, portable analysis system for industrial and environmental applications.

(703) **Drug Product Identification using 1064 nm Handheld Raman Spectroscopy**; Joseph Stoltz[1,2], Claire Dentinger[1, 2]; [1]Pfizer, Inc; [2]Rigaku Technologies

Typically use of handheld Raman instruments have been confined to the realm of raw material or excipient testing within the pharmaceutical industry.Use of a Rigaku 1064 nm Progeny handheld Raman has been shown to be capable of distinguishing active Drug Product (DP) tablets and capsules with Active Pharmaceutical Ingredient (API) / excipient blends that contain API loading in the range of approximately 5 – 28% from corresponding matching placebos for two of three DP formulations.

The ability of the Raman analyzer to collect spectra through containers, e.g. plastic bags and clear or amber bottles reduces potential analyst exposure issues experienced with traditional Attenuated Total Reflectance (ATR) FTIR and wet chemical techniques.

(704) **Handheld Raman for Real-Life Chemical Detection and Identification**; Philip Zhou[1], Katherine Bakeev[1]; [1]B&W Tek, Inc.

As a more and more widely recognized technology, Raman spectroscopy is used to analyze unknown chemicals compounds including narcotics, explosives, toxic materials and common chemicals. There is rising demand of instrumentation to be used for on-site quick screening with light weight, compact dimension and battery power as well. As now many common analytical techniques are transitioning from laboratory to the field, handheld Raman is a successful example of being an ideal solution to this request. A specific library is carried on the handheld Raman in terms of different applications and thus the obtained spectrum of unknown sample will be analyzed against it by well-developed search algorithm. In this way the identification of sample can be made to connect its Raman signature to unique chemical structure of particular chemical. Benefits of handheld Raman are proven and implemented to the field for law enforcement department and homeland security to process suspicious samples as quickly as possible with limited sample handling. More applications are extended such as use by the Environmental Protection Agency to verify unsure toxic materials and customs logistics for safety screening of packaged goods.

(705) **Novel Approaches to Overcoming Obstacles in Conducting Handheld Raman Measurements**; Thomas Tague[1]; [1]Bruker Corporation
A novel handheld Raman spectrometer (Bravo) has been designed and built to overcome the traditional challenges of using Raman in a portable format. The spectrometer utilizes a temperature controlled diode laser, which allows the instrument to operate in a sequentially shifted excitation (SSE) mode to eliminate fluorescence backgrounds, fixed pattern noise, and room lights, while keeping the Raman data in true spectral space. Even with incorporation of SSE into the system, the data collection is fast with high signal-to-noise. The instrument also incorporates a dual laser configuration to further mitigate fluorescence and provide greater spectral range. The system is always calibrated ensuring very high wavelength accuracy, which is vital for successful library searching and spectral subtractions. Application specific smart tips are encoded and linked to the acquisition parameters, so potential user errors are significantly reduced. These tips allow easy switching between sample types, such as vials, powders, tablets, and almost any object. An intuitive LCD graphical user interface is built into the Bravo for easy control of the unit. The Bravo can also be controlled remotely using the Bruker OPUS software platform. Lastly, the Bravo is Class I laser safe and 21CFR part 11 compliant.

(706) **Advances & Applications of Handheld Raman & FTIR spectrometers**; Michael Hargreaves[1]; [1]Thermo Fisher Scientific
Advances in handheld instrumentation continue to expand the capabilities for chemical identification in the field. Deployed globally by military, customs, law enforcement, and pharmaceutical companies, handheld analyzers continue to evolve to meet the changing needs – and stringent requirements – of users in the field.

This presentation addresses the latest evolution of handheld Raman and FTIR analyzers, from a hardware and software perspective, to the varied applications, with several examples presented.

(707) **Dopant-Assisted Atmospheric Pressure Chemical Ionization for Gas Chromatography High Resolution Mass Spectrometry: Metabolomic Analysis of Arabidopsis thaliana**; Carolyn Hutchinson[1], Rebecca Hansen[1], D. Paul Cole[1], Young Jin Lee[1]; [1]Department of Chemistry, Iowa State University
Traditional GC-EI-MS is very effective for well-studied compounds, but fragmentation can severely limit identification capabilities. Databases are critical but can miss or incorrectly identify fragile compounds. GC-APCI-TOF MS is more suited to the analysis of fragile or unidentified compounds due to softer ionization, low limit-of-detection, and wide applicability. However, some compounds such as sugars still fragment extensively. The novel addition of dopant gas in the APCI source reduces fragmentation and enhances ionization for a variety of compounds. An Agilent 7890A gas chromatograph is coupled to an Agilent 6200 time-of-flight mass spectrometer (resolution of ~7,000 at m/z 230) using a GC-APCI interface. The APCI source is operated in positive mode. Optional pre-heated ammonia (500ppm in helium) is introduced into the source as sheath gas. A modified Bligh-Dyer extraction was performed on wild-type *Arabidopsis thaliana* leaves. N,O-Bistrifluoroacetamide with 1% trimethylsilyl chloride was used to derivatize samples. A mixture of representative metabolite compounds (0.1mM each in methanol) containing dicarboxylic acids (citric acid, succinic acid, malic acid), amino acids (phenylalanine, alanine, isoleucine), phenols (coniferyl alcohol, cinnamic acid), flavonoids (quercetin), sugars (ribose, sucrose, glucose, shikimic acid), and other small metabolites (methyl jasmonate, glucose-6-phosphate) was prepared. Underivatized mixture was injected to test the effect of the dopant gas. Without dopant, stable compounds (e.g., amino acids, flavonoids) display a protonated ion with negligible fragmentation (<5%). Most compounds exhibit protonated ions and heavy fragmentation; very fragile compounds (sugars and dicarboxylic acids) fragment extensively. When dopant is introduced, nine underivatized compounds exhibit dominant ammoniated peaks; four (quercetin and amino acids) exhibit enhanced protonated peak signals. The significantly fragmented peaks are sucrose, which exhibits a small ammoniated molecular ion and a dominant fragment corresponding to a loss of one sugar unit, and glucose-6-phosphate, with a dominant peak corresponding to the loss of the phosphate group and water. Derivatization did not dramatically change the fragmentation behavior. A derivatized nonpolar extract displays 43 peaks without and 59 peaks with ammonia dopant. 24 peaks are unique to the dopant-assisted chromatogram, and 8 peaks are unique to the non-dopant-assisted chromatogram. The unique dopant-assisted peaks are likely sugar compounds which fragment extensively without dopant.

(708) **Identification of the Splicing Regulatory Factors using Mass Spectrometry**; Toru Takarada[1], Ken-ichi Yoshino[2], Masafumi Matsuo[3], Atsuko Takeuchi[1]; [1]Kobe Pharmaceutical University; [2]Kobe University; [3]Kobe Gakuin University
Introduction: Alternative splicing is regulated by splicing regulatory factors that bind to specific motifs located within pre-mRNA. The collapse of this regulation causes a variety of genetic diseases. Myotonic dystrophy type 1 (DM1) is a multisystemic disorder caused by splicing deregulation in tens to hundreds of genes. The insulin receptor (IR) gene consists of 22 exons and exon11 is alternatively spliced to produce two isoforms; isoform A (IRA) that lacks the exon11 sequence, and isoform B (IRB) that retains exon11. IRA has lower insulin sensitivity than IRB. In DM1, the predominant production of IRA causes glucose intolerance. In this report, we attempted to search for the chemical that enhances splicing of the exon11 and identify the splicing regulatory factors related to exon11 splicing.

Methods: 1) Screening of candidate chemicals that enhance exon11 splicing.

We examined candidate chemicals for their ability to enhance IR exon11 splicing in cultured cells by RT-PCR amplification. RT-PCR products were separated and semi-quantitated by high-resolution capillary electrophoresis.

2) Identification of splicing regulatory factors bound to IR exon11.

To identify the splicing regulatory factors involved in exon11 splicing, we performed an *in vitro* RNA binding assay. RNA probe, which consists of exon11 sequences, was incubated with HeLa nuclear extract and this reaction mixture was then separated by Native-PAGE. The separated band was excised and in-gel digested with trypsin. The extracted peptides were analyzed by LC-MS/MS (LTQ Orbitrap Discovery, Thermo Scientific).

Results; Among the examined chemicals, trans-Resveratrol (RES), a polyphenolic compound, promotes the exon11 inclusion in fibroblasts from DM1 cases. This result is encouraging that RES could be used to improve glucose intolerance in DM1. Next, we tried to elucidate the regulatory mechanisms of the alternative splicing of exon11 by identifying the related splicing regulatory factors. A separated band was observed on Native-PAGE following in vitro binding reaction. From the results of LC-MS/MS analysis, it was confirmed that hnRNPA1 (heterogeneous nuclear ribonucleoprotein A1) forms a complex with IR exon11 RNA sequence.

(709) **Analysis and Its Application of Urinary Prostaglandin D2/E2 Metabolites**; Atsuko Takeuchi[1], Yoshihiro Urade[2], Masafumi Matsuo[3]; [1]Kobe Pharmaceutical University; [2]Tsukuba University; [3]Kobe Gakuin University

Prostaglandin D2 (PGD2) has been implicated in both the development and resolution of inflammation. PGD2 is synthesized by PGD synthase (PGDS) from PGH2 that is produced from arachidonic acid by the action of cyclooxygenase. One kind of PGDS in skeletal muscles was found immunohistochemically stained in some of Duchenne muscular dystrophy (DMD) patients but not in controls. This indicated that the inflammatory mediator PGD2 plays a role in DMD pathology. However, detailed time-course studies of PGD2 metabolism in DMD patients are incomplete. Recently, urinary PGDM was shown to be a major urinary metabolite of PGD2 and to reflect its biosynthesis. DMD is a progressive muscle wasting disease caused by muscle dystrophin deficiency. Downstream of the primary dystrophin deficiency is not well elucidated. Here, the hypothesis that PGD2-mediated inflammation is involved in the pathology of DMD was examined by measuring PGD2 metabolite (PGDM), a major PGD2 metabolite, in urine of DMD patients. We measured PGDM, together with prostaglandin E2 metabolite (PGEM) in urine using a selected reaction monitoring mode of LC-MS/MS. The monitored product ion peaks of PGDM and PGEM were clearly separated in the mass chromatogram with each single peak but no interference peaks. The concentration of PGDM and PGEM was quantitated using the peak area ratios to the respective internal standard, on the basis of the calibration curve. First morning urine samples were collected from genetically confirmed DMD patients and age-matched healthy controls aged 4 to 15 years old. The mean concentration was significantly higher in DMD patients than in controls ($p<0.05$). Remarkably, urinary PGDM concentrations in DMD patients showed chronological changes: it stayed slightly higher than control until 7 years old but surged at the age 8 years old to a significantly higher concentration. Urinary PGDM concentrations were increased in DMD patients and became higher with advancing age. It was confirmed that PGD2-mediated inflammation plays a role in the pathology of DMD and PGDM levels are influenced by physical activity, while PGEM levels are more stable.

(710) **High-sensitivity Capillary Electrophoresis Nanoelectrospray Ionization Mass Spectrometry using Tapered-tip Emitters: Toward Single-cell Proteomics**; Sam (Bok Dong) Choi[1], Peter Nemes[1]; [1]George Washington University

With a remarkable peak capacity and compatibility with miniscule amounts of sample, capillary electrophoresis (CE) is attractive for proteomic measurements on volume/mass-limited specimens, such as small tissue biopsies and single cells.Although multiple interfaces have been developed to couple CE to microflow (>1 µL/min) electrospray ionization (ESI) for mass spectrometry (MS), fewer designs exist that utilize nanoflow ESI (nanoESI, <350 nL/min) that leads trace-level measurements in proteomics. Here, we present a CE-nanoESI interface that is simple to construct and able to detect trace-level peptides and proteins in single cells by MS.

The CE-nanoESI source was constructed based on the broadly successful co-axial sheath-flow design. As the electrospray emitter, we selected a tapered-tip metal capillary made of stainless steel because our earlier studies showed that this emitter provided high stability in both the nano- and micro-flow regimes. A CE separation capillary (20/90 µm ID/OD, 100 cm length) was fed through the tapered-tip emitter (100/350 µm ID/OD)and set to protrude ~15 [redacted] nanoESI source was positioned ~400 µm from the sampling plate orifice of a time-of-flight mass spectrometer. Optical inspection and monitoring of the ion current confirmed that stable cone-jet spraying regime was maintained when 50% methanol (0.1% formic acid) was supplied through the emitter at 150–350 nL/min as sheath-flow, and 25 kV was applied across the capillary ends during CE separation.

Analytical performance metrics of CE-nanoESI-MS were encouraging for single-cell investigations. By measuring 1 nL of a mixture of peptide standards, reproducibility was established at 2% relative standard deviation (RSD)for migration time and <10%RSD for peak area. The lower limit of detection was ~2 amol for angiotensin II.Ina digest of 2 pg standard bovine serum albumin, the protein was identified with 12.2% sequence coverage (Mascot), raising a potential for measuring proteins in single cells. At present, we are testing this platform to monitor protein expression between single embryonic cells at different stages of embryonic development. We anticipate that CE-nanoESI-MS is adoptable to quantitative and qualitative analysis of broad types of single cells.

(711) **Analysis of the Loss of Efficiency in the Confines of LC-ESI-MSn while Testing Drugs of Abuse in Urine Samples**; Ross Carter[1], Anjali Alving[1]; [1]Bruker Daltonics

The number of both known and unknown drugs of abuse is continually increasing, which demands screening methods that are accurate and capable of growing with the increasing number of products. In this experiment, a urine sample spiked with ten known drugs of abuse will be analyzed using the Compass Open Access Toxtyper software, designed for rapid walk-up identification of drugs of abuse. LC-MSn will be used to provide data for MS, MS2, and MS3 spectra. The unique combination of these spectra will be matched against the Toxtyper library to provide an unambiguous identification of the compound with more confidence than traditional methods. One sample will be prepared using standard SPE, and the other will remain unprepared. The two samples will be analyzed for the relative loss of intensity to ascertain whether SPE is effective as a preparation method. Additionally, multiple trials of each sample will be run in order to assess the reproducibility of the sample preparation method. Concentration curves will then be generated for methamphetamine using methamphetamine-d5 as an internal standard. This data will be used to analyze the change in concentration after SPE and the original accuracy for an unprepared sample.

(712) **Development of a New Versatile Instrument for Complementary Analysis Combining Laser Ablation Mass Spectrometry and Laser Spectroscopy**; Andreas Bierstedt[1], Knut Rurack[1], Jens Riedel[1]; [1]BAM Federal Institute for Materials Research and Testing

In recent years, hyphenated techniques have received increasing attention to approach complex analytical problems. The combination of different orthogonal analytical methods such as laser ablation mass spectrometry and laser spectroscopy yields complementary information on the same sample spot.

The individual techniques reveal specific but limited analyte characteristics: for instance Raman spectroscopy alone can often not identify a sample since superposition of spectral information can be present. In contrast, MS is known for its high molecular specificity but is invariant regarding the chemical structure. Thus, a combination of LA-MS and optical spectroscopy could be highly beneficial for an unambiguous identification of complicated analytical samples: MS gives the exact molecular mass, while laser spectroscopy reveals structural information.

A hyphenated system for laser ablation and laser emission spectroscopy was coupled to an orthogonal time-of-flight mass spectrometer with an atmospheric pressure inlet capillary. Radiation for both techniques was obtained by using the second harmonic of a shared diode pumped solid state laser, with a tunable repetition rate from 1 Hz - 500 kHz. For each experiment a suitable repetition rate was chosen to maximize the S/N ratio and shorten the acquisition time. Emitted radiation was detected using a Czerny-Turner spectrograph.

To enhance the ionization efficiency for MS analysis, a versatile dielectric barrier discharge probe was added to the system as a post-ionization source. Without further post-ionization no ions could be observed. Our used plasma jet design allows for selectively switching between the formation of ammonium cations and protonated ions as reagent-ions.

MS spectra were obtained in less than 5 s. The absence of any fragmentation pattern indicates the softness of the used ionization technique. Optical spectroscopy was performed at higher repetition rates. The pulse energy used is below the ablation threshold. Depending on the sample properties, the set-up is capable of performing laser-induced fluorescence, Raman scattering or laser induced breakdown spectroscopy (at higher pulse energies). Due to the high repetition rates, the acquisition time for spectroscopic experiments could be as short as 2 s.

(713) **Investigating Electrospray Ionization Using a Pulsed Nanospray Emitter**; William P. McMahon[1], Carina S. Minardi[1], Arjuna Subramanian[1], Kaveh Jorabchi[1]; [1]Georgetown University

Electrospray ionization (ESI) is a soft-ionization technique that has enabled mass spectrometry of non-volatile analytes. There has been great interest in various fundamental factors dictating the electrospray process such as charge, hydrophobicity, size, and conformation of the analyte. Yet, the details of how analytes transform from highly charged droplets to gas-phase ions are still under discussion. This understanding is paramount for maximizing the instruments' analytical capabilities. To gain a better understanding of the ESI process, we have coupled a pulsed nanospray ESI emitter to an ion-mobility cell to capture the timing of ionization for variety of analytes. The ionization timing for analytes is manifested in shape of the ion clouds that emerge from the plume of charged droplets emitted by the pulsed spray. Therefore, we characterize axial and radial distribution of analyte ions and relate them to analyte properties to map the ionization pathways. We have observed that charge and hydrophobicity are fundamental factors affecting both radial and axial expansion of ion clouds. We have established that increasing the amount of charge on analyte ions promotes an ion cloud with larger radial distribution and smaller axial expansion. This approach is applied to a series of primary amines and their quaternary equivalents to further establish the methodology.

(714) **Plasma-Assisted Reaction Chemical Ionization Time of Flight Mass Spectrometry for Identification and Quantification of Halogenated Compounds**; Kunyu Zheng[1], Peter Haferl[1], Haopeng Wang[1], Hamid Badiei[2], Feven Gezahegn[1], Kaveh Jorabchi[1]; [1]Georgetown University; [2]Perkin Elmer, Inc.

Elemental mass spectrometry (MS) offers excellent quantitative analysis especially when individual standards are not available. We have recently developed plasma-assisted reaction chemical ionization (PARCI) for high-sensitivity elemental quantification of halogenated compounds. However, the stand-alone elemental analysis falls short in identification of unknown compounds. Here, in order to achieve comprehensive speciation analysis, we evaluate the capability of PARCI for formation of molecular ions in addition to atomic ions. An oxygen-helium microwave induced plasma with over 50% oxygen concentration is utilized in these studies. In elemental mode, high plasma power is applied for complete analyte breakdown. Compound-independent response factors are achieved for fluorinated and chlorinated compounds. In molecular mode, on the other hand, the plasma power is reduced to supply sufficient helium metastable atoms for ionization of oxygen to form O2+ as reagent ions. Low plasma power and high concentration of oxygen significantly reduces the plasma temperature, leading to softer ionization conditions. Molecular ions (M+) and other reaction products, such as oxidized species, are detected for potential identification of both halogenated and non-halogenated compounds.

(715) **Soft µs Mid-IR Laser Desorption Ionization of Acoustically Levitated Liquids**; Aleksandra Michalik-Onichimowska[1,2], Carsten Warschat[1], Toralf Beitz[2], Ulrich Panne[1], Hans-Gerd Loehmannsroeben[2], Jens Riedel[1]; [1]BAM Federal Institute for Materials Research and Testing Division; [2]Physical Chemistry, University of Potsdam

Analysis of liquid samples by gas phase ion detection methods has become a standard procedure. Therefore, the necessary liquid to gas phase transition is a topic of continuous research. Especially for the interrogation of small sample fractions, this vaporization can be established by laser desorption. For this task, typically nanosecond laser pulses are being used. Microsecond pulsed lasers may offer an attractive alternative. Due to their performance and affordability microsecond ablation has already been established as a standard tool in medical and industrial applications. In stark contrast, their analytical potential is yet far from being exhausted.

This contribution utilizes an acoustically levitated droplet as a containerless sample coupled to a time-of-flight mass spectrometer (TOF-MS), allowing direct interrogation of the ablated ion plume. Desorption of a small fraction of the liquid droplets is performed by the output of a diode pumped Er:YAG laser at $\lambda = 2.94$ µm. This wavelength is absorbed directly by excitation of OH and NH groups, allowing for the use of pure solvents without further addition of chromophores. The produced spray plumes are temporally resolved imaged by nanosecond shadowgraphy utilizing the laser induced fluorescence of a Rhodamine B solution as the ultrafast light source. The plume dynamics following a microsecond and a nanosecond pulse laser excitation are directly compared. Counterintuitively, the temporal and spatial spread of the vapor cloud produced by the longer pulse is smaller compared to the short pulse ablation. This can be rationalized by a large contribution of postpulse ablation and a pronounced reflection of the vapor plume on the formed shock wave in the surrounding air in the case of nanosecond irradiation. Furthermore, microsecond ablation is found to yield in a better defined distribution of secondary droplet radii, recoil velocity and recoil direction. All these findings indicate a better suitability for desorption/ionization applications as direct liquid mass spectrometry. The results are corroborated in additionally recorded mass spectra. While the observed ion patterns are identical for nanosecond and microsecond desorption, the total ion yield is greatly improved by the longer pulse duration.

(716) **Multistage Mass Spectrometry of Phospholipids Using Collision-Induced Dissociation (CID) and Metastable Atom-Activated Dissociation (MAD)**; Pengfei Li[1], William Hoffmann[2], Glen Jackson[1,2]; [1]C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV; [2]Department of Forensic and Investigative Science, West Virginia University, Morgantown, WV

Three phospholipids, PC(16:0/18:0) (PSPC), PC(16:0/18:1(9Z)) (POPC) and PC(16:0/18:2(9Z, 12Z)), were fragmented in their 1+ charge state via helium metastable atom-activated dissociation (MAD). The resulting radical cation ($[M]^{+\bullet}$) was further isolated and subjected to collision-induced dissociation (CID) (MS^3). The MS^3 CID spectrum of the $[PSPC]^{+\bullet}$ ion contains a series of fragment ions from *m/z* 550 to *m/z* 732, which differ by 14 Da increments and correspond to the loss of CH_2 units from the fatty acid chain. Two of the most abundant ions are at *m/z* 537 and 565, which are McLafferty rearrangement product ions. CID fragmentation of the $[POPC]^{+\bullet}$ ion resulted in near-complete fragmentation along saturated and unsaturated fatty acid chains. The McLafferty rearrangement-type fragment at *m/z* 537 was observed as well, but it is far less abundant than the fragment at *m/z* 550, which corresponds to gamma-cleavage of carboxyl group. Another interesting fragment is at m/z 620, which is produced from the cleavage of the single bond on the carboxyl side of the C=C double bond. A similar fragmentation pattern was

observed in PC(16:0/18:2(9Z, 12Z)). Fragments at *m/z* 660 and 620 were also observed, which correspond to the cleavage of vinyl bond on the carboxyl side. A prominent fragment at *m/z* 550 was observed as well, corresponding to the gamma-cleavage of carboxyl group. This experiment induces the cleavage of vinyl bond, which is a good indicator for site of unsaturated bond(s) of fatty acid chain; the number of vinyl cleavages is equal to the number of C=C double bonds, which can help determine the position and degree of unsaturation in in-tact phospholipids.

(717) **Selective Separation of Metalloproteins using Aqueous Two-Phase System**; Maria C. Hespanhol da Silva[1], Anna Donnell[2], Julio A. Landero[2], Joseph A. Caruso[2]; [1]Universidade Federal de Viçosa; [2]University of Cincinnati

A simple aqueous two-phase system (ATPS) of polymer/ammonium sulfate was proposed for selective separation of metalloproteins. The combination of ATPS with ICP-MS was applied to identify the partition of metalloproteins in two phases of the ATPS. The metalloproteins cyanocobalamin, myoglobin and carbonic anhydrase were partitioned in different ATPS. The copolymer triblock/salt system was first applied to the separation of metalloproteins. The partitioning of metalloproteins was compared using two different ATPS: copolymer L35 + ammonium sulfate + water and poly(ethylene oxide) (PEO) +ammonium sulfate + water. The effects of the type macromolecule and composition of the ATPS on the formation of two phases and partition of metalloproteins cyanocobalamin, myoglobin and carbonic anhydrase were investigated. The results showed that cyanocobalamin was enriched mainly in top phase in copolymer L35/(NH4)2SO4 and PEO/(NH4)2SO4 at room temperature. The partition coefficient (K) increase when macromolecule concentration in the system increase (K= 8 to 1000). On the other hand, myoglobin and carbonic anhydrase were enriched in bottom phase in both ATPS, since the partition coefficient was lower than 1. The copolymer ATPS is more hydrophobic than PEO ATPS. The cyanocobalamin coefficient partition in PEO + ammonium sulfate + water ATPS was 1000. However, the K for the cyanocobalamin in L35+ammonium sulfate + water was 40. This shows that there are specific interactions between cyanocobalamin and EO segments of the polymers. The copolymer L35 has less EO segments than PEO, causing the cyanocobalamin K to be higher than in PEO ATPS.

(719) **Blood Sample Preparation using Gradient Insulator-based Dielectrophoresis (g-iDEP) Device**; Jie Ding[1], Christine Woolley[1], Mark Hayes[1]; [1]Arizona State University

Immunoassay techniques are commonly used in clinical diagnostic methods since it allows for quantitative testing that is based on the specific interaction between target the molecule and antibody. However, for a whole-blood or plasma sample, cross-reactivity may restrict the limit of quantification to the nM to μM range without prior sample simplification. Consequently, in recent bio-analytical research and applications, developing rapid, efficient and low-cost blood sample preparation methods attract considerable attention considering it is the prerequisite for the high-sensitive detection and analysis.

Microfluidics, as an advanced analytic family of techniques, has the potential to be used in blood sample preparation to separate blood cells from plasma. Among all microfluidics strategies, dielectrophoretic force is one of the most promising since it is able to separate neutral bio-particles in a label free system rapidly and selectively.

In this work, effective removal of red blood cells (RBCs) from solution containing cardiac biomarkers has been achieved using gradient g-iDEP device with sawtooth pattern. The result shows that RBCs can be captured and removed from its mixture with either human fatty acid binding protein or myoglobin, which are two key biomarkers for cardiopathy. This provides a practical microscale purification method for the pretreatment of blood samples in medical diagnostics as well as providing the capability of further integration into a μTAS for the quantification of multiple biological targets.

(720) **Developing New Trapping Efficient Designs for Gradient Insulator-based Dielectrophoresis (g-iDEP) Devices**; Claire V. Crowther[1], Mark A. Hayes[1]; [1]Arizona State University

The ability to separate analytes with increasingly similar properties drives the field of separation science. One way to achieve such separations is using trapping and streaming dielectrophoresis (DEP). Similar analytes can be separated using trapping DEP by manipulating the analytes based on their physical properties. One example of separations of similar analytes currently achievable by insulator based DEP is the work of Jones et al., where the separation of two strains of *Staphylococcus epidermidis* was achieved (*Analyst*, **2015**, *140*, 5152-5161).

The non-uniform fields necessary for DEP are formed using various insulator shapes in microchannels. Current insulator shapes used include: triangles, diamonds, circles, and rectangles. However, all of these insulators pose problems for trapping as they do not have laterally uniform electric fields and gradients of the electric field. This leads to analytes experiences different forces depending on their pathline in the channel, ultimately resulting in poor resolution separations. Based on an iterative process testing approximately 40 different insulator shapes a new design was developed to improve trapping efficiency in insulator based microfluidic channels. The new insulator, using different length-scale structures, streamlines the analyte to improve homogeneity in the lateral electric field, while still achieving high gradients necessary for analyte separation using DEP, and minimizes the presence of extraneous trapping zones. Utilizing this new insulator design improved resolution separation should be attainable. Comparisons of the forces and capture locations in microfluidic channels for current and the improved geometry will be presented.

(721) **Electrophoretic Exclusion Based on a Microdevice**; Fanyi Zhu[1], Mark Hayes[1]; [1]Arizona State University

The Hayes research group focuses on the development of novel separation techniques for complex sample analysis. The ultimate goal is to make a point-of-care device integrating separation methods and detection techniques developed by the group for fast diagnosis. To make a step towards our ultimate goal, we first develop an array-based electrophoretic exclusion microfluidic device and seek its potential as a novel separation technique.

Electrophoretic exclusion is a novel counter-flow gradient focusing technique, which exploits hydrodynamic flow velocity and electrophoretic velocity to exclude, enrich, and separate analytes.

At current stage, the microdevice was designed to have one large entrance reservoir and three parallel separation components, with a specific voltage configuration for each. Qualitative studies focused on channel area with fluorescent dye molecules, demonstrating exclusion phenomenon when electric field was applied. When voltage was released, a bright bolus passed through the channel under hydrodynamic flow, which further verified the exclusion. Moreover, for this microdevice, finite element analysis models using commercial COMSOL software were built to aid our understanding of this technique, especially the placement of electrodes. Snapshots from simulations at different time point matched well with experimental data. Centerline concentration profiles were also generated within both the simulation results and experimental images, presenting a

good match and significant difference for each placement. More factors are under investigation in microdevice both by simulations and experiments to further explore the temporal and spatial dynamics, the resolving power, the degree of concentration and so forth, thus providing useful information for the improvement of next generation design aiming at better resolution, higher capacity and easier manipulation. In addition, protein samples, labeled with two different fluorescent dyes, were also tested using this microdevice, giving rise to a good, clear exclusion, indicating a broad potential usage of our device for future separation development and a step closer to our ultimate goal of developing a point-of-care device.

(722) **Influence of Metal Cations on the EOF of Phospholipid Coated Capillaries**; Christopher Harrison[1], Shane Wells[1], Eduardo de la Toba[1], Srilatha Vydha[1], Katherine Cortell[1]; [1]San Diego State University

Phospholipid bilayers have been used for some time, as protective coatings in capillary electrophoretic (CE) separations of proteins and biomolecules. Phospholipids are attractive for this purpose as they self assemble on the surface of the capillary with minimal effort and they act as an effective barrier to most hydrophobic biomolecules. An added influence of the phospholipid bilayers is that they are capable of altering the electroosmotic flow (EOF) in the separation capillary. Specifically, it was revealed in some of the earliest work with the phospholipid 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) that the presence or absence of calcium in the separation buffer results in revered or forward EOFs respectively.

This work seeks to apply and expand upon the variability in EOF for phospholipid coated capillaries. The phospholipid DLPC will be used for capillary coatings, however, in place of calcium, other divalent and even trivalent metals will be explored as buffer additives. One focus of this work is to examine how the variations in charge, size, and likely affinity for the phosphocholine head groups, by the different metal cations is impacts the magnitude of the reversed EOF. Additionally, we are seeking to use the ability for metal cations to alter the direction of the EOF to perform selective sample stacking of analytes such as proteins. By including or excluding the metal cations from the sample solution, and/or separation buffer, we can locally, and temporarily, modify the direction and magnitude of the EOF, in the hopes of enhancing analyte concentration and detected signals.

(723) **Biophysical Differentiation and Separation of Staphylococcus epidermidis Strains Based on Antibiotic Resistance**; Shannon Huey Hilton[1], Paul V. Jones[1], Mark A. Hayes[1]; [1]Arizona State University

Antibiotic resistance first occurred soon after the introduction of the first antibiotic, and the problem continues to grow today (*Microbiol. Mol. Biol. Rev.* **2010**, *74* (3), 417-433). As new antibiotics are developed, resistance follows shortly after (*Front. Microbiol.* **2010**, *1*, 1-7). This resistance can result in longer lasting illnesses and higher mortality rates. The ability to differentiate and separate susceptible strains would help with this problem by informing treatment decisions.

Subtle physical differences between strains of bacteria, such as those between a resistant and susceptible strain, can be probed with dielectrophoresis (DEP) to bring about such a separation. DEP, which describes the motion of a polarizable particle in the presence of an electric field gradient, has previously been used to separate numerous targets, including separating cells of different bacterial species and cancer cells from blood cells (*Anal. Chem.* **2007**, *79* (12), 4552-4557; *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92* (3), 860-864); we have shown the differentiation of *E. coli* serotypes (*Anal. Bioanal. Chem.* **2014**, *406* (1), 183-192). Here, we show the differentiation and separation of resistant and susceptible *Staphylococcus epidermidis* in a microchannel device with sawtooth shaped insulating features used to create the necessary electric field gradients. The two strains show different behaviors in the channel, including capturing under different conditions, which leads to the ability to separate these two strains.

(724) **Gold Nanoporous Membranes for Tunable Protein and DNA Separations**; John Orlet[1], Daniel McCurry[2], Ryan Bailey[2]; [1]Truman State University, [2]University of Illinois at Urbana-Champaign

Developing a means by which a wide variety of biological analytes can be selectively resolved with minimal supplies is crucial to cost-effective diagnostics and analyses. Recent advances in synthetic nanopores have allowed for single molecule detection and separations, though most exhibit charge-selective behavior dependent on pH. The separation of biomolecules remains a highly conditional process, often requiring specific solution compositions which limit the ability to modulate the separation through the pore. Nanoporous gold (NPG) membranes have pore sizes below 60 nm, combining the small sample volumes and charge-selective properties of traditional, synthetic nanopores with a surface that is externally manipulated. With high surface area and channels with intricate geometry, the NPG surface inherently induces varied size-specificty for further dynamic tuning. The modulation of NPG surface charge is explored through electrokinetically driven transport of proteins and DNA through the membrane.

The membrane is simply prepared by de-alloying a predominately gold alloy in concentrated nitric acid, leaving behind a exterior with a typical surface area on the order of 1500 cm2. NPG membranes are shown to exhibit magnitudes of transport dependent on the electrical potential applied to the surface. Under conditions where a cationic protein is subjected to a negative potential applied to the NPG, flux is shown to be greater than at a positive potential. Additionally, application of thiol self-assembled monolayers (SAMs) to the surface of the gold are also shown to affect the transport of proteins, allowing for further selectivity based on polarity and chemical reactivity with the monolayer while creating a surface that is resistant to protein fouling. Polar thiols like 6-hydroxy-1-hexanethiol demonstrate different degrees of protein transport than do nonpolar alkanethiols. The facile fabrication of NPG membranes allows for future incorporation of tunable biomolecular separations in microfluidic devices for subsequent analysis downstream.

(725) **New Generation Raman Imaging for Correlative Microscopy: Confocal 3D Raman Imaging Meets Highest Spatial and Spectral Resolution**; Ute Schmidt[1], Wei Liu[2], Thomas Dieing[1], Olaf Hollricher[1]; [1]WITec GmbH, Ulm Germany, [2]WITec Instruments, Knoxville, TN

The combination of several imaging techniques in one instrument facilitates the acquisition of varied data from the same sample area, enabling the advantages of correlative microscopy. As each microscopic technique uses a different type of interaction with the sample, complementary information can be derived. Confocal Raman imaging contributes to correlative microscopy by revealing the chemical and/or molecular composition of a heterogeneous material. In the past decade confocal Raman imaging has gained in importance in the characterization of heterogeneous materials and is now applied in almost all fields of research in which chemical or molecular identification, purity, and stress-strain states are of importance. Until recently the acquisition of the highest lateral resolution 3D Raman images was limited by computer memory and data acquisition routines. In addition, for high spectral resolution only long focal length spectrographs with low light throughput were available.

New developments in computer memory management and data acquisition routines facilitate the acquisition of millions of Raman spectra in one data file, allowing the acquisition of Raman images from a large sample area at higher pixel density, thus higher spatial resolution from large area scans. Furthermore the acquisition of confocal 3D Raman images can be expanded to larger sample volumes with higher pixel density, resulting in more detailed information from the sample. Through a complete redesign, a long focal length spectrometer with an extremely high throughput allowing the acquisition of complete Raman spectra with integration times as low as 2 ms, and with a spectral resolution below one wave-number has become available.

The aim of this contribution is to present the latest achievements in confocal Raman imaging and its integration with AFM and SEM. The highlights will be demonstrated with examples from various fields of application.

(726) **Multi-modal Molecular Imaging of Chemically Communicating Bacterial Communities**; Nameera Baig[1], Sage Dunham[, 2], Nydia Morales-Soto[1], Jonathan Sweedler[2], Joshua Shrout[1], Paul Bohn[1]; [1]University of Notre Dame; [2]University of Illinois at Urbana-Champaign

Bacteria primarily exist as complex, matrix enclosed and, surface-associated communities called biofilms. Bacteria that are part of a biofilm community are much more resistant to antibiotics and the host immune system than their free-floating counterparts. *Pseudomonas aeruginosa* is a gram-negative opportunistic human pathogen. *P.aeruginosa* biofilms are associated with persistent and chronic infections in diseases such as cystic fibrosis. *P.aeruginosa* synthesizes and secretes secondary metabolites called quinolones which are implicated in processes such as biofilms formation, virulence and cell-to-cell signaling. Our current work seeks to use a multi-modal imaging platform combining the powerful label-free imaging capabilities of Confocal Raman Microscopy (CRM) and Secondary Ion Mass Spectrometry (SIMS) for studying the spatiotemporal distribution of quinolones in *P.aeruginosa* biofilm communities. We also shows how CRM a non-destructive molecular imaging technique, in combination with multivariate statistical analysis tools such as Principal Component Analysis (PCA) can be used to guide SIMS analysis for characterization of co-localized isobaric analytes in heterogeneous biological systems.

(727) **Optimizing Operative Parameters for Endosperm Purity and Yield for a Newly Constructed Commercial Flour Mill with Quantitative Spectroscopic Chemical Imaging**; David Wetzel[1,2], Mark Boatwright[, 2], Elieser Posner[3]; [1]Microbeam Molecular Spectroscopy Lab, Department of Grain Science, Kansas State University; [2]Department of Biochemistry & Molecular Biophysics, Kansas State University; [3]ESP International, Israel

Direct spectroscopic determination of flour purity from individual unit processes in flour milling we have previously employed for both commercial and experimental milling. A newly constructed state of the art commercial scale mill has provided the opportunity to conduct experiments immediately following 10 hour production shifts prior to 24 hour, seven day operation. The 21st century quantitative spectroscopic imaging enables direct product (endosperm) purity determination in place of measuring the mineral content from the bran as a negative quality indicator. By combining the intermediate stream flow rate with direct endosperm purity values, the cumulative endosperm yields for select unit processes is readily calculated. The recently developed method utilizes quantitative spectroscopic imaging by the individual pixel from an indium antimonide focal plane array of 81,920 pixels. A partial least squares treatment applied to individual spectra from each pixel enables a summation of endosperm within the optical field of view. We report the overall efficiency of milling operation before and after adjustment of various operational parameters for successive sections of the operation starting with the initial breaking of individual kernels between corrugated rolls. The expertise of an experienced miller was employed to select intermediate key test points within the process that with proper equipment adjustment, guided by the optical technique that we use, can make an economic difference. With a 24 hour production of 710 tons with a product value of $275 per ton, a relatively small increase in high quality product enjoys large multiplicative amplification.

(728) **Efficiency of an Alternate Mill Stream Configuration Assessed via Quantitative Endosperm Content Spectroscopic Imaging with 81,920 Individual Pixels**; David Wetzel[1], Mark Boatwright[, 2]; [1]Microbeam Molecular Spectroscopy Lab, Department of Grain Science, Kansas State University, Manhattan, KS, [2]Department of Biochemistry & Molecular Biophysics, Kansas State University, Manhattan, KS

Quantitation of endosperm in solid mixtures from the wheat milling process (e.g. flour millstreams) of known flow rates enables calculation of both the purity and subsequently the product yield resulting from individual unit processes. The objective is to measure the efficiency of the separation of wheat endosperm from other botanical parts of the kernel through a refining process consisting of many successive operational stages that separate product fractions based on particle size. As the operational parameters of key unit processes are adjusted individually or in concert, the summation expression: $\sum$(% Endo1)(Wt1) + (% Endo2)(Wt2) + …(% Endon)(Wtn) enables an objective measure of the impact of fine tuning to maximize the yield of high value product. The pixel by pixel quantitative chemical imaging procedure uses an array of 81,920 individual spectra to reveals the endosperm distribution in the selected field of view. Each resulting near infrared spectrum enables identification and estimation of the prevalent chemical species for that x, y coordinate of the image by a partial least squares data treatment. The summation of the PLS analyte scores within the FOV results in direct quantitative chemical imaging. Mass balance of endosperm is subsequently reported for intermediate products of individual or successive unit processes using the summation expression described. The practical purpose is to demonstrate optimization of the physical process by objective measurement of the chemical composition of intermediate product streams. Presently, we report the utility of this technique that has been recently applied to more than 20 individual sampling sites in a modified wheat milling operation preceded by debranning. Optimization and relative efficiency of this operation is revealed in this paper.

(729) **New Applications Enabled by Ultra-miniaturized Hyperspectral Imagers**; Owen Wu[1]; [1]BaySpec, Inc.

A self-contained, miniaturized hyperspectral imager in handheld or ultra-compact form eliminates expensive, moving, bulky and complex components that have been used for decades. The result is a spectral imager that can be used everywhere easily such as simply point-and-shoot, on small, low-cost UAVs, or conveyor belts in production lines, with dramatically increased convenience, reduced cost and enhanced sample measurability. Small and low-cost devices pave new avenues to numerous applications in biology, precision agriculture, remote sensing, quality control, art, archeology, and eventually be adapted for much broader use such as in point-of-care diagnostics and other new in-field applications.

(730) **Fluorescence Imaging of Apurinic/Apyrimidinic Endonuclease 1 (APE1) Activity in Living Cells**; Meiping Zhao[1], Junqiu Zhai[1], Simin Fang[1]; [1]Peking University

Apurinic/apyrimidinic endonuclease 1 (APE 1) plays crucial roles in the base excision repair (BER) pathway in human cells.1 Currently available approaches for APE 1 measurement are either discontinuous or susceptible to degradation by other nonspecific nucleases2, making it a difficult issue to directly measure the activity of this nuclease complex biological samples. In our previous work, we have constructed two chimeric

oligonucleotide fluorescent probes for in-situ visualization of the activities of DNases and 3'-5' exonuclease in living cells3. In this study, we further demonstrate a novel magnetic nanoparticle conjugated DNA probe for fluorescence imaging of the activity of APE 1 in living cells.

The probe was prepared by covalently immobilization of a fluorescently labelled dsDNA substrate containing an abasic site onto the surface of silica coated magnetic (Fe3O4@SiO2) nanoparticles (NPs). By optimization of the thickness of the silica layer, the magnetic core itself may serve as a quencher for the fluorescent DNA and guarantee low initial fluorescence background. Upon addition of APE 1, the abasic site in the probe was cleaved and the fluorophore was separated from the NP, giving out strong fluorescence signals. The obtained nano-probe exhibited good dispersion stability in water, very low cellular toxicity and high sensitivity for APE 1 with an LOD of 0.05 U/mL. Moreover, the probe showed high selectivity for APE over other non-target endonucleases, indicating the unique property of the NPs in selective protection of the DNA substrate from being degraded by other non-specific enzymes. With the assistance of external magnetic field, the nanoprobe could be rapidly transferred into living cells under mild conditions. The approach has been successfully applied for in situ fluorescence imaging of the variation of APE 1 activity in living cells.

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(731) **A Surface Plasmon-Coupled Tunable Wavelength Filter for Wide-Field Hyperspectral Imaging.**; Ajaykumar Zalavadia[1], John F. Turner II[1]; [1]Cleveland State University

In the work presented here an electronically tunable wide-field wavelength filter has been developed for hyperspectral imaging applications in the visible and near-infrared region. Conventional electronically tunable wide-field imaging filters technologies include liquid crystal-based filters, acousto-optic tunable filters, and electronically tuned etalons; each having its own set of advantages and disadvantages. The construction of tunable filters is often complex and requires elaborate optical assemblies and electronic control circuits. In the work presented here, we describe a novel surface plasmon-coupled tunable filter (SPCF) with a simple optical design and control. The SPCF employs surface plasmon coupling in metal films separated by a tunable dielectric layer. The advantages of the SPCF are its large spurious free spectral range (450nm-1000nm), appreciable throughput , compact design and low cost of production. In addition, the SPCF is not susceptible to the unwanted harmonic bands that lead to spurious diffraction in Bragg-based devices. Hence its spurious free spectral range covers a broad region from the blue through near infrared wavelengths. The compact design and rugged optical assembly make it suitable for hand-held hyperspectral imagers. The underlying theory and SPCF design are presented. In addition, widefield hyperspectral imaging using the SPCF is demonstrated on both model substrates and real-world samples.

(732) **Combined NIR Imaging and Mapping Approach to Study Large Samples with High Spatial Resolution**; Patrick Wray[1], John Gamble[1], Magnus Hoffmann[1], Gary McGeorge[1]; [1]Bristol-Myers Squibb

Often when using chemical imaging processes it is necessary to study the sample with a high spatial resolution to understand the distribution of the components in the sample on the smallest scales. This can usually best be done by using high magnification optics to study a small area of the sample in great detail. However, samples often have variation which extends over a much greater scale. In the case of an NIR imaging system with a 320 x 240 pixel focal plane array and magnification optics providing an effective pixel size of 20 µm, the image size would be approximately half a centimetre. This is not a sufficient field of view to image the entire face of a tablet or to reveal longer order variations across a ribbon sample. This lack of imaging area can be overcome by using an imaging and mapping approach. This work uses a Malvern Sapphire NIR imaging system in combination with a motorised stage and some custom control software to automate the acquisition of large tiled arrays of chemical imaging data. Custom data processing algorithms were also implemented to improve image stitching by minimising the effects of non-uniform reflections from curved samples. This information is used to investigate thin subsurface layers in the cross sections of multiple large tablets, density variations and particle distributions in ribbon samples simultaneously and to gather information on particle sizes in powder blends over a large enough area to be statistically significant. The work has shown that this method allows NIR imaging and mapping to provide high quality detailed data over a large ample area.

(733) **HPTLC Method for Simultaneous Estimation of Aliskiren, Amlodipine and Hydrochlorothiazide in Synthetic Mixture using Quality by Design Approach**; Mehul Patel[1]; [1]Faculty of Pharmacy, Dharmsinh Desai University

A HPTLC method for the simultaneous estimation of Aliskiren and Amlodipine and Hydrochlorothiazide in the combination dosage form is proposed. Silicagel 60F254 precoated plates were used as the stationary phase, while Quality by design approach was applied for the efficient separation of the drugs through which a simple mobile phase composition Ethyl acetate: Methanol: Ammonia (7.5:2.8:0.22,v/v) was finalized. The RF values optimized were 0.43±0.01, 0.29±0.01 and 0.65±0.01 for Aliskiren and Amlodipine and hydrochlorothiazide, respectively. The densitometric analysis was carried out at 229 nm. The method was linear in the range of 1500-5250 ngspot−1 for Aliskiren, 50–175 ngspot−1 for Amlodipine and 125-432.5 ngspot−1 for Hydrochlorothiazide. The method was validated as per the International Conference on Harmonization guidelines and the %RSD was within the acceptable criterion indicating the method is accurate and precise. The LOD and LOQ were found to be 133.38 ng spot−1 and 404.19 ng spot−1 for Aliskiren, 14.19 ng spot−1 and 43.01 ng spot−1 for Amlodipine and 11.76 ng spot−1 and 35.65 ng spot−1 for Hydrochlorothiazide, respectively. The proposed method was successfully applied for the simultaneous estimation of the drugs in bulk and synthetic mixture indicating suitability for routine analysis of drugs in combination dosage forms.

(734) **Microwave Spectroscopy: Matrix Effects and Interferences on Water Determinations in Pharmaceutical Formulations**; Anders Sparén[1], Halldis Thoroddsen[2], Álvaro Díaz-Bolado[1], Olof Svensson[1]; [1]AstraZeneca R&D Mölndal, [2]Chalmers University of Technology

Pharmaceutical products may be sensitive to water and therefore, it is important to measure and control the water content of pharmaceuticals during manufacturing and storage. The traditional method for determining water content is Karl Fischer titration, but this method is both time consuming and sample destructive. Near-infrared (NIR) spectroscopy is a well-known, fast and non-destructive alternative to Karl Fischer

titration, but requires more advanced calibrations, in order to account for matrix effects that may disturb the water signal in NIR spectra. Microwave spectroscopy, based on microwave resonators, is another fast and non-destructive alternative method that is believed to be more selective to water than NIR spectroscopy and more robust towards matrix effects. There is, however, limited in-depth knowledge of the selectivity and robustness of microwave spectroscopy used for water determination in pharmaceutical formulations.

In this work, we investigated the robustness of microwave spectroscopy for water determination in a pharmaceutical tablet formulation, using experimental design. In the first part of the project, the effects of changing the tablet matrix, such as the drug substance, the particle size of the filler, the size and tensile strength of the tablet were investigated. Tablets of 36 different types plus 12 replicates were manufactured in a lab-scale tablet press and conditioned in four different climates, in order to spread out the water content in the tablets. All tablet types were measured with microwave spectroscopy and with NIR spectroscopy, for comparison. For microwave spectroscopy, the tensile strength and the filler particle size of the tablets had an impact on the microwave signal. The drug substance and the tablets size did, however, not significantly affect the microwave signal. For NIR spectroscopy, the tensile strength, the filler particle size and the drug substance of the tablets interfered with the water determination, while the tablet size did not.

In the second part of the study, the water selectivity of the microwave signal was investigated in liquid phase by adding a mixture of four polar solvents; acetone, acetonitrile, ethanol and dimethylformamide, to a dioxane-water (90/5%) mixture. All solvents tested had some effect on the microwave signal.

(735) ***In situ*** **Near Infrared Imaging and Raman Mapping to Study the Disproportionation of an API HCl Salt During Dissolution**;
Patrick Wray[1], John Jones[1], Graham Clarke[1], Douglas Both[1]; [1]Bristol-Myers Squibb

NIR imaging and Raman mapping were used as complementary approaches to study the dissolution of model pharmaceutical formulations containing the HCl salt of a developmental compound. A custom designed flow through cell was used, which had a flexible design enabling it to be used with either a Raman mapping or NIR imaging system without modification. NIR images were gathered as a function of time over the duration of the experiment showing the whole tablet dissolving. A custom mapping routine was used for the Raman microscope which gathered Raman spectra in a profile from the centre of the dissolving tablet to the edge of the cell. The routine also gathered tiled video images of half of the tablet, allowing details in the video data to be assigned to features in the spectra. This work has shown that NIR imaging and Raman mapping are capable of monitoring the distribution of the components in a formulation during dissolution, while also revealing any form changes which may occur in real time. The NIR and Raman data revealed that the drug underwent conversion to the free base when water was used as the dissolution medium. However, in 0.1 M HCl this conversion was no longer seen as the medium was below the pHmax (the pH of saturation of both unionised and ionised species and above which the free base can form) of the drug. The data from both approaches broadly agreed demonstrating the applicability of these methods to studying and enhancing our understanding of the complex physical and chemical processes which occur during dissolution in real time.

(736) **GTI Control Strategy Based on Fate and Purge Study by HPLC for a Sulfonyl Chloride Compound used as a Starting Material Precursor for API Development**;
Xin Fang[1], Yanqun Zhao[1], James Marek[1], David Hill[1]; [1]AbbVie Inc.

Potential genotoxic impurities (GTI) need to be evaluated and controlled to an appropriate level during API (active pharmaceutical ingredient) process development in order to ensure patient safety. In one such API process, a Sulfonyl Chloride containing compound is used as a non-isolated starting material precursor to form the proposed regulatory starting material (RSM). Sulfonyl Chloride has a class 3 structure alert from In Silico analysis. It is known that Sulfonyl Chloride is highly water reactive and can be hydrolyzed easily to form Sulfonic Acid when exposed to water. Subsequently, Sulfonic Acid can react further with the alcohol/HCI used in the following step (step 2) during the crystallization to form Sulfonate Ester, a known GTI.

Since both Sulfonyl Chloride and Sulfonate Ester levels are linked to the concentration of Sulfonic Acid, an HPLC method was developed for the determination of Sulfonic Acid, to monitor the residual Sulfonyl Chloride in RSM and evaluate the formation of Sulfonate Ester as well. Four representative production batches of RSM were analyzed using the HPLC method and the residual Sulfonic Acid level detected was from ND (< 3 ppm) to 13 ppm. Then, partitioning of Sulfonic Acid between aqueous phases and Ethyl Acetate was studied in the lab using the HPLC method by simulating the aqueous work-ups in step 2 manufacturing process. The total rejection of Sulfonic Acid determined through the work-up process was 93%. Therefore, the level of Sulfonic Acid can be reduced to less than 1 ppm after the aqueous washes and Sulfonate Esters formed during the following crystallization would be less than 1 ppm. The analytical monitoring and spike/purge study demonstrated that adequate control of Sulfonyl Chloride and Sulfonate Esters in the API is achieved using the current process.

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(737) **Analysis of Pharmaceutical Bilayer Tablets Using Transmission Raman Spectroscopy**;
Yan Zhang[1], Gary McGeorge[1]; [1]Bristol Myers Squibb

Transmission Raman Spectroscopy (TRS) has become an increasingly applied technology in the analysis of pharmaceutical tablets for quality control purposes and developing formulation and process understanding. One area that has received only cursory attention to date is that of bilayered tablets that represents an unusually challenging situation. A bilayered tablet is composed of two discretely unique formulations that are sequentially compressed, one after the other to create a layered composite tablet. Traditional absorption techniques are unable to quantitatively assess the tablet composition without imposing constraints on several of the tablets manufacturing variables.

This presentation will provide an understanding of the relationship of the API content and the transmission Raman spectral response in bilayered pharmaceutical tablets to facilitate development of quantitative models for the prediction of API content in bilayer tablets. The Raman intensity was considered as a function of the Raman photon generation and decay in a layer of interest and Raman photon decay from a second layer. The complex spectral response was effectively modeled according to a modified Schrader, Kubelka-Munk model where both the Raman photon generation factor and photon losses were accounted for. The combined results of these studies yields a comprehensive approach for modeling multi-component bilayer tablets. The addition of a beam enhancer on the bottom surface allowed for a selective over-enhancement of the bottom layer, which helps in the analysis of thin layers or coatings.

(738) **Determination of Residence Time Distribution for a Hot Melt Granulation Process using NIR and Raman Probes**; Patrick S Wray[1], Keely Bergqvist[1], John W Jones[1], Martin Vernon[1], Gary McGeorge[1]; [1]Bristol-Myers Squibb

It is necessary to understand the residence time of a material inside a process unit as this can have a large effect on the properties of the final product. Hot melt granulation is a continuous process, in which the goal is to convert the powdered input into a granulated product. If the material spends too long in the granulator it is possible that over granulation may occur or for example if the material is thermolabile there may be some thermal degradation of the sample. Often tubular reactors are approximated as having plug flow conditions, meaning that it is assumed that there is no longitudinal mixing. For hot melt granulation this may not be a valid assumption and therefore it is necessary to assess the residence time distribution for a system. The most common method by which to do this is to spike the input. For the NIR and Raman methods used here, this was another drug with distinct spectral bands. The system used a polymer blend for the phase being granulated. A caffeine based blend was used for the spike. The spikes were added to the input to the granulator and the probes were used to measure the delay and concentration profile as a function of time as the spike reached the other end of the granulator. The spread of the spike at the end of the system served to reveal the mixing behavior of the powder within the granulator. This experiment was performed for different screw designs and speeds in order to understand how these parameters affected the system. Data collection for hot melt granulation can be problematic as the discrete nature of granules means that the sensor may be measuring the empty screws for a large fraction of the measurement time. As a result of this several optimisation methods including decreasing measurement time and a range of data processing methods were applied. The work has established that despite the inherently discrete nature granules it is possible to generate a good assessment of the residence time distribution by using spectroscopic methods.

(739) **Structure of Biologics with Cutting Edge Vibrational Spectroscopy**; Carolina Carballo, Rina Dukor; [1]BioTools Inc

Biopharmaceutical industry has evolved in the last few years to include not only biosimilars but also peptides, ADC's, and fusion proteins. As molecules evolve so should the techniques to analyze them. Vibrational Spectroscopy in particular has many advantages to study secondary and higher order structures in liquid and solid forms. In this presentation, we will demonstrate examples of the combined use of the four vibrational techniques - FTIR, Raman, VCD & ROA.

(740) **Surface Interaction of Nitrogen-Containing Aromatic Molecules with Gold Investigated with Surface Enhanced Raman Spectroscopy (SERS)**; Ashish Tripathi[1], Erik Emmons[1], Augustus Fountain[2], Jason Guicheteau[2], Martin Moskovits[3], Steven Christesen[2]; [1]LEIDOS Inc.; [2]U.S. Army Edgewood Chemical Biological Center; [3]Department of Chemistry and Biochemistry, University of California, Santa Barbara

Surface-enhanced Raman scattering (SERS) is potentially a capable tool for detecting trace concentrations of analytes. In order to predict the SERS activity of relevant toxic compounds, it is important to understand the nature of the binding of these compounds to the noble metal surfaces. In this effort we have studied the binding of several nitrogen containing aromatic chemicals to nanostructured gold surfaces from aqueous media. These studies have revealed that a complex equilibrium state exists between the molecules and the gold surface that cannot be explained by a simple Langmuir isotherm. The peak area of selected Raman features versus concentration data for each of the chemicals analyzed suggests two equilibrium states. The two equilibrium states could be attributed to concentration dependent arrangements/orientation and/or two types of adsorption sites. The Raman spectral data also shows observable differences between the two equilibrium states. Our studies were expanded to investigate the causation of the observed complex equilibrium state between these molecules and nanostructured gold surfaces.

(741) **The Effect of Molecular Polarity and Solubility on Adsorption Rates and Equilibrium Constants for Molecules on Noble Metal Surfaces Using Surface-Enhanced Raman Spectroscopy**; Erik Emmons[2], Ashish Tripathi[2], Neal Kline[3], Jerry Cabalo[1], Jason Guicheteau[1], Augustus Fountain[1],Steven Christesen[1]; [1]Research and Technology Directorate, Edgewood Chemical Biological Center; [2]LEIDOS Inc; [3]Oak Ridge Institute for Science and Education at Research and Technology Directorate, Edgewood Chemical Biological Center

Adsorption of molecules on surfaces plays an important role in many different technical applications, and can be influenced by many factors. Molecular parameters such as polarity and solubility are expected to play an important role in adsorption. We are studying the effect of polarity and solubility on the adsorption of aromatic molecules on noble metal surfaces using the technique of surface-enhanced Raman spectroscopy (SERS). Detection of low concentrations of analytes of interest such as explosives and toxic chemicals is possible using SERS, but only if the molecule adsorbs to the surface. To reduce the relative lack of information on what causes molecules to adsorb, we have studied different series of aromatic thiol and nitrogen-containing molecules with different substituents that lead to varying levels of overall molecular polarity and solubility. These factors have been shown to have an effect on adsorption rates, equilibrium constants, and binding energies. These measurements show trends which can provide predictive power for determining the SERS response.

(742) **Electroless Gold Plating as an Adaptable Tool to Fabricate Custom Surface Enhanced Raman Spectroscopic (SERS) Substrates**; Buddini Karawdeniya[1], Y. M. Nuwan Bandara[1], Caitlin Masterson[1], Julie Whelan[1], Brian Velleco[1], Jason Dwyer[1]; [1]University of Rhode Island

We are interesting in developing low-cost, disposable, surface-enhanced Raman (SERS) substrates. Our goals extend to being able to customize the physical nature of the SERS-active metal film while being flexible in our choice of underlying support structure to create custom SERS sensing platforms. We fabricated such a substrate with an electroless plating technique we developed that does not require any sophisticated deposition equipment yet is capable of forming thin gold films on silicon nitride—a frequently used nanofabrication material with properties favorable for creating devices for chemical analysis. The ~20nm diameter grain size of these gold films was effective in creating a substrate for surface enhanced Raman spectroscopy (SERS). Initial experiments using nitrobenzenethiol (NBT) as a probe molecule confirmed that the electroless gold film allowed us to record a SERS spectrum, while less nanostructured gold films did not display a detectable signal. The same electroless plating technique is operable on a number of silicon-based substrates while both the grain size and film structure can be modulated through various chemical and physical approaches. We used NBT in more comprehensive experiments in which we explored the limits of detection, ease of use and reproducibility. It is possible to further tailor the SERS performance by chemical modification of the semiconductor surface with selected organic layers, prior to electroless plating. Furthermore, the plating chemistry we use has allowed us to gold-plate paper substrates, allowing us to record SERS spectra from paper substrates—an approach which provides tremendous benefits of in terms of low material cost, flexibility and easy disposability. We expect our SERS substrates to have utility in standard lab-based analysis as well as in applications such as environmental monitoring.

(743) **Gold-based Multi-layered Probes for Enhanced SERS**; Pietro Strobbia[1], Alex Henegar[1], Theodosia Gougousi[1], Brian Cullum[1]; [1]University of Maryland Baltimore County

Sensors based on surface enhanced Raman spectroscopy (SERS) have several advantages over other types of sensing platforms, due to their sensitivity and potential for highly multiplexed detection. Over the years, SERS probes have been developed to be capable of extreme sensitivities, with single molecule detection having been achieved in "hot-spots" of colloidal aggregates. However, these structures suffer from significant irreproducibility, due to the randomness of the aggregation. Alternatively, strategies such as ordered 2D arrays or enhancement based on single probes (e.g. immunno-nanosensors, nanostars) have high reproducibilities but limited enhancement factors.

In our laboratory a widely applicable enhancing geometry has been developed based on thin films of silver interleaved with native oxide spacers that enhance the signal independent from the underlying structure. Using this geometry is possible to amplify the sensitivity of a previously developed SERS-based sensing platforms up to an order of magnitude over their current enhancement factors. This multi-layer architecture has been extended to the fabrication and optimization of gold structures, resulting in sensors with improved stability in addition to the enhanced sensitivity. Preliminary evidence into the principle of this enhancement mechanism suggests that the spacer material and thickness play a key role in the magnitude of the resulting enhancement. In this paper we investigate the relationship between the enhancement dependence and spacer thickness using atomic layer deposited ultrathin oxides. Additionally, results from these studies are used to construct a predictive model relating multilayer enhancement magnitudes to bulk properties of the spacer materials employed.

(744) **Rapid Monitoring of Biocatalytic Processing using UVRR and SERS Spectroscopies**; Heidi Fisk[1], Jason Micklefield[1], Roy Goodacre[1]; [1]The University of Manchester (UK)

Raman spectroscopy is a non-destructive, label-free and information-rich analytical technique that arises due to the inelastic scattering of light. Raman spectroscopy has a wide range of applications and has become increasingly popular within biological applications as water is a weak scatterer, enabling analysis of biological samples. Although it is a powerful technique that can uncover detailed structural information, it is an inherently weak phenomenon and detection may not always be possible, particularly when low sample concentrations are required. Two methods of enhancement are surface enhanced Raman scattering (SERS) and UV resonance Raman (UVRR).

The main aim of this work is to develop a rapid high-throughput enzyme assay screen based on SERS and/or UVRR spectroscopy that could be used within direct evolution projects in order to assess the most appropriate clones to take forward for further enzyme evolution. The enzyme class of choice are tryptophan-dependent halogenases which halogenate small molecules, including the chlorination of tryptophan and fenamic acid; an intermediate used in the synthesis of a number of nonsteroidal anti-inflammatory drugs. Traditional chemical synthesis of halogenated compounds often involve the use of toxic reagents and frequently sufferer from a lack of chemoselectivity and regioselectivity. Halogenases overcome this problem as benign chloride sources are employed and halogenation occurs in a regioselective manner. It has been shown that the introduction of one or more selectively positioned halogen atoms to a biologically-active compound can have a significant effect on the inherent biological activity, therefore selective halogenation is of great interest to the pharmaceutical industry.

This presentation will report progress in: (1) UVRR for the successful real-time monitoring of the biocatalytic conversion of small molecules to chlorinated and/or brominated products using tryptophan-dependent halogenases; (2) a SERS-based solution for the quantification of substrate and reactants by chemical modification by the addition of thiol groups to enable enhanced interaction of the small molecule with the silver nanoparticle surface.

(745) **A Sheath-Flow Microfluidic Device for Combined Surface Enhanced Raman Scattering and Electrochemical Trace Detection**; Matthew R. Bailey[1], Amber Pentecost[2], Asmira Selimovic[2], R. Scott Martin[2], Zachary D. Schultz[1]; [1]University of Notre Dame; [2]Saint Louis University

The combination of hydrodynamic focusing with embedded capillaries in a microfluidic device is shown to enable both surface enhanced Raman scattering (SERS) and electrochemical characterization of analytes at nanomolar concentrations in flow. Linking multiple detection methods allows for the unique advantage of obtaining complimentary information about analytes. The approach utilizes a versatile polystyrene chip that contains an encapsulated microelectrode and fluidic tubing, which is shown to enable straightforward hydrodynamic focusing onto the electrode surface to improve detection. A polydimethylsiloxane (PDMS) microchannel positioned over both the embedded tubing and SERS active electrode (aligned 200 μm from each other) generates a sheath flow that confines the analyte molecules eluting from the embedded tubing over the SERS electrode, increasing the interaction between the analyte and the surface. The microfluidic device was characterized using finite element simulations, amperometry, and Raman experiments and demonstrates a SERS and amperometric detection limit near 1 and 25 nM, respectively. Combining SERS and amperometry onto a single platform provides an improved method to identify and quantify electroactive analytes over either technique independently and suggests a straightforward route to improving trace detection both spectroscopically and electrochemically. The device is currently being used to investigate a number of different species, including a series of neurotransmitters.

(746) **SERS Detection of Glucose Phosphate Isomers**; Colleen Riordan[1], Zachary Schultz[1]; [1]University of Notre Dame

Glucose phosphates are important in metabolism, from glycolysis and gluconeogenesis to glycogen storage and breakdown. Changes in glucose metabolism can be tracked by monitoring the ratio of glucose 1-phosphate to glucose 6-phosphate, which will give information about the functioning of the cell under different conditions. Isomeric structures of carbohydrates, including glucose phosphates, are difficult to identify by many methods; however, Raman spectra provide distinct spectra associated with subtle changes in chemical structure. Raman spectroscopy is an opitical technique where the observed signal corresponds to vibrational modes of molecules. These vibrational frequencies are unique to each structure, allowing for the differentiation of isomeric analytes. One disadvantage of Raman is low sensitivity, since only $1x10^{-13}$ photons are stokes and anti-stokes scattered meaning that small sample sizes are hard to detect. Surface-enhanced Raman spectroscopy (SERS) allows for signal enhancements that are typically 10^6 or better, which enables detection of analyte at low concentrations. The biggest disadvantage of SERS is that to achieve this enhancement the analyte must be close enough to the SERS substrate to interact with the electric field. One solution to the issue of analyte proximity to the surface is to use a self-assembled monolayer (SAM) on the SERS surface. The basic idea is that the SAM interacts with the analyte of interest, which brings the analyte into close enough proximity to the surface that the Raman enhancement is achieved. Preliminary results I have obtained show that a SAM of 4-mercaptophenylboronic acid (4-MPBA) or 3-MPBA are both able to bind glucose 6-phosphate and show a marked change in SERS spectra. I also found that a rinse with acetic acid ($1x10^{-5}$ M) led to the disappearance of the glucose 6-phosphate peaks, suggesting that the acid rinse removes the sugar from the surface by changing the interaction with the SAM

because of the change in pH. Optimizing the MPBA SAM for glucose detection by SERS and incorporating the MPBA SAM into a flow cell may enable high-throughput glucose phosphate characterization.

(747) **Development of a Stable, Gold Nanoparticle SERS Substrate**; Md Shah Alam[1], Mary M. J. Tecklenburg[1]; [1]Central Michigan Univ., Dept. of Chemistry & Biochemistry, Science of Advanced Materials

The project aims at developing a stable sensor suitable for field testing based on noble nanoparticles in order to identify very low quantities of hazardous chemicals and pharmaceuticals in the environment. The sensor must protect the nanoparticles from exposure to the air and other elements that lower their stability. Owing to unique properties e.g. optical, sensing, electronic, and catalytic, functionalized metal nanoparticles have been extensively utilized for surface enhanced Raman scattering (SERS) - a surface responsive technique that enhances the Raman scattering of a molecule adsorbed on nanoparticles. Most of these applications require definite particle size and shape, uniform distribution as well as stability. We are working on incorporating as prepared gold nanoparticles into polymer matrices to stabilize them for better performance in detecting chemicals by the SERS method.

We have synthesized surfactant-assisted gold metal nanoparticles whose surface plasmon resonance wavelength ranges from 500 nm to 700 nm in UV-Visible absorption spectra. In addition, nanoparticle characteristics have been investigated by transmission electron microscopy (TEM). These nanoparticles are used to optimize the SERS detection limit for a test molecule, 4-aminothiophenol (4-ATP), a mercaptan which is very toxic and hazardous at high exposure. Further studies relate to embedding of the optimized nanoparticles into a polymer matrix to produce a stable sensor for SERS detection of environmental chemicals. Furthermore, the interaction of nanoparticle-polymer matrix and the potential of polymer embedded gold metal nanoparticles will be studied for SERS application.

(748) **Green Photochemical Synthesis of Plasmonically Tunable, SERS-Active Y2O3@Ag Hybrid Nanomaterials**; Aaron Crookes[1], Casey Gallagher[1], Jonathan Scaffidi[1]; [1]Miami University

Plasmonic core-shell nanomaterials have attracted intense interest in recent years, but traditional syntheses of these core-shell materials typically use potentially cytotoxic compounds such as cetyltrimethyl ammonium bromide (CTAB), formaldehyde, or polyvinylpyrrolidone (PVP). Here, we describe an alternative photochemical approach that allows rapid formation of silver shells around non-toxic yttrium oxide cores. The overall size, core-shell ratio and plasmon wavelength of the resulting core-shell particles can be tuned from ~400 nm to the NIR through selection of the non-toxic dispersant, the concentration of silver nitrate during shell formation, and the duration of light exposure. We present preliminary surface-enhanced Raman scattering data to highlight the plasmonic nature of the resulting Y_2O_3 @ Ag hybrid nanomaterials.

(749) **Evaluation of Sensitivity and Selectivity in Quantitative SERS-based Determination of Heavy Metal Concentrations**; Jenny DeJesus[1], Ji Li[1], Audrey Hoffmann[1], Alyssa Meier[1], Jessica Krandel[1], Jonathan Scaffidi[1]; [1]Miami University

The nature of interactions between heavy metals and various metal binding groups (MBGs) have been extensively investigated, but these interactions have only rarely been applied to the quantitative determination of heavy metal concentrations in surface-enhanced Raman spectroscopy (SERS). Here, we present results describing the sensitivity and selectivity of various MBGs during SERS-based determination of heavy metals including Cd^{2+}, Cu^{2+}, Pb^{2+}, and Hg^{2+}. In some cases, limits of detection meet or exceed EPA maximum contaminiant levels (MCLs). This raises the possibility of on-site or continuous *in situ* heavy metal monitoring using SERS.

(750) **A Stable Nanostructured Substrate for Surface Enhanced Raman Scattering detection of Benzotriazole**; Brandon Russell[1], Mary Tecklenburg[1]; [1]Central Michigan University

Surface-enhanced Raman scattering (SERS) is a powerful spectroscopic technique that has a potential for high-throughput characterization and sensing of a variety of trace-level environmental and biological analytes when adsorbed on nanometer-sized metallic colloidal particles. A most critical aspect of an SERS measurement is the structure that is used to enhance the Raman signal. Much of the research being carried out shows a large number of methods that have been used to manufacture structures for SERS. Among the many substrates, colloidal silver nanoparticle synthesis is easy and is preferred for the convenience of monitoring the nanoparticle solutions by UV spectroscopy. However, the utility of this type of structure is limited by poor control of the nature and extent of particle aggregation which results in a poor stability and reproducibility. To this end, our aim is to implement an easy and robust approach to overcome these limitations for the production of long-term stable silver nanoparticle dispersions with narrow size distribution.

In our approach, we have utilized the unique inverse micellar behavior of amphiphilic hyperbranched polyethyleimine with a hydrophilic core and a hydrophobic shell. The polar domains of these micelles effectively stabilize the silver nanoparticles in a non-polar environment. Such a polymer encapsulated nanoparticle solution is made into films to be used as a SERS surface for testing. Our research is also focused on the influence of the density of the hydrophobic shell with respect to the stability of the silver for the SERS signal and also on the homogeneous distribution of silver in the polymer to attain a consistent SERS signal. The SERS effect of benzotriazole (a drug precursor) on our silver nanoparticle encapsulated surface will be examined.

(751) **Multiplexed Homogenous SERS Immunoassay Based on Antigen-Mediated Aggregation of Gold Nanoparticles**; Seth Filbrun[1], Yen Lai[1], Arielle Lopez[1], Jeremy Driskell[1]; [1]Illinois State University

Surface enhanced Raman spectroscopy (SERS) immunoassays have been increasing in popularity in the past several years due the ability to provide low limits of detection, high sensitivity, and multiplexed detection. While SERS-based assays have led to improvements in the analytical performance of immunoassays relative to conventional assays, e.g., ELISAs, greater efforts are needed to develop convenient and rapid SERS-based assays that are suitable for point-of-need applications. To this end, we show the development of a multiplexed immunoassay utilizing antigen-mediation aggregation of gold nanoparticles (AuNP). This sandwich immunoassay was performed using extrinsic Raman labels (ERLs), which consist of a AuNP modified with a mixed monolayer of a Raman dye and an antibody. In order to obtain a multiplexed assay ERLs were made using unique combinations of Raman dye and antibodies specific for mouse, rabbit, and human IgG. Several Raman reporter molecules were systematically studied to select three reporters that provided the highest signals while simultaneously giving unique Raman bands. Sample solutions were then added to the mixture of ERLs, and when the antigen of a particular antibody was, present it caused aggregation of that ERL.

The sample/ERL mixture was drawn through a 0.2 µm porous filter that had been previously incubated in a 1% bovine serum albumin (BSA) solution to minimize non-specific interactions. The filter pore size was selected to allow the non-aggregated ERLs to pass through the filter while

the aggregated ERLs were captured. SERS spectra were collected on the filters, and the specific vibrational bands were used to identify the antigen in the sample while the band intensities were used to quantify the antigen. Our current limit of detection for this method is 1.9ng/mL, ~20-fold lower than that achieved with an ELISA utilizing the same antibody-antigen system. Moreover, the filter-based assay takes 60min to complete detection while EILSA takes 24 hrs. This method was validated using whole mouse serum to accurately quantify IgG. This method lays the groundwork for the development of powerful point-of-care diagnostics, or field portable defense applications.

(752) **Surface-Enhanced UV Fluorescence and Raman Scattering from Electrochemically Roughened Aluminum Substrates**; Danielle Montanari[1], Nathan Dean[1], Tyson Davis[1], Natascha Knowlton[1], Joel Harris[1]; [1]University of Utah

Interest in label-free detection of biomolecules has given rise to the need for UV plasmonic materials. DNA bases and amino acid residues have electronic resonances in the UV which allow sensitive, label-free detection of these species by UV fluorescence or resonance-enhanced Raman spectroscopy. Detection of sub-monolayer coverages of molecules on surfaces is essential for the development of sensor technologies. Electrochemical roughening has been used extensively to generate plasmonically active metal surfaces that produce localized enhancement of excitation and emission of electromagnetic radiation from surface-bound molecules. Electrochemical roughened gold and silver surfaces produce enhancement in the visible and near-IR regions, but to the best of our knowledge, application of this processing for UV-enhancing substrates has not been reported. Using electropolishing or anodization of aluminum, we are able to generate surfaces that produce enhanced spectroscopic detection of molecules in the UV. We have fabricated electropolished aluminum films with sub-nanometer RMS roughness, as well as aluminum films patterned electrochemically on a 100nm scale by anodization followed by removal of the oxide layer. These nanostructured aluminum surfaces exhibit a plasmon resonance at wavelengths between 200 and 300nm. We have studied UV-excited fluorescence enhancement from sub-monolayer coverage of tryptophan on both the electropolished and patterned (anodized) aluminum substrates using a UV-laser based spectrometer. Quantitative dosing by dip-coating was used to deposit known surface concentrations of tryptophan, so that fluorescence enhancement can be evaluated. Compared to a quartz substrate dosed in the same manner, we demonstrated a 4.5-fold enhancement of fluorescence on the anodized substrates, and a 65-fold enhancement of fluorescence on the electropolished substrates. Preliminary UV-SERS data shows a similar trend, with the electropolished aluminum substrates yielding significantly greater enhancement of scattering intensity from pyrene compared to an anodized aluminum surface.

(753) **Exploring the Potential of Commercially Available Gold Nanoparticles for Surface Enhanced Spatially Offset Raman Spectroscopy (SESORS) for Tissue Diagnostics**; Louise Clark[1]; [1]University of Exeter

Deep Raman techniques have been demonstrated previously for subcutaneous identification of the biochemical composition of tissue. Thus, allowing deeply buried malignant lesions and calcifications to be detected. The use of gold nanoparticles (AuNPs) in combination with deep Raman: SESORS (surface enhanced spatially offset Raman Spectroscopy) have been found to amplify the Raman signal by many orders of magnitude. By labelling and functionalizing them it may be possible to measure varying expressions of disease from outside the body. This non-invasive approach has the potential for sensitive cancer diagnosis allowing tailored treatment options to be considered more swiftly, making it an exciting biomedical tool.

The overall aim of the project is to use the SESORS technique for intramammary carcinoma diagnosis. For in vivo tumour targeting, the AuNPs must be biocompatible, non-cytotoxic and designed to produce the largest optical enhancement achievable. Specific, minimally invasive cancer diagnostic techniques which can detect early stage cancerous lesions are very important in ensuring reduced mortality rates from easily treatable breast cancers.

Hence, the viability of different commercially available AuNPs has been explored. Work considering the most favourable wavelength, the optimisation of the size of the AuNP, investigation into PEGylated AuNPs and labelling of the NPs using Raman labels is discussed here, with results from the evaluation presented. Further work will be looking into the functionalization of these AuNPs with the intention of striving towards breast disease specific NPs.

(754) **A Wet Synthetic Method Yielding Plasmonically Tunable Solid State SERS Substrates**; Seth Filbrun[2], Jennifer Fasciano[1], Jonathan Scaffidi[1]; [1]Miami University; [2]Illinois State University

Solid-state plasmonic substrates provide an appealing alternative to colloidal gold or silver nanoparticles in surface-enhanced Raman spectroscopy (SERS) because they avoid a host of instability and aggregation issues. Most methods used to prepare solid-state substrates, however, at some stage rely on small-batch, high-vacuum evaporative deposition of gold or silver. This requires expensive instrumentation that is typically housed in a clean-room environment, and the resulting substrates are often poorly suited to use outside the research laboratory.

Here, we present results for a recently-developed wet chemical approach to preparing solid-state SERS substrates. Morphologically, these substrates consist of a dense network of closely-spaced, irregularly shaped islands similar to gold- or silver-island films generated by high-vacuum evaporative deposition. Control of reaction conditions allows the surface plasmon of these substrates to be tuned from ~525 nm to >800 nm. Strong SERS signals are observed for a range of common excitation wavelengths.

(755) **Aluminum Substrates for UV-SERS**; Maria Fernanda Cardinal[1], Bhavya Sharma[1], Michael B Ross[1], Alyssa Zrimsek[1], Sergei V. Bykov[2], David Punihao-le[2],Sanford A. Asher[2], George C. Schatz[1], Richard P. Van Duyne[1]; [1]Department of Chemistry, Northwestern University; [2]Department of Chemistry, University of Pittsburgh

Here we present UVSERRS data obtained with 229 nm excitation for three molecules on: Ag and Al film over nanosphere (FON) substrates, Ag and Al mirrors, on quartz, in solution, and in the solid state. Additionally, we discuss experimentally observed enhancements with theoretical calculations to understand the plasmonic effects of Ag and Al in the deep UV wavelength region.

For this work, we specially adapted the methodology[1, 2] to create silver and aluminum FONs of tunable localized surface plasmons resonance in the UV spectral range. In particular, we focused our discussion on two different spheres sizes used to prepared Al and Ag FONs and three common analytes: adenine, tris(bipyridine)ruthenium(II) and trans-1,2-bis(4-pyridyl)-ethylene. For each substrate and analyte, we estimate an enhancement factor and then generalize rules concerning the ideal substrate composition based on theoretical predictions[1, 3, 4] and excitation wavelength.

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(756) **Lab-on-a-Chip Instrumentation and Methods for Detecting Trace Organic and Bioorganic Molecules in Planetary Exploration**; Richard Mathies[1]; [1]Chemistry Department, University of California at Berkeley

In situ organic analysis is a powerful approach for detecting molecules of relevance for chemical and biochemical evolution in our solar system. Over the past 15 years we have developed a microfabricated capillary electrophoresis (CE) system integrated with a multivalve sample processor that enables the autonomous analysis of organic molecules including amino acids, carboxylic acids, amines, aldehydes, thiols and polycyclic aromatics with part-per-billion sensitivity (Skelley et al., PNAS 102, 1041, 2005). Organic molecules are labeled with a fluorescent reagent according to their functional group in a programmable microfluidic processor and then separated in a CE system followed by laser-induced fluorescence detection to determine molecular size and concentration (Kim, et al., Anal Chem, 85, 7682, 2013).

We are currently focused on the use of this technology to detect organic molecules in comets, in comet tails, and in ejecta from icy moons of Saturn such as Enceladus because these sources provide perhaps the best opportunity for detecting extraterrestrial biomolecules. Enceladus is idea because it ejects water plumes from cracks in its salt water ice surface that are rich in organic molecules. The Enceladus Organic Analyzer (EOA) will fly through such a plume and use a special open door for ice-particle capture. After closing the capture door, the material in the capture chamber is then dissolved, labeled and analyzed by the microfabricated CE system. If amino acids are detected, their chirality is determined because chirality is the best indicator of a biologically produced molecule. The EOA is a 16x16x12 cm, 3 kg instrument with low power usage that is currently proposed for a Discovery Mission to Saturn.

(757) **Nanowires, Nanoelectronics and the Interface with Biological Systems**; Charles Lieber[1]; [1]Harvard University

Nanoscale materials enable unique opportunities at the interface between the physical and life sciences, for example, by integrating nanoelectronic devices with cells and/or tissue to make possible communication at the length scales relevant to biological function. In this presentation, the development of nanowires, nanowire-based nanoelectronic devices and their application as powerful tools for the recording and stimulation from the level of single cells to tissue will be discussed. First, a brief introduction to the synthesis of complex modulated nanowires will be highlighted as a central material in nanoscience for enabling scientific studies across a broad range of fields. Second, we will introduce key constraints for developing electronic devices for biological studies. We will discuss our focus on 'active' nanoelectronic devices and compare these to other electrophysiology tools to illuminate strengths/weaknesses and opportunities enabled by active devices. Third, the creation of new nanoelectronic probes capable of intracellular recording and stimulation at scales heretofore not possible with existing techniques will be discussed. Recent results from ongoing studies of neuronal systems will be described. Last, we will take an 'out-of-the-box' look and consider merging nanoelectronic arrays and circuits with tissue in three-dimensions (3D). We will describe a new method by which freestanding macroporous nanoelectronic networks can be delivered by syringe injection and subsequently 'unfold' to their originally-designed geometric configuration. Studies focused on targeted injection into rodent brains, including the minimally-invasive integration with neural networks. Multiplexed recording of brain activity from the addressable nanodevices within the nanoelectronic scaffolds will be described, as well as detailed histology studies demonstrating unique neurophilic nature of the these new nanoelectronic networks. The prospects for broad-ranging applications in the life sciences as the distinction between electronic and living systems is blurred in the future will be discussed.

(758) **Extreme Separations-Cells: Antibiotic Resistance as a Differentiator in Staphylococcus epidermidis**; Mark Hayes[1]; [1]Arizona State University

Our group is developing better separation methods for virtually all biological targets (metabolites, peptides, proteins, organelles, bioparticles, cells, etc.). Using gradient techniques for separations of complex mixtures allows for the effects diffusion and other dispersive factors to be minimized. An especially attractive mix of forces for common biological samples is electrophoresis and dielectrophoresis in an open channel. In this channel, there are unique capture points for various values of electrophoretic and dielectrophoretic properties of the analytical targets, described by characteristic mobilities (electrokinetic and dielectrophoretic mobilities, respectively). With this technique, very similar particles and biological materials can be efficiently separated. Under optimal conditions a one micron particle can be separated from one that varies by only one nanometer (one part in a 1000-2000). Other characteristic resolution factors include minimal resolvable dielectrophoretic mobility at 10^{-26} $m^4/V2^s$ (one part in 10^9) and minimal resolvable Clausius-Mossotti factor at 10^{-9}. These are extraordinary figures of merit. We have already shown extremely positive results by separating gentamicin resistant Staphylococcus epidermidis from susceptible strains, reflecting extremely subtle alterations in cellular structure or function. A theoretical framework and resulting predictions will be presented along with key data supporting the models.

(759) **Miniaturization of Liquid Chromatography**; Milton Lee[1], Sonika Sharma[1], Paul Farnsworth[1], Dennis Tolley[1], Alex Plistil[2], Hal Barnett[2], Stanley Stearns[2]; [1]Brigham Young University; [2]VICI Valco

The desire for immediate chemical analysis at the sampling point is one of the main driving forces for the development of small instruments. Analyzing samples at the point of collection rather than in the laboratory is useful in many applications, such as space missions, forensics, homeland security, environmental contamination, and point-of-care analysis, to name a few. Over the last four decades, liquid chromatography (LC) has experienced an evolution to smaller columns and particles, and even to microchips. However, the miniaturization of LC instrumentation has not followed, mainly due to difficulties encountered in miniaturizing pumps and detectors, and in reducing solvent consumption.

In this work, a novel splitless nano-flow gradient generator integrated with a stop-flow injector and on-column 260-nm deep UV LED-based absorption detector was developed and evaluated. The gradient pumping system consisted of two nano-flow pumps controlled by micro stepper motors, a mixer connected to a serpentine tube, and a high-pressure valve. The gradient system weighed only 4 kg (9 lbs) and could generate up to 55 MPa (8000 psi) pressure. The system could operate using a 24 V DC battery and required 1.2 A for operation. The total volume capacity of the pump was 74 µL, and a sample volume of 60 nL could be injected. The system provided accurate nanoflow rates as low as 10 nL/min without

employing a splitter, making it ideal for capillary column use. Retention time reproducibility was very good in run-to-run experiments (RSD < 1.42%, n = 4).

The detector was small (5.2 × 3.0 cm) and weighed only 85 g (without electronics), and it was specifically designed and optimized for on-column detection to minimize extra-column band broadening. No optical reference was included due to the low drift in the signal. Two ball lenses, one of which was integrated with the LED, were used to increase light throughput through the capillary column. Stray light was minimized by the use of a band-pass filter and an adjustable slit. Signals down to the ppb level (nM) were easily detected with a short-term noise level of 4.4 µAU, confirming a low limit of detection and low noise.

(760) **Microfluidic Sample Preparation, Separation and Delivery for Ultrasensitive MS-Based Bioanalyses**; Ryan Kelly[1], Yongzheng Cong[1], Tao Geng[1], Shanta Katipamula[1], Sachin Jambovane[1], Erin Baker[1], Keqi Tang[1]; [1]Pacific Northwest National Laboratory

Mass spectrometry (MS) provides information-rich, label-free analysis of biomolecules and is the workhorse technology in the fields of proteomics and metabolomics. Coupling microfluidics with MS enables new applications and a reduction in required sample sizes. In particular, active microfluidic devices with integrated pneumatic microvalves allow automated, multistep biochemical analyses to be performed using subnanoliter volumes of sample and reagents. We will describe microfluidic interfaces for electrospray ionization (ESI)-MS that enable stable operation at low-nanoliter-per-minute flow rates, enabling sub-attomole detection limits to be reached. We will then describe the coupling of both droplet-based and droplet-free platforms with an optimized ESI interface and their application to (1) real-time monitoring of high-rate, solution-phase reactions, (2) preconcentration, injection and capillary electrophoresis separation of protein and peptide mixtures, and (3) preparation of individual cells for MS analysis.

(761) **Combined Fluorescence and Raman Spectroscopy for Tumor Bed Assessment in Soft Tissue Sarcomas**; Anita Mahadevan-Jansen[1], John Nguyen[1], Zain Gowani[1], Margaret O'Connor[1], T. Quyen Nguyen[1], Xiaohong Bi[2],Ginger Holt[1]; [1]Vanderbilt University; [2]University of Houston; [3]Northwestern University

Soft tissue sarcoma (STS) is a broad term that describes a group of over 50 subtypes of rare and aggressive tumors. Despite their heterogeneity, STS's often physically manifest as localized masses within the body that are primarily managed through surgical resection. Inadequate resection of these tumors can lead to local recurrence and decreased survival rates. Current intraoperative methods for tumor margin assessment are time consuming and suffer from sampling errors. Here, we will present the use of a combined near infrared fluorescence and Raman spectroscopy system for the differential diagnosis of STS and normal tissues within the surgical bed. Near-infrared spectra was acquired from in vivo human patients undergoing STS resection at the Vanderbilt University Medical Center and analyzed using logistic multivariate techniques in order to distinguish different STS subtypes from different normal tissues with high specificity and sensitivity. Evaluation of the effect of the signal on normal and diseased fat and normal and disease muscle will be further discussed.

(762) **ALA-Induced Fluorescence Imaging of Breast Cancer Margins Detects Tumors Otherwise Occult to the Surgeon**; Ralph DaCosta[1,2,3], Kristina Blackmore[1], Kathryn Ottolino-Perry[1], Stephanie DeLuca[1], Susan Done[1], Alexandra Easson[1],Wey-Liang Leong[1]; [1]University Health Network; [2]University of Toronto; [3]Techna Institute

Purpose: Breast cancer is often treated by breast conservation surgery (BCS), where tumor is removed while preserving the maximum amount of healthy tissue. Detecting positive tumor margins intraoperatively under white light is challenging because of suboptimal tumor-normal tissue contrast - sometimes necessitating re-excision when positive margins are found. We developed a new fluorescence imaging technology capable of assessing surgical margins in real-time when combined with a clinically-approved tumor localizing fluorescence contrast agent called 5-aminolevulinic acid (5-ALA).

Methods: In an ongoing clinical trial at the Princess Margaret Cancer Centre, we are investigating the diagnostic efficacy of the imaging device with 5-ALA in differentiating healthy and cancerous breast tissues in real-time across three patient cohorts (n=45 total): control, low dose (15 mg/kg) and high dose (30 mg/kg) of 5-ALA. The device detects ALA-induced red protoporphyrin IX (PpIX) fluorescence that preferentially accumulates in tumor cells using 405 nm excitation light. Lumpectomy or mastectomy specimens were resected according to best clinical practice and fluorescence images of the specimens were captured by the device. Differentiation between cancerous and normal tissue was validated by punch biopsy and quantitative fluorescence spectroscopy for correlative histopathology and spectral characterization of PpIX concentrations, respectively.

Results: To date we have completed recruitment of 22/45 patients. Imaging data from the first 22 ex vivo samples showed fluorescence imaging spectrally discriminated between healthy breast adipose and connective tissues while detecting primary tumor-specific PpIX fluorescence (with both ALA doses). The accuracy of PRODIGI to identify tumor on ex-vivo samples was 73% (n=41 biopsies) using blinded histological correlation. In addition, ex vivo imaging identified a positive DCIS margin on a single lumpectomy specimen and the device was sensitive to low PpIX concentrations, successfully identifying disease outside the palpable tumor mass in 6 samples. Results also demonstrated the technical feasibility of the approach with minimal disruption to surgical workflow.

Conclusion: ALA-induced fluorescence imaging can detect subclinical breast tumors in surgical samples as well as differentiate adipose from connective tissues. The next phase of our study will investigate this approach in imaging the surgical bed margins after tumor resection for the detection of positive margins intra-operatively.

(763) **Tethered Capsule Endomicroscopy for Barrett's Esophagus Screening**; Rohith Reddy[1,2], Michalina Gora[1, 2], Robert Carruth[2], Tim Ford[1,2], Jing Dong[1,2], Guillermo Tearney[1,2]; [1]Harvard Medical School; [2]Massachusetts General Hospital

Optical coherence tomography (OCT) is an imaging technology that forms depth-resolved images by using interferometry to measure the optical delay of backscattered light from a sample. OCT is commonly performed with near-infrared illumination and is frequently used to reveal tissue morphology and structure. It has been shown in prior studies to be capable of accurately diagnosing Barrett's Esophagus (BE), a metaplastic change that conveys an increased risk of developing esophageal adenocarcinoma (EAC).

Our laboratory has developed a new technology termed tethered capsule endomicroscopy (TCE) that is capable of being utilized to screen for BE in the primary care setting. TCE involves swallowing a tethered, opto-mechanical capsule that is 11mm x 24.5mm in size that obtains 10 µm

resolution cross-sectional OCT images of the entire esophageal wall as it traverses the esophagus via peristalsis. The images acquired by the TCE device are sensitive to the microscopic changes that characterize BE.

The current method of diagnosis of BE is endoscopy with biopsy followed by microscopic analysis of the excised specimen. One of the main drawbacks of this process is that samples are collected from small areas relative to the size of the esophagus. There is a high likelihood of missing entire regions of diseased tissue by virtue of insufficient sampling. Moreover, endoscopic biopsy is a complex and inconvenient procedure that requires sedation and is expensive. TCE using the proposed OCT imaging capsule overcomes these problems. We obtain three-dimensional, microscopic images of the entire esophagus. The TCE device is easy to use and the entire procedure can be performed by a trained nurse in about 15 minutes without sedation.

The current generation of TCE technology, while robust is expensive both in terms of fixed and disposable costs. In this work, we propose a new generation of TCE device that is significantly less expensive in both fixed and disposable costs while simultaneously providing better, quantitative data. This would enable TCE to be used in a primary care setting and help diagnose BE in patients more effectively.

(764) **Developing Infrared Biofluid Diagnostics**; Matthew Baker[1], James Hands[1], Lila Lovergne[1], Caryn Hughes[1], Graeme Clemens[1], Ganesh Sockalingum[2],Benjamin Bird[3]; [1]University of Strathclyde; [2]Universite de Reims; [3]Daylight Solutions

Spectroscopic analysis allows for the label-free objective classification of biological material on the molecular scale. This technique has been applied to histology, cytology and surgical pathology and can detect subtle changes in the proteome and metabolome. This new biochemical information has the potential to improve patient outcome through the identification of earlier stages of disease, drug resistance, disease states and high-risk populations.

Recently, IR serum analysis has been shown to be capable of rapid, specific and sensitive analysis of disease. Capable of diagnosing cancer severity as well as pre-symptomatic diagnosis of sepsis. However a full understanding of sample preparation effects and the effect of the coffee ring effect upon a spectrum is not fully understood which is standing in the way of clinical translation and development. In addition the recent combination of broadly tunable laser sources (QCLs), refractive based high numerical aperture objectives and a large format detector system has enabled high-definition diffraction-limited resolution, and new opportunities for data collection including real-time and data collection for discrete frequency infrared (DFIR) imaging capable of speeding up our analysis into clinically relevant times.

This paper will discuss recent applications in the development and validation of FTIR and DFIR spectroscopic serum diagnostics with a focus on cancer and sepsis diagnosis from serum. In particular it will discuss a large patient study for cancer diagnostics, pre-symptomatic study for sepsis diagnostics and proof of principle study on hand applied 1 microlitre drops to investigate the impact of DFIR on the spectrum and the ability of spectral diagnostics to discriminate samples based upon discrete frequencies of the most salient information obtained from the dataset, including data analysis techniques to enable this. Importantly we will discuss the use of piezojetted serum spots combined with DFIR imaging to enable rapid spectral diagnostics observing the reproducibility and repeatability obtained when implementing different levels of a DFIR regime

(765) **Multiplexed Detection of Breast Tumor Antigen with Nanprobe-Amplified Spectro-Immunoassay**; Ishan Barman[1], Ming Li[1]; [1]Johns Hopkins University

Circulating biomarkers have emerged as promising non-invasive, real-time surrogates for cancer diagnosis, prognostication and monitoring of therapeutic response. Emerging data, however, suggest that single markers are inadequate in describing complex pathologic transformations. Architecting assays capable of parallel measurements of multiple biomarkers can help achieve the desired clinical sensitivity and specificity while conserving patient specimen and reducing turn-around time. Here we describe a plasmon-enhanced Raman spectroscopic assay featuring nanostructured biomolecular probes and spectroscopic imaging for multiplexed detection of disseminated breast cancer markers cancer antigen (CA) 15-3, CA 27-29 and cancer embryonic antigen (CEA). In addition to offering extensive multiplexing capability, our method provides higher sensitivity than conventional immunoassays and demonstrates exquisite specificity owing to selective formation of conjugated complexes and fingerprint spectra of the Raman reporter. The strong proof-of-concept data generated here provides the needed momentum to pursue clinical feasibility studies for metastatic cancer diagnosis and longitudinal monitoring of chemotherapy response in breast cancer patients.

(766) **Integration of Higher-Order Gap Derivatives**; Stephanie DeJong[1], Zhenyu Lu[1], Brianna Cassidy[1], Stephen Morgan[1], Michael Myrick[1]; [1]University of South Carolina

Fourth-order gap derivatives are well known for their ability to enhance spectral resolution and reduce baseline effects, consequently improving multivariate calibrations based on the transformed data. Recently, our lab has demonstrated how gap derivatives can move beyond the typical derivative, with the appropriate selection of gap sizes allowing specific feature recognition approximating a matched filter. To further understand how these GDs work to improve multivariate calibration, we developed a method to integrate fourth-order gap derivatives to obtain the zero-order derivatives (or original spectra). We further extend this method to the integration of regression vectors formed in partial least squares regression. Integrating regression vectors can aid in understanding the models obtained from derivative-transformed data, particularly in understanding how the gap size selected can influence calibration. Further, examining the derivative regression vector in zero-order spectral space can further enforce the validity of using higher-order derivative transforms as a preprocessing tool. The method of differentiation and integration is presented here along with examples of its application to diffuse reflection infrared spectral data.

(767) **Optimal Preprocessing and Similarity for Automatic Whole-Spectrum Matching**; CJ Carey[1], M. Darby Dyar[2], Thomas Boucher[1], Stephen Giguere[1], Sridhar Mahadevan[1]; [1]College of Information and Computer Sciences, University of Massachusetts - Amherst, [2]Department of Astronomy, Mount Holyoke College

Many applications in the spectroscopic research domain depend on matching data from unknown samples to those in spectral libraries. Our own and other recent work on Raman spectroscopy has demonstrated the utility of whole-spectrum matching as an alternative to peak-fitting methods for spectrum analysis. These methods exploit all available spectral data, without the need for sensitive fitting or dimensionality reduction. While the individual components of whole-spectrum matching algorithms vary, common subtasks include spectrum preprocessing and pairwise similarity computations. Previous work has highlighted the importance of user choices about specific preprocessing steps and similarity metrics, which exhibit a strong influence on matching accuracy.

While making the correct choices of preprocessing steps and similarity metrics yields significantly better performance in spectral matching, finding this optimal configuration is a manual, time-intensive task. This project addresses this shortcoming by generalizing the preprocessing and similarity metric choices as parameterized functions that are amenable to a variety of efficient, automated optimization methods. This reduces the amount of time spent tuning the model and often results in improved performance on a given whole-spectrum matching task.

In this project, two models of generalized preprocessing functions are proposed based on cubic polynomial and quadratic Bezier functions. These functions are constrained to be concave and monotonically increasing for all normalized spectral intensity values, reducing the parameter space and resulting in better overall performance. A unified model of spectrum similarity is proposed, in which a tradeoff parameter blends the popular cosine and L1 metrics. This blend is shown to capture the properties of overlapping spectral peaks, which have similar intensities at relatively high amplitudes.

The resulting framework for whole-spectrum matching is parameterized by three real-valued numbers in bounded ranges. On a mineral identification task using Raman spectroscopy, a randomized parameter search was able to outperform the best accuracy results from previous methods without any manual intervention. The resulting accuracy changes smoothly as each parameter is varied, enabling the use of sophisticated optimization methods for efficient, automatic tuning.

(768) **Hotelling Trace Criterion as a Figure of merit for the optimization of chromatogram alignment**; Edward Soares[1], Gopal Yalla[1], John O'Connor[1], Kevin Walsh[1], Amber Hupp[1]; [1]College of the Holy Cross

We present a methodology for optimization of chromatogram alignment using a class separability measure called the Hotelling trace criterion (HTC). This metric is a multi-class distance measure that accounts for within-class and between-class variation. We chose the correlation optimized warping algorithm as our alignment method and used the HTC to judge the effectiveness of the alignment based on algorithm parameters called segment length and max warp.

Biodiesel feedstock samples representing classes of soy, canola, tallow, waste grease, and hybrid were used in our experiments. Fatty acid methyl esters in each biodiesel were separated using gas chromatography-mass spectroscopy. The entire data set was baseline corrected, aligned, normalized, and mean-centered prior to principal components (PCs) analysis. The aligned, baseline corrected data sets were used to compute a figure of merit called warping effect, while the PC-transformed data sets were used to evaluate the HTC. The segment length and max warp parameters that maximized the warping effect and/or HTC were then determined. Scores plots of pairs of PCs, along with 95% confidence ellipses, were created and analyzed.

The results demonstrated that the parameters derived from maximizing the HTC more effectively aligned the data, as evidenced by better clustering of the biodiesels in the scores plots. This behavior was robust to the number of PCs used in the computation of the HTC. We conclude that the HTC is an objective measure of alignment quality that allows for optimal class separability and can be applied to optimize other methods of chromatogram alignment.

(769) **Design of Experiments in Spectral Space for Efficient Development of Near-Infrared Methods in Tablet Analysis**; Md Anik Alam[1,2], James Drennen III[1, 2], Carl Anderson[1,2,3]; [1]Graduate School of Pharmaceutical Science, Duquesne University; [2]Duquesne University Center for Pharmaceutical Technology; [3]Duquesne University

Designing a calibration set is the first step in multivariate spectroscopic calibration development for quantitative analysis. This step is critical because successful model development depends on the availability of appropriate data. Typical calibration methods designed in concentration space do not follow the classical idea of experimental design for multivariate calibration. Moreover, the current concentration based techniques for designing calibration sets are prone to have redundant information in terms of the variance spanned by the spectral data. At the same time, calibration sets developed by these techniques may lack necessary information for a successful calibration model. Experimental design in spectral space is consistent with the purpose of design of experiments. This work presents a novel method for designing calibration sets in spectral space. This method was expected to maximize information content of measurements and minimize sample requirements to provide an efficient means for developing multivariate spectroscopic calibration.

A comparative study was conducted between the traditional concentration based technique and newly developed spectral space based technique. These techniques were used to develop calibration model for predicting a model drug Acetaminophen in pharmaceutical compacts using Near Infrared Spectroscopy. Similar prediction performances were achieved using newly developed spectral space based technique compared to the traditional technique with fewer sample requirement. Designing experiments in spectral space can provide an efficient means for developing spectroscopic multivariate calibration technique.

(770) **Development of Multiple Merit Ranking Methods for Automatic Selection of Multiple Tuning Parameters in Multivariate Calibration and Maintenance**; Alister Tencate[1], John Kalivas[1], Alexander White[2]; [1]Department of Chemistry, Idaho State University; [2]Department of Physics and Optical Engineering, Rose-Hulman Institute of Technology

Many multivariate calibration applications, including model maintenance, involve selection of multiple tuning parameters to determine the final calibration model. Optimization of multiple tuning parameter values across many different model merits is challenging. The best combinations of tuning parameter values are often ambiguous making the final calibration model choice almost certainly empirical. Three different ranking methods are investigated: a consensus ranking approach called sum of ranking differences (SRD), and two data fusion methods. All three methods allow for the simultaneous comparison of any number of calibration model merits to select the optimal combination of tuning parameter values based on those merits. These methods are evaluated by including a pre-threshold on the merits, sectioning each merit relative to tuning parameter sets and then evaluating each section, or using the ranking processes iteratively. A near infrared pharmaceutical data set, including primary lab samples and secondary full batch samples, in conjunction with a Tikhonov regularization calibration maintenance approach is presented as an example. Another example presented consists of model development for a smooth regression vector using several different types of spectroscopic data sets.

(771) **Exhaled Breath Condensate Cystic Fibrosis Markers Through the Eye of High Resolution Mass Spectrometry**; Facundo Fernandez[1], Xiaoling Zang[1], Maria Eugenia Monge[2], Nael McCarty[3,4], Arlene Stecenko[3,4]; [1]Georgia Institute of Technology; [2]Centro de Investigaciones en Bionanociencias (CIBION); [3]Emory+Children; [4]Emory University School of Medicine and Children

Progressive lung function decline from in cystic fibrosis (CF) often does not proceed in a linear fashion, rather is punctuated by acute pulmonary exacerbations (APEs). The frequency of APEs severe enough to require hospitalization is a crucial factor that may contribute to death from CF. APE diagnosis remains challenging due to changes in patient symptoms over time, as observed by the attending clinician. Reliable measurements to predict oncoming APEs would enable early clinical intervention and potentially prevent loss of lung function.

Herein we address this issue using ultra performance liquid chromatography-quadrupole-time-of-flight mass spectrometry and a supervised classification model to profile and identify metabolites in exhaled breath condensate (EBC) samples, thereby facilitating discrimination of pre-APE patient samples from stable CF patient samples.

EBC samples from 4 pre-APE patients (CF patients 1 to 3 months before an APE) and 19 stable CF patients (EBC samples from CF subjects who are clinically stable without an APE for ≥3 months) were profiled using a Waters ACQUITY Ultra Performance liquid chromatograph coupled to a Xevo G2 quadrupole-time-of-flight mass spectrometer (QTof-MS) .

A total of 389 spectral features (Rt, m/z pairs) were quantitatively detected in EBC metabolome profiles from the cohort. Out of the 389 spectral features, 174 were present in at least 50% of pre-APE or stable CF patient samples. Twenty-seven out of these 174 spectral features had tentative endogenous identities after searching through databases. Reverse interval PLSDA feature selection was performed on the 27 tentatively identified spectral features, which resulted in the selection of 8 discriminant features. The 8 discriminant features tentatively identified comprised a metabolic profile that distinguished the 4 pre-APE EBC samples from the 19 stable EBC samples with excellent accuracy, specificity and sensitivity using the oPLSDA model.

Three of the key differentiating metabolites were tentatively identified as lactic acid, pterins and their derivatives, and eicosanoids and their metabolites. These 3 metabolites were all elevated in pre-APE patient EBC samples, compared to stable CF patient EBC samples. Interestingly, lactic acid has been reported to be elevated in the bronchoalveolar lavage fluid (BALF) of CF patients, relative to control subjects.

(772) **Ultrasensitive and Accurate Quantification of Oncogenic microRNAs using Nanoplasmonic Sensors**; Rajesh Sardar[1], Gayatri Joshu[1], Samantha Deitz-McElyea[2,3], Sonali Mali[1], Murray Korc[2,3]; [1]Indiana University-Purdue University Indianapolis; [2]Indiana University School of Medicine

MicroRNAs (miRs) are noncoding, short (20 -22 nucleic acid) RNAs that have many functional roles in cellular roles in plants and animals, including gene regulation. They have also have been classified as important diagnostic biomarkers for diseases and therefore their detection and quantification have received considerable attention using various detection methods. We have designed, a reusable, solid-state, localized surface plasmon resonance (LSPR) sensor based on highly sensitive nanostructures (gold nanoprisms) without the need for labeling or amplification of miRs is required in other techniques. The direct hybridization-based sensor displayed the ability to detect femtomolar concentrations of miR-21 and miR-10b with high selectivity. The sensor was further successfully applied to the detection of these miRs in human plasma samples collected from pancreatic cancer patients, and the results were compared with quantification by qRT-PCR. The sensor eliminates the drawback of current techniques, which are unable to detect the miRs in their native environment due to lack of a high degree of sensitivity and selectivity, and can be applied to other types of cancers. This will allow a rational approach for testing several disease markers for early diagnosis and will expedite individualized therapy.

(773) **Plastic antibodies for SERS detection**; Amanda Haes[1], Wenjing Xi[1], Anna Volkert[1]; [1]University of Iowa

Molecular imprinted polymers (MIPs) and plasmonic nanomaterials are synthesized, characterized, and incorporated into a device to better understand the effects of nanomaterial incorporation into a new class of small molecule biosensors. Specifically, acetaminophen, aspirin, and caffeine templated MIPs are synthesized, characterized, and used to quantitatively and directly detect drugs in complex samples using Raman microscopy, localized surface plasmon resonance spectroscopy, and surface-enhanced Raman scattering (SERS). Unique Raman bands for caffeine, acetaminophen, aspirin, and non-imprinted polymer are identified. Shifts in these unique Raman vibrational modes are used to identify drug molecules that are bound to the MIP or free in solution. The magnitude of the SERS response correlates with the number of binding sites in the MIP and electrostatic and hydrogen bonding interactions between the MIP and nanorod. Future studies could focus on exploiting the hydrophilic driven interactions between the nanomaterials and MIP for increased SERS enhancements for complex sample analysis and SERS-based MIP biosensor development.

(774) **SERS on Core-Shell Substrates**; Christy Haynes[1], Zhe Gao[1], Antonio Campos[1]; [1]University of Minnesota

Surface-enhanced Raman scattering has many advantageous qualities that make it a good candidate technique for in vivo sensing applications. From a practical standpoint, injectable colloidal substrates would be preferred over implantation of a solid SERS substrate into a biological organism. However, colloidal substrate stability limits the use of this sensing platform in part because aggregated colloids have non-ideal plasmonic properties and in part because biomolecules bind non-specifically to the colloidal surface, blocking high enhancement sites. This work demonstrates the synthesis of SERS-active colloids with mesoporous silica shells and demonstrates both the colloidal stability of these nanoparticles and SERS sensing of analyte species transported through the mesopores.

(775) **Single Hotspot Raman Spectroscopy of a Self-Assembled Monolayer using Scanning Near-Field Optical Microscopy Excitation**; Camiel van Hoorn[1], Freek Ariese[1], Arjan J.G. Mank[2]; [1]Faculty of Sciences and LaserLaB, VU University, Amsterdam, The Netherlands; [2]Philips Innovation Services, HighTech Campus, Eindhoven, The Netherlands

Raman spectroscopy is an important technique to study chemical reactions in bulk samples. However, molecular interactions and kinetics strongly depend on the local environment. Therefore, to be able using the advances of Raman spectroscopy at the (near-)single molecule level is beneficial. To increase the Raman sensitivity by several orders of magnitude, surface and tip enhanced methods can be used. TERS, in contrast to SERS, is a single-hotspot technique and uses a metal coated tip which also provides spatial information. In this technique, the resonance enhancement strongly depends on the tip shape and tip sample distance, hence small variations in tip orientation can cause large signal deviations.

In a different approach, the tip can be replaced by silver or gold nanoparticles deposited on the surface [1]. In this way it remains a single hotspot technique providing high resolution spatial information, but avoids the complication of a moving tip. This approach can be used to study chemical interactions in a monolayer of molecules, which would not be possible with normal Raman spectroscopy due to weak signals.

In our research, we combine this single hotspot Raman technique with scanning near-field optical microscopy (SNOM) to study chemical interactions in self-assembled monolayers (SAM). As a model sample we use a p-nitrothiophenol (pNTP) SAM grown on a gold substrate with silver nanoparticles distributed over the surface. pNTP can be photo activated with green light to start a dimerization reaction. By using a SNOM fiber as a miniaturized Raman excitation source, chemical activity occurring at the optical hotspots can be studied in detail.

Reference

[1] Van Schrojenstein Lantman, E. M.; de Peinder, P.; Mank, A. J. G.; Weckhuysen, B. M. ChemPhysChem 2015, 16 (3), 547–554.

(776) **Spectral Line Shapes in Atomic and Molecular Laser-Induced Breakdown Spectroscopy**; Christian Parigger[1]; [1]University of Tennessee Space Institute

Fundamental to spectroscopy is a detailed theoretical understanding of phenomena that contribute to the specific shapes of atomic lines and/or molecular bands. The appearance of spectral features can be associated with various rates of processes. Time resolved measurements of laser-induced atomic spectra and bands involve deconvolutions due to the instrument characteristics. In consideration of irradiance levels utilized in laser-induced breakdown spectroscopy, spectral broadening mechanisms may significantly affect atomic line appearances, e.g., Stark broadening may be significantly stronger than Doppler broadening. Self-absorption is also expected to modify the line shapes, and of course spectral line shifts are also expected to occur. Other radiating species may cause contributions in the spectral region of interest, and such contributions are frequently labelled background radiation. Spectral fingerprints of atomic the species hydrogen, H, and aluminum, Al, are discussed in detail, but also commonly encountered molecular spectra of the carbon Swan bands, C_2, cyanide, CN, aluminum monoxide, AlO, and titanium monoxide, TiO, will be of interest including a recently communicated package for the computation of diatomic molecular spectra. Comments will be included about continued research interests in spectral line shapes.

(777) **Issues and Advances of Calibration Transfer in LIBS**; Jean-Baptiste Sirven[1,2], Jessica Picard[1, 2], Cécile Maury[1,2], Maria El Rakwe[1,2]; [1]CEA; [2]DEN, DANS, DPC, SEARS, LANIE

As a fast, direct and remote technique, laser-induced breakdown spectroscopy is particularly relevant for online or in situ analysis, or to measure samples with limited access, like in the nuclear sector or in planetary exploration. In these cases, quantitative analysis can be difficult for different reasons. First, environmental conditions are not completely controlled and instrumental drifts may occur. Secondly, matrix-matched calibration samples are not always available. Thirdly, the samples matrix is known to a certain extent, with a possible high variability that can induce unexpected spectral interferences. In addition, due to practical or cost constraints, feasibility studies are frequently performed on matrices and/or analytes and/or experimental conditions other than that of interest, and the question of analytical performances that would be obtained in the real situation is raised.

Calibration transfer could be helpful to cope with those issues. In the field of NIR spectroscopy, where it is widely studied, this concept generally relates to the use of a multivariate calibration model obtained with an instrument to predict samples measured with another instrument. In LIBS, this definition is somewhat restrictive and can be broadened in different ways. Thus, we could consider transferring a calibration from one matrix to another, which is equivalent to compensating for matrix effects. But it can also be related to the calibration transfer for a given matrix from one element to another, or for a given element, from one line to another. Furthermore, a change in experimental conditions can also be dealt with by calibration transfer approaches.

In this talk we will review the interest of calibration transfer in LIBS as well as the existing works on the subject. Recent results obtained in our laboratory will be presented to illustrate the different definitions of calibration transfer given above. Issues and perspectives of such approaches will be discussed.

(778) **Understanding the Complex Mechanisms Leading to Signal Enhancement in Double Pulse LIBS**; Prasoon Diwakar[1], Patrick Skrodzki[1], Jason Becker[1], Tatyana Sizyuk[1], Ahmed Hassanein[1]; [1]Center for Materials Under eXtreme Environment, School of Nuclear Engineering Purdue University

Laser-induced breakdown spectroscopy (LIBS) provides robust, non-invasive and real-time analysis of variety of samples and analytes, however there are certain challenges related to signal intensity and sensitivity which need to be addressed. Double pulse LIBS (DPLIBS) studies have shown enhanced emission intensity and improved detection limits. Various DPLIBS approaches have been used by varying geometry (collinear, orthogonal, angular), laser wavelength, pulse duration (nanosecond, picosecond, femtosecond, etc.). It has been shown that signal enhancement mechanisms are related to reheating of the plume, increase in mass ablation, rarefication of the ambient, or a combination of multiple mechanisms. These mechanisms are complex and difficult to understand given the number of variables, which could affect signal enhancement. In this work, fundamental mechanisms that lead to signal enhancement for different DPLIBS schemes are explored. Emission properties of collinear NIR femtosecond-femtosecond and NIR nanosecond-nanosecond double-pulse laser induced breakdown spectroscopy are discussed in detail. In particular, the role of excitation energy, inter-pulse delay times, energies of the reheating pulses and target material properties on LIBS sensitivity improvements are studied. Target samples chosen for varying properties include metals of varying Z: Al, Mg, Co, Fe, Sn, Cu, Cr, Ta, and W. Signal intensity, signal-to-noise and signal-to-background as well as plasma persistence, were correlated to material properties such as atomic weight, melting and boiling point, thermal conductivity, optical reflectivity, and ionization potential to elucidate the role of material properties on signal enhancement. Ultimately, mechanisms involved for signal enhancement in DPLIBS are discussed in context of laser-material interaction and material physical properties.

(779) **Standoff LIBS using a Spatial Heterodyne Spectrometer**; Patrick Barnett[1], Nirmal Lamsal[1], S. Michael Angel[1]; [1]University of South Carolina

For the first time, a spatial-heterodyne spectrometer (SHS) has been used for standoff laser-induced breakdown spectroscopy (LIBS) measurements. The SHS is a diffraction grating based interferometer with no moving parts. The SHS design offers advantages over dispersive spectrometers including 10-100 times larger acceptance angle and subsequently a much larger field of view, 100-10,000 times higher light throughput, high spectral resolution, and a relatively wide spectral range in a small package. In previous work our group described a spatial heterodyne Raman spectrometer (SHRS) for standoff Raman measurements at visible and UV wavelengths, however, a standoff spatial heterodyne LIBS spectrometer (SHLS) has not been previously described. In the case of standoff LIBS measurements, the wide field of view of

the SHLS design minimizes issues of laser pointing stability and alignment. Also, because spectral resolution is not a strong function of entrance aperture size, high spectral resolution can be achieved in a very small spectrometer using small diffraction gratings. In the described work 10 mm diffraction gratings were used to provide ~0.3 nm spectral resolution. The high light throughput of the system allows LIBS measurements to be made using no collection optics, other than the 10 mm gratings, at distances up to 20 meters, which corresponds to a collection solid angle of less than one microsteradian. In this paper, stand-off LIBS spectra of minerals and other materials will be shown, collected at distances up to 20 m, without collection optics. Measurements using a small telescope will also be described.

(780) **Characterization of Protein and Nucleoprotein Complexes by Surface Induced Dissociation Coupled to Ion Mobility**; Vicki Wysocki[1]; [1]Ohio State University

Many proteins carry out their biological functions as part of a complex of many proteins. Tools for characterizing the structure of these complexes can help define how structure relates to function. Surface-induced dissociation is an appealing activation method because it provides substructure information on protein complexes. This holds true for protein-protein, protein-ligand, and protein-nucleic acid systems. Relative interfacial surface areas define which interfaces will cleave at the lowest energies and define the competition between different possible fragmentation pathways. Recent results will be presented to illustrate progress made as more complex systems are investigated. Ion trapping and charge changing through ion ion reactions are providing comparisons of the ion mobility and dissociation characteristics of the same charge states prepared in solution vs in the gas phase.

(781) **Structural Biology in the Gas Phase: New Approaches for Conformationally-selective Inhibitor Screening and Multiprotein Topology Mapping**; Brandon Ruotolo[1]; [1]University of Michigan, Department of Chemistry

Within each living organism proteins are at work, carrying out activities which impact every aspect of cellular function from synthesis to cell death. A key factor in varied protein functionality is their ability to self-assemble, creating vast molecular 'machines' capable of performing intricate, highly specialized tasks. To rapidly discover the structural properties of such assemblies, especially when bound to therapeutic small molecules, is a grand challenge for structural biology which is nearly insurmountable with current tools. In this presentation, recent developments surrounding ion mobility-mass spectrometry (IM-MS) methods, aimed at bridging this gap in basic technology, will be discussed. Our approach exploits a series of highly-accurate, gas-phase measurements of protein size and mass to rapidly determine multiprotein architectures and stabilities when bound to small molecules, without the need for covalent labels or tagging, and consuming10-100 times less protein than almost any other label-free technology. Recent IM-MS applications, including multi-protein topology construction, conformationally-selective kinase inhibitor discovery efforts, and the rapid structural characterization of intact biotherapeutics will be discussed.

(782) **Protein Structure in Solution and in the Gas Phase: Insights from Ion Chemistry, Ion Mobility, and Mass Spectrometry**; Matthew F Bush[1]; [1]University of Washington

Native mass spectra of proteins and protein complexes often contain narrow charge-state distributions and wide peaks, which makes assigning charge states challenging and can limit the accuracy of native mass spectrometry experiments. These complications are exacerbated when samples contain mixtures of proteins or oligomeric states. Here we use Cation to Anion Proton Transfer Reactions (CAPTR) reactions to generate charge-reduced product ions of protein complexes. CAPTR is analogous to ion/ion proton transfer reactions pioneered by McLuckey and coworkers to aid in the analysis of peptides and other analytes (Chem. Commun. 2013, 49, 947–965). Using CAPTR, we have detected the lowest charge state below m/z 100 000 for a series of protein complexes ranging from 12–468 kDa. Charge states are assigned unambiguously using the additional charge-reduced peaks, which enables accurate oligomeric state and mass assignments. Additionally, we demonstrate the ability to separate proteins in a mixture by reacting ions selected from a congested m/z region containing contributions from multiple protein complexes. Using this approach, we observed a >2000 fold increase in the resolution of two components from that mixture. The improved resolution of the CAPTR products is attributable to selecting precursor ions of similar m/z but different z; thus, the resolution increases following each proton transfer reaction. Lastly, collision cross sections of several protein complexes were determined. These results show a reproducible, but subtle range of collision cross sections (-3% to +3%) for protein complexes as a function of charge state. Our results show that CAPTR is an efficient method to manipulate the charge states of native-like ions, which we use to accurately assign charge states, separate complex protein mixtures, and probe the relationship between charge state and protein structure.

(783) **What Multiplexing Can Do for Your Experiment: Tangible Enhancements for Ion Mobility Spectrometry;** Brian Clowers[1], Austen Davis[1], Kelsey Morrison[1]; [1]Washington State University

It is well known that the duty cycle of common drift-tube ion mobility experiments is often below 1%. However, multiplexing approaches such as Fourier and Hadamard transforms have been shown to independently enhance the throughput of ion mobility spectrometry (IMS) experiments to levels that approach 50%. While challenges remain to their broad scale implementation we have recently implemented a new Fourier transform (FT) IMS experiment that is directly compatible with standard drift tube ion mobility mass spectrometers (DT-IMMS). Assessment of our initial results highlights an increase in SNR relative to both previous implementations FT-IMS experiments and signal averaged (SA) experiments. Because both FT and HT techniques both adopt a multiplexing approach there is an outstanding analytical question as to which technique yields the highest levels of analytical performance including resolving power, signal to noise ratio, and by extension instrumental detection limits.

Using an atmospheric pressure DT-IMMS system equipped with an electrospray source we contrast the performance of FT and Hadamard approaches to measuring gas-phase mobilities. This comparison focuses on simple mixtures of tetralkylammonium salts, peptides, and multiply charged gas-phase proteins. Additionally, our direct comparison highlights the biases associated with each technique and the circumstances in which each is appropriate. When focusing on experimental speed, the Hadamard IMMS technique has a distinct advantage, however, there are specific cases in which the FT technique remains superior. More specifically, FT-IMMS methods demonstrate a unique ability to eliminate spectral contributions from gas-phase intermediates in the final stages of spectral transformation. Such information proves especially useful for systems with rapidly changing gas-phase conformations. To build on these modes of instrumental operation we will present the key aspects, considerations and minimum resource necessary for other IMS researchers to incorporate these operational modes into their research. Multiplexing offers a tractable mechanism to enhance sensitivity for IMMS measurements and its broad-scale adoption by IMMS researchers promises to enhance the level of mobility measurements using hybrid instrumentation.

(784) **Developing IMS-HDX-MS/MS Techniques for Structural Proteomics Investigations**; Mahdiar Khakinejad[1], Samaneh Ghassabi-Kondalaji[1], Gregory Donohoe[1], James Arndt[1], Stephen Valentine[1]; [1]West Virginia University

Over the last several decades, tremendous progress has been made in the characterization of the three-dimensional structures of proteins. Evidence of this progress is demonstrated by the fact that the number of protein structures added to the protein databank (PDB) structural database has grown from ~150 in 1990 to over 9000 by 2013.1 Despite this progress, structure characterization is inaccessible for a number of proteins due to limitations in the analytical methodologies used to obtain detailed structures. For example, many proteins do not readily form crystals amenable for x-ray diffraction studies. The work presented here describes a new approach combining ion mobility spectrometry (IMS) with gas-phase hydrogen deuterium exchange (HDX) techniques and mass spectrometry (MS) characterization. The HDX measurements help to refine the structural information afforded by the IMS measurements. Results for IMS-HDX-MS experiments of peptide and protein ions are discussed with respect to this refinement process as well as their potential value in the study of protein complex structure. 1 http://www.rcsb.org/pdb/statistics/contentGrowthChart.do?content=total&seqid=100

(785) **Study of Therapeutic Monoclonal Antibodies under Thermal Stress using Deep-UV Resonance Raman Spectroscopy**; Sergey Arzhantsev[1], Justin Bueno[1], John Kauffman[1]; [1]US FDA

Quality control of formulated protein drugs presents unique analytical challenges compared to small molecule pharmaceuticals. Proteins are complex macromolecules with secondary and tertiary structures that may easily be perturbed, potentially resulting in loss of therapeutic efficacy. Deep ultraviolet Resonance Raman (DUVRR) spectroscopy is distinctively advantageous for quality assessment of formulated proteins because it is sensitive to changes in protein secondary structure, and is capable of analyzing proteins at concentrations of approximately 1 mg/mL, lower than the typical concentrations of formulated products. The deep ultraviolet resonance Raman enhancement from proteins (approximately 104) typically permits analysis in the presence of excipients in aqueous media, which exhibit relatively weak nonresonant Raman signals. These attributes have allowed us to analyze the secondary structure of formulated monoclonal antibodies (mAbs) with minimal sample preparation. In this study, DUVRR spectra of formulated Rituxan and Avastin will be presented. These products were degraded with chemical and thermal stress to effect changes in protein higher order structure. Changes were observed in the DUVRR spectra and multivariate statistical models were applied to analyze data. The results indicate that the DUVRR method may be applicable as a rapid, sensitive and specific tool to detect degradation of formulated mAb pharmaceuticals.

(786) **In In Formulation: Developability and Predictive Stability Techniques**; Deniz Temel[1]; [1]Biogen

When a biomolecule progresses from discovery to development, it should meet certain criteria. It should possess favorable storage stability and solubility profiles. Expressibility of the molecule should be good. It should be able to reach to high concentrations with low aggregation and viscosity. Last but not least; low immunogenicity is required. According to these criteria, it is crucial to identify the physicochemical risks associated with the biomolecular candidates before they leave the discovery and progress to development. Developability assessment may therefore play an extremely important role in evaluating their behavior and prevention of technical failure during preclinical and clinical development. Developability studies are integrated with and would bridge between the discovery/design and development/delivery.

To improve developability assessment requires improved data interpretation, prediction and improved criteria for delineation of the best candidates. What drives our project is the goal of understanding which techniques are high through put, fast, easy to interpret, and most predictive towards the best drug candidate selection.

My research focuses on various measured and calculated properties of monoclonal antibodies that may then serve as predictors and indicators of the suitability of these important biomolecules over a wide range of concentrations.

(787) **Design of Pharmaceutical Formulations: ATR-FTIR Spectroscopic Imaging to Study Drug Release and Tablet Dissolution**; Andrew Ewing[1], Graham Clarke[2], Sergei Kazarian[1]; [1]Imperial College London, [2]Bristol-Myers Squibb

Tablet compacts are the most widely used method to deliver active pharmaceutical ingredients (APIs) to patients. Innovative designs of pharmaceutical formulations are essential to overcome the undesirable dissolution properties of many APIs which are poorly water soluble.

Our studies have shown the potential of ATR-FTIR spectroscopic imaging to investigate tablet dissolution experiments by collecting spectral measurements as a function of time, revealing spatially resolved and chemically specific information about the samples. Previously, we have obtained spectroscopic images to determine dynamic polymorphic and structural changes, study the effect of pH modified environments and even to validate mathematical models of tablet dissolution, to name but a few.

We have built upon this research to further develop the approach to: 1) study the effects of polymeric excipients on API crystallisation, 2) observe disproportionation of an ionised API during dissolution experiments, 3) validate our imaging approach with magnetic resonance imaging and 4) investigate the effect of API carriers on release kinetics.

Here we present this robust and reproducible approach, in combination with UV/vis detection to quantify the amount of API dissolved, to study the release of indomethacin formulated with model drug carriers in situ. Two different mechanisms of drug release were identified using ATR-FTIR spectroscopic imaging, which could not be obtained from the UV/vis data alone. The first resulted from maintained drug-carrier interactions in solution, whereas the second was caused by a disintegration effect of the tablet where such interactions were not detected. This demonstrates further development of in situ ATR-FTIR spectroscopic imaging for pharmaceutical applications.

(788) **Using Spectroscopic Methods to Probe the Effects of Formulation Excipients on Protein Aggregation and Structure**; Julie Wei[1]; [1]Biogen Inc.

Many challenging obstacles can be encountered during the development of high concentration biopharmaceutical formulations. One of the most intriguing properties of therapeutic proteins is its ability to self-associate, which can result in protein aggregation and high viscosity that could limit product stability and its very viability as a usable drug. In this presentation, we present a case study of applying spectroscopic analysis to characterize a model protein that is known to self-associate and exhibit high viscosity. Using a variety of methods, we explored possible aggregation sites on the protein and the effects of different formulation excipients on the self-association and structure of the protein. Using Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy, we investigated the effects of different formulation excipients on the protein's secondary structure. Sedimentation velocity analytical ultracentrifugation (SV-AUC) was used to study the oligomeric state of the protein under

different conditions, and near-UV Circular Dichroism spectroscopy provided valuable data regarding concentration dependent changes of the hydrophobic environment of the protein. Together, these methods provide insight into self-association behavior of the biopharmaceutical product and suggest formulation approaches to mitigate these effects.

(789) **Introduction of Raman to Raw Materials Testing**; Sanjeev Johar[1]; [1]Genzyme

Raw Material testing in the pharmaceutical and biotechnology industry is key to ensuring the reliability and efficiency of manufacturing processes and quality of finished product. Many raw materials are currently tested using old methods detailed in compendia such as USP, EP, JP. Identification tests in particular are usually performed using wet chemistry tests that are time consuming and potentially use hazardous chemicals. The introduction of handheld Raman to the QC Raw materials department has changed the way raw materials identity tests are performed, improved ID testing efficiency by as much as 80% all while enhancing specificity and reducing the use of hazardous materials.

(790) **Analysis of Drugs in Saliva during Treatment of Military Veterans Suffering from Post-Traumatic Stress Disorder**; Kathryn Dana[1], Chetan Shende[1], Stuart Farquharson[1], Albert Arias[2]; [1]Real-Time Analyzers, Inc.; [2]Veterans Affairs Hospital of Connecticut

Some 476,000 military personnel with post-traumatic stress disorder (PTSD) have developed drug or alcohol dependence. Treatments are available for drug and alcohol dependence including medications to reduce their use. Veterans Affairs hospital physicians must frequently test patients to identify the discontinuation of medications or any recurrence of drug use and then adjust treatment appropriately. These tests most often involve collecting and sending a urine sample to a clinical lab for analysis by gas chromatography coupled mass spectrometers. Results are usually returned in 1-2 weeks, a delay that makes timely adjustment of treatment difficult. In an effort to provide an analyzer that can determine drug use at the time of VA hospital or clinic visits so that treatment can be adjusted, we have been developing a surface-enhanced Raman spectroscopy (SERS) based assay that can detect and identify numerous drugs in saliva at ng/mL concentrations within 10 minutes. This technology represents a major advancement in drug analysis, allowing for real-time at-site analysis, which will likely lead to improvements in clinical care as well as cost benefits. Measurements of lab and patient samples will be presented.

(791) **Hyperspectral Imaging of Crystalline Domains in Biopolymers**; Venkata N K Rao Bobba[1], John F. Turner II[1]; [1]Cleveland State University

Advances in hyperspectral imaging systems are providing turnkey solutions for Raman analyses. While the Raman modality is effective at distinguishing regions of a sample that differ in chemical composition, effective multivariate strategies for image classification are needed in the absence of a priori information. This is especially true for samples that exhibit only subtle changes in the spectrum across the field of view. In the work presented here, an important class of environmentally biodegradable polymers and biocompatible implant substrates based on polylactic acid (PLA) and poly-L-lactic acid (PLLA) is investigated using Raman spectroscopy and Raman hyperspectral imaging. The biodegradation rate of PLLA in the body, for example, is strongly dependent on its degree of crystalline content. Non-destructive imaging methods are needed to assess polymer purity and crystalline content during manufacture. As part of this work, a novel multivariate method is described for Raman imaging of PLLA that returns the degree of crystallinity at each pixel location in the image. The Raman results are correlated to differential scanning calorimetry results from PLLA for samples exhibiting a range of crystalline content. The Raman instrumentation and a complete theoretical treatment of the multivariate approach are described.

(792) **Statistical Developments for In-Line Sensitivity and Selectivity Improvement in Single Molecule Dynamic-SERS**; Thibault Brulé[1,2], Alexandre Bouhelier[2], Hélène Yockell-Lelièvre[1,2], Aymeric Leray[2], Alain Dereux[2], Jean-Francois Masson[1],Eric Finot[2]; [1]Département de Chimie, Université de Montréal; [2]Laboratoire Interdisciplinaire Carnot de Bourgogne, Université de Bourgogne, Dijon, France

The Raman intensity fluctuations are the initial source of a lack of accuracy or reproducibility of SERS based nanosensors to reliably estimate the molecular concentration in microfluidics.

In this contribution, we report how single molecule Dynamic-SERS coupled with statistical analyzes should improve the selectivity and the sensitivity in-line. To that purpose, we design an active Surface Enhanced Raman Spectroscopy (SERS) template based on surfactant-less Chebyshev nanoparticles operating in a microfluidic platform.

We show that the statistical analysis of the weighting of the Raman scattering of the probed molecules compared to the photoluminescence of gold nanoparticles is particularly effective as concentration indicator in single molecule regime. We present a novel approach in the Fourier domain that is not limited by a multipoint pre-calibration or even the knowledge of the enhancement factor of the transducer. The analysis of pink noise in the frequency domain reveals a subdiffusion motion of individual adsorbed molecules in the hot-spot. The cut-off frequency between the white and pink noise is used to determine the in-line molecular concentration over a wide range between 10 pM and 1 microM.

We report also the identification of the principal reference Raman spectra of a single cystein molecule. A principal component analysis is obtained from fluctuating spectra to sort the reference spectra of cystein. The assignment of Raman bands brings new insight into the conformation of an amino acid adsorbed onto gold nanoparticle.

(793) **Towards Quantitative Lipid Characterization in Cellular Matrices using Raman Microspectoscopy**; Nils Kristian Afseth[1], Ingrid Måge[1], Zdenek Pilat[2], Ulrike Böcker[1], Jens Petter Wold[1], Volha Shapaval[1],Silvie Bernatova[2], Ota Samek[2]; [1]Nofima - Norwegian Institute of Food, Fisheries and Aquaculture Research; [2]Institute of Scientific Instruments of the Academy of Sciences of the Czech Republic

Lipids play essential roles in biological systems, and the use of Raman microspectroscopy for characterisation of lipids and lipid metabolism in cellular matrices is gaining interest from both fundamental and applicational points of view (1). Whereas qualitative lipid characterisation with Raman spectroscopy and subsequent spectral interpretation are readily achievable, quantitative lipid analysis is still a major challenge. The high spatial resolution obtained in Raman microscopic systems often enable more or less pure lipid Raman spectra to be obtained from lipid bodies. This opens the possibility for calibration-transfer, i.e. obtaining fatty acid calibrations on pure lipid fractions and then transferring this calibration to microscopic spectra or images obtained from cell matrices. Examples of this approach have been shown for beta-caroten-contents in algal lipid bodies (2), and for iodine value predictions in lipid droplets (3). However, quantitative estimations of individual fatty acid features have so far rarely been obtained. Hosokawa et al.(4) used least squares fitting together with pure fatty acid profiles to estimate the contents of individual fatty acids present in lipid bodies. In the present study, the authors propose an alternative approach that might provide more robust fatty acid predictions. When making calibration models for fatty acid composition, a major challenge is the intrinsic correlation between individual fatty

acids. In order to minimize this problem and obtain a calibration set which spans all the fatty acid concentrations as independently as possible, we have used optimal design theory to select samples from a large candidate set based on mixtures of vegetable and marine oils of known fatty acid composition. The oil mixtures were then analyzed with the same optical settings as used for the cell systems. In this way, calibration-transfer to "real" cell systems was possible. In the presentation, results illustrating the feasibility of this approach will be provided along with a discussion on alternative approaches for quantitative lipid characterization using Raman microspectroscopy.

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(794) **Application of Coherent Raman Techniques for the Screening of Oesophageal Cancers.**; Kelly Curtis[1], Julian Moger[1], Catherine Kendall[2], Hugh Barr[2], Oliver Old[2], Nick Stone[1]; [1]University of Exeter, UK; [2]Gloucestershire Hospitals NHS Trust

The survival rates for oesophageal cancers in the UK are poor, with only 15% of patients surviving 5 years or more. This is as a result of a late diagnosis, whereby the cancer has progressed to the advanced stages. Therefore there is a clinical need for a label-free, minimally invasive and rapid technique.

The potential for spontaneous Raman spectroscopy to be able to discriminate between different pathologies in epithelial tissues including the oesophagus, has already been demonstrated. Furthermore as it probes the biochemistry of the tissue it has the potential to identify cancerous changes before they are visible using the current histological approach. The major disadvantage of this approach however are the long acquisition times and even with the introduction of rapid mapping techniques, which has decreased mapping times in comparison to point mapping techniques, it can still take several hours to map areas of tissues in sufficient detail. For this reason we are exploring the application of coherent Raman (CR) techniques, which include Coherent Anti-Stokes Raman Scattering (CARS) and Stimulated Raman Scattering (SRS). These techniques are able to produce images of a higher spatial resolution, in a matter of seconds for a selected molecular vibration. In addition we are able to extract spectral information that matches that of spontaneous Raman, by performing a SRS hyperspectral stack.

We present results from a study of 20 oesophageal samples from 4 different pathology groups, measured using spontaneous and coherent Raman techniques. Oesophageal samples were removed from patients, who have undergone an endoscopic mucosal or oesophageal resection, at Gloucester Hospital, UK. The tissues are frozen and sectioned to a thickness of 10µm. Contiguous sections are used for hematoxylin and eosin staining, Raman mapping and coherent Raman imaging respectively. The pathology group of the tissue and the region of interest were identified using the H&E sections. We use these measurements to develop a spectral classification model, using multivariate statistics, to assess the potential for disease discrimination of each technique.

(795) **Low-frequency Raman Spectroscopy as a Probe or Order: From Pharmaceuticals to Organic Solar Cells**; Keith Gordon[1]; [1]University of Otago, Dunedin, New Zealand

The utility of the low frequency region is shown in a study of OPV materials. The low-frequency spectrum of annealed polythiophene shows an amorphous spectrum; however upon heat treatment at 110 oC, the appearance of phonon modes are clearly distinguishable. The interpretation of these bands is complicated by the nature of the material and rather than attempt that directly we have undertaken a systematic study of OPVs in which the cell performance has been measured at a range of temperatures.

Using chemometric analysis we have developed correlations between spectral response and performance. Raman spectroscopy may be used in this scenario as a quality control and a screening method for the most interesting materials which are then subjected to more intensive investigation using synchrotron-based techniques.

(796) **Raman Spectroscopy of Low Energy Phonons**; David Tuschel[1]; [1]HORIBA Scientific

Raman spectroscopy of low energy phonons is a useful method of probing crystal structure of solid state materials. We will present Raman spectra obtained between 5 cm^{-1} and 50 cm^{-1} of organic and inorganic compounds and demonstrate the effect of crystal orientation on the relative Raman band strengths of these modes. Furthermore, we will present the results of a Raman study of the low energy phonons of nanocrystal MoS_2 consistent with previously published theoretical predictions of a correlation between the number of metal dichalcogenide molecular layers and low energy Raman band position.

(797) **Novel Brillouin-Raman Microspectroscopy of Hydrated Connective Tissue**; Francesca Palombo[1], Ryan S. Edginton[1], Ellen Green[1], Nick Stone[1], C. Peter Winlove[1], Daniele Fioretto[2]; [1]University of Exeter, UK; [2]University of Perugia, Italy

The extracellular matrix (ECM) is an intricate network formed predominantly by the structural fibrous proteins collagen and elastin. Collagen forms the framework of the ECM in connective tissues such as bone, tendon and skin, whilst elastin provides elasticity and resilience to tissues, e.g. arteries and lungs, subjected to repeated distension and physical stress. Characterisation of the individual contribution of elastin and collagen fibres to the biomechanics and structure of connective tissue as a function of hydration has been the focus of our study, using a multimodal microspectroscopic approach. Recently [1], we used macroscopic tensile stress-strain testing to determine the Young's modulus of elastin fibres from bovine nuchal ligament (27 to 1 MPa) and type I collagen fibres from Sprague Dawley rat tail (383 to 193 MPa) as a function of hydration. In a previous analysis [2], Brillouin light scattering (BLS) spectroscopy was applied to measure the complete elasticity tensor of purified elastin and collagen fibres. The obtained longitudinal modulus was found to be 6.1 and 10.2 GPa for dried elastin and collagen fibres, respectively, a few orders of magnitude larger than the macroscopic Young's moduli. In this work, a new combined Brillouin-Raman microscope has been applied to measure the elasticity and composition of hydrated connective tissue, rat tail tendon (type I collagen) and equine joint cartilage (type II collagen). These show that at the molecular level, the mechanics are dominated by the influence of water, reducing the stiffness of the material by around half of the dry value. A key role of water is observed in the reduction of mechanical anisotropy, particularly within type I collagen fibres.

Furthermore, we identified an inverse correlation between the level of water content (Raman) and molecular stiffness (Brillouin) of these samples.

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(798) **Ligand Chemistry and the Low-Frequency Vibrations of Semiconductor Nanocrystals**; Anna Jolene Mork[1], Elizabeth Lee[1], Nabeel Dahod[1], William Tisdale[1]; [1]Massachusetts Institute of Technology

A variety of phonon-mediated processes centrally contribute to heat dissipation in colloidal quantum dot (QD) solids, and a method to tailor the QD vibrational spectrum may allow engineering of more efficient QD devices. Inorganic shells surrounding the quantum dot core, as well as organic ligands attached to the quantum dot surface, affect optical and electronic properties of quantum dots but their effect on the QD vibrational spectrum has been heretofore unknown. We use low-frequency non-resonant Raman spectroscopy to non-destructively probe the acoustic phonon vibrational structure of CdSe QD cores with a variety of different attached ligands and different shell thicknesses. We develop a mathematical model based on vibrations of an elastic sphere to understand shell- and ligand-dependent shifts in the QD Raman spectrum. These data further our understanding of the factors affecting phonon energies and heat transport in QD solids.

(799) **Low Frequency Raman Spectroscopy for the Structural Analysis of Polycyclic Aromatic Hydrocarbons**; Anjan Roy[1], James Carriere[1], Randy Heyler[1], Peter Larkin[2], Eric Chan[3]; [1]Ondax Inc; [2]CytecIndustries; [3]Bristol-Myers Squibb

Low frequency Raman spectroscopy is a particularly useful technique for exploring the structural properties of materials because the low energy vibrational transitions in this spectral region provide contributions from both selected molecular vibrations and crystal lattice vibrations. The crystal lattice vibrations involve movements of the entire molecule with respect to other molecules in the crystalline solid, thus providing a more direct probe of intermolecular interactions and structure. The low wavenumber region of the Raman spectrum is sensitive to conformational changes of the molecule such as with different polymorphic forms of the same material or the effect of co-solvents/co-crystals.

To better understand the relationship between the physical structure of the material and these low energy vibrational modes, we present our observations of the corresponding spectral changes for a series of polycyclic aromatic molecular species that vary systematically in their molecular form. We monitor the changes in the structural vibrational degrees of freedom of the organic molecular crystals as the complexity of the molecule increases. The polycyclic aromatic hydrocarbon family of materials containing compounds such as Naphthalene, Biphenyl, Anthracene etc. were chosen as good candidates for this study because they are both well understood and modeled in the literature.

While historically this low wavenumber region has been difficult to access, recent advances in volume holographic grating (VHG) based Rayleigh blocking filter technology have enabled simultaneous measurements to ~5cm^{-1} from the laser line in both the Stokes and anti-Stokes spectra.

To measure these materials, a high resolution spectrometer was combined with several configurations of the Ondax SureBlock XLF family of low frequency Raman spectroscopy products that incorporate this VHG filter technology. The optimal excitation frequency was highly dependent upon the fluorescence properties of the samples.

The corresponding measured peak positions for each material were compared with the crystalline structure parameters of the organic molecular crystals and theoretical calculations from the literature. We differentiate the bands which derive from molecular vibrations and crystalline lattice vibrations and identify trends for these structurally related aromatic organic crystals

(800) **Extended Proteomics-Bioinformatics to Characterize Metalloproteins**; Joseph Caruso[1], Anna Daigle Donnell[1], Aleksey Porollo[2], Julio Landero-Figueroa[1], Kavitha Subramanian[1], George Deepe[1]; [1]University of Cincinnati; [2]Cincinnati Children's Hospital Medical Center

MS/MS data with database searching will not specifically identify the peptides or protein as metal associated. Using an extended bioinformatic approach a method has been developed to better annotate proteins for *Histoplasma capsulatum (Hc)*, but one that may be extended to multiple organisms. The Hc disease, Histoplasmosis, may be progressive, especially for immuno-compromised individuals. Macrophages (Mϕ) are one of the first lines of defense. However, *Hc* has the ability to avoid innate and adaptive immune defenses by residing and replicating within the Mϕ (1). Understanding how the fungus is able to survive within the host Mϕ under low Zn stress is an ongoing project, a part of which is to characterize the *Hc* metalloproteome.

A custom database has been developed that leads to greater annotation certainty through an alternative proteomics-bioinformatics approach. This is based on the predicted putative proteins of *Hc* G217B strain, (11,220), generated from the Genome Sequencing Center at Washington University and incorporated into HistoBase (2). In this process, we have developed a web-based annotation server, which provides a simple user-friendly interface for a quick homology based annotation of protein sets obtained from a MS report. It operates with gene ontology (GO) annotation of the *Hc* G217B transcriptome and includes categories including molecular functions. With our annotation server, using the zinc-ion binding GO sub-category we are able to be more confident of the metalloprotein annotations from the 11,220 proteins predicted.

However, studies with this method can translate to metalloproteins in other organisms such as viruses; for example, with bacteriophage λ. This virus transports DNA via a protein enclosure known as a 'capsid.' This protein structure is composed primarily of three sulfur rich proteins. While these have been annotated, no metal ions are shown in the annotation report. However, to the chemist seeing all the sulfur heteroatoms, strong metal-ion binding must be considered; therefore, metalloproteins await discovery. With the proteomics/bioinformatic method, this becomes a much more likely possibility.

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(801) **Interfacing Nanofluidic Devices to the Real World: Analyzing Drug-Induced Damage in Single DNA Molecules Isolated from Circulating Tumor Cells**; Steven Soper[1]; [1]University of North Carolina, Chapel Hill

Doxorubicin, cisplatin, paclitaxel, and tamoxifen are examples of chemotherapeutic drugs used for treating breast cancer with selection of therapy based on the classification and staging of the patient's cancer. While treatments assigned to some patients may be optimal using the current classification model, others within certain breast cancer sub-types may fail therapy. Thus, new assays, the associated hardware and biomarkers must be developed to determine how a patient may respond to therapy to realize precision medicine for breast cancer patients. In this presentation, we will discuss a mixed-scale fluidic system that quantifies response to chemotherapy using three pieces of information secured from circulating tumor cells (CTCs); (1) CTC number; (2) CTC viability; and (3) the frequency of DNA damage (abasic (AP) sites) in single DNA molecules harvested from the CTCs. The fluidic system consists of task-specific modules integrated to a fluidic motherboard. All modules and the associated fluidic motherboard and made from thermoplastics using replication-based technologies. Micro-scale modules are used for CTC selection directly from whole blood, CTC enumeration and viability determinations on single CTCs using an impedance measurement, lysing CTCs, and purifying the DNA. The module to read AP sites in DNA is a nanofluidic device made and contains a nanochannel with dimensions less than the persistence length of double-stranded DNA (~50 nm). Labeling the AP sites with a fluorescent dye and stretching single DNA molecules in the nanochannel allowed for direct readout of the AP sites, even from a few CTCs. The system has been used to monitor chemotherapy response in patients with triple negative breast cancer.

(802) **SERS in Live 3D Cell Cultures as a New Tool for Drug Discovery**; Colin Campbell[1], Lauren Jamieson[1], Pierre Bagnaninchi[1], David Harrison[2]; [1]University of Edinburgh; [2]University of St Andrews

3D cell cultures are emerging as a more physiologically meaningful alternative to monolayer cultures for applications such as drug discovery and drug safety studies. 3D cell structures such as tumor spheroids are attractive because they develop gradients of pH and redox potential that create a microenvironment that is a better mimic of real tissue than monolayer culture – as a result they make better models for drug efficacy and toxicity.

Measuring pH and redox potential with spatial resolution in live tumor spheroids is complicated by factors such as autofluorescence, light scatter and a lack of suitable probes.

We have developed a system that uses SERS nanosensors to probe pH and redox potential in live tumor spheroids. Our system requires the controlled delivery of nanosensors to distinct parts of the spheroid and we have verified this using optical coherence tomography.

Spatially resolved SERS signals can be measured using a simple fibre-based spectrometer and shed new light on both the physicochemical heterogeneity of tumors and how it changes on drug treatment.

(803) **Five-dimensional Single Particle Tracking in Live Cells**; Ning Fang[1,2,3]; [1]Georgia State University; [2]Iowa State University; [3]Ames Laboratory, USDOE

A novel single particle tracking (SPT) technique has been developed to visualize the intracellular transport of cargos in five dimensions (5D): the three spatial coordinates (x, y, z) and two orientation angles of a probe's transition dipole over a series of time steps. Tracking rotational probes, such as gold nanorods, in the z-axis is challenging because translational motions in the z-axis can be easily confused with rotational motions as both types of motion result in similar changes in signal intensity. The 5D-SPT method overcomes this long-standing challenge by employing an automatic feedback loop algorithm to control the objective's focal plane and bring the target object back to focus repetitively. In such an implementation, the target object is kept in focus to provide the highest possible S/N for 2D localization and orientation determination, while the z positions are recorded from the vertical movement of a high-precision objective scanner. Through the live-cell SPT experiments, we demonstrate that the 5D-SPT technique has potential to significantly improve the capacity to study the intracellular transport of cargos in a cellular environment.

(804) **Mid- and near-infrared spectroscopy**; Peter Griffiths[1]; [1]Griffiths Consulting LLC

It took almost 100 years from the discovery of infrared radiation by Sir William Herschel before William Coblentz, by measuring spectra of hundreds of compounds point-by-point, showed that certain functional groups absorbed IR light of a certain wavelength. (This generalization had been hinted at in earlier work by others, but not with such a large amount of supporting data as Coblentz presented.) Over the next 60 years, advances in the instrumentation for mid -infrared spectroscopy made this technique invaluable for elucidating the structure of small molecules. This situation changed with the development of nuclear magnetic resonance (NMR) and, to a lesser extent, X-ray diffraction (XRD). There were, however, some functions for which neither NMR nor XRD was unsuitable, including the measurement of gases and surface species, the study of nanogram and sub-nanogram quantities of materials, the discrimination of chiral molecules, most hyphenated techniques, medical diagnosis, and on-line and field measurements. For the next 50 years, remarkable results in all these topics have been obtained with mid-infrared spectroscopy. Until the 1970s, near-infrared (NIR) spectroscopy was thought to be a technique with few important applications. Then, Kart Norris showed that it was possible to use NIR spectroscopy to estimate the water content of grains by applying linear regression techniques to the NIR spectra of ground grains. Shortly thereafter, Norris showed that the protein, oil and water content of grains could be estimated. His work changed the perception of NIR spectroscopy from an academic pursuit to an important technique for rapidly monitoring the composition of (among other commodities) agricultural crops and pharmaceuticals. Because NIR spectra cannot be interpreted as readily as mid-infrared spectra, many different chemometric algorithms were developed to aid in both quantitative and qualitative analysis by NIR spectrometry. It is ironic that the techniques that were first developed for NIR spectroscopy are now being applied equally successfully to mid-infrared spectra. In this talk, I will attempt to give an overview of many of the important developments in mid- and near-infrared spectroscopy and to forecast possible future developments in these fields.

(805) **X-ray Spectroscopy with Compact X-Ray Sources**; Christoph Rose-Petruck[1,3], Petr Bruza[4], Bernhard Adams[2], Yishuo Jiao[1]; [1]Brown University; [2]Argonne Natl. Laboratory; [3]Research Instruments Corporation; [4]Czech Technical University

The presentation will give an overview over recent research on ultrafast, hard x-ray spectroscopy of chemical dynamics in solution using accelerator-based and laboratory-based x-ray sources. Examples discussed are ligand substitution reactions as well as the recent observation of

the coherent oscillations of solvation shells around photoexcited anions in aqueous solution. The laboratory-based x-ray sources used above can also be used as compact soft-xray sources in the water window. Prospects for the analysis of organic trace compounds using these compact, table-top sources will be discussed.

(806) **Raman Spectroscopy as a Versatile Tool for Fundamental Research and Practical Applications**; Igor Lednev[1]; [1]University at Albany, SUNY

Since the discovery of the Raman effect almost ninety years ago, many different techniques have been developed that utilize the general phenomenon of inelastic light scattering. These include surface-enhanced Raman spectroscopy (SERS), coherent anti-Stokes Raman spectroscopy (CARS), spatially offset Raman spectroscopy (SORS), Raman optical activity (ROA), tip-enhanced Raman spectroscopy (TERS), and hyper Raman spectroscopy, to name a few. Both significant advancements in laser technology and the development of novel light detectors have dramatically improved Raman instruments and provided excellent opportunities for numerous practical applications. In this presentation, the application of deep UV resonance Raman spectroscopy, TERS, and Raman microspectroscopy, combined with advanced statistics, for biochemical research, disease diagnostics, and forensic purposes will be discussed.

(807) **Shedding Light on Terahertz Radiation**; Richard Temkin[1]; [1]MIT

Terahertz (THz) radiation covers the range from about 0.3 to 10 THz (wavelengths of 30 microns to 1 mm). Nature has dealt a cruel blow to this spectral region since atmospheric transmission is very poor. Nevertheless, there are many exciting applications in the THz frequency range. THz radiation is non-ionizing and it passes through most dielectric materials, such as plastics, ceramics and clothing. This allows for “T-Rays” to be used for inspecting such valuable items as pills on a conveyor belt and humans attempting to board airplanes. Other exciting applications exist in the realm of biology, medical diagnostics, space sensing, radar, spectroscopy, plasma science and measurements of the cosmic background radiation. Sources of THz radiation are often based on extending microwave techniques to higher frequency or optical laser techniques to lower frequency. Still, power and bandwidth of practical sources is limited, while cost is apparently unlimited. Transmission of THz radiation falls into the regime of quasi-optics, intermediate between diffraction optics and geometrical optics. This talk will attempt to “shed light” on the “last unexplored portion of the electromagnetic spectrum.”

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