CONFOCAL LASER SCANNING MICROSCOPY - OBSERVATION OF THE MICROSTRUCTURE OF BITUMEN AND ASPHALT CONCRETE

Florian Handle, Hinrich Grothe, Susanna Neudl

Institute of Materials Chemistry, Vienna University of Technology, Vienna, Austria

ABSTRACT

Confocal Laser Scanning Microscopy (CLSM) has gathered interesting new results regarding the microstructure of bitumina as part of a comprehensive study on bitumen and asphalt concrete. Compared to normal fluorescence microscopy, the CLSM technique has been proven to be the method of choice for bitumen. The main advantage is a much stronger fluorescence signal due to the higher intensity of the excitation laser, which is higher by several orders of magnitude compared to conventional lamps (e.g. Mercury-vapour lamps). Also, the smaller aperture used to scan the surface creates much better special resolution.

Since the definition and improvement of asphalt concrete is the great challenge the ever rising expectations and requirements of roadworks puts in front of the bitumen industry. Thus, the search for techniques allowing an assessment of the adhesive and rheological properties of bitumen is very essential. The CLSM technique makes the detailed observation of the fluorescent centres in bitumen accessible, and thus provides important information on the microstructure and on the asphaltene micelles. Additionally, it is possible to observe the changes implied by the introduction of mineral aggregates into the bitumen, and thus to draw conclusions about the interaction of bitumen and the mineral components inside the asphalt concrete. Especially for polymer modified bitumina these experiments have shown intriguing results regarding structural phenomena that influence and practically define their improved mechanical properties.

Keywords: Bitumen, Fluorescence, Microstructure, CLSM, Microscopy

1. Introduction

The nature of bitumen puts great challenges in front of chemists and physicists. The composition of bitumen is simply too diverse to be easily accessible. As part of a comprehensive study on asphalt, the microstructure of bitumen was investigated and discussed. The micelle theory [1, 2] and the model of the 5+1 scales of asphalt observation (see Figure 1) were used as the theoretical basis for this study. The theory assumes that bitumen is in fact a colloidal system of asphaltene micelles dispersed in a maltene matrix. CLSM was found to be an imaging technique capable of visualising the actual microstructure of bitumen, by showing the spatial distribution of fluorescent molecules. Additionally, integrated fluorescence spectra of bitumen were gathered, offering a different perspective on the same physical qualities. In combination, these two methods were used to analyze the distribution and the origin of fluorescent particles in bitumen.
Many previous CLSM studies focused on the microstructure of SBS modified bitumina [2, 3, 4, 5], while only few researchers used this method on pure bitumen [6, 7]. The identification of the fluorescent centres is rather difficult and led to contradictory conclusions. Ranging from waxes to asphaltenes varied explanations for the origin of fluorescence were found. In principle, waxes are not capable of fluorescent emission, but asphaltenes are on the top list of suspects, so the first tests were aimed at proving their responsibility for fluorescent centres found in all bitumina studied.

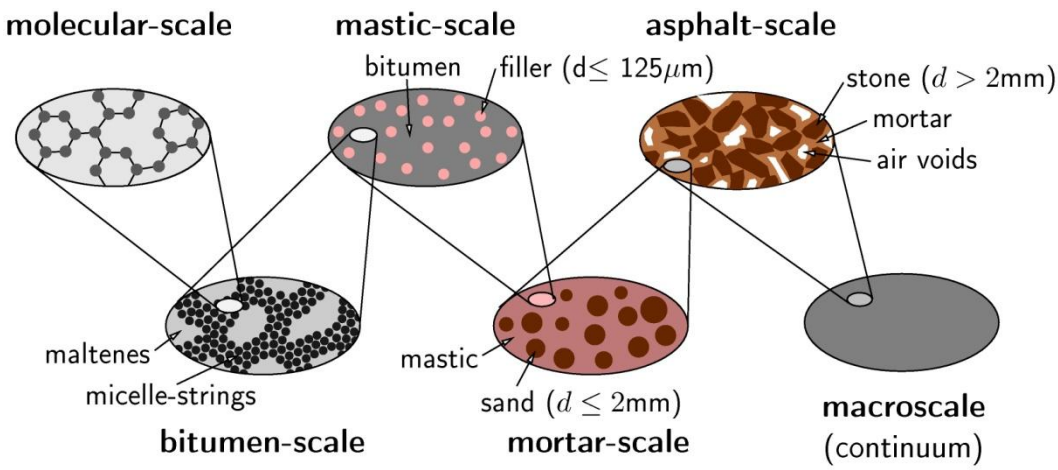

Figure 1: The 5+1 scales of observation for asphalt (cp. [9]), based on the micelle theory on the bitumen scale [1, 2]

2. Methods

CLSM provides many advantages compared to conventional fluorescence microscopy, most prominent the enhanced signal strength, the narrower focus, and the scanning procedure allowing a far better spatial resolution. For these experiments, an inverted confocal fluorescence microscope was used, an ECLIPSE TE2000 (Nikon Corporation, Tokyo, Japan). The microscope hosts both a transmission and a CLSM (Confocal Laser Scanning Microscope) array. The light source for the transmission array is a T-DH 100W Illumination Pillar (Koehler Type). An Argon-ion laser is used as source of excitation radiation. Typical for CLSM, laser optics is configured to allow a scanning of the surface and the image is created point by point and line by line. The advantages mentioned above in combination with the highly sensitive detector are key features for the successful application. However, the capability to scan volumes below the surface, a key advantage of the CLSM technique, cannot be applied here, because of the high absorption cross section in the visible range of all bitumina.
For sample preparation, the bitumen was heated to about 150-200°C as necessary for melting the sample. Then a small quantity of bitumen was applied to a glass slide with a piece of wire and a second glass slide was placed onto the sample. After a short period of cooling, the sample was measured. Early experiments showed that this procedure had to be modified. The first step towards a significant improvement in picture quality is the replacement of the standard object carriers by extremely thin glass slides (<0,5mm). Second, an additional heating period for the samples was implemented, allowing the bitumen film to spread and become thinner. These thin films could then be examined by CLSM.
Also experiments on mixtures of bitumina and mineral aggregates were conducted. The samples were prepared in a similar fashion. First, the bitumen film was applied to a glass slide, but before putting the second slide on top, mineral aggregate powder was dusted on the bitumen. Afterwards, the glass slide was put on top and a few minutes on the heating plate assured that the mineral aggregates sunk through the thin bitumen film. After a short cooling period, the samples were measured by CLSM.

For fluorescence spectroscopy, an Edinburgh Instruments FSP920 photoluminescence spectroscopy setup was at our disposal. All samples were examined using both excitation and emission scans. The combination of these methods was used to find the optimal setup for microscopy. After the CLSM experiments were concluded, fluorescence spectroscopy was used to search for the origin of the fluorescent signals in combination with fractionation experiments.
For sample preparation, the bitumina were heated to 150°C and a drop of bitumen was applied to a standard microscopic slide. This slide was stored at the same temperature for 15 minutes to allow the drop to spread and increase its surface. Afterwards the samples were cooled to room temperature and subjected to fluorescence spectroscopy. For the extraction experiments, about 15g of bitumen were tightly wrapped with filter paper, to avoid the loss of any n-heptane insoluble material. Then the filter was placed inside a Soxhlet extractor and the sample was subjected to a 48h period of continuous extraction with distilled hot n-heptane, guaranteeing that any soluble fractions were extracted. The n-heptane was then gently removed by a rotary evaporator. The oily residuum was taken up with 5ml of toluene and the flask was washed three times with another 5ml of toluene. The filter was removed from the Soxhlet extractor, put into a beaker, immersed in 20ml of toluene and heated to 70°C for 10 minutes. The toluene based solutions were than applied drop by drop to a microscopic slide that was heated to 150°C to ensure the complete evaporation of toluene before measurement at either the CLSM or the fluorescence spectrometer. Five bitumina and bitumen precursors were examined (see Table 1) this way.

Table 1: Overview samples and types studied

Sample	Bitumen type
50/70	Standard bitumen used in roadworks
70/100	Base bitumen for the production of PmB
45/80-65	SBS based PmB
PE1005	Bitumen precursor (vacuum flashed, cracked residuum)

3. Results

3.1 CLSM of pure bitumina

The CLSM pictures obtained from bitumina exhibit the typical bitumen microstructure as predicted by the micelle model. The fluorescent centres are of roughly ellipsoid shape, vary in size and amount, and their spatial distribution seems to be statistical and unordered (Figure 2). The exact size and population density varies from one sort of bitumen to the other. These images provide perfect evidence for the micelle model and put a solid scientific background to the most prominent model capable of explaining the rheological properties of bitumen [2].

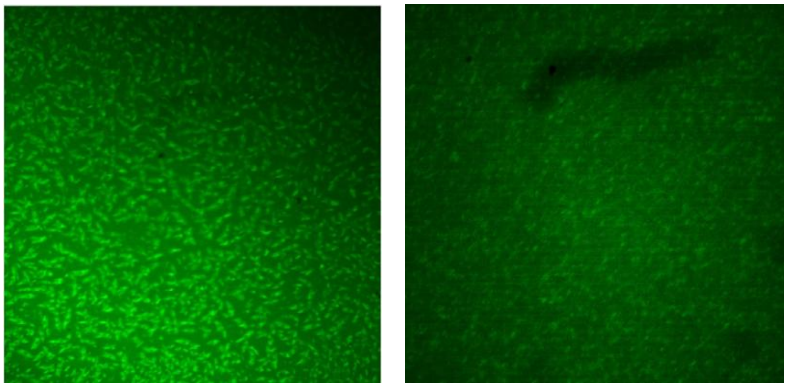

Figure 2: CLSM images of PE1005 (left) and 70/100 (right)

Since the micelle model describes bitumen as a colloidal system of dispersed asphaltenes in a continuous maltene phase, the spatial distribution of the fluorescent centres is an important issue and a strong connection to the mechanical and rheological properties of the bitumen can reasonably be assumed. Thus, preliminary experiments with picture analysis software have been conducted to assess the particle size distribution for the tested bitumina and whether significant differences can be found. Although the necessary picture processing is a rather complicated task, the results are quite promising and future experiments and process automation could provide an important and powerful tool for bitumen analysis.

3.2 CLSM of bitumen/mineral aggregate mixtures

The measurement of mixtures of bitumen and filler, which is essentially finely powdered mineral aggregate, was particularly interesting because it is reasonable to assume that the most polar molecules, be they asphaltenes or resins, might interact with the polar surface of the mineral aggregate.
As a preliminary experiment, the fluorescent capabilities of the mineral aggregates were determined. It was confirmed that the fluorescence signal of the studied fillers is weaker than the fluorescent centres in bitumen by several orders of magnitude. Early experiments show fluorescent halos around the mineral aggregates. These halos surly stem from fluorescent molecules accumulated at the surface of the mineral component, although it is not certain whether this accumulation is promoted by the preparation process or is actually the result of a chemical or physical phenomenon. Since this effect could be observed using mixtures of different bitumina and fillers, it was strongly suggested, that these "halos" were caused by actual interaction of the filler and the fluorescent molecules. To verify this theory, a further experiment was designed. An additional resting period of 20 minutes at 200°C was included after the sample preparation process, to allow any reforming or migration processes to take place. This allowed exclusion of mechanical strain as a reason for the formation of the halos. However, the resulting images show no halos – en contraire, they show the typical bitumen microstructure of small fluorescent centres distributed evenly across the surface. The assumption that the mixing process, meaning the implantation of the mineral aggregates into the bitumen, is the reason for the formation of these halos (see arrow in Figure 3, middle) is strongly supported by these pictures. The extended resting period on the heated plate allows the bitumen structure to relax and the fluorescent particles return to a statistical and unordered distribution of small dispersed particles. This essentially indicates that the formation of high concentrations of fluorescent molecules at the surface of the mineral aggregates is only a transitional state, which on the other hand indicates a lack of interaction between the filler and the fluorescent centres in bitumen. As discussed earlier, the fluorescent centres could originate from three different fractions capable of fluorescent emission. The asphaltenes and the resins are the most polar fractions in bitumen, and hence, most likely to play an important role in the chemical or physical bonding in the interface. If these compounds return to their original agglomerated state after a short period of resting essentially leaving the surface of the mineral aggregates, this indicates that there are no significant or only very weak chemical or physical interactions between bitumen and the minerals.

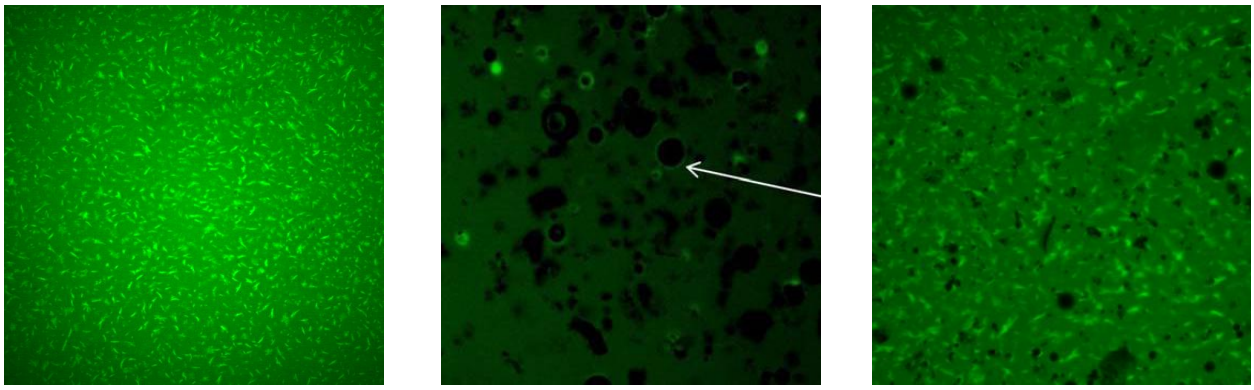

Figure 3: CLSM image of PE1005 (left), mixture of PE1005 with filler (middle) showing the halo effect (arrow), mixture of PE1005 with filler after storage at 150°C for 20 minutes (right)

3.3 CLSM of SBS modified PmB

The addition of polymers to bitumen is a popular strategy to increase the durability of the road by enhancing its resilience to deformation under strain. Adding an elastomer like SBS (Styrene-Butadiene-Styrene) to bitumen, as has been performed in sample 45/80-65, strongly improves the capability of elastic reformation. SBS is a block copolymer and an elastomer. It is one of the most popular polymers used in bitumen production [10], since it is available in sufficient quantities, relatively cheap and has proven itself to be capable of improving the mechanical properties of bitumen greatly, notably adding to the elastic deformability of the material. As a negative side effect of this modification, the loss of ductility and the vastly increased viscosity of the material have to be noted. Thus, higher processing temperatures are necessary and at the construction sites, the PmB based asphalt concretes have to be stored at higher temperatures, which in turn promotes bitumen oxidation.
Fluorescent microscopy is an important and often used tool to explore the microstructure of polymer modified bitumina. Though it has to be noted that in SBS (Figure 4, top) only the styrene monomers, the benzene rings, are capable of exhibiting fluorescence. Furthermore, the polymer amounts only up to 5wt% of the whole material. Figure 4 (bottom left) is an image of the base bitumen for the PmB, 70/100, and exhibits the typical bitumen microstructure of small dispersed fluorescent centres. The picture of the PmB 45/80-65 (Figure 4, bottom right) on the one hand shows agglomerations that are by several orders of magnitude larger than in the base bitumen, while on the other hand there

are few small particles still visible. It is difficult to explain why the polymer would generate such an intense signal, effectively masking the fluorescent centres detected in the base bitumen. A possible explanation can be found in the chemical similarities of the styrene blocks and the aromatic fractions of the bitumen, opening the possibility for the formation of agglomerated structures between the styrene blocks and the asphaltenes/resins/aromatics of the bitumen. This would explain the lack of small fluorescent centres as well as the high intensity of the fluorescent effect of the SBS agglomerates and network. This theory is supported by the experiences of the production process. The admixture of the polymer is often problematic due to agglomeration and phase separation problems. If the SBS granulate is added untreated into bitumen, these phenomena have been reported to frequently occur. These effects can be avoided by either using very fine SBS powder, thus ensuring a good phase contact between bitumen and SBS, or by beforehand soaking of the SBS granulate in a special oil. This indicates stability problems of the SBS/bitumen mixture, which is not surprising, as the macromolecules of SBS are hardly soluble in the bitumen itself. It is thus reasonable to assume that the SBS needs to be stabilized in a similar fashion as the asphaltenes.

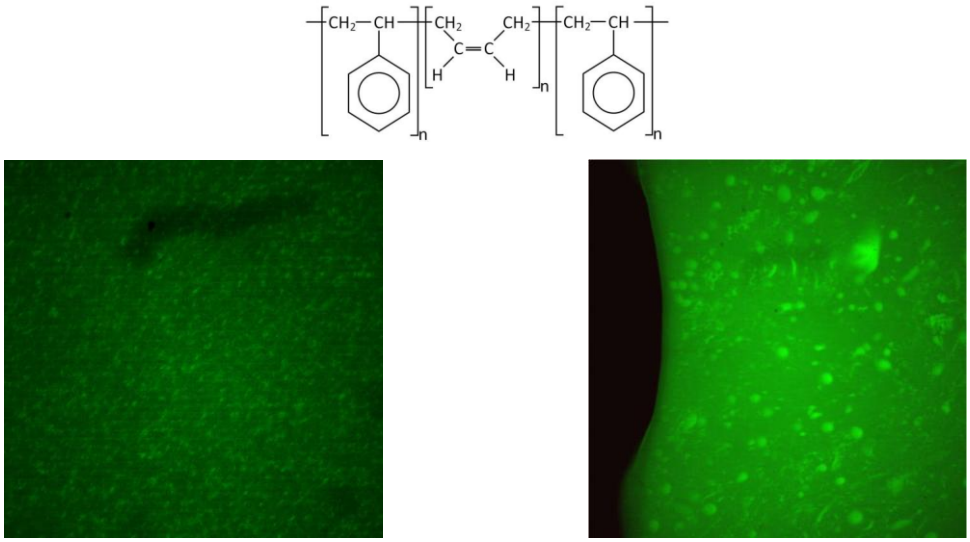

Figure 4: SBS block-copolymer (top), CLSM image of bitumen 70/100 (left), CLSM image of PmB 45/80-65 - SBS modified 70/100 (right)

Concerning the improved mechanical properties, it is important to note that the effectiveness of the polymer modification is highly dependent on a good spatial distribution of the polymer chains throughout the bitumen phase to confer the enhanced mechanical properties to the bulk of the material. The procedure used for application of filler to the normal bitumina/bitumen precursors does not change the microstructure of the PmB significantly (see Figure 5, left). Even after extended periods of resting at high temperatures (200°C), allowing even the macromolecules in bitumen a reasonable mobility, will not result in a change or adaptation of the SBS agglomerates. This indicates a lack of interaction between the SBS and the mineral aggregate. To improve the phase contact, the sample preparation was modified in two steps: The first experiment included a gentle mixing of bitumen and mineral aggregates on the microscopic slide, which showed slight changes in the microstructure (Figure 5, middle). In a second step, the sample was mixed before the application to the glass slide at high temperatures, while thorough stirring ensured good phase contact. The result was the formation of a SBS network (Figure 5, right).

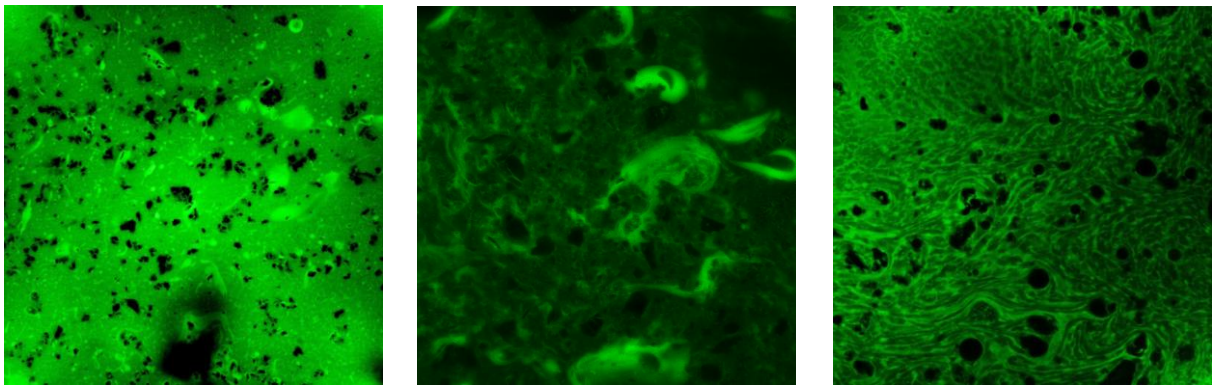

Figure 5: CLSM images of mixture of PmB with filler, sprinkled and pressed (left), gently stirred on microscopic slide (middle), violent stirred before application to microscopic slide (right)

3.4 Fluorescence spectroscopy

Fluorescence experiments have been conducted to support the use of CLSM as an imaging technique on bitumen. Besides the distribution of fluorescent centres, the general fluorescent behaviour is of particular interest. However, fluorescence spectroscopy yields an integrated spectrum that is not capable of visualising spatial distributions. The identification of the fluorescent centres is hence not trivial, since a closer look on the basic physico-chemical properties of the bitumen fractions reveals that bitumen basically contains three species that could be the origin of the fluorescent signal. The asphaltenes, the resins, and the aromatics phase are based on their general description capable of fluorescence, while only the fraction of saturates is not supposed to be fluorescent. There are two kinds of spectra presented here, excitation scans (variable excitation, fixed detection wavelength) and emission scans (fixed excitation, variable detection wavelength). For the excitation scans, the same detection wavelength as used at the microscope was chosen, 515nm, and a spectral range of 200-500nm was observed. Likewise, for the emission spectra, an excitation wavelength of 488nm was selected. A range from 300-480nm and from 500-750nm was measured. The 20nm gap was bridged through interpolation to provide a continuous spectrum (Figure 6, Figure 7).
The first results of the fluorescence spectroscopy were the identification of four wavelength of particular interest (see Figure 6, right): 210nm (1), 270-280nm (2), 370nm (3), 480-490nm (4) at a detection wavelength of 515nm. These are both local and absolute maximum, as well as the two shoulders visible in the excitation scan of the pure bitumen. The distribution of the fluorescent centres of these would be of particular interest. For excitation radiation, a wavelength of 488nm was chosen, since it is very close to the local minimum at 480-490nm. Also it is one of the strongest spectral lines available at the Argon-ion laser and is commonly used for CLSM. The technique was capable of revealing the origin of the fluorescence signals of the different species spatially distributed.
Investigating the source of the fluorescence, usually a detailed interpretation of the fluorescence spectra could be attempted. For bitumen, this is a rather difficult task, since it has been shown that fluorescence phenomena are greatly dependent on the chemical vicinity and the effects of the surroundings are hardly determinable/predictable for bitumen [11]. Rather, an empirical approach was chosen. The fractionation of bitumen is a standard test method and often used to characterize bitumina by their relative contents. The fractions are again mixtures of molecules of similar chemical/physical behaviour. Since literature in general suspects the origin of the fluorescence being the asphaltenes, this theory was put to a test. Asphaltenes are defined as insoluble in n-heptane or n-pentane [12, 13]. Most extraction protocols for standardized testing are based on a simple extraction with n-heptane [13]. For these experiments, a more refined n-heptane based extraction method was chosen, i.e. a Soxhlet-extractor, which guarantees as pure asphaltenes as possible. The results of fluorescence spectroscopy on the fractions gave surprising results, since contrary to other researchers, our experiments on the insoluble residuum (asphaltenes), the soluble extract (maltenes: resins, aromatics, saturates), and the pure bitumen strongly indicate that the origin of the fluorescent centres is rather to be found in the maltenes than in the asphaltenes. The spectra of the residuum show almost no fluorescent capabilities at either the detection wavelength or the excitation wavelength used by the CLSM. On the other hand, the spectra of the extract phase exhibit intense fluorescence in these areas. The absolute intensity ratings, although being only general tendencies, suggest that the residuum somehow dampens the fluorescence of the pure bitumen 50/70 as it dilutes the extract (Figure 6, left, Figure 7, right). Since the fluorescent centres are spatially distributed and the fluorescent species seem to agglomerate, they can be interpreted as a mantle, composed of resins and aromatics, surrounding and stabilizing the asphaltenes. The normalization of the emission spectrum shows the relations between residuum, extract, and pure bitumen (Figure 7, left). In the range of 300nm to 450nm and again above 700nm, the influence of the residuum is very strong and the pure bitumen sample behaves like the residuum. However, between 450nm and 700nm the pure bitumen behaves more like the extract, although the maximum of intensity is shifted towards higher wavelengths caused by the influence of the residuum. In general it can be said that the fluorescence of the asphaltenes seems to be shifted to wavelengths higher than 750nm, into the near infrared region, which is not accessible to the fluorescence spectroscope used in this study.

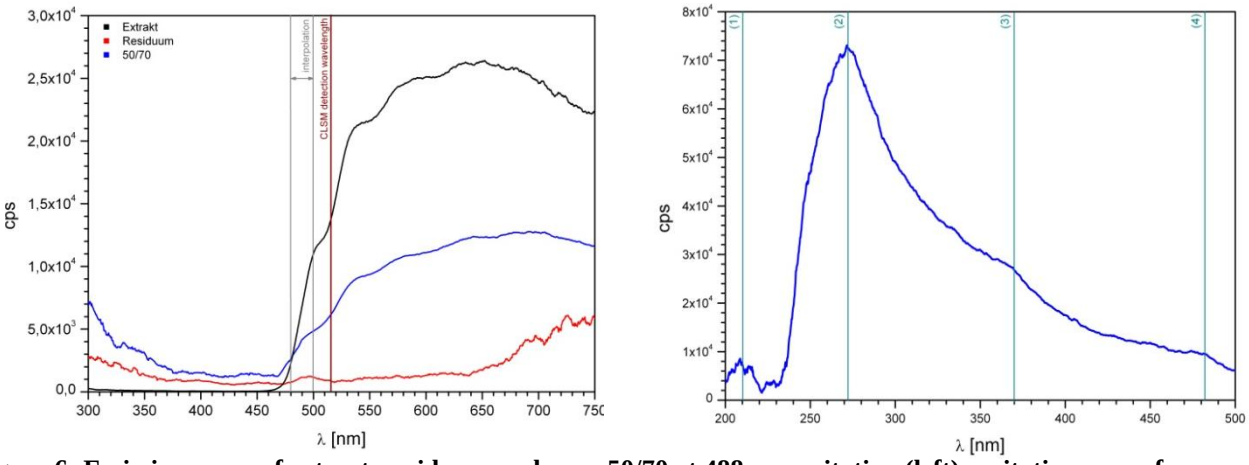

Figure 6: Emission scans of extract, residuum and pure 50/70 at 488nm excitation (left) excitation scan of pure 50/70 for 515nm detection (right)

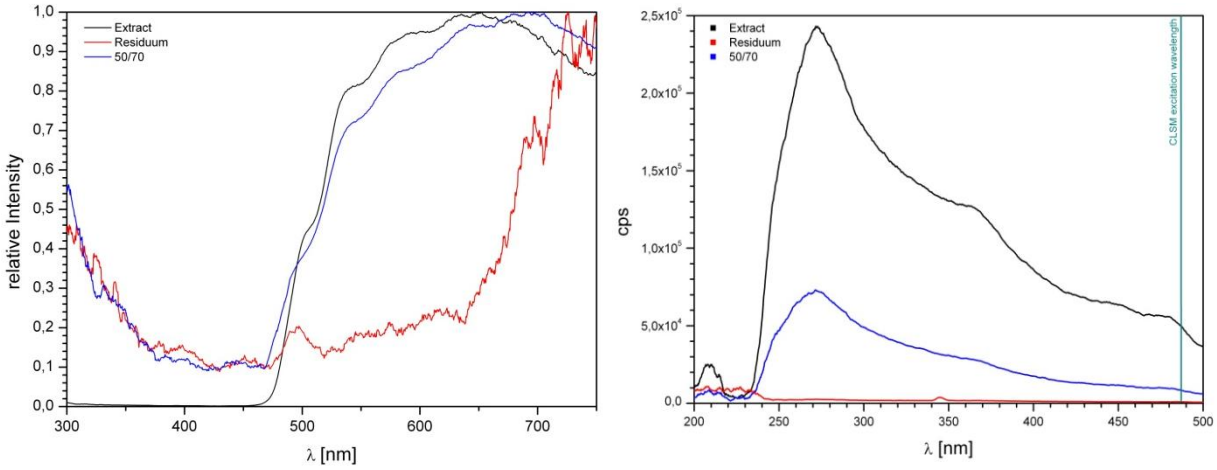

Figure 7: Normalized emission scans of extract, residuum and pure 50/70 at 488nm excitation (left) excitation scans of extract, residuum and pure bitumen for 515nm detection (right)

4. Conclusion

CLSM can be used as an imaging technique on bitumen. The technique allows the confirmation of the micelle theory, as it is capable to visualize a microstructure of agglomerated fluorescent particles. These have been interpreted by many researchers as asphaltene micelles, attributing the fluorescent signals to asphaltenes themselves. Our results of the fractionation experiments presented herein indicate that the samples behave differently. As proven by fluorescence spectroscopy, the capability of the asphaltenes for fluorescence at 488nm excitation is limited at best. Likewise, the emission signal strength at 515nm detection wavelength is very low. Combining both facts, the fluorescent centres originate from the maltene phase and not from the asphaltenes. The fluorescence of the latter can be found looking to the near infrared region. Further fractionation experiments will be used to narrow the point of origin down. The educated guess points toward the resins or the more polar aromatics. Both groups amass in the vicinity of the asphaltene agglomerates, according to the micelle theory.

For the SBS modified bitumen, the microstructure is perfectly visible. The results of the experiments strongly suggest that the challenges encountered in production are closely related to the fluorescent species, as they also stabilize the SBS in the bitumen. Problems of storage times at high temperatures at the construction sites have to be pointed out. Given enough time and diffusion rate, meaning essentially enough temperature, SBS will agglomerate and a phase separation might occur.

Particle size analysis is also a prospect for further studies, since the possibility of assessing material characteristics beforehand is intriguing. If the automation of the image processing can be achieved and the data correlates with mechanical properties, this method could be a great asset in bitumen analysis.

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