# $CH_2$-Symmetric/$CH_2$-Antisymmetric Stretch Ratio Sensor for Cell Analysis

S. van den Driesche[1], W. Witarski[2], and M.J. Vellekoop[1]

[1] Institute of Sensor and Actuator Systems, Vienna University of Technology, Vienna, Austria
[2] Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

*Abstract*— **Here we report on a novel infrared sensor system for measuring the $CH_2$-symmetric/$CH_2$-antisymmetric stretch ratio of cell samples. Based on IR absorbance spectra of healthy and malignant breast [1], blood [2] and brain [3] cells found in literature we hypothesized the possibility of disease stage cell discrimination by only comparing a few absorbance peaks in the lipid absorbance wavelength region between 3 and 4 µm. By comparing the lipid $CH_2$-symmetric and $CH_2$-antisymmetric stretch ratios (with baseline correction and normalization) of three defined epithelial kidney cell lines, healthy MDCK and carcinoma A-498 and Caki-1 with the developed sensor, significant stretch ratio differences have been found between healthy and tumor cell types (and even between the two tumor types). The developed LED-photodiode based infrared absorbance sensor could be used for quick pre-screening of biopsy samples which, compared to labeling and staining techniques, does not require highly trained personnel and is much cheaper than liquid nitrogen cooled FTIR spectroscopes.**

*Keywords*— **Infrared absorbance sensor, Label-Free, Cell analysis**

## I. Introduction

In recent years cancer is becoming the number one disease of dead causes [4]. The development of diagnostic tools plays an important role in understanding the fundamentals of tumor development and in selecting the proper treatment. Normally the screening of biopsies for possible malignant cells is done by visual inspection of slides with labeled or stained cells. The labeling and staining techniques are expensive and the visual inspection is time consuming, performed only by highly trained personnel and also results in false positives and negatives (e.g. for cervical tumor screening the interobserver reproducibility is very low [5]).

Infrared spectroscopy, IR absorbance due to specific molecular vibrations, is an interesting diagnostic tool without the need of added labels with the ability to analyze cell components such as DNA, RNA, proteins and lipids. By comparing the IR absorbance spectra of healthy and malignant cells, published in literature (e.g. in breast [1], blood [2] and brain [3]), we hypothesized a new concept to distinguish cell types. It concerns a few-wavelength based cell type discrimination concept in the wavelength region between 3 and 4 µm. In this region specific lipid absorbance peaks [6] can be found ($CH_2$-and $CH_3$-symmetric and antisymmetric stretch).

To test the few-wavelength hypothesis we recorded and compared our own data set of healthy (MDCK) and carcinoma (A-498 and Caki-1) epithelial kidney cell lines with a Fourier transform infrared (FTIR) spectroscope. The IR absorbance ratio $CH_2$-symmetric/$CH_2$-antisymmetric stretch (3.51/3.42 µm) differs between the cell types. This ratio is increased in the carcinoma cell lines compared to the healthy MDCK cell line (Fig. 1). This confirms that it is possible to distinguish between healthy and carcinoma epithelial kidney cell lines by only measuring the IR absorbance at a few wavelengths. To compare the lipid $CH_2$ stretch ratio of different samples baseline correction and normalization is required. This is normally done with the software package supplied with the IR spectroscope. Instead of recording the whole IR spectra between 2 and 20 µm with an expensive FTIR spectroscope, comprising of an liquid nitrogen cooled detector, we developed a smaller, faster and cheaper sensor system based on LED light sources, narrow bandpass filters and a room temperature operable photodiode detector which could be used to discriminate between healthy and tumor cell types.

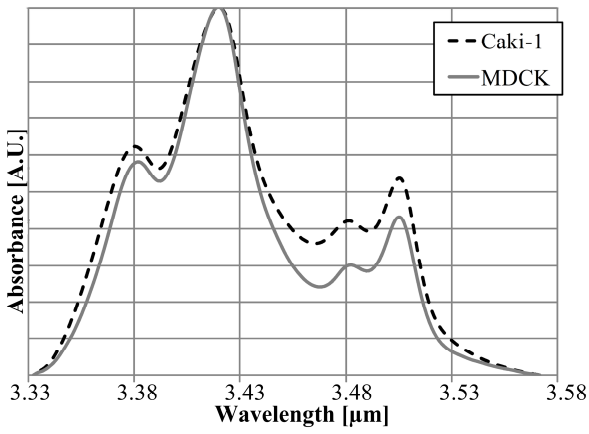


Fig. 1 Normalized and baseline corrected IR Absorbance spectra of epithelial kidney cells MDCK and Caki-1 recorded with a Bruker Equinox 55 spectrometer (240 scans per spectrum, 4 cm-1 resolution and 1 mm beam diameter). The absorbance peak at 3.51 µm is increased in the Caki-1 carcinoma cell line compared to the healthy MDCK cell line.

## II. Materials and methods

### A. Sample preparation

The investigated epithelial kidney cell lines, healthy canine MDCK (ATCC CCL-34) and two human carcinoma A-498 (ATCC HTB-44) and Caki-1 (ATCC HTB-46), were cultivated under the same conditions in monolayer on IR transparent calcium-fluoride ($CaF_2$) slides at 37°C and 5% $CO_2$. The culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM), 2 mM L-glutamine, 10% fetal calf serum (FCS) and antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml Amphotericin B) all obtained from Lonza. Before the IR absorbance measurements the sample slides were washed twice in phosphate buffered saline (PBS) to remove detached cells and medium components and dried for 3 hours in a sterile laminar flow cabinet to remove the water fraction.

### B. Detection method

To determine the IR absorbance ratio between $CH_2$-symmetric and $CH_2$-antisymmetric stretch (3.51/3.42 µm) out of the total absorbance signal and to compare different samples baseline correction and normalization is required. For normalization and baseline correction the IR absorbance values at two additional wavelengths (at 3.33 and 3.57 µm) are recorded (Fig. 2). This way the non-functional absorbance values under the base line can be calculated and subtracted from the total absorbance signal resulting in the functional values. By dividing the functional absorbance value from the $CH_2$-symmetric with the antisymmetric stretch band the normalized ratio will be obtained which can be used for sample comparison.

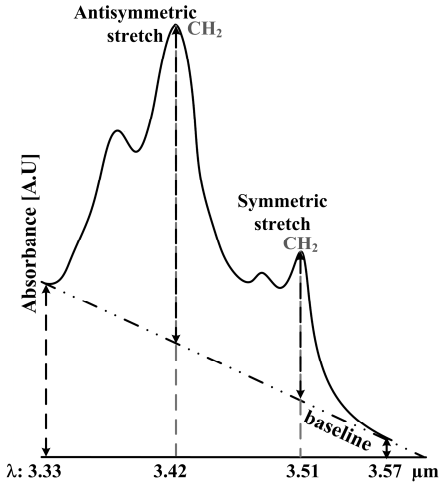


Fig. 2 Ratio determination. From each sample the absorbance at 3.33 and 3.57 µm is used for the base line (dashed black arrows) calculating the absorbance ratio 3.51/3.42 µm (dashed black grey arrows).

### C. Sensor

The developed sensor (Fig. 3) measures the infrared absorbance values at four specific wavelengths (3.33, 3.42, 3.51 and 3.57 µm). The emitted light of two LED IR sources (center wavelength at 3.4 and 3.6 µm) are collimated by plano-convex lenses and directed at a filter wheel with narrow bandpass filters (NBP). This way, from each LED multiple wavelengths can be selected (in our case 3.33 and 3.42 µm from the 3.4 µm LED and 3.51 and 3.57 µm from the 3.6 µm LED). After passing the NBP the selected wavelengths are focused on a 1.5 mm aperture by a beam-splitter and another plano-convex lens.

To detect the transmitted light through a sample by a room temperature operable photodiode (PD) two additional plano-convex lenses are used.

By manually rotating the filter wheel the two other filters can be selected. To prevent the LEDs and PD shifting their center wavelength, they are all built into housings containing a thermocooler. The two LEDs are emitting 8 µs pulses alternately at a carrier frequency of 2 kHz (one LED at the up flank the other at the down flank of the carrier signal). The amplified photodiode signal (PD connected to a current to voltage amplifier) is only recorded when an LED is emitting. This is realized by connecting the carrier signals of both LED drivers and the amplified photodiode signal to a data acquisition board which is programmable with a Matlab script. This method is used to measure the IR absorbance values at two wavelengths simultaneously.

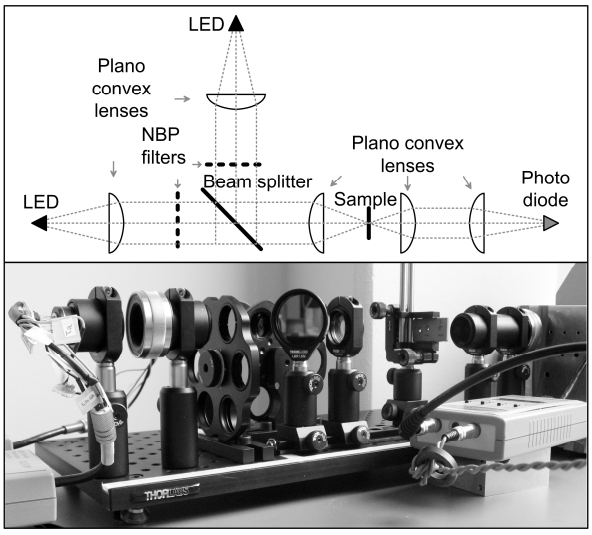


Fig. 3 Sensor concept. Two LEDs operated in alternating pulse mode both emitting in a broad range so that with changeable narrow band pass filters (NBP) two wavelengths can be used (3.33 and 3.42 µm for LED1 and 3.51 and 3.57 µm for LED2). A beam-splitter is used to focus the two beams on the same spot of the sample. The photodiode (PD) is used as detector and can be operated at room temperature.

*D. Data analysis*

The absorbed IR light by the sample is determined by comparing the transmittance difference of an empty calcium-fluoride slide and one containing the sample of interest. 8 µs LED pulses are sampled at a rate of about 10 sample points per pulse. To get the proper information out of a pulse the mean voltage of the four second highest values is used (Fig. 4). By averaging the values of multiple pulses more accurate absorbance values can be obtained.

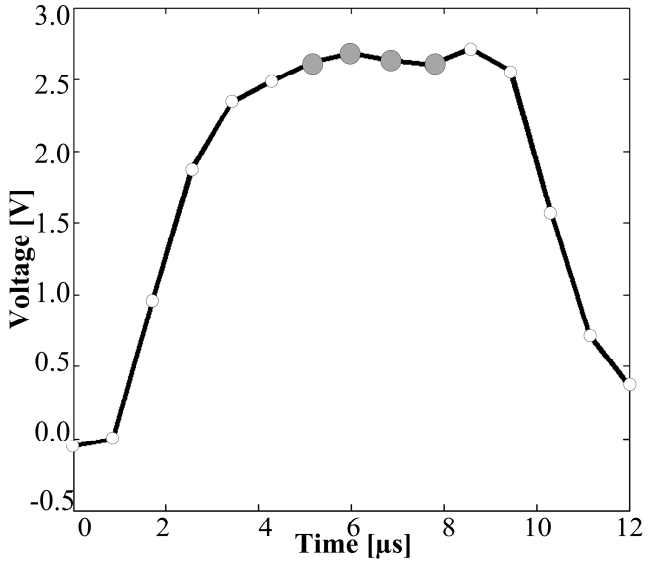


Fig. 4 Amplified photodiode signal of a single 8 µs LED pulse. By averaging the voltage values of the four second highest sample points (gray dots) one voltage value will be assigned to the pulse for further comparison.

## III. Results

The developed sensor system, measuring the infrared absorbance at four wavelengths to determine the lipid $CH_2$-symmetric / $CH_2$-antisymmetric stretch ratio, has been used to distinguish healthy from carcinoma epithelial kidney cell lines. The absorbance measurements were made after 3 hours of sample drying. Fig.4 shows the baseline corrected and normalized $CH_2$ stretch ratio of MDCK, A-498 and Caki-1. For every cell line the average value of four 1.5 mm diameter spots measured four times each were compared. Because the pulsed LEDs were operating at a frequency of 2 kHz it was easy to record multiple pulses to get more accurate data values. For these measurements 500 pulses per wavelength were recorded, resulting in a total of 8000 recorded pulses per wavelength per cell line.

As can be seen in Fig.5 there is a significant ratio difference between the healthy MDCK and carcinoma, A-498 and Caki-1, cell lines. Even between A-498 and Caki-1 a ratio difference of about 20% is detected.

Directly after the measurements the samples were stored in a closed polystyrene box at room temperature. Six days later the same samples were measured again for their $CH_2$ stretch ratios (the same way, four spots, measured four times). This resulted in comparable lipid $CH_2$ stretch ratios as measured earlier (0.49, 0.59 and 0.71 for MDCK, A-498 and Caki-1, respectively).

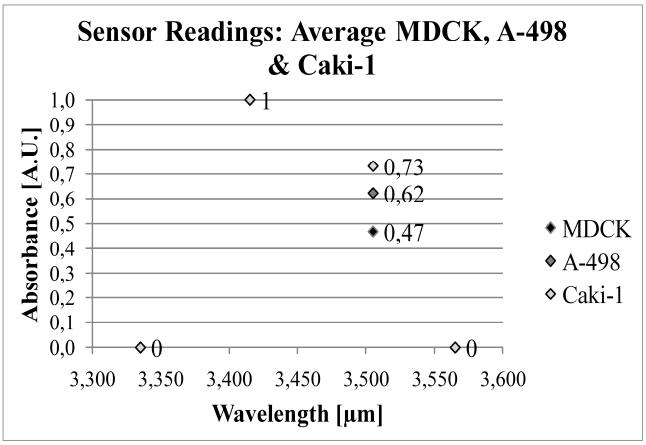


Fig. 5 $CH_2$-symmetric / $CH_2$-antisymmetric stretch ratio of three epithelial kidney cell lines MDCK (healthy) and two carcinoma A-498 and Caki-1. The absorbance values at wavelengths 3.33 and 3.57 µm are used as baseline points (set to zero). Next, the baseline corrected absorbance points at 3.42 and 3.51 µm are normalized so that different measurements can be compared. The stretch ratios of the carcinoma cell lines are higher compared to the healthy cell line (0.62 and 0.73 compared to 0.47).

## IV. Discussion

When cytology or histopathology techniques are used for cancer prevention programs the outcome of such tests depends on the judgment of the cytotechnologist and pathologist. A study of the interobserver reproducibility for cervical cancer [5] showed strong irreproducibility and false negativity.

Infrared spectroscopy has the potential to detect components such as DNA, RNA, proteins and lipids label-free but due to the requirement of a liquid nitrogen cooled detector and high price it not a potential device for easy prescreening purposes.

With the developed LED-photodiode based infrared absorbance sensor it is possible to determine the ratio between lipid $CH_2$-symmetric and antisymmetric stretch with baseline correction and normalization.

The compared samples used for the presented experiments were all epithelial kidney cell lines but not originated from the same species instead we compared healthy canine cells with two human carcinoma cell types. From the results it can be concluded that the developed sensor has the potential for detecting the lipid $CH_2$ stretch ratio which otherwise only could be detected with an infrared spectroscope. By comparing FTIR spectroscope recorded absorbance spectra of healthy and malignant blood, breast and brain cells found in literature differences in lipid $CH_2$ stretch ratios between healthy and malignant cell type can be derived. This indicates the potential of the developed sensor. Because of the relatively cheap design and the lack of necessary expensive chemicals the sensor could be interesting for fast prescreening of biopsy material (e.g. blood, breast and kidney).

## V. Conclusions

A significant difference between $CH_2$-symmetric and $CH_2$-antisymmetric stretch ratios of healthy versus carcinoma (and even between two carcinoma) epithelial kidney cell lines have been detected with a novel infrared sensor system based on two LED light sources and a room temperature operable photodiode as detector.

The developed sensor could be used for quick prescreening of biopsy samples which compared to labeling and staining doesn't require highly trained personnel and is much cheaper than liquid nitrogen cooled FTIR spectroscopes.

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## References

1. Fabian H, Thi NA, Eiden M, et al. (2006) Diagnosing benign and malignant lesions in breast tissue sections by using IR-microspectroscopy. Biochim Biophys Acta 1758(7):874-882
2. Maziak DE, Do MT, Shamji FM, et al. (2007) Fourier-transform infrared spectroscopic study of characteristic molecular structure in cancer cells of esophagus: an exploratory study. Cancer Detect Prev 31(3):244-253
3. Krafft C, Thümmler K, Sobottka SB, et al. (2006) Classification of malignant gliomas by infrared spectroscopy and linear discriminant analysis. Biopolymers 82(4):301-305
4. CDC/National Center for Health Statistics at http://www.cdc.gov/nchs
5. Stoler MH, Schiffman M, (2001) Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 285(11):1500-1505
6. Tamm LK, Tatulian SA, (1997) Infrared spectroscopy of proteins and peptides in lipid bilayers. Q Rev Biophys 30(4):365-429

Author: Sander van den Driesche
Institute: Institute of Sensor and Actuator Systems, Vienna University of Technology
Street: Gusshausstrasse 27-29/366
City: Vienna
Country: Austria
Email: sander.driesche@tuwien.ac.at