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Novel Method for Controlled Wetting of Materials in the Environmental Scanning Electron Microscope

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Abstract: Environmental scanning electron microscopy has been extensively used for studying the wetting properties of different materials. For some types of investigation, however, the traditional ways of conducting *in situ* dynamic wetting experiments do not offer sufficient control over the wetting process. Here, we present a novel method for controlled wetting of materials in the environmental scanning electron microscope (ESEM). It offers improved control of the point of interaction between the water and the specimen and renders it more accessible for imaging. It also enables the study of water transport through a material by direct imaging. The method is based on the use of a piezo-driven nanomanipulator to bring a specimen in contact with a water reservoir in the ESEM chamber. The water reservoir is established by local condensation on a Peltier-cooled surface. A fixture was designed to make the experimental setup compatible with the standard Peltier cooling stage of the microscope. The developed technique was successfully applied to individual cellulose fibers, and the absorption and transport of water by individual cellulose fibers were imaged.

Key words: environmental scanning electron microscopy, *in situ*, cellulose fiber, manipulator, water transport, swelling

Introduction

The interaction of different materials with water is central to many applications surrounding us in daily life and therefore constitutes an important field of study. Over the past few decades, environmental scanning electron microscopy has become an established technique for observing and analyzing this type of interaction. As opposed to a conventional scanning electron microscope, the environmental scanning electron microscope (ESEM) can be operated in a gaseous environment. The presence of the gas enables the imaging of electrically insulating materials without the need for deposition of a conductive coating. Using water vapor as the imaging gas, we arrive at a special case where the gas may also be utilized for stabilizing water-containing specimens and performing dynamic experiments involving the wetting or drying of materials (Stokes, 2008).

In the ESEM, the state of hydration of the specimen is determined by its temperature and the water vapor pressure in the microscope chamber. In the phase diagram of pure water, equilibrium conditions at a given relative humidity are represented by the saturated vapor pressure curve (Stokes, 2003). *In situ* dynamic hydration or dehydration of the specimen is usually accomplished by changing pressure or temperature to induce condensation or evaporation.

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To date, most studies, if not all, involving the wetting of a material in the ESEM employ the method of humidity control described above (see, e.g., Liukkonen, 1997; Roman-Gutierrez et al., 2002; Montes-H et al., 2005; Wei et al., 2006). However, when a relative humidity of 100% is desired, the degree of control offered by this standard approach is sometimes insufficient. As water condenses on the surface of the specimen, it will form droplets or a continuous film depending on the nature of the surface. Since the water is relatively opaque to the electrons in the beam used for imaging, this may obscure the underlying structure of the material (Jenkins & Donald, 2000). In addition, some studies require precise control of the point of interaction between the water and the specimen. This cannot be achieved by letting water condense on the specimen surface.

One approach that gives improved control is to use a microinjector to place a droplet of water on the specimen. If the microinjector is combined with a high-precision manipulation system, a site of interest on the specimen surface can be chosen with high accuracy. Another advantage is that it enables the use of liquids other than water. Several studies have employed microinjection in the ESEM (see, e.g., Wei et al., 2002; Camacho-Bragado et al., 2011). However, this approach does not address the problem of the liquid obscuring the structure of the specimen.

In this work we present a new method for controlled wetting of materials in the ESEM. A sample holder has been designed, which utilizes a piezo-driven nanomanipulator to bring a specimen in contact with a water reservoir. The

water reservoir is established by local condensation on a cooled surface in the ESEM chamber. This method offers improved control of the point of interaction between the water and the specimen and renders it more accessible to the electron beam used for imaging. The purpose of developing the method was to provide insight into the structure-property relationships of materials for which the interaction with water is of importance, and the information gained may aid the design of new materials. The usefulness of this approach stems from the combination of high-resolution imaging, *in situ* manipulation, and the ability to maintain a localized water reservoir in the sample chamber. It enables the investigation of wetting phenomena with a resolution significantly higher than that of an optical microscope, with an emphasis on the processes taking place in the initial stages of contact at the interface between water and specimen.

A further advantage of our method is the ability to observe freestanding structures; the specimen can be mounted and observed without the influence of an underlying surface. This enables direct imaging of the transport of water through a material, allowing the water to permeate at one end of the sample and progress into drier parts of the specimen. Reingruber et al. (2007) accomplished direct imaging of water transport in the ESEM using a different technique that did not involve an *in situ* manipulator. To our knowledge, no other methods for direct imaging of water transport in the ESEM are currently available.

There are several potential applications of this technique. For instance, studies of wetting properties of different materials may be performed at a much smaller length scale compared to what is possible with conventional contact angle measurement techniques, both with regard to the size of the water droplet and the size of the specific materials structures of interest. Another interesting aspect is the ability to follow the uptake of water by a material, along with resulting processes such as swelling and/or water transport. In its present form, the method can be used to probe structural changes on the surface of a material using the secondary electron signal from the specimen, while internal structures remain hidden. Future possibilities include the use of transmitted electrons to study subsurface processes and changes in the internal structure.

To demonstrate the possibilities of the developed technique, dynamic *in situ* wetting experiments were conducted using individual cellulose fibers. A number of studies have previously addressed the wetting properties of cellulose fibers using ESEM (see, e.g., Jenkins & Donald, 1997; Karlsson et al., 1998; Jenkins & Donald, 1999, 2000; Gellerstedt et al., 2000). However, they all employ the traditional method where *in situ* hydration is accomplished by pressure increase or temperature decrease. Also, few attempts have been made at isolating an individual fiber to avoid influence from either an underlying surface or other fibers. Water absorption in a network of cellulose fibers is complex, with contributions from the fibers themselves and from the capillaries between them (Karlsson et al., 1998). Therefore, isolating the contribution from an individual, freestanding fiber may increase the understanding of the mechanisms involved.

Materials and Methods

Environmental Scanning Electron Microscope

The microscope used throughout the experimental work was an FEI Quanta 200 FEG ESEM (FEI Company, Hillsboro, OR, USA). The microscope was equipped with a Peltier cooling stage that can operate in a temperature range of −25°C to 55°C. An acceleration voltage of 8 kV was used for imaging during the experiments because previous studies had shown that this voltage did not result in visible beam damage in the specimens at the magnifications used.

Materials

Wetting experiments on cellulose fibers were performed to test and evaluate the new method. The cellulose fibers originated from softwood kraft pulp and were provided by Södra Cell AB (Värö, Sweden). The study included fibers treated with sodium hydroxide (NaOH), reducing the hemicellulose content to 9% by weight, as well as untreated fibers. After the pulping process, the material was stored in a moist and cool (below 8°C) environment to prevent hornification prior to specimen preparation. For the wetting experiments, the following specimen preparation was employed. A single fiber was extracted from pulp and cut with scissors to expose the lumen, i.e., the cavity running through the fiber. To be able to mount the sample on the manipulator, the fiber was attached to an Al wire of diameter 0.3 mm using epoxy resin. It should be noted that during the subsequent drying of the epoxy resin, the fibers were exposed to dehydrating conditions. Hence, at the start of the wetting experiments, the fibers were no longer moist.

Nanomanipulator

The positioning system used for the new *in situ* sample holder has been developed and produced by NanoFactory Instruments AB (Gothenburg, Sweden). It consists of a nanomanipulator with PC-controlled electronics. The manipulator is shown in Figure 1. Designed for use in the transmission electron microscope (TEM), it is compact enough to fit in a regular TEM side-entry holder. It involves a sapphire ball attached to a piezoelectric tube and a movable part connected to the ball by springs. The movable part, which will henceforth be referred to as the "tip holder," holds a metal wire onto which the specimen is glued. This construction enables three-dimensional movement on several length scales. An inertial sliding mechanism, capable of 0.1 μm steps and a total movement of approximately 1 mm in three dimensions, is used for the coarse alignment of the specimen with respect to the water reservoir. The expansion/contraction of the piezoelectric tube can be used for fine-tuning the alignment on the order of nanometers. A more detailed description of the positioning system is provided by Svensson et al. (2003).

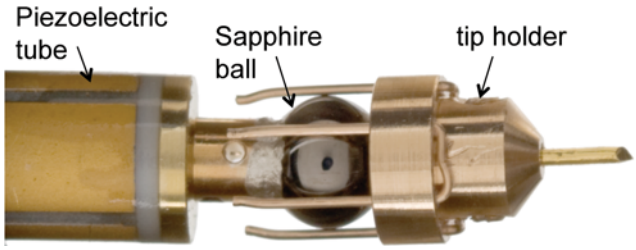

Figure 1. The manipulator used in the *in situ* sample holder, consisting of a piezoelectric tube, a sapphire ball, and a "tip holder." The latter, which holds the specimen, is attached to the sapphire ball by springs and is thus movable.

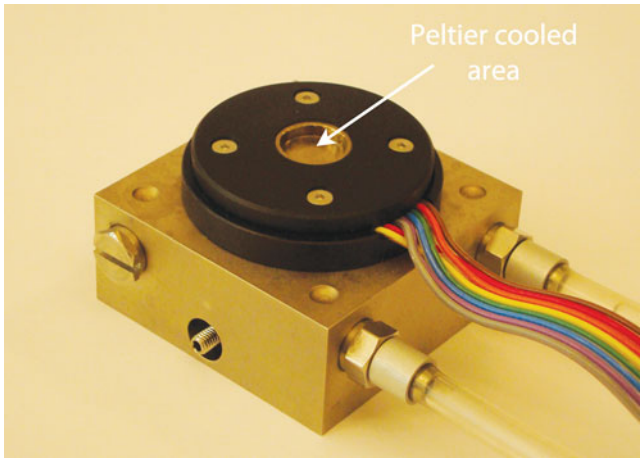

Figure 2. Photo of the standard Peltier cooling stage of the FEI Quanta 200 FEG ESEM with the Peltier-cooled area highlighted.

Results and Discussion

Design of the *In Situ* Sample Holder

The aim in developing the *in situ* sample holder was to achieve a geometry where the interaction between the specimen and the water was confined to a well-defined site that was readily accessible for imaging. Until making contact with the water, the sample should remain relatively dry. In other words, we must keep the sample at a low relative humidity while maintaining a reservoir of condensed water at 100% relative humidity. This requires a difference in temperature between the sample and the site of the water reservoir. Instead of cooling the sample, as is the normal practice for wetting experiments in the ESEM, local cooling was applied to a surface that could act as a support for the water reservoir. The positioning system provided a way to make the comparatively dry specimen and the water meet and interact; the task at hand was to render it compatible with the standard Peltier cooling stage of the microscope, shown in Figure 2. To this end, a fixture allowing the nanomanipulator to be placed in the ESEM chamber together with the cooling stage was designed and manufactured.

Several aspects had to be taken into consideration when designing the fixture. First, it should accommodate the manipulator and its housing and, second, it should provide access to the Peltier-cooled area of the stage. Third,

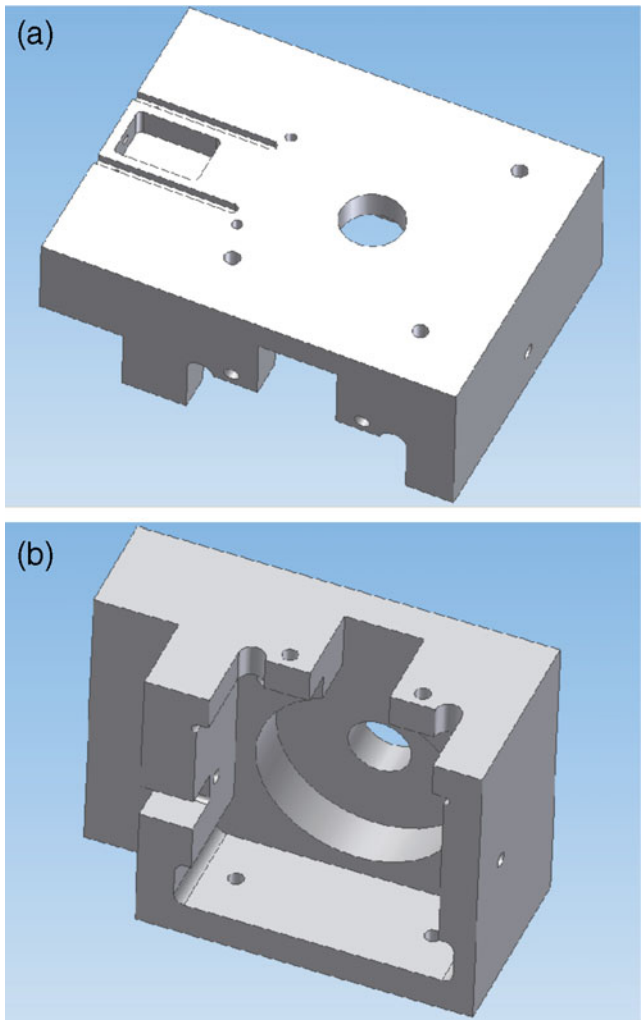

Figure 3. CAD drawings of the fixture used to accommodate the manipulator and Cu block in the *in situ* sample holder. (**a**) The fixture is viewed from the top, i.e., the side facing the detector when mounted in the ESEM. (**b**) It is viewed from the bottom, i.e., the side facing the Peltier cooling stage.

it must be thermally insulated from the stage to avoid excessive thermal loading of the Peltier element. A CAD drawing of the fixture can be seen in Figure 3, and the resulting *in situ* sample holder is displayed in Figure 4. Tailored to the shape of the cooling stage, the fixture can be fitted over the stage and locked into place by screws. Thermal insulation is accomplished by allowing an air gap between the two components and using screws made from nylon.

As a support for the water reservoir, a cylindrical block of solid copper (Cu) was placed in contact with the Peltier-cooled area through the opening in the fixture. The block had a height of approximately 20 mm to match the level of the nanomanipulator and a diameter of 9.5 mm in order to fit directly into the Peltier cooling stage. Before experiments could be initiated, the condensation point on top of the Cu cylinder had to be checked to ensure thermal equilibration of the block. The temperature of the Peltier cooling stage was brought to 1°C. Water condensed on the upper surface

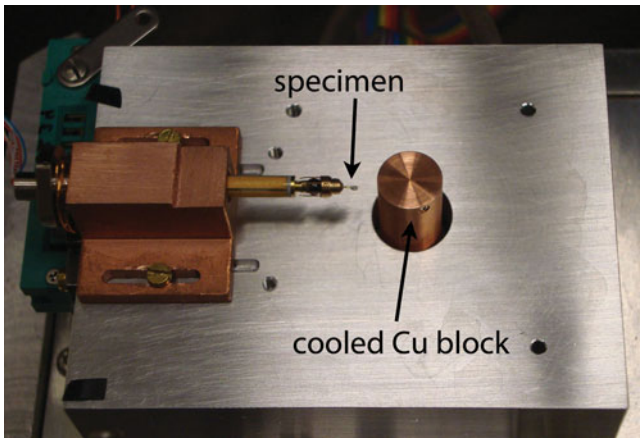

Figure 4. Photo of the *in situ* sample holder. It consists of an Al fixture that accommodates a piezo-driven manipulator holding the specimen, as well as a Cu block that is cooled through its contact with the Peltier cooling stage underneath the fixture.

of the block after a few minutes at 657 Pa (4.93 torr), which, according to the phase diagram for pure water, corresponds to the saturated vapor pressure at the chosen temperature. This indicated thermal equilibration throughout the block. At water vapor pressures slightly above the saturated vapor pressure, the condensation rate was sufficiently slow so that the water droplets formed on the vertical surface of the block could be used as reservoirs for dynamic *in situ* wetting experiments.

Wetting of Individual Cellulose Fibers

To test the new method, dynamic wetting experiments on individual cellulose fibers were performed using the *in situ* sample holder. The aim was to obtain qualitative results that demonstrated the possibilities of the developed technique. The cellulose fibers were chosen as specimens due to their relatively high capacity for water uptake. An ESEM image of a cellulose fiber with an exposed lumen is shown in Figure 5. It was estimated, based on the saturated vapor pressure curve for water vapor and the temperature data presented later in this article, that the relative humidity at the portion of the fiber visible in the image was approximately 30%. This particular fiber has a fairly circular cross section, and the lumen appears not to be collapsed but rather open. The lumen plays an important role in the transport of water, and so a fiber whose lumen has collapsed suffers a reduction of its transport capability (Karlsson et al., 1998). It should be noted that the fibers used in the experiments described below, although originating from the same batch and subjected to the same pulping process and specimen preparation, showed significantly different morphologies. Consequently, the degree of collapse of the lumen varied.

Prior to the wetting experiments, the Al wire holding the single cellulose fiber was fixed in the tip holder, which was subsequently mounted on the sapphire ball of the manipulator. The temperature of the Peltier cooling stage was brought to 1°C and imaging was initiated at a pressure

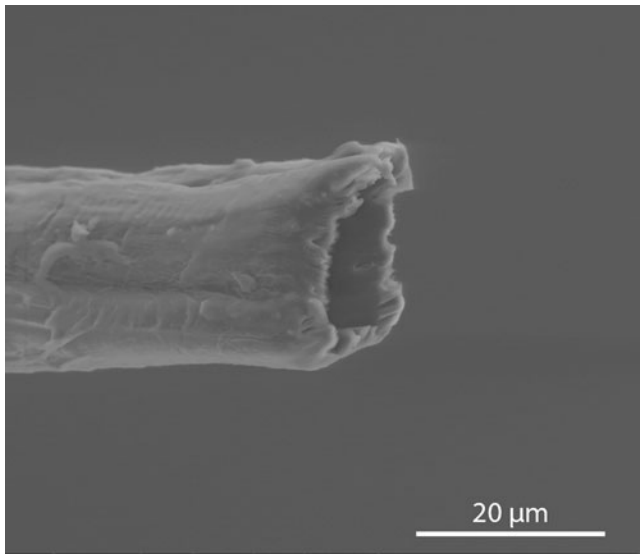

Figure 5. ESEM image of an individual cellulose fiber. The fiber, treated with NaOH for hemicellulose removal, was cut to expose the central cavity (lumen).

of approximately 500 Pa (3.75 torr). Subsequently, the pressure was increased to 660 Pa (4.95 torr), and after a few minutes water had condensed on the wall of the Cu block and produced droplets of suitable size.

A cellulose fiber was brought in contact with a water droplet on the vertical wall of the Cu block as shown in Figure 6, and the point of contact between the fiber and the water could be clearly observed. This experiment was repeated several times using different fibers. In general, swelling was observed as an increase in thickness and a decrease in surface roughness of the fibers. When a fiber was left in contact with a droplet for an extended period of time, the droplet was seen to shrink until it disappeared entirely from the surface of the Cu block. Figure 7 shows a sequence of images captured during such an event. Although only a few selected frames are displayed here, the image sequence recorded during this process is of sufficient temporal resolution to show that the swelling of the fiber appears to progress along the portion of the fiber that is visible in the image, away from the point of contact with the water droplet.

These observations suggest that the entire droplet is absorbed by the fiber and transported along it, and there are several arguments to support this conclusion. First, the smaller droplets that can be seen in the foreground and background of the image stay constant in size during the absorption of the larger droplet. This indicates that the environmental conditions in the ESEM chamber do not change during the course of the experiment. Hence, the disappearance of the large droplet from the surface of the Cu block is not an artifact due to unstable environmental conditions. Second, the arrows in Figure 7 indicate that the fiber changes its shape and seems to swell during the process but reverts back to its original shape once the entire droplet has been absorbed. This reversibility shows that the

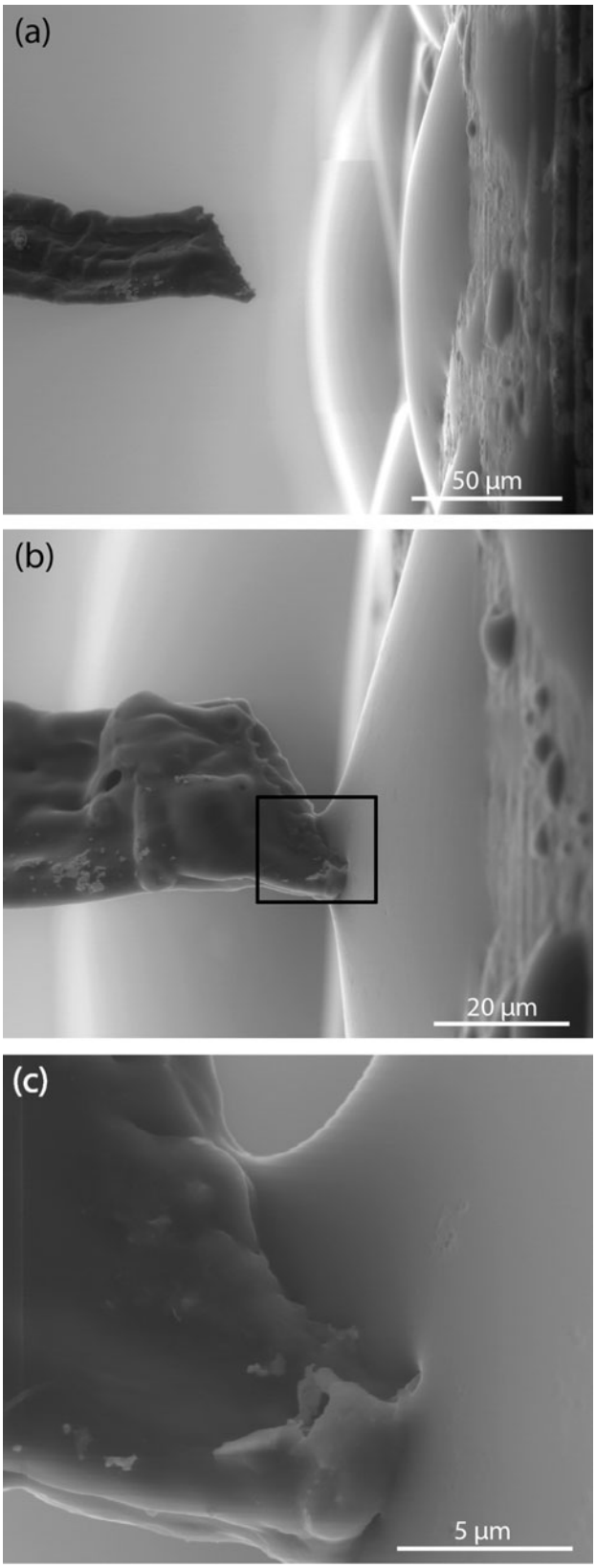

Figure 6. ESEM images of an individual cellulose fiber during a wetting experiment. This fiber had not been treated with NaOH. The fiber (**a**) approaches a water droplet and (**b**) makes contact with it. The point of contact, indicated by a rectangle in image **b**, is clearly visible at higher magnification in image **c**.

swelling is not an artifact of beam damage, something that can otherwise complicate the image interpretation especially when it comes to experiments involving hydration and swelling (Jenkins & Donald, 1997). Ultimately, the evaporation of water from the fiber can be regarded as a minor influence on the process. This assumption is supported by results that will be shown below, indicating that the temperature near the cooled copper block is relatively low.

For the process shown in Figure 7, the time required for absorbing the droplet was approximately 15 s. The size of the droplet may be estimated by considering its shape to be a spherical cap. The formula for the volume of a spherical cap then gives an estimated volume of 0.02 nL in this case. However, the droplet size and time for absorption varied when repeating the experiment with different fibers. In some cases, the process was much faster, e.g., 0.13 nL in 5 s. Moreover, most fibers could absorb several droplets in consecutive wetting experiments before a saturation level was reached and the absorptive capability lost. We believe that the differences observed with respect to wetting properties among individual fibers stem from differences in structure. Properties such as length, width, tortuosity, and accessibility of lumen may be relevant factors in this context.

To our knowledge, this is the first time that the absorption and transport of water by an individual cellulose fiber has been studied by direct observation in the ESEM. The roles of the fiber wall and the lumen with respect to the mechanisms of water transport in single cellulose fibers are, however, still unclear, and further studies need to be undertaken to address that question.

Evaluation of the Developed Technique

The above experiments enabled us to evaluate the performance of the new *in situ* sample holder. Due to the spatial constraints imposed by the size of the tip holder, the minimum working distance that could be used without risking a collision with the detector was approximately 5 mm. It was found that the movement of the specimen could be accurately controlled by means of the manipulator. Alignment of the fiber and the water droplet in the direction of the electron beam was managed by adjusting the height of the fiber until the droplet and the fiber were both in focus. The size of the water reservoir used for wetting of the specimen could be controlled to an extent, either by choosing a particular droplet out of those available on the Cu surface or by changing the pressure for a limited amount of time to achieve a general growth or shrinkage of the droplets.

Another issue concerns the relative humidity of the specimen before it is brought in contact with the water reservoir. During the wetting experiments, the single cellulose fibers were seen to swell to some extent on approaching a water droplet but before making contact with it. This indicated an increase in humidity with decreasing distance to the cooled surface of the Cu block. In the ESEM, the relative humidity is determined by temperature and water vapor pressure. The latter can be assumed to be constant

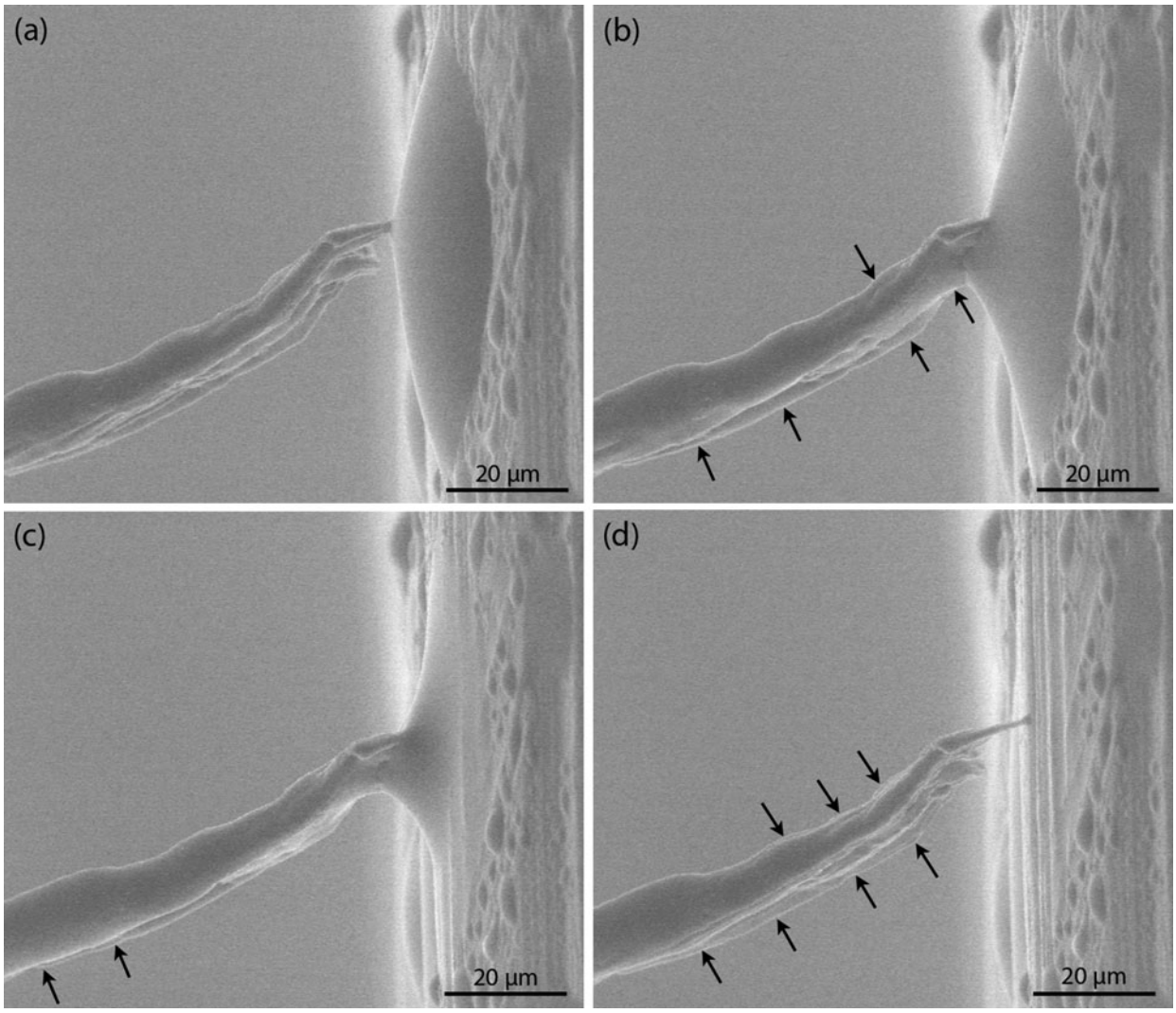

Figure 7. ESEM images of an individual, NaOH-treated cellulose fiber absorbing a water droplet during a wetting experiment. Changes in the fiber shape during the absorption process are highlighted by arrows.

throughout the chamber [except for the immediate vicinity of the final pressure limiting aperture, where some instabilities may occur (Stokes, 2008)], since a pressure gradient cannot be maintained without having a flow of gas in the chamber. Therefore, the precontact swelling of the fibers suggested a gradient in temperature near the cooled surface.

To characterize the temperature gradient, the temperature was measured as a function of the distance to the Cu surface. Prior to the measurements, the copper block was equilibrated at 1°C and water was observed to condense on its surface. A type K thermocouple was mounted on a support inside the ESEM and positioned so that its wire junction made contact with the copper block. Using the ESEM stage control, the copper block was then retracted from the thermocouple wire junction in discrete steps and the thermocouple reading was recorded at each interval. The temperature was allowed to stabilize for 1 min before each reading was taken. The result is displayed in Figure 8. There is a clear decrease in temperature as the copper block is approached, and the gradient increases with decreasing

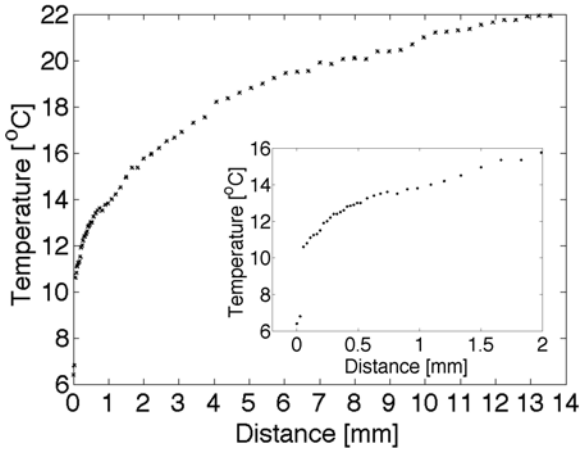

Figure 8. The temperature as a function of the distance from the Cu block, which is kept at 1°C. The inset shows the details of the temperature profile in the region 0–2 mm from the Cu block. The error in temperature was measured for each data point and varied between 0.1 and 0.4°C.

distance. As shown by the graph, the temperature at contact was measured to slightly higher than 6°C. This is probably due to the fact that only a small portion of the surface of the thermocouple wire junction made contact with the copper block. This, in turn, is a consequence of the spherical shape of the thermocouple wire junction.

During a wetting experiment, the maximum distance between the copper surface and the tip of the cellulose fiber is typically about 1 mm. Hence, the specimen experiences a considerable change in temperature and thus humidity, when approaching a water droplet sitting on the copper surface. Whether this should be seen as a limitation of the developed method depends on the purpose of the experiment at hand. For the wetting experiments reported in this work, the precontact swelling of the cellulose fibers must be considered a deviation from the conditions expected to prevail in the application of the material. When used in absorbents, the fibers are not likely to be gradually hydrated but rather to go from dry to wet in an instant when exposed to a water front. That being said, the developed technique gives a considerably more realistic representation of the real wetting process than offered by the traditional method, where water droplets are gradually formed on the specimen surface through pressure increase or, equivalently, temperature decrease.

Beside the limitation discussed above, there is another aspect of the temperature gradient that may actually be seen as an advantage in this particular experiment. Since the fiber stays relatively cold in the vicinity of the copper block, the evaporation of water can be considered a minor influence on the cellulose fiber in this region. Regarding the absorption process shown in Figure 7, we can therefore be confident that water is actually being transported along the fiber rather than evaporating from it.

Aspects of Sample Preparation and Geometry

There are certain aspects of the method that should be kept in mind when considering its applicability to different materials. As always when it comes to *in situ* wetting experiments in environmental scanning electron microscopy, the condition of the specimen at the start of the experiment must be carefully considered to ensure that it reflects the purpose of the study. Accurate handling of the sample prior to investigation as well as the choice of pumpdown sequence are crucial, whether the specimen is supposed to be moist or dry at the beginning of the investigation in the microscope. Another point to consider is the fact that the water reservoir maintained at the cooled surface will have a relatively low temperature. As determined by the saturated water vapor pressure curve, the temperature of the water is limited by the maximum operating pressure that can be tolerated with respect to beam scattering and signal-to-noise ratio.

Furthermore, the sample geometry may impose certain constraints. A fiber is well suited for observing the processes occurring at the point of contact with the water and the resulting morphological changes. A suspended film geometry is more challenging but also poses more possibilities. The film can be oriented in different ways to reveal different aspects of the specimen-water interaction. For example, when viewed in cross section from the side, the progress of the water front through the film can be studied. Complementary information can be obtained from a plan view of the film. In this case, the film approaches the cold copper cylinder from above, making contact with the water on its horizontal bottom surface. The water is absorbed at the contact point and penetrates through the film to the opposite surface, which is available for observation. In a similar way, different arbitrary geometries have to be considered individually in order to provide access to desired information.

Conclusions

A new method for *in situ* dynamic wetting experiments in the ESEM was developed. It uses a piezo-driven nanomanipulator to bring a specimen in contact with a water reservoir formed by local condensation in the ESEM chamber. Due to the favorable geometry resulting from this setup, the point of interaction between the water and the specimen is well defined and readily accessible for imaging. Moreover, since only one side of the specimen is in contact with the water reservoir, the water may progress into the material as a single front. This better mimics the conditions in some real life materials applications compared to the traditional approach to *in situ* wetting. To demonstrate the possibilities of the developed technique, wetting experiments were carried out on individual cellulose fibers. For the first time, the absorption and transport of water by a single cellulose fiber were imaged. The conducted experiments were used to evaluate the performance of the method. The specimen was found to absorb some amount of water from the vapor phase due to a temperature gradient near the Peltier cooling stage. The implications of the temperature gradient must be considered separately for each new application depending on the aims of the investigation. For the wetting experiments conducted here, it may be regarded as both a limitation and an advantage. It is a limitation when it comes to accurately representing the conditions in the materials application, but it is useful in preventing evaporation from the specimen in the vicinity of the water reservoir.

Acknowledgments

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