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Application of Atomic Force (AFM), Environmental Scanning Electron (ESEM) and Confocal Laser Scanning Microscopy (CLSM) in bitumen: A review of the ageing effect

Georgios Pipintakos [a], Navid Hasheminejad [a,*], Caitlin Lommaert [a], Anastassiya Bocharova [a] and Johan Blom [a]

[a] *University of Antwerp, EMIB research group, Groenenborgerlaan 171, Antwerp 2020, Belgium*

Abstract

Undoubtedly bitumen's viscoelastic performance has received much attention in the literature. Especially, the oxidative ageing phenomenon of bitumen has been studied by several scholars from different physicochemical and mechanical perspectives due to its direct impact on asphalt performance. The microstructural patterns observed with ageing utilising different microscopic techniques have not remained unexplored, and an increasing interest has been expressed to understand the bitumen's architecture by coupling it with different theories. This review aims to provide a useful guide for the road engineer by collecting all the existing microstructural trends that have been reported upon ageing by utilising some of the most promising microscopic techniques. The study demonstrates the changes being observed for the size of the so-called bee structures via Atomic Force Microscopy (AFM). The apparent fibril microstructure captured with Environmental Scanning Electron Microscopy (ESEM) consistently reported in the literature to become denser and coarser with ageing. The existing findings of Confocal Laser Scanning Microscopy (CLSM) revealed the conflicting observations that exist for the fluorescent centres of bitumen upon oxidation, concerning their size and number. Finally, this paper provides a comparative analysis of the three techniques for bitumen applications and recommends a systematic sample preparation protocol to move towards more consistent observations between the different research groups.

Keywords: ageing, bitumen, AFM, ESEM, CLSM

1. Introduction

Bitumen is a material derived from crude oil and acts predominantly as the binding medium in asphalt pavements due to its superior viscoelastic and water-proofing performance [1]. At the same time, bitumen exists as a natural deposit, with apparent differences existing in terms of composition between refined and naturally occurring bitumens [2]. Generally, bitumen consists of about 85% carbon, 10% hydrogen, heteroatoms in lesser amounts such as nitrogen (0-2%), oxygen (0-2%), sulfur (0-9%), and traces of metals such as vanadium, iron and nickel [3,4].

Bitumen undergoes alterations not only in the physicochemical [5–11] and mechanical properties [12–14] but also in microstructural patterns due to the effect of different processes occurring during its production and service life. Among other existing processes (physical hardening, volatilisation, condensation), the diffusion-reaction of oxygen with bitumen, known as oxidative ageing, is an irreversible phenomenon that has been studied thoroughly over the past years. A common practice to mimic and accelerate ageing of bitumen in the lab is to use standardised procedures to capture the oxidation taking place in the initial production, mixing, transportation and paving phase and during service life due to the environmental conditions. Usually, the rolling thin-film oven test (RTFOT) [15] is used to simulate the first phase known as short-term ageing and the pressurised ageing vessel (PAV) [16] to mimic the effect of ageing after paving in situ (long-term ageing). Doubts remain whether these simulations realistically anticipate the environmental conditions in situ due to the high temperature and increased pressure that are used [17].

Hitherto, a number of studies suggested that chemistry and microstructure are the key-elements to unwrap the association of ageing with the bitumen's performance [18–23]. Understanding of the fundamentals is considered crucial since ageing of bitumen is one of the major causes of distress types of asphalt in situ, such as ravelling and cracking [12,21,24,25]. Coupling of the possible interactions of polar compounds produced with ageing [6,8,26–29] and the microstructural changes, may assist in understanding their role in bitumen's performance. A rather simplistic but pragmatic approach is to characterise bitumen's structure based on solubility classes by utilising different solvents. This technique was introduced already in the 70s [30] and is known nowadays as the SARA fractionation due to the four main derived solubility-based categories namely Saturates, Aromatics, Resins and Asphaltenes. Different techniques have been proposed for this classification, which can also result in slight differences even for the same bituminous samples [31,32]. Many studies have highlighted that due to ageing bitumen exhibits a shift of these fractions from aromatics to resins and finally to asphaltenes, whereas saturates are considered in general unreactive and thus relatively constant employing their percentage value [20,33–36]. Moreover, asphaltenes are the largest and most aromatic constituents which account primarily for the overall bitumen's viscous behaviour [37,38], and correlations of asphaltene content with viscosity have been reported [18]. Resins are believed to contribute as a stabilising medium in bitumen, by means of asphaltenes micelles surrounded by them [37,39]. Controversy still exists in the bitumen community with regard to this colloidal model as researchers worldwide have questioned the validity of this theory counter-proposing different ones [40,41].

In parallel, microscopic applications have been considerably improved to such an extent able to gain deeper insights into the bitumen microstructure, not only in the surface but even in the bulk of bitumen upon ageing. However, obstacles still exist to understand completely what is captured on a microscale level which accounts for the bitumen's microstructure or morphology and its association with crystallising substances. For example, the lack of universal sample preparation, non-reporting of the sample's substrate nature, exact thermal history, storage time, and thickness as well as essential compositional characteristics such as the presence of crystallisable components have hampered the direct comparison of a variety of outputs published by different research groups. Difficulties in the comparison of different studies can also be generated as a result of long heating times and high temperatures during sample preparation, which can induce additional ageing. Other factors such as remaining water drops after samples' freezing, dissolved samples, insufficiently flat surfaces of the bituminous films and destruction of the sample due to the use of a highly energetic electron beam can introduce additional artefacts.

2. Objectives and outline

Since it became obvious during the last decades how microscopic techniques can assist in understanding the microstructural patterns in bitumen, the need to highlight the most important techniques and accompanying changes in microstructure upon ageing is of utmost importance. One able to dive into the microstructure of bitumen choosing the correct tool can afterwards consult towards a systematic modification (addition of polymers, rejuvenators, nanoparticles or other agents [42]). Furthermore, as bitumen is a non-renewable organic material, a disciplined usage is required against its depletion which is stimulated additionally due to ageing. It is believed that by providing this overview, potential policy-makers will be able to make the correct decisions, knowing the material by its basics.

Hence, this review paper provides the framework of the three most promising microscopic applications to investigate surface microstructural traits upon ageing in bitumen. To that end, the working principles of the atomic force microscopy (AFM), environmental scanning electron microscopy (ESEM) and confocal laser scanning microscopy (CLSM) are presented herein so that the potential reader, especially the pavement engineer, can become familiar, as there is an increasing demand for them. The paper aims to gather all the reported ageing-related microstructural observations reported until now for these three techniques. It provides a comparison between them and makes recommendations for sample preparation instructions.

The review is outlined in the following way: first, the theoretical background of the existing microstructural theories is briefly revisited in subsection 3.1. The reader then is invited in subsection 3.2. to strengthen the knowledge concerning the wax presence in bitumen, which is believed to have an association with the microscopic observations. Section 4 is divided per microscopic technique for which the general working principles to perform a measurement are provided, followed by the reported bitumen–related applications. The remainder of each subsection is devoted to the ageing-induced microstructural changes that have been observed utilising the different microscopies. Next, section 5 provides an overview of the limitations and advantages of each microscopy when it comes to identifying ageing-related microstructural changes and attempts to propose a comprehensive protocol to increase reproducibility and repeatability between different research groups. The remainder of the paper (Section 6) provides the main conclusions and recommendations that can be drawn from this review for capturing the ageing effect in bitumen's microstructure.

3. Theoretical background

To gain a better understanding of the main findings by the use of microscopies in bitumen and the effort to link them with possible microstructural models the governing theories and the effect of wax regarding these observations are briefly reviewed.

3.1. Microstructural theories

Already in the dawn of the 20th century, a colloidal structure for bitumen was proposed [43], with more detailed descriptions of this assumption documented by Nellensteyn a decade later [44]. The latter study supported that asphaltenes constitute a colloidal suspension together with the maltenes, which consists of aromatics, resins and saturates, while resins play a stabilising role in bitumen [45]. This idea was developed further based on the association with the rheological response which was believed to vary between sol bitumens (Newtonian behavior) and non-linear gel ones (non-Newtonian behavior), while most of the bitumens behaved in a situation between the two cases, known as sol-gel [46,47]. The difference between the two extrema was attributed to the interconnectivity of the asphaltenes micelles in the gel phase, whereas the full dispersion and successively lack of interaction of the asphaltenes micelles was responsible for a sol type of bitumen's structure. The intermediate situation implied the co-existence of the two [46]. Relationships between the different colloid types in bitumen and the ratio of asphaltenes and saturates content over the sum of resins and aromatics were established, to characterise bitumen's colloidal stability [48]. It is widely accepted nowadays that the gel character is apparent for ratios greater than 1.2 and the sol one for values smaller than 0.7 [48,49].

The main opponents of the colloidal model argued already in the 90s that bitumen is a purely homogeneous fluid known as dispersed polar fluid, which gave its name in this theory concerning the intrinsic architecture of bitumen [40,41,50]. The main argumentation in disregarding the colloidal theory was generated around the lack of an elastic plateau for the gel bitumens [37] and the lack of a thermodynamic basis of separation of bitumen in different phases. More specifically, a hypothesised phase separation would reduce the system's entropy and would require an enthalpy compensation which is contrary to the micelle theory where asphaltenes are considered to be gathered. In the homogeneous fluid theory, the bitumen molecules are considered to be in a mutual solution including a range of solubility parameters in such a way that everything is kept soluble. Additionally, they believed that the monotonic time dependence and the unimodal relaxation spectrum with regard to the viscoelastic response of bitumen were the basis to support such a homogeneous theory.

The most recent colloid theories, known as the Yen-Mullins model, support that asphaltenes play a dominant role as 'island' structures in the molecular architecture of crude oils and therefore also of bitumen [51,52]. Asphaltenes are believed to appear in 'islands' which in sufficient concentration can form nanoaggregates and in higher concentration clusters [52,53]. Moreover, the role of resins as stabilising medium of asphaltene nanoaggregates is no longer acceptable. Validation of disk-shaped core-shell structures for the asphaltene nanoaggregates has also been provided experimentally with advanced techniques of Small-Angle Neutron and X-Ray Scattering [54] as well as with Nuclear Magnetic Resonance studies [55].

3.2. Waxes in bitumen

Bitumen, apart from the reported solubility-based SARA fractions, can include another specific fraction which is called wax. Petroleum wax can appear in the type of paraffinic or microcrystalline wax [56–58]. The former refers to the linear n-alkanes with a few or no branches, whereas the latter to the aliphatic hydrocarbons with a significant amount of iso- and cycloparaffins [4]. The paraffinic wax is usually crystallised in flat plates and is known as macrocrystalline wax. Microcrystalline wax, on the other hand, crystallises in small needles.

Microscopic studies have also revealed different geometries for the formed crystals of apparent size 1-10 µm [59,60]. Among the factors that affect the crystallisation of the wax in bitumen, as well as the shape and size of crystals, are the thermal history, cooling conditions and rate, as well as the storage time of the samples.

In practice, the Differential Scanning Calorimetry (DSC) is used as an effective tool to detect the wax content in bitumen based on the occurrence of melting signals and derived melting enthalpies [61]. From various DSC studies, it has been measured that paraffinic waxes in waxy bitumens account for about 5% wt or less and their effect on rheology has been identified mainly to the low-temperature physical hardening [62]. In some bituminous binders, the effect of waxes on rheology has also been seen as a loss in time-temperature superposition in the melting range of the wax [63].

Wax has also been reported to display interrelationships with microscopic findings on the bitumen surface. A previous study supported the hypothesis that crystalline structure will move towards the surface in order to reach an equilibrium with the wax molecules still in solution in the bitumen matrix [26]. In parallel, it was believed that ageing creates a thin film of less soluble hydrocarbon molecules at the surface of a bitumen film, preventing the microstructures from floating to the surface.

4. Microscopic techniques

4.1. AFM

4.1.1. Working principles

Imaging with the AFM microscope has evolved over the past years but the general working principles remain the same, rooted back in its invention in 1986 [64]. More specifically, the imaging of the target uses a cantilever, with a sharp tip (colloidal probe) that is used to scan the sample surface by interacting with it. When the cantilever experiences a force between the tip and the sample, measured by a laser beam that is placed at the end of the cantilever, it deflects and shifts up according to the Hooke's law. This results in a deviation of the laser beam from its original position, which is measured as a voltage. This voltage is translated into a variety of forces (e.g. mechanical contact force, capillary forces etc.) or relative height and an image can be made based on all the measured differences [64,65].

There are different operational modes of an AFM, but among others (near-contact, pulsed force, lateral force mode) the most common ones are discussed here, namely the dynamic (tapping) and the static (contact) mode [22]. With contact mode, one refers to the mode where the tip stays in contact with the sample at constant load, while the surface is moving in the plane directions. This mode is able to generate both frictional and topographic data. Tapping mode, on the other hand, occurs when the cantilever, oscillating up and down at its resonance frequency, is gently tapped on the surface to reach contact and then in the plane directions when the tip is lifted away from contact. This mode provides phase and topographic data and generally reduces the chance to destroy the surface compared to the static mode where the developing forces are higher [65].

4.1.2. Bitumen application

The microstructure of bitumen is still a subject that is not fully understood and completed. Over the years, there were a lot of attempts to examine the microstructure of bitumen, with the most commonly used technique being the optical microscope. AFM proved to be a promising alternative and was adopted by bitumen researchers soon after its invention [66]. The results give information about the topography and phase contrast of the sample and are therefore especially useful for multi-phase materials such as bitumen. Using AFM, experts aimed to characterize the topography, stiffness, tackiness and molecular interaction at the micro-level of bitumen [67]. In parallel, mechanical properties of bitumen such as adhesion, rigidity, hardness and modulus of elasticity can be estimated employing AFM with the nanoindentation technique [68]. This technique penetrates the bitumen surface with a tip of defined geometry [69].

The fact that AFM is time-efficient and experiments can be performed at different temperatures, even at room temperature, makes its use favorable. However, it was proven that the handling temperature has a significant impact on the final results, and as such for a fair comparison between AFM findings by different research groups, the thermal history, among other factors, during the sample preparation must remain the same. Some limitations of AFM bitumen application are that it delivers surface images, possibly not depicting the microstructure in the bulk of bitumen [70] and that the surface of the prepared sample should be relatively smooth and of sufficient thickness to exclude surface-driven effects [71,72]. Moreover, tacky and liquid samples cannot be measured with an AFM in the tapping mode.

Another appealing reason for the AFM application in bitumen is the relatively simple sample preparation. Attention should be though given in the consistency of the preparation procedures as it may introduce differences if the handling procedure and storage conditions vary significantly between the samples. In general, sample preparation can be done by two different techniques for bituminous binders, either the spin or the heat casting method. Spin casting includes a spinning plate, where the sample is cast on. The centrifugal forces of the plate provoke the sample to spread evenly so that it eventually results in a thin film for the imaging. Beforehand, the bitumen is dissolved in a solvent which evaporates after the spinning. Allen and his coworkers suggested the elimination of the residual solvent to preserve the sample in an airtight heated vacuum desiccator with a purification step with dry nitrogen after [73].

For the heat casting method, the bitumen is heated and stirred at a temperature T=110-130 ^{o}C, according to the bitumen type to be workable, and profoundly mixed so that a specimen has no more oxidants or dust compared to a replica of the same bitumen tank [74]. Next, a bitumen drop of 15-30 mg is placed on a conductive sample holder and held horizontally on a heating plate (set at the same temperature with the heating T of bitumen) so it would spread evenly and become a flat surface. The samples are normally tested directly in the AFM and then held in a dust-free environment before testing again at different time intervals so that the microstructures can settle and their evolution can appear on the bitumen's surface.

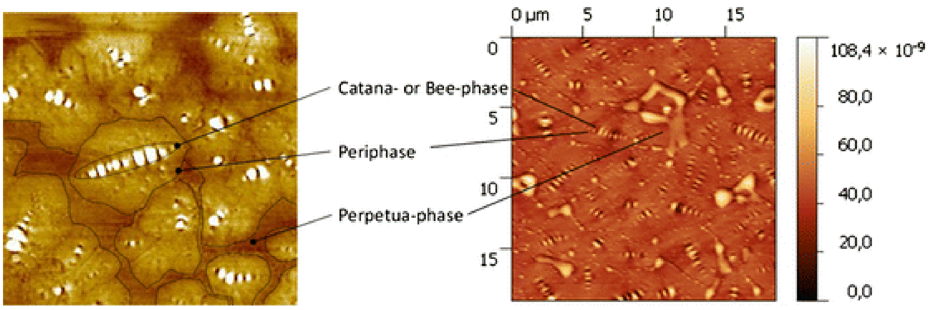

Figure 1: Typical phases of surface bitumen microstructure before [left] and after ageing [right] in phase contrast and topography respectively [74].

Concerning the observed AFM microstructure in bitumen is generally accepted that rippled, wavy-like structures exist on the bitumen surface, widely known as 'bees' due to the yellow and black color with which they were first represented by Loeber and his colleagues [66]. Typically, 3 to 4 phases can be identified with AFM imaging: the catanaphase (bee-structure), the periphase (around catanaphase), and the perpetua phase which can be distinguished in the paraphase (solvent regions) and the salphase (high phase contrast spots) [74,75]. A simplified example of this phase detection is given in the example Figure 1 before and after ageing. Whether the bee-shape structures have an association with the bitumen fractions was a matter of debate for many years, with lots of studies to believe that asphaltenes are responsible for the bees as they appear as a result of this fraction [76,77]. The opponents of this claimed that the paraffinic wax crystals present in bitumen are the reason behind the bee-structures by providing support via DSC measurements and the addition of waxes to a wax-free bitumen [74,78,79], while other scholars also supported this theory with the wrinkling of the surface [80–82]. The wax crystallisation seems to gain support from the most recent studies as the main reason behind the bee structure formation [83,84].

4.1.3. Ageing-induced microstructure

Since the main goal of this review is to use the microscopes as a tool to trace ageing-related microstructures, a summary of past AFM works reporting mainly the consequence of ageing on the bee-structure as well as on other air-cooled surface-related properties, such as phase stiffness, are presented in Table 1.

It becomes obvious from most of the past works that ageing affects the bee-like structures, whereas limited studies supported that ageing has no effect on the bee microstructure [85]. Yet controversy exists whether prolonged and more severe ageing increases [68,86–90], decreases [91,92] or fluctuates [73,93,94] the length and area of the bee-structures. Part of the studies having investigated ageing-related AFM microstructures argue that the bee structures are attributed to asphaltenes [87,89,93,95] while others associated them with the waxy molecules that bitumen may contain [26,86,92,93], which is also the governing theory nowadays. It is worth mentioning here that relationships of the microstructural changes with the SARA fractions have also been reported as a result of ageing [85,93,96]. Moreover, a number of studies [68,73,85,89–91,95–97] found changes with ageing in the surface roughness and stiffness, adhesive and cohesive strength and force, with most of them claiming an increase of these properties, while the rigidity of bitumen was related to the bitumen morphology and its components.

Table 1: Summary of previous works with regard to AFM bitumen observations with ageing.

Research work	Bitumen information	Ageing treatment	AFM mode	Testing temperature	Key conclusions
[93]	A virgin binder 60/80, aged and rejuvenated with different rejuvenator percentages	Thin-film oven test (TFOT) at 163ºC for 3, 12 and 15 hours	Tapping mode	Room temperature	▪ The bee phase is coated by a thick layer which is getting thinner with ageing and this is related to the changes of maltenes ▪ Bee structures are attributed both to asphaltenes and wax crystallisation ▪ The microstructure varies at diverse ageing stages ▪ Rejuvenation does not affect the microstructure (size, quantity of bee-structures) compared to the aged samples
[86]	Two styrene-butadiene-styrene (SBS) commercial bitumens from different plants of penetration grade around 50, modified with waste engine oil (WEO)	Standard RTFOT, PAV and 2 x PAV	Tapping mode	Not specified, prior cooling with nitrogen gas was used for 6 hours	▪ The bee structure proportion and length increased with ageing as a possible result of volatilisation and polymerisation ▪ WEO alters the microstructure of bitumen vanishing the bee-structure.
[91]	Three bitumens with penetration grade of 50, 70 and 90	Self-developed ageing equipment including UV at 65 ºC at different time intervals up to 720 hours	Peak force mode	Room temperature	▪ Two parts were identified in aged samples: the valley areas and the aged film areas ▪ Ageing reduced the surface roughness of the valley areas of the bitumen ▪ Ageing decreases the area and length of the bee-structure appeared in bitumen with penetration around 50 ▪ The maximum adhesion force in the valley areas was 2-3 times greater than in aged film areas

					▪ Hardness in the aged film area was 2-4 times greater and modulus measured by AFM was 1-2 times greater than in the valley areas
[87]	One base bitumen with penetration grade around 65 and its SBS modified bitumens with penetration grade around 60 and 55	RTFOT, PAV at 60 ºC at different time intervals up to 2000 hours	Static mode	Room temperature	▪ The unmodified bitumen appeared bigger tubers with ageing compared to the aged SBS-modified bitumen in 3D AFM images ▪ The size of the bee-structure was increased with ageing for the conventional bitumen and appeared for the SBS only after PAV. ▪ The bee-structures were attributed to asphaltene micelles
[88]	One conventional bitumen with penetration grade around 90	TFOT at 163ºC up to 20 hours	Tapping mode	Room temperature	▪ The height of the bee-structure is fluctuating with ageing whereas the areas away from the 'bees' keep relatively stable height with ageing ▪ The bee-structure aggregate and become bigger with ageing (area) ▪ The roughness and maximum amplitude gradually increase with ageing ▪ There is an negative relationship with the increase of microstructural and technical indexes i.e. increasing area of bee-structure with decreasing penetration
[97]	Three bitumens: one commercial of penetration grade 60/70, one recovered after 7 years in situ with original penetration 60/70 and a recovered after 36 years with original penetration 80/100	For the non-recovered: standard RTFOT and PAV	Tapping and contact mode	Controlled environment (24ºC, 71% humidity)	▪ Ageing increases the adhesive and cohesive strength of the samples ▪ Ageing increases the spatial variations of the nanoscale sample properties i.e. the plasticity index during the indentation process
[96]	Two bitumens with penetration grade of 63 and 87	Standard RTFOT and PAV, UV aged at 80 ºC after TFOT	Tapping mode	Room temperature	▪ The overall surface stiffness increased with ageing ▪ PAV ageing effect was bitumen-dependent with regard to the single phase trend in contrast to TFOT which contributed clearly to a single-phase trend for both bitumens ▪ UV radiation resulted in a contrasted matrix and dispersed phase (phase separation) ▪ Relationships between microstructural changes and SARA compositions were found
[92]	One unmodified and one modified with organo-montmorillonite (OMMT) bitumen of penetration grade 63	TFOT followed by UV aged at 80 ºC	Tapping mode	Room temperature	▪ Correlations were found between the waxy molecules of the separated asphaltene fraction and the bee-structure of the unmodified bitumen

					▪ The dimension and amount of bee-structures were reduced upon ageing as the result of crystallisation of waxes ▪ The contrast between the matrix and dispersed phase was reduced with ageing ▪ These changes were prevented in the OMMT-modified bitumen, demonstrating a bitumen ageing enhancement
[26]	A wax-including 70/100 bitumen (5,4% wax)	UV aged, unaged samples conditioned in argon environment for 15 and 30 days at 20ºC	Tapping mode	Room temperature	▪ Water soluble thin films are formed with ageing ▪ Ageing creates a thin surface film acting as a barrier for the microstructures to freely float on it, thus under UV and oxygen the wax-induced microstructures decrease until they disappeared ▪ Through washing of the aged surface, microstructures reappeared
[89]	Conventional bitumen 50/70	Standard RTFOT and PAV	Tapping and contact mode	Room temperature	▪ Ageing increases the average size of the asphaltene micelles which are in sol state in the unaged sample ▪ After PAV these micelles become interconnected in a network with open voids covered by the hydrocarbon matrix ▪ The hydrocarbon matrix has higher loss modulus than the asphaltene micelles without ageing while the opposite trend holds upon ageing ▪ The friction coefficient reduced in half after RTFOT and remained unchanged with PAV ▪ Ageing increased the stiffness of the bitumen film and its apparent viscosity
[85]	Conventional bitumen PG64-22 and field aged for 6 months after RTFOT	Standard RTFOT and PAV	Contact mode	Room temperature	▪ AFM-derived adhesion and stiffness increased with ageing ▪ Bee-structures were not considered to appear due to ageing ▪ Bee-structures were correlated with the aromatic fraction ▪ Adhesion and rigidity were related with the bitumen components ▪ The catana-phase is associated with the stiffness and adhesion
[68]	Two conventional bitumens PG64	Standard RTFOT and PAV	Contact and pulsed force mode	Room temperature	▪ Adhesion and stiffness increased with ageing ▪ Ageing increased the length of catana-phase ▪ AFM modulus of elasticity was increasing with ageing
[95]	Four conventional bitumens with penetration grades 30, 50, 70 and 90	Standard RTFOT and PAV	Tapping mode	Room temperature	▪ AFM-derived cohesion and adhesion force increased with RTFOT ageing

					▪ The adhesion force decreases with PAV whereas the cohesion keeps increasing with PAV as a result of the asphaltene association ▪ The ageing-induced reduction in adhesion force was found in the catana, peri and para phase
[73]	Three SHRP bitumens of different performance and crude oil [3]	Standard RTFOT and PAV	Force measurement mode	Room temperature	▪ Prior to ageing two phases are detected a continuous and a dispersed one ▪ Long-term ageing introduces microstructural changes (phase dispersion, phase clustering and materialisation) which are bitumen-dependent ▪ A third phase in long term ageing can be observed; the bee-structure ▪ Ageing increases the stiffness of both the continuous and dispersed phase
[94]	One virgin bitumen, one blended virgin - extracted from reclaimed asphalt bitumen (50/50), one extracted bitumen from reclaimed asphalt pavement (RAP) and two extracted from laboratory aged mixtures	Extracted after ageing the mixture with short-term oven ageing (4 hours at 135ºC) and long-term oven ageing (5 days at 85ºC) [98]	Tapping mode	Room temperature	▪ The extracted recycled and aged binders showed no bee-structures compared to the virgin and RAP binders ▪ The bee-structures were different in height and size
[90]	One SBS-modified bitumen with penetration grade 54	Standard RTFOT and PAV	Tapping mode	Room temperature	▪ The overall roughness increased with ageing possibly as a result of polymer degradation ▪ Longer structures occur with ageing ▪ PAV ageing increases the number of microstructures

4.2. ESEM

4.2.1. Working principles

Another promising microscopic technique, invented in the mid-80s, is the environmental scanning electron microscopy (ESEM) [99]. The advantage of this microscopy versus traditional scanning electron microscopy (SEM) is the choice of sample environment by means of temperature, pressure and gas flow, while simultaneously the necessity of high vacuum conditions is overcome [100]. It is even possible to investigate samples containing volatile components without using any additional conductive coating. Additionally, the examined sample can be tested in different states (wet, oily) and environments with gas flow and pressure up to 50 torr and temperature up to 1500 ºC [101,102].

The working principles and manufacturing are based on the classic SEM, however, the target pressures in the electron beam tube are ensured in ESEM by isolation valves and differential pumping. Moreover, in ESEM multiple Pressure Limiting Apertures are used and the sample chamber is separated from the column which is maintained in high vacuum to preserve the electron beam from scattering [103]. A gaseous environment is used around the sample, whereas the electron gun is maintained at standard pressures around 10^{-6} torr [102]. An environmental secondary detector (ESD) is used to monitor the variety of signals resulting from the beam-sample interaction which has the capability of performing also in a non-vacuum environment. The ESD collects the emitted secondary electron signal which is gathered to an electron amplifier. Finally, the output signal can be constructed to a virtual image of the sample surface.

4.2.2. Bitumen application

Soon it became obvious that the advantages of ESEM in bitumen samples were numerous compared to SEM, as the sample could be tested in its natural state without de-oiling or conductive coating. As such, the benefits of the handy sample preparation allow a greater number of possibilities to be captured with ESEM under sample freezing, heating and other effects [104]. ESEM allows the microstructural analysis of the fracture, the distribution of possible containing particles, the determination of the adhesive properties, of the healing effect and others [103]. The most apparent ESEM bituminous microstructures, as a result of the subjection to the electron beam, look like fibrils. The density, size and shape, as well as the required time for the fibrils to appear and stabilise, have been studied with ESEM greatly the last decade [105,106]. An example of the fibril microstructure in ESEM is illustrated in Figure 2.

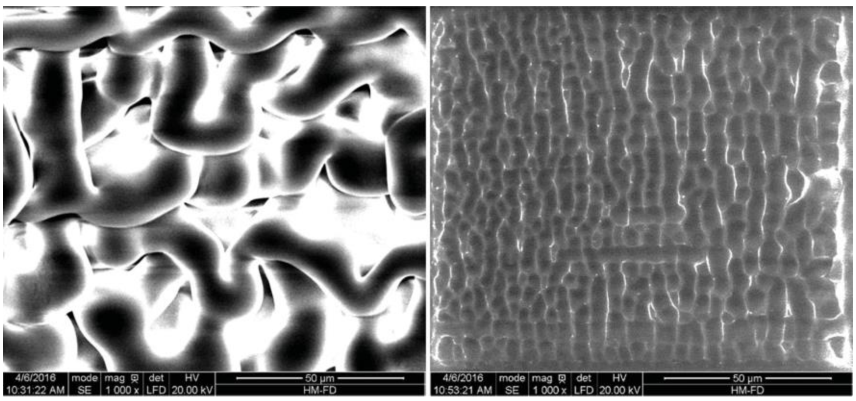

Figure 2: ESEM fibril microstructure of an unaged bitumen [left] and upon oxidation [right] [104].

Rozeveld and his coworkers found that the alignment of the fibrils with the direction of tensile stresses subjected to a bitumen sample is clearly due to the bitumen microstructure and not the electron beam [107]. This was understood as the volatilisation of the lighter molecules by localised heating of the sample by the electron beam. Based on this hypothesis, the apparent ESEM bitumen fibrils were initially interpreted by the scientific community to have an association with asphaltenes [107], the heaviest bitumen SARA fraction, while later it was assumed that it can also be assigned to a part of the maltene fraction [108]. In the dissertation of Gaskin, it was reported that the radiolysis and not the localised heating is most probably the reason for the fibril microstructure, taking place in two steps: precipitation of paraffinic wax and diffusion of aromatics [108].

For the sample preparation itself, a kind of heat-casting method has been developed in [104] and was adopted in [34,109] where the bituminous samples are placed prior in covered containers about 1 hour at 110 °C, then a quantity of 0.1 grams was placed with a spatula in a cylindrical sample holder of a diameter of 8 mm and height of 2 mm. Then the sample holder is placed on a heating plate for 10-30 seconds (depending on the ageing state) at 150 °C to ensure flattening of the surface. The prepared samples are suggested to be stored for 24 hours at 7-8 °C before the ESEM analysis.

4.2.3. Ageing-induced microstructure

Until now ESEM observations for the changes in bitumen microstructure upon ageing are rather limited compared to the wider use of AFM. Nevertheless, this review suggests that even these few works, provided in Table 2, report a consistent evolution of the captured fibril network with oxidation. More specifically, the studies so far support that the fibril network formation time is increasing with ageing [104,105,108–112], whereas it was observed that this three-dimensional network is becoming also denser and coarser [104–107,109–111]. Processing of the ESEM images in aged bituminous samples also showed that the fibril size (area, diameter, length) varies differently based on the examined bitumen which can either increase [106,107,112] or decrease [104,105,108–111]. More extensive research is needed in the near future for the effect of ageing in modified bitumens with thermosets or crumb rubber [113]. Finally, these studies reported the association of the heavier SARA fractions with the fibril ESEM microstructure [106,108,112].

Table 2: Summary of previous works with regard to ESEM bitumen observations with ageing.

Research work	Bitumen information	Ageing treatment	ESEM conditions	Testing temperature	Key conclusions
[107]	Three modified binders : SBS, styrene-ethylene-butylene-styrene (SEBS) and styrene-butylene-rubber (SBR)	TFOT at 163 °C up to 5 hours	Chamber pressure: 2.0 torr water vapor, 20keV acceleration voltage, 250x magnification	25 °C	▪ A three-dimensional entangled network was observed in both the unaged and aged bituminous films after several minutes in beam exposure ▪ The fibril network became coarser with ageing ▪ The fibril diameter increased with ageing
[109–111]	Four bitumens of different crude sources of similar penetration grade and softening point.	RTFOT at 123 and 163 °C followed by standard PAV	Chamber pressure: 0.8 mbar in low vacuum, 20keV acceleration voltage, 1000x magnification	Room temperature	▪ RTFOT at 123 °C did not provoke any fibril evolution, while at 163 °C caused a denser structure ▪ The fibril microstructure became highly denser with PAV ▪ The fibril diameter was getting smaller with PAV for the three bitumens ▪ The fibril formation time with ESEM irradiation increased with ageing ▪ The fibril density increased with RTFOT ▪ The fibril area and formation time correlated well with changes in physical properties
[106]	One conventional and one SBS modified bitumen	Standard RTFOT and PAV	Chamber pressure: 0.6 torr, 20keV acceleration voltage	Not specified	▪ The random fibril (string-like) network was assigned to the heavier molecular SARA fractions of resins and asphaltenes ▪ The fibril network structures became coarser with increasing ageing

					▪ The fibril diameter increased by 50% with RTFOT and decreased significantly with PAV ▪ The packing density (the distance of adjacent fibrils) increased with the ageing severity
[112]	Two modified bitumens: one SBS and one SEBS	TFOT followed by standard PAV	Water vapor atmosphere of 1 to 20 torr	Room temperature	▪ A highly entangled three-dimensional network was observed for all the bitumens ▪ The network structure was hypothesized to appear due to evaporation of the lighter molecular oil phase ▪ The aged bitumen appeared a less defined and entangled network ▪ The network formation time was 5-10 times longer in the aged sample ▪ The network chains (17 to 20 μm) was slightly higher in the aged samples
[113]	Crumb rubber modified asphalt (CRMA) binder (10% crumb rubber w/w)	Standard RTFOT and PAV	Chamber pressure: 40 Pa 5-10keV acceleration voltage, up to 2000x magnification	Room temperature	▪ Unaged CRMA binder presented a single-phase continuous non-uniform structure ▪ The aged CRMA binder appeared two phases; a broad dark region and a lighter one attributed to the rubber-bitumen interaction
[108]	Two bitumens with different asphaltene fraction percentages	Standard RTFOT and PAV	Chamber pressure: 0.6-1.4 torr water vapor, 5 to 10keV acceleration voltage, high magnification	40 ºC	▪ Ageing decreased the fibril diameter of the low asphaltene binder ▪ The fibril structure was considered a combination of the lighter maltenes and asphaltene SARA fractions ▪ The network formation appeared more quickly in higher testing temperatures
[105]	Bitumen PG 58-28	Oxidised with lab-scale air bower at 260ºC for 5 hours	Chamber pressure: 0.8 mbar in low vacuum,, 20keV acceleration voltage, 1000x magnification	Various testing temperatures	▪ Ageing doubled the network formation time compared to the unaged bitumen ▪ The fibrils decreased in size and increased in number with ageing, resulting in a tighter and organized microstructure ▪ Oxidised bitumen, stored priorly at 7 ºC for 14 days, appeared an intermediate micelle-like structure associated with the AFM 'bee' structures
[104]	Bitumen PG 58-28, and three blends of 25, 50 and 75% of the same bitumen oxidised with PG 58-28 as base bitumen	Oxidised with lab-scale air bower at 260ºC for 5 hours	Chamber pressure: 0.8 mbar in low vacuum,, 20keV acceleration voltage, 1000x magnification	Room temperature	▪ Ageing increased the fibril network formation time ▪ The structure was more dense and stable, with more fibril interconnections in terms of number upon ageing ▪ The average fibril diameter decreased with oxidation

4.3. CLSM

4.3.1. Working principles

The confocal laser scanning microscopy (CLSM) has become a popular method in biology, biomedical, and material sciences in the past few decades [114,115]. The basic concept of confocal microscopy was first developed and patented by Marvin Minsky in 1957 to image the neural networks in unstrained preparations of brain tissues [116]. Unlike conventional optical microscopes, confocal microscopes focus the light on a specific depth and eliminate any information away from the focal plane, using a spatial pinhole in front of a detector. It was during the 70s and 80s that the advances in computer and laser technology led to the development of the CLSM [117]. In CSLM, the light source, which is a laser beam, based on depth selectivity allows for optical sectioning. The information gained from this focal point is projected on a pinhole in front of the detector, which ensures that only the light from the small area of the sample, which is irradiated, is detected [118]. Apart from the reflectance or transmission mode, CLSM can also operate in fluorescence mode. This is done by using a different laser light source with a lower wavelength. In fluorescence mode, the molecules absorb the high energy (short wavelength) light and after a short lag period (fluorescence lifetime) emit a lower energy (longer wavelength) light.

Comparison of images taken by conventional optical microscopy and CLSM in reflected light mode has indicated the higher resolution and thus the better quality of the images obtained with CLSM [119]. Finally, in a CLSM, the image is created by scanning the surface point by point. If this is done in the x-y plane for different depths in z-direction, the 2D-images can be reconstructed to a 3D-representation, which is the biggest advantage of CLSM over other microscopy techniques [119].

4.3.2. Bitumen application

CLSM is a relatively new technique to investigate the microstructure of bitumen. This technique has been used both in reflectance and fluorescence mode on bitumen. Next to its capability of creating 3D-reconstructions, using CLSM to investigate bitumen requires little pre-treatment. This is in favor of the obtained results since changes in temperature or chemical composition may cause shifts in the molecular structure of the sample, which could lead to differences in the observed microstructures [120].

To prepare the bitumen samples, the general process used by researchers is as follows: the bitumen is heated between 120-160°C, then a small drop is placed on the microscope slide. A coverslip is placed onto the drop, and the sample is heated further until the top slide can be pressed against the bottom slide. The second heating period causes the drop to form a very thin film. Then the sample is kept at room temperature and atmospheric pressure so that it can cool down before placing it under the microscope [120]. The comparison of obtained images, between tests on a thin sample squeezed between two slides and a thick sample in a metal cup covered with glass, can be found in [59]. In this research, it was observed that the sizes of the crystals are larger for the thicker samples. Therefore, it was concluded that the thicker samples in metal cups are more representative of bitumen in bulk, and this procedure was recommended for observation of the wax morphology using CLSM.

For observations on epoxy asphalt rubbers, a different sample preparation procedure has been used. In this case, the sample is dissolved in a solvent, and the solution is spin-coated onto a microscopic slide with a speed of 3000 rpm for 1 min. Afterwards, the slide is heated at around 110-115°C for 3 min to evaporate the solvent, and a cover slide is placed on top [121].

CLSM in reflected mode has been used by researchers to determine the size, distribution, and shape of the asphaltene particles [119] or classification of wax morphology [59]. Furthermore, in the last few years, researchers started to use this technique to observe fluorescence centers in bitumen. It was demonstrated that bitumen exhibits fluorescence when irradiated with 488 nm wavelength light [122]. Therefore, to investigate bitumen using CLSM in fluorescence mode, the samples are typically irradiated with 488 nm wavelength laser light, while observation of emitted signals is mostly done in the 500-550 nm wavelength range [120,123,124].

The origin of fluorescence centers in bitumen has been the subject of debate among researchers, and the conclusions over the nature of these fluorescent centers were somewhat contradictory in different articles [119,120,125]. Li & Wan found that asphaltene gives strong fluorescence when studied by CLSM. They allocate this observation to the highly conjugated aromatic structures that are a major part of asphaltene composition. It should be noted that in this study, they have separated the asphaltene fraction from the original bitumen sample by dissolving the bitumen sample in n-heptane. This precipitation process could influence the structure of the molecules, hence it is a distinct possibility that their results have been compromised [119].

A study of fluorescence signals in bitumen was also conducted by Bearsley and his group. In this study, the bee-like structures, observed with AFM, and the fluorescent particles, observed with CLSM, were both of the same dimensions. The geometrical similarities cause a presumption of the latter being asphaltenes. To verify this hypothesis, separation of asphaltene and maltene fractions was done according to ASTM D4124-01 (2002). When both major phases were evaluated using CLSM, asphaltenes seemed to exhibit a uniform fluorescence signal. For this reason, they concluded that asphaltene is in fact, the cause of the fluorescence signals in bitumen [120].

However, Handle and his peers showed that the aromatic mantle, serving as a stabilizing agent around the micelle, is responsible for the high-intensity fluorescent emissions in the visible range and not the asphaltenes themselves. To prove their hypothesis that asphaltenes are incapable of producing fluorescence and the fluorescence signals come from the maltene phase, they studied the different bitumen fractions separately. They observed that asphaltenes emit barely any fluorescent signals, and maltenes, on the other hand, produce very strong signals. By fractioning the maltene phase, they found the aromatics to be the most fluorescent fraction. However, they did not neglect the similarities with the bee-like structures by comparing their conclusions on the source of fluorescence and the micelle theory. Interestingly, they stated that the fluorescence signals are still derived from the asphaltene micelles, however not from the inner core, as presumed previously, but from a surrounding mantle of aromatics [125–127].

Other than using CLSM to investigate virgin bitumen, this technique was also used to study the morphology of polymer modified bitumen [107,128,129] and recycled asphalt shingle blended with asphalt binder [123]. Moreover, CLSM has been widely used to explore the morphology of epoxy asphalt binder [130], phase-separated microstructure and dispersion of the asphalt rubber in epoxy asphalt [121,131], as well as morphology and phase separation of polymer-modified epoxy asphalt binders [132–134].

4.3.3. Ageing-induced microstructure

Up until now, only a few researchers have used CLSM to investigate the microstructure of bitumen upon ageing. Most of these studies use CLSM in fluorescence mode, to observe the fluorescence centers and propose ageing models by relating these centers to different fractions of bitumen.

Groβegger used CLSM to investigate ageing of bitumen, discovering fluorescence centers in both unaged and laboratory aged samples. The sizes of the centers in both samples were in the same range of 1 to 2 µm, and apart from the induced artefacts at the edges of the images, the fluorescence centers remained constant [124]. These findings are in-line with the observations of Bearsley in which the structure and fluorescence of the asphaltene phase did not alter radically upon ageing [120].

However, these conclusions were in contrast with the research previously done by Li & Wan [119]. Their research with CLSM on asphaltenes showed that the fluorescence almost vanishes after 48 hours from the time when the sample is exposed to air. However, when the sample was kept in a dry box filled with nitrogen, the fluorescence signal stayed invariable. They concluded that the difference in evolution due to ageing could be a consequence of numerous factors, including oxidation. Possible explanations for these contradictions between studies can be found in the difference in temperature or time passed between the preparation of the sample and performing the test. Furthermore, an important factor that could have influenced the results is the difference in the studied samples.

In more recent research, Handle and his colleagues proved the existence of two separate phases in bitumen using CLSM and proposed an enhanced micelle theory for ageing of bitumen that can explain the different effects of short- and long-term ageing, as well as healing due to temperature elevation [125]. In another study, CLSM was used to investigate the ageing properties of PMB by measuring the fluorescence emission of the samples. The results showed a decreased fluorescence intensity for the aged samples compared with unaged samples [129].

A list of different studies using CLSM to investigate the ageing of bitumen is presented in Table 3. Most of these studies use CLSM in fluorescence mode or conduct measurements with a lower resolution than AFM. However, since recently developed CLSMs can acquire images with the same resolution as AFM, further studies in this field are still needed.

Table 3: Summary of previous works with regard to CLSM bitumen observations with ageing.

Research work	Bitumen information	Ageing treatment	CLSM mode	Testing temperature	Key conclusions
[120]	Two bitumens obtained from a Saudi Arabian, Safaniyan crude with penetration grades of 180/200 and 80/100. A third bitumen, AR4000 viscosity graded bitumen manufactured from a Californian, San Joaquin crude.	Artificially aged as 1 mm films in a forced draft convection oven at 60 ± 2 °C for 3024 ± 8 h.	Fluorescence mode	Room temperature	▪ Possibility to observe the asphaltene phase of bitumen ▪ Fluorescence is influenced by the chemical nature of the bitumen. Therefore optimum conditions for observing bitumen of different sources could vary ▪ The observed structure does not change significantly upon ageing

[135]	Unmodified 70/100 pen bitumen.	RTFOT, RTFOT + PAS, exposure in road	Fluorescence mode	Not specified	▪ The spatial distribution, size and shape of the fluorescence centers vary with origin, treatment state and ageing.
[124]	Unmodified 70/100 pen bitumen.	Standard PAV	Transmission and fluorescence mode	Room temperature	▪ Observation of typical fluorescence centers on both unaged and aged bitumen samples. ▪ The centers were evenly distributed and in the same range of 1 to 2 μm for both aged and unaged samples.
[119]	Cold lake bitumen samples with n-pentane insoluble asphaltene content of 17.8%.	Exposure to air for 24-48 h.	Reflected light and fluorescence mode	24-48 hours exposure to air & two weeks under dry nitrogen atmosphere inside a box.	▪ Fluorescence on asphaltene samples almost vanishes after 48 hours from the time the sample is exposed to air. ▪ Fluorescence on asphaltene samples remains the same when the sample is kept in a dry box filled with nitrogen
[125]	Five samples were used: A typical 50/70 bitumen, two SBS-modified bitumen produced from a 70/100 bitumen, precursor sample 1 (vacuum flashed, cracked residuum), and precursor sample 2 (residuum of vacuum distillation)	No ageing treatment	Fluorescence mode	Room temperature	▪ Observation of two separate phases in bitumen, as predicted by the micelle theory. ▪ Development of an enhanced micelle theory for ageing, capable of explaining the different effects of short term and long term ageing as well as thermal healing of asphalt concrete.
[129]	SBS block copolymer modified bitumen	Standard PAV	Fluorescence mode	Room temperature	▪ Phase morphology of PMB samples alter depending on the mixing time and/or shear rate applied. ▪ Shear rate is more influential than mixing time in achieving a highly dispersed SBS phase in bitumen. ▪ Ageing of PMB samples caused a decrease in fluorescence intensity.

5. Comparative overview for bitumen application

In this research, the application of three microscopic techniques to analyse the obtained microstructural changes upon oxidative ageing of bitumen was reviewed. The summary of the main information of these commonly used instruments for these observations is presented in Table 4. Each instrument has its own advantages and limitations, which means that the choice of microscopy is dependent on which microstructural characteristics and degree of detail is required as well as the possession and costs of such a microscope, especially in small-scale laboratories.

Table 4: Comparison of the three microscopies for bitumen application.

Microscope	Radiation source	Working medium	Specimen mounting	Best resolution	Cost of equipment
AFM	Micro cantilever probe	air	Aluminum stubs or glass slides	0.5 nm	++
ESEM	Electrons	gaseous environment	Cylindrical sample holder	1.3 nm	+++
CLSM	Laser light	air	Glass slide	1 nm	++

5.1. Limitations and advantages

There is a wide range of microscopic imaging techniques available to evaluate the microstructure of bitumen. Currently, AFM is the most common instrument used to investigate the ageing phenomenon in bituminous binders. AFM requires minimum sample preparation, can operate in ambient conditions and provides better resolution than ESEM. Other than the microstructure, AFM is also able to provide information on the mechanical properties of the sample in nanoscale. However, this method is highly sensitive to vibrations during the measurements, can only conduct measurements on a limited scanning area, and has limitations while conducting measurements on material with high viscosity or large height differences.

Another popular instrument to examine the effect of ageing on bitumen is ESEM. This microscope is designed based on SEM but permits measurements in a near ambient environment in which the sample does not need to be coated with gold or carbon, and there is no need to have a high vacuum in the sample chamber. Even though this feature makes measurements on bitumen samples possible, ESEM is still not favored by many researchers to observe the bitumen microstructure. This is due to the fact that the fibril network observed on the surface of the samples by ESEM is caused by the microscope itself after several minutes of electron beam exposure and does not correspond with the observations of other microscopic techniques.

A rather recent microscopic technique used by researchers to investigate the ageing of bitumen is CLSM. By using these instruments in fluorescence mode, it is possible to observe the fluorescence centers on the surface of the samples and further improve the available ageing models. The application of CLSM on bitumen in the literature is mostly limited to fluorescence imaging. However, the exceptional resolution (as low as 1 nm in ideal conditions) of the recently developed commercial CLSMs in reflection mode, which are comparable with the resolution of AFM, provides an opportunity to observe the detailed microstructure of bitumen in future research. Furthermore, in contrast to AFM, the multi-focus imaging capability of the CLSM makes acquiring non-contact images of non-smooth surfaces with large height differences possible. Another advantage of CLSM that has not been fully employed in this field is its ability to map the 3D surfaces and produce images of a sample in multiple scales. These abilities, combined with some advanced image processing techniques, can lead to a detailed analysis of a time-dependent phenomenon such as ageing.

5.2. Need for a common preparation protocol

Sample preparation is a crucial step before acquiring images with any microscope. The microstructural properties of bitumen are strongly dependent on the temperature when the sample is extracted, isothermal annealing, and cooling rate. The sample preparation method is important since bitumen can age or undergo steric hardening during or after the sample preparation stage, so that the examined sample might not be a good representation of the bulk material, or bitumen samples might collect dust particles before the images are acquired. Various preparation methods have been developed and used by researchers, but a standard method has not yet been established to investigate the microstructure of bitumen using the microscopes reviewed in this article. Comparison between different sample preparation methods on the morphological properties of polymer modified bitumen samples using fluorescence microscopy is available in [129,130].

The most common sample preparation method used in the literature that is recommended by the authors is as follows:

I. The preparation should start with a high-temperature treatment to create a workable and homogenous material. The temperature and heating time are chosen based on bitumen type and ageing state and is often between 110 and 160 °C. Even though higher temperature have also been used in the literature, this can cause additional ageing in the material and is not recommended.

II. A sample holder (microscopic slide) is placed horizontally on a heating plate set at temperature 140-160 °C. A bitumen drop is placed on the sample holder and left until a smooth surface (1-2 minutes) with sufficient thickness is obtained.

III. The sample is left inside a dust-free environment for two hours to cool down to the ambient temperature. If applicable, the cooling rate is kept between 2 °C/min and 3.4 °C/min to obtain similar cooling conditions of bitumen during asphalt mixture compaction.

IV. Finally, images can be taken at different time intervals, using a non-contact microscopic technique, to keep the surface of the samples free of any artefacts.

6. Conclusions and recommendations

The conclusions that have been extracted by the present literature review for the three most promising microscopic techniques with respect to ageing of bituminous binders are summarised herein:

- There is a transition from the classic colloidal theory of bitumen's microstructure to an asphaltene nanoaggregation model.
- There is still controversy about the source of commonly observed microstructural patterns. The wax crystallisation gains recently more acceptance against the belief of asphaltenes being the source of certain microstructures.
- AFM is the most widely utilised microscopy to capture changes in bitumen microstructure with ageing. Typical bee-like structures are captured with AFM which either increase or decrease with short- and long-term laboratory ageing in terms of average size, based on the used binder.
- ESEM is capable of imaging fibril-like microstructural patterns in bituminous samples. This fibril network is getting denser and coarser with ageing, while its formation time is increasing. A clear association of ageing with changing trend (increasing or decreasing) of fibril size is not possible based on the existed literature.
- CLSM has been utilised to capture the fluorescent centres in the different bitumen fractions. Fluorescent centres have been linked with the asphaltenes' fraction in bitumen. They have been reported to be either unaffected in terms of size by ageing or to disappear upon oxidation.
- Based on a comparative analysis, the future of microscopy in small-scale asphalt laboratories seems to belong to CLSM since CLSM in reflection mode is rather underutilized and can offer, among others, imaging of high resolution and mapping in 3D surfaces.
- A simplistic common preparation procedure for bituminous microscopic samples is suggested to be adopted in order to reduce variations between different research groups.

Yet, despite the understanding of the overwhelming benefits of microscopic techniques to capture ageing-induced microstructures in bitumen, the efforts to link them with upper scales can be somehow biased when the investigated binders are rather limited. Therefore it is recommended that the bitumen research community turns towards a more systematic investigation of bituminous binders, of different origin and performance, to verify certain trends and microstructural patterns reported upon ageing. It remains to be seen if the microstructural theories proposed up to now will be able to explain the formation and change of these microstructural patterns with ageing. Epitomising, it is believed that this review will consist a useful guide for the pavement engineer who is concerned not only for the bitumen's performance but also for the reasons and links behind this.

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