Is Atomic Force Microscopy suited as Tool for fast Screening of Bituminous Materials? 
An Inter-laboratory Comparison Study

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ABSTRACT

Bituminous binders are known to have microstructures at typical length scales of micrometers. This microstructure can be probed with Atomic Force Microscopy (AFM). Now that worldwide several research groups are reporting AFM results on bitumen, it is becoming important to improve the understanding of the reproducibility and objectivity of the technique for studying bituminous samples. When reproducibility and stability are proven, AFM can be a tool for asphalt professionals to rapidly screen bituminous binders. In this context two independent laboratories have developed a standard method for preparing and conditioning bitumen for AFM imaging. By means of an inter-laboratory comparison of independently imaged specimen, the reproducibility of microstructure measurements was investigated. A quantitative comparison on different microstructures was developed, and the consistency of independently obtained results was confirmed. The results from both labs were comparable: the microstructural properties were found to be randomly distributed within a 5% interval. Also the influence of temperature on the microstructure was demonstrated to be reproducible and consistent. With the increase of temperature, the microstructure gradually disappeared, however traces of the microstructure remained visible up to the highest measurement temperature of 60°C. It is concluded that given well defined sample preparation and measurement procedures, the microstructure of bitumen can be reproducibly imaged by AFM from room temperature up to temperatures where bitumen has become soft and too sticky to be probed by the same setup as used for lower temperatures.
INTRODUCTION
The distillation residue of crude oils is a highly viscous and complex molecular fluid named bitumen, that is usually applied as a sealant or as a binder in asphalt pavements. Solidification of these fluids leads to a visco-elastic (semi)solid with a two (or more) phase morphology on a micrometer length scale. Some researchers did observe micro structuring in bitumen by small angle X-ray scattering techniques [1]: this was clustering of molecules at the nanometer length scale. Because of the opacity of bitumen optical techniques have not been used extensively to study the morphology of bitumen at larger length scales, though the presence of a crystalline fraction allowed for some polarized light microscopy studies [2].

The introduction of the atomic force microscope (AFM) made possible that also opaque, non-conductive surfaces could be imaged. The technique was since then used to study and characterize phase separated systems such as polymers [3]. Later it was found by AFM that also bituminous materials exhibit a variety of microstructures [4, 5, 6, 7, 8, 9]. The bitumen microstructure appeared to have a unique fingerprint: ellipsoidal particles with a regular, transverse pattern (bee-shape), FIGURE 1. The microstructure was found developing to different degrees and forms depending on the crude origin of the bitumen and its thermal history. The chemical composition of the phases have since been topic of discussion. Some authors concluded that the bee-shaped structures should consist of the most polar fraction in bitumen, namely the asphaltenes [6, 10]. More recently, other authors attributed the bee-structures mainly to clustering of non-polar waxes, based on chemical extraction studies [8] and neutron scattering evidence [11].

FIGURE 1 Typical bitumen microstructure as observed in this study, with i) microstructural inclusions with transverse pattern (‘bees’), ii) phase that encloses the ‘bees’ and iii) a tertiary phase.

The Significance of Microstructure on Mechanical Properties
A material with a microstructure (M) has a typical length scale at which a sudden change in physical properties emerges. In the case of bitumen this is evident: the ‘phase contrast’ observed by AFM (see the section on AFM) is a consequence of a contrast in viscoelastic properties between the phases, hence at the microstructure length scales the continuum description of the material’s properties ceases to be valid. There may be one or more microstructural length scales present in a material, e.g. in composite materials like tendon, but also in bitumen where structuring at the nanometer and at the micrometer scales has been observed. The physico-chemical and mechanical properties (P) of a material will relate to the
material’s microstructure, i.e. \( P = P(M) \). The details of this relation might be very complex and hard to obtain. Nevertheless, these relations are known and utilized in materials science. For instance in metallurgy it is common knowledge that microstructural dimensions relate to a materials yield strength [12, 13, 14]: a coarser microstructure will lead to a mechanically weaker material (lower yield strength). This so-called Hall-Petch relation reads in a simplified way as \( \sigma_y \propto d^{-1/2} \) (with \( \sigma_y \) the yield stress, and \( d \) the typical dimension of a microstructural grain). This is a typical example of the general structure-property relationship \( P(M) \).

Similar relations can be found in asphalt as composite material where the bitumen, filler particles and the aggregates can be considered as various microstructures \( M \) emerging at different length scales. But also for the binder such relation holds, though its details are yet not known: softer bitumen usually possess larger microstructural features and a higher surface coverage by these features. Likewise, other physico-chemical properties like micro-crack healing have been related to the microstructural properties of the material [15].

The relation between microstructures and material properties is the key to attribute a material with desired properties: material appraisal. The first step towards this goal is to establish phase diagrams that relate the microstructure to various processing parameters. Also the application of additives like polymers or zeolites may be considered in the context of such phase diagrams. For practical engineering the establishment of these phase diagrams would be an indispensable tool. The first step towards this goal will be the reproducible, robust and fast acquisition of bitumen microstructure \( (M) \). Therefore this study aims at inter-laboratory comparison of the reproducible measurement of the microstructure of bitumen as obtained by atomic force microscopy. The thermal stability of the measurement will be investigated, and at the same time an exemplary structure-property relationship for bitumen will be obtained: the relation of microstructural changes with increase of temperature and softening of the material. As many researchers in the asphalt community are using, or considering to use, atomic force microscopy as one of their research tools of choice, a first assessment of issues related to reproducible imaging of microstructure should fulfill a common need. Finally, this study was inspired by the Dutch Road Authorities Rijkswaterstaat, who desired to have an independent assessment of the possible usability of the AFM technique for rapid assessment of bituminous materials.

**ATOMIC FORCE MICROSCOPY**

Atomic force microscopy (AFM) is a scanning probe technique that is designed to image surface topography and heterogeneity of materials with high spatial resolution [16, 17, 18, 19]. In AFM imaging, a cantilever with an extremely sharp tip (nominal tip radius of 8 nm) located on its free end is scanned over the sample surface utilizing a piezoelectric scanner. The changes in tip-sample interactions result in deflection of the cantilever which is measured by an optical-lever detection system. In this system a laser beam is focused onto the back side of the cantilever and the reflected beam is detected with a position sensitive photodiode. While scanning, a specific operating parameter is kept constant by a feedback loop between the optical detection system and the piezoelectric scanners. Measurements are being recorded electronically. The data acquired build up a map of the surface topography and other parameters representative of variations in the tip sample interaction.

One of the dynamic modes of AFM, tapping-mode [20], was used in this study. In tapping-mode the probe is modulated near its first resonant frequency while it is scanned across the sample. Thus the tip maintains an intermittent contact over the sample surface, keeping the tapping force low and the lateral forces negligible. This moderate force exerted
on the surface leads to scanning in a non-destructive manner, which is ideal for soft material surfaces such as bitumen [18, 20].

Since the probe is oscillating, it experiences attractive and repulsive forces depending on its position in the cycle. As the tip approaches the sample, the tip-sample interactions alter the amplitude, resonance frequency, and phase angle of the oscillating cantilever. During scanning, the amplitude at the operating frequency is maintained at a constant level, called the set-point amplitude, by adjusting the relative position of the tip with respect to the sample.

The oscillating cantilever dissipates various amounts of energy as it interacts with material heterogeneity on the sample surface. In tapping-mode the instrument provides three different types of data simultaneously: topography, phase-contrast, and amplitude error. Each of the image types provides specific information with respect to the sample surface. Topography images provide information of relative height of the various features as the probe tip is raster scanned across the sample surface. Phase imaging creates images of the phase of the tapping response, which is a function of the forces that the tip is experiencing; in other words the relative damping of an oscillating cantilever tip as it experiences heterogeneity in material response of the surface. Error images provide a record of any variations from constant deflection of the cantilever as the tip is scanned over the surface which represent the high frequency contrast of the topography signal indicating areas where topography is changing. All of these image types are obtained simultaneously and need all to be considered for the interpretation of tapping-mode AFM results.

DESIGN OF THE INTER-LABORATORY COMPARISON STUDY

Materials

The first step in this comparative study was to select the bitumen grades to be imaged. For reasons of synergy with another research project, which was aimed at a phenomenological bitumen healing study by a four point bending test with rest periods, it was decided to investigate the same bitumen as in this study, i.e. a virgin hard PEN 10/20 bitumen and a softer virgin PEN 70/100 bitumen. Besides, the pure bitumen; to study the same bitumen recovered from fatigue test was also included in this context. The 10/20 and 70/100 PEN graded bitumen were used to make the asphalt concrete beams which undergone the four point bending test. After the mechanical tests, bitumen was recovered from the beams by cold extraction; NEN 3971 and subsequent rotatory evaporation; EN 12697-3. This allows for comparing bitumen microstructural properties before and after a fatigue test. Thus, the starting point for this study were four materials: virgin 10/20, ‘fatigued and recovered’ 10/20, virgin 70/100 and ‘fatigued and recovered’ 70/100 bitumen. The differences between virgin and ‘fatigued’ materials were not subject of this particular study, and will be published separately. Both laboratories, TNO and Delft University of Technology received four batches of bitumen to prepare AFM samples (the partners in this study will be consistently designated LAB1 and LAB2 from here on, where the exact affiliation of LAB# is not revealed here, but consistently used throughout this study).

Sample Preparation

Both laboratories prepared from each batch of bitumen two identical samples by heat casting the material on 12 mm steel sample disks. One of the samples was exchanged between labs and the other was for the control measurement. Thus, every lab performed the AFM analysis (at 25 °C) on two samples per bitumen type which were prepared following the same procedure. Each specimen was prepared by applying an amount of 20-30 mg of bitumen with
a spatula on the steel sample disk (⌀ 12 mm, 0.5 mm thickness) which was kept at 100 °C on a heater plate for 40 seconds. The conductive heating allowed the bitumen to melt and spread over the sample disk, providing the sample thicknesses ranging from 300 µm to 500 µm bitumen film. After heat casting, the samples were kept in an oven for one hour at 100°C and subsequently ambient air cooled, and stored in dust free environments until AFM measurement took place (see FIGURE 3). Sample thicknesses were obtained by assuming a bitumen layer of constant thickness covering the sample disk completely. Though an appearance of curvature was observed at the edge of the sample holder, the assumption of flatness holds extremely well for the small area probed by AFM (order of micrometers). Given the exact area of the sample disk (12mm in diameter) and the density of bitumen (assumed 1 g/cm³), the sample thickness was calculated with an uncertainty of ± 5%, given the curvature and other unknowns. For each batch, one sample was transported to the other laboratory, and the other was kept in place.

A second batch of two sets of four samples was later prepared by one of the laboratories for the thermal stability study (see FIGURE 2 and 3).

FIGURE 2 Two sets of similar bitumen samples for the thermal stability study.

Instrument Settings and Measuring Conditions
Prior to AFM imaging, the samples were allowed to thermally equilibrate at 22°C for a time period of 48 hours. The time \( t_{\text{rest}} \) between the thermal conditioning of the samples at 100°C in the oven, and the time of measuring the microstructures with AFM was kept the same between both laboratories within a margin of 4 hours. The time \( t_{\text{rest}} \) was 48 hours for all measurements and within 3 hours the same between the labs. it is known that the microstructure, immediately after cooling, is not in equilibrium but in a quasi-stable state. No mass loss of the samples was measured during conditioning, so the thickness of the bitumen films has remained the same.

For the measurement of the microstructure by AFM, the sample was first heated to 25°C, then allowed to equilibrate for 30 minutes, and then the measurement was started. For the thermal stability study the same procedure was followed with the AFM data acquisition and further the sample was heated to the next temperatures (see FIGURE 3).

In order to have a fair comparison between the laboratories and to account for the experimental reality that different laboratories possess different instrumentation, it was decided not to select a common cantilever. The only parameters that were synchronized between the labs were scan sizes (15µm and 30µm), scan frequency (1 Hz) and the number of data points to be stored per scan line (512). Further it was agreed that both teams would strive for optimal AFM image quality. It should be recognized that the ultimate decision on what qualifies as a good images is a matter of skill and professional judgment of the experimenter.
FIGURE 3 Sample preparation (conditioning) and subsequent measurement of the bitumen microstructure as function of temperature.

Some typical settings of the AFM-setups in both laboratories are given in TABLE 1. Further it was agreed that imaging would take place in air that should be in the range 20-23 °C, at normal ambient humidity (40% relative humidity) conditions. Each laboratory followed a similar image post processing procedure, using Gwyddion open source SPM software [21]:

i) Data leveling by plane subtraction;
ii) Line correction by matching height median;
iii) Scars (parts of the image that are corrupted by a scanning error caused by local faults of the closed loop) removal by using neighboring lines to ‘fill-in’ the gaps.

TABLE 1 Settings of the AFM-Setups for both labs

<table>
<thead>
<tr>
<th>AFM Setting</th>
<th>LAB1</th>
<th>LAB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM-system</td>
<td>Bruker Multimode V</td>
<td>Nanosurf Easyscan 2</td>
</tr>
<tr>
<td>Scan direction</td>
<td>Trace</td>
<td>Down</td>
</tr>
<tr>
<td>Setpoint</td>
<td>75%</td>
<td>60%</td>
</tr>
<tr>
<td>P-Gain (feedback parameter)</td>
<td>3-5</td>
<td>10899</td>
</tr>
<tr>
<td>I-Gain (feedback parameter)</td>
<td>2-5</td>
<td>900</td>
</tr>
<tr>
<td>Cantilever</td>
<td>Bruker (RTESPA)</td>
<td>Nanosensors XYNCHR</td>
</tr>
<tr>
<td>Resonance frequency</td>
<td>285 kHz</td>
<td>321 kHz</td>
</tr>
<tr>
<td>Measurement environment</td>
<td>Air</td>
<td>Air</td>
</tr>
</tbody>
</table>

RESULTS

The results of one series of measurements (i.e. same material but samples exchanged between the labs.) are shown in FIGURE 4, The microstructure is as explained in FIGURE 1: consistently there are three phases visible in all images, one of them being the bee-phase.
FIGURE 4 Topography and phase images (30µm×30µm) of the four selected bitumen as measured by both laboratories. Images in the left column labeled (i) are imaged by LAB1, while the right column images, labeled (ii) are imaged by LAB2. a) 10/20 virgin bitumen, b) 10/20 recovered bitumen, c) 70/100 virgin bitumen and d) 70/100 recovered bitumen. In the upper right of the topography images z-scale (min-max) signifies the height in z-direction and on the phase images ϕ (min-max) is the phase lag of the tapping response.

As every laboratory imaged for each bitumen two samples, produced in different labs, first the influence of sample preparation on the imaging results was checked. No differences were observed other than those that could be expected because of local fluctuations of sample
properties (qualitatively). Hence, the influence of the sample preparation procedure on the results was minimized by the initial choices of sample preparation and treatment.

**Method of Quantitative Image Comparison**

The AFM images obtained by both laboratories were compared qualitatively and quantitatively. The qualitative comparison was used as a first check of the mutual consistency of the data retrieved. The processed data were then quantitatively analyzed by researchers of LAB1. In this way it was assured that all images were quantitatively interpreted in the same way. Although, as visible in FIGURE 1 and FIGURE 4, there are three distinct regions (phases) visible from the AFM phase images, it was decided to focus the analysis on the ‘bee-phase’ i) in FIGURE 1, because distinguishing the other two phases appeared to be cumbersome. The following parameters were obtained from the images for later comparison:

- Shape of the bees, i.e. in the approximation that the bees are elliptical: the long and short axes size distribution and particle aspect ratio;
- The total number of bee particles present in the scanned area;
- The surface area covered by the bee particles;
- The spatial distribution of the particles over the scanned surface, expressed by the pair correlation function \( g(r) \) for the bees [22].

**TABLE 2  Numerical details for microstructural comparison as obtained from image analysis of the AFM-images measured by LAB 1 and LAB 2 respectively (all measurements were done at 25 °C).**

<table>
<thead>
<tr>
<th>Bitumen type</th>
<th>Total Microstructures</th>
<th>Microstructure Size</th>
<th>Microstructural shape</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>count N</td>
<td>µm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scan size (30×30µm)</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>10/20</td>
<td>LAB 1</td>
<td>160</td>
<td>2.9</td>
</tr>
<tr>
<td>(virgin)</td>
<td>LAB 2</td>
<td>153</td>
<td>2.9</td>
</tr>
<tr>
<td>10/20</td>
<td>LAB 1</td>
<td>167</td>
<td>2.1</td>
</tr>
<tr>
<td>(recovered)</td>
<td>LAB 2</td>
<td>173</td>
<td>2.4</td>
</tr>
<tr>
<td>70/100</td>
<td>LAB 1</td>
<td>82</td>
<td>6.5</td>
</tr>
<tr>
<td>(virgin)</td>
<td>LAB 2</td>
<td>86</td>
<td>5.8</td>
</tr>
<tr>
<td>70/100</td>
<td>LAB 1</td>
<td>118</td>
<td>3.2</td>
</tr>
<tr>
<td>(recovered)</td>
<td>LAB 2</td>
<td>111</td>
<td>3.1</td>
</tr>
</tbody>
</table>
Microstructural Shape and Density Comparison

The comparative results for the measured shape and density (i.e. particle count and surface coverage) parameters are presented in FIGURE 5. Microstructural, shape and density data are very well comparable between the labs.

![Figure 5](image.png)

**FIGURE 5** Comparative results for the measured shape and density: a) number of microstructures, b) length of ellipsoidal long axes (standard deviation is stated at the top of each column) and c) aspect ratios. In d) it is shown how the long axis has been defined, and e) shows a typical distribution that leads to a column in b).

For clarity the same data have been summarized in TABLE2. Extra details about the distribution of microstructural parameters can be found by comparing the maximum and minimum of the long axes of the bees. Meanwhile the mean gives a measure for the distribution of bee dimensions.

Spatial Distribution of the Microstructure

The local and long range order in liquids and solids is most easily described by a function that accounts for the correlation between individual particles. This function is called the pair correlation function $g(r)$, which is related to the probability of finding the center of a particle a given distance from the center of another particle [22]. For short distances, this function quantifies how particles are packed together. For larger distances, the probability of finding two spheres with a given separation is essentially constant. In that case, it is related to the
density - a more dense system has more particles, thus it is more likely to find two of them at a given separation $r$. For highly ordered structures like crystalline materials $g(r)$ will have sharp peaks at multiples of the lattice vectors: the Fourier transform of the pair correlation function is the (static) structure factor $S(k)$ which is simply the pattern that is measured in any diffraction experiment.

The 2-dimensional version of the pair correlation function is considered as a tool to quantify the spatial distribution of an AFM microstructure, and it can serve as a basis for comparing two independently measured microstructures. An important property of $g(r)$ is its relation to the structure factor $S(k)$, here given for the 2-dimensional case [22]:

$$S(k) = 1 + n \int (g(r) - 1) \frac{\sin(kr)}{kr} r^2 dr,$$  \hspace{1cm} (1)

where $k$ is the wavenumber and $n$ is the average particle density. For small wave numbers $S(k)$ can be approximated by [22]:

$$\lim_{k \to 0} S(k) \approx e^{-k^2 R_G^2},$$  \hspace{1cm} (2)

with $R_G$ the Guinier radius of the microstructural features, which is another measure for the average microstructural size which is readily obtained from the pair distribution function. Moreover, whenever there is microstructural order, for example orientation correlations between the bees (cf. liquid crystals), this will become apparent from $g(r)$, while it may be difficult to observe this correlation visually.

For the microstructure of the 70/100 bitumen the density-density correlation function, which is proportional to the pair correlation function, has been calculated (FIGURE 6).

![FIGURE 6 Density-density correlation function for the 70/100 bitumen microstructure.](image)

The pair correlation looks very similar for both labs. The initial part of the graph relates to the correlations within a bee: it measures the average bee size. The results of both labs are very similar with respect to the initial part of the graph. The inter-particle correlation range
(larger $r$) shows weaker correlation than anticipated, though it is clear that there are inter-particle correlations present in the range of 3 to 6 $\mu$m.

**Thermal Stability of Microstructure**

Finally the microstructure was studied as a function of temperature. It is known that there are issues related to drift of the AFM stage when samples are heated. This problem is usually only important when the temperature is changing, once the desired temperature is reached the drift becomes negligible. Another issue is condensation on AFM components (and sample) while cooling. To prevent this, the thermal study was performed by heating the sample, subsequent 30 minutes equilibration.

**TABLE 3** Microstructural details for 70/100 bitumen as function of temperature

<table>
<thead>
<tr>
<th>70/100 Bitumen Temperatures</th>
<th>Total microstructures count N</th>
<th>Microstructure Size $\mu$m</th>
<th>Microstructural Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/100 Bitumen</td>
<td>Scan size $(15\times15\mu m)$</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>25 $^\circ$C</td>
<td>25</td>
<td>5.7</td>
<td>0.9</td>
</tr>
<tr>
<td>35 $^\circ$C</td>
<td>25</td>
<td>5.9</td>
<td>0.3</td>
</tr>
<tr>
<td>40 $^\circ$C</td>
<td>20</td>
<td>7.0</td>
<td>0.4</td>
</tr>
<tr>
<td>50 $^\circ$C</td>
<td>16</td>
<td>6.3</td>
<td>0.6</td>
</tr>
<tr>
<td>60 $^\circ$C</td>
<td>14</td>
<td>4.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Samples were prepared in two-fold in one lab, and then both laboratories imaged the samples at 25$^\circ$C, 35$^\circ$C, 40$^\circ$C, 50$^\circ$C and 60$^\circ$C. For one sample, the 70/100 bitumen, the microstructural parameters as function of temperature are presented in TABLE 3. In FIGURE 7 the phase fraction, i.e. the percentage of the surface that is covered by the bee-structures, is presented as function of temperature for the four materials that were considered in this study.
DISCUSSION

Various microstructural parameters have been compared based on the AFM images obtained by two separate laboratories on four types of bitumen. The sample preparation procedure was coordinated between the labs and the results did not depend on the place of sample preparation, hence the preparation procedures were at least sufficient. The influence of varying these parameters has not been part of this research.

The microstructures of the same sample measured at both labs were very consistent, within one standard deviation the results were in agreement. This is about the accuracy one can obtain in measuring two similar samples in the same lab. This can be seen by comparing the thermal stability data of 70/100 bitumen at 25°C with the data for the same material in TABLE 2.

A measure for the representativeness and stability of an AFM image is the comparison of the trace and retrace (forward and backward) images. These were very similar, hence the images were considered to represent the surface structure accurately. Further, AFM is a local probe, and may image local surface disturbances that do not represent the overall surface structure. Therefore, all measurements were done at two distinct scan sizes. Comparison of these scan sizes demonstrated that the 15 µm × 15 µm surface structures were still representing the global surface features well (although statistical fluctuations are of course larger at smaller imaging scales). Thus, for future studies 15 µm scan size suffices as far as a fair representation of the overall surface is concerned.

For the spatial ordering of the microstructure a method based on the correlation function formalism has been designed and programmed. There is overall good agreement in spatial distribution characteristics, but the correlations at larger distances \( r \) are weaker than expected. This may be due to the small scan-size (30 µm ×30 µm ) that was processed by a custom-made correlation function program (implemented in Matlab). For small images boundary effects play a prominent role. In a next version of the program these boundary
effects will be addressed. Nevertheless, there were clearly correlations on the 3-6 µm length scale, but orientation correlation was not found.

The thermal stability study had similar results as the earlier laboratory comparison: results were consistent between the labs, hence it may be concluded that the sample preparation and measurement protocols have been adequate. Some additional difficulties with imaging at higher temperatures such as thermal expansion, increased stickiness of the samples and increase of capillary forces were overcome independently by both labs.

The thermal study also allows for exploring structure-property relationships as presented in the introduction. Clearly, the microstructural shape parameters decrease with temperature for all samples, and it is also clear that the microstructure did not disappear up to temperatures as high as 60°C. The disappearance of microstructure with increase of temperature is (slightly) larger for the two recovered bitumen samples.

CONCLUSIONS
The following conclusions can be drawn from this comparative study on the microstructure of bituminous materials using atomic force microscopy:

1. The microstructure of bituminous materials are stable and representative, hence they are reproducible;

2. The microstructure of bitumen as obtained by 15 µm×15 µm scan size AFM images is representative for the surface as a whole, thus it represents a global rather than a local material property;

3. The conditioning of the AFM samples, i.e. thermal annealing procedure, sample storage and time between annealing and measurement, does influence the microstructural details observed by AFM; therefore these parameters should be controlled closely and always be reported together with AFM datasets that represent the microstructure of bituminous materials;

4. New methodologies for quantifying the microstructure (shape and spatial distribution) have been developed and they have proven to be successful;

5. The microstructures obtained by two laboratories were statistically similar (within one standard deviation for the selected structure representing parameters);

6. Virgin as well as chemically reclaimed bitumen (from an asphalt test beam) have a microstructure that gradually disappears with increase of temperature, though not completely up to 60 °C;

7. The disappearance of microstructure at elevated temperatures was more significant for the reclaimed materials, thus they may be considered less stable.
By specifying a standard measurement procedure for the microstructure determination of bituminous samples by AFM, an inter laboratory reproducibility of ±5%, was obtained. Thus, Atomic Force Microscopy is suited as tool for fast screening of bituminous materials. AFM will become an even more useful tool in engineering, when the relation between microstructure and mechanical, physical and chemical properties of bitumen has been established.

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