APPLICATION OF THE ESEM TECHNIQUE IN WOOD RESEARCH: PART I. OPTIMIZATION OF IMAGING PARAMETERS AND WORKING CONDITIONS

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ABSTRACT

A study using the ESEM (Environmental Scanning Electron Microscopy) technique was performed on wood objects in order to assess the particular advantages, possibilities, and limitations of this microscopic tool. In contrast to conventional high vacuum SEM, in ESEM specimens can be investigated in a gaseous atmosphere, usually of water vapor. This enables the observation of non-conductive, polymeric, composite, and porous materials (such as wood) in their natural state, without drying, evacuating, or sputtering them with a layer of carbon or metal. Further advantages include observations in a wide range of temperatures (−15° to 1000°C), conduction of dynamic processes such as condensation, freezing, and thawing of the specimen during observation, or mechanical testing.

The imaging quality of ESEM for natural samples, however, is inferior to that of conventional SEM, and the specimens are liable to beam damage. The process of acquiring an image in ESEM is more complex than in SEM, demanding the optimization of a number of interacting parameters. These include the physical conditions of the specimen, conditions of the chamber environment, and electronic parameters of the formation and optimization of the image.

The work on the ESEM can be performed through several operational modes that offer various sets of environmental and imaging conditions. This article presents guidance for assessment of influential operating parameters and their selection for the optimization of the ESEM work with wood.

Keywords: Wood, SEM, ESEM, structure, microstructure, fractography.

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INTRODUCTION

The aim of this paper is to present optimized parameter settings of the ESEM (Environmental Scanning Electron Microscopy) technique and operational routines for observation of wood objects. A second paper in a series brings details of specific wood experiments and compares different operational ESEM modes (Turkulin et al. 2005).

ESEM imaging is more complicated than conventional SEM imaging because of numerous and complex interactions between the sample properties, microscope chamber conditions, and imaging parameters. An excellent overview of the ESEM principles and applications has been presented by Johnson (1996). Considering microstructural investigations of wood, it must be emphasized that wood is a difficult material for investigation with the scanning electron microscope (SEM). Charging is a major problem due to its non-conductivity and high porosity. In addition, wood specimens are sensitive to beam damage, especially at high magnifications. However, the advantages of the environmental SEM are promising. Several working groups have demonstrated the great potential of the ESEM technique for wood research, e.g. Kamdem et al. 1998 in Michigan; Shaler et al. 1996 in Orono; Stehr et al. 1999, Rosenquist 2001, and Kifetew et al. 1998 in Stockholm; and Frühmann et al. 2003 in Vienna.

Our findings demonstrated in this paper and in a more extensive report including a thorough literature review (Turkulin et al. 2004) should form a sound basis for other ESEM operators who specifically work with wood and other biological materials.

Specifics of the ESEM

The high qualities of electron microscope images are related to a strong light-optical analogy and three-dimensional visual effect, as well as their good contrast, resolution, and depth of field. Application of field emission (FE) cathodes has made a great impact on both SEM and ESEM technology, but the latter are still not widely used in wood research. The ESEM instrument basically differs from its conventional counterpart (SEM) inasmuch as the specimen in ESEM need not be observed under conditions of high vacuum. In ESEM, the high vacuum electron column is separated from a low vacuum environment of the sample chamber by means of two closely spaced and vigorously pumped pressure limiting apertures (PLA). Therefore, pressures up to 20 torr (≈ 26 mbar) can be maintained in the chamber, while keeping the column under high vacuum. Water vapor proved to be the most advantageous gas for most ESEM operations because it is convenient, cheap, nontoxic, and it ionizes easily to provide good imaging results (Johnson 1996). Moreover, water is a basic constituent of many sample materials such as wood. A "moist" climate for such materials can be established within the chamber by cooling the sample and using increased vapor pressures up to 10 torr at the same time. The relative humidity (RH) can be controlled via temperature and vapor pressure according to the phase diagram of water. The triple point of H_2O (common boundary of the stability fields of ice, water, and vapor) is at 0°C and 4.5 torr. Above 4.5 torr, and 0°C, water can be condensed and wet samples can be kept stable at 100% RH.

In ESEM the primary electron beam and the emitted secondary electrons pass a gaseous atmosphere on their way between the final lenses, through the PLA apertures, to the sample surface and finally back to the detector. Part of the electron beam current is lost through dispersion caused by collisions of the electrons with gas molecules. This is called the skirt effect. The remaining unscattered component of the beam, focused to the beam spot on the sample, is relevant for the image resolution and for contrast. However, the distance that the electron beam travels through the gaseous space (*beam gas path length, BGPL,* i.e. the distance between the final aperture and the specimen) should be kept at minimum in order to minimize the scattering, which strongly affects signal intensity and imaging contrast (Fig. 1).

Since conventional Everhard Thornley detectors require high vacuum conditions, special sec-

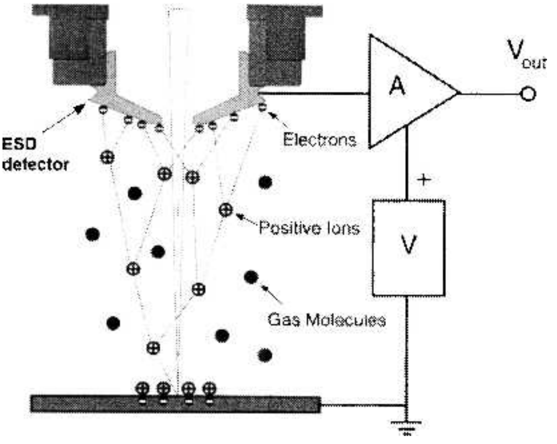

FIG. 1. Electron-gas interactions in ESEM chamber (Courtesy Philips Electron Optics FEI). Collisions of secondary electrons with molecules of ionized gas (e.g. water) contribute to greater electron signal at the ESD detector and to suppression of negative charging at the specimen surface. ESD opening forms a final aperture of the column.

ondary electron (SE) detectors are necessary for the ESEM. There exist various types of ESEM detectors: the Environmental SE Detector (ESD, Fig. 1), the Gaseous Secondary Electron Detector (GSED), and the Large Field Detector (LFD), which are described in great detail elsewhere (Johnson 1996). The principle for all three detectors is the same. Secondary electrons (SE) emitted from the sample are attracted by the ESD, GSED, or LFD, due to a positive potential. ESD and GSED operate at vapor pressures up to 10 torr, whereas LFD is used in low vacuum (up to 2 torr). GSED is a newer detector generation which, due to a different architecture with a smaller surface area, reveals a smaller contribution from back scattered electrons (BSE).

On their way from the sample to the detector, the emitted electrons collide with gas molecules. This results in an avalanche of newly created electrons and cations. The avalanche of electrons leads to an amplification of the detected signal. On the other hand, the cations accumulate on the surface of the negatively biased sample and effectively suppress the charging. This is considered as one of the greatest benefits of the ESEM (Cameron and McDonald 1994). Obviously, there is a compromise between the positive effects of gas concentration (increased signal and reduced charging) and negative consequences of beam interaction with the gas (beam skirt, which lowers contrast and signal to noise ratio). The image optimization can be achieved through adjustments in a range of operating conditions, i.e. vapor pressure, beam gas path length, temperature, accelerating voltage, and spot size (Turkulin et al. 2004).

Comparison of SEM and ESEM

ESEM operating systems and advantages.—Table 1 represents a general comparison of operational features of ESEM and conventional SEM. The specific advantages of ESEM can be listed as follows:

Specimen preparation can be omitted for a number of specimens (including wood), and the potential for artifacts due to drying, impregnation, and excessive metal deposits on the surface is eliminated. Wet, dirty, oily, and evaporating samples and even living objects can be observed in their natural, moist state.

Possible wood defects during drying and vacuuming, such as excessive shrinking and col-

TABLE 1. *General comparison of functional characteristics of conventional and environmental SEM techniques on wood samples.*

Conventional (FE) SEM	ESEM
Advantages	
High resolution	New objects
High magnification	-Moist wood
(up to 50.000×)	-Bio-objects
	No specimen preparation
	A range of operational modes/ working conditions
	Dynamic experiments
	-Moisture
	-Temperature
	-Load
Limitations	
Specimen preparation	Complex operation
(only dry samples)	-Optimization of working/ imaging conditions
Artifacts	-Time and skill requirements
-Shrinkage defects	Limited magnification and contrast
-Coating deposits	

lapsing, are minimized. Stehr and co-workers performed a study using UV laser ablation technique for the preparation of wood surface for microscopic observation (Stehr et al. 1998), or for gluing investigations (Stehr 1999, Stehr et al. 1999). Stehr and Oestlund (2000) showed that ESEM is an essential tool in studying surface condition and fine machining deformations without a risk of artifacts in specimen preparation.

Using the ESEM as an experimental microscope, dynamic processes (e.g. fracturing, elastic/plastic deformations, or phase transformations) can be followed within the ESEM chamber. In combination with heating and cooling stages, the reactions of the material under investigation may incorporate condensation, freezing, melting, shrinkage or swelling, hydration or dehydration. These aspects are of great importance in studies of water interactions with fiber, paper, or wood. Sheenan and Scriven (1991) and Forsberg and Lepoutre (1994) demonstrated how *in situ* moistening and re-drying of paper can be analyzed in ESEM for observation of interfiber contacts, fiber-rising, or migration and orientation of coating particles on the paper. Gu et al. (2001) successfully measured the anisotropy of shrinkage of transversal cell walls during drying in ESEM.

X-ray analysis of non-conductive specimens, such as wood, is free from X-ray interference with conductive coatings. Crystalline solids deposited on cell walls of preservative-treated wood were clearly seen; and EDXA (Energy Dispersive X-ray Analysis) was determined without metal sputtering (Craciun et al. 1997; Kamdem et al. 1998; Maldas and Kamdem 1998; Kamdem and McIntyre 1998; Dawson-Andoh and Kamdem 1998). Rosenquist (1999, 2001) used the advantage of coating-free analysis of silver particles in wood micro-autoradiographs.

Charging on the sample is suppressed as a characteristic of the physical process involved; this alleviates the imaging of non-conductive materials (such as wood), or combinations of materials of different conductivity, without sputtering with metal such as gold or platinum. The imaging of the wood-polymer composites was presented by Razi et al. (1997) and Nitz et al. (2000). Fractographic and morphological studies of glue bonds on wood were performed in ESEM by Stehr et al. (1999) and Stehr (1999).

Imaging at large range of temperatures (−20°C to above 1000°C) is possible due to heat insensitivity of the gaseous secondary electron detector (GSED). Pulido-Novicio et al. (2001) studied physical and anatomical characteristics of wood carbonization within the ESEM chamber.

Constant or variable load can be applied to the specimen by means of a micro-fracture stage within the chamber for studies of mechanical behavior (e.g. crack propagation in tension or compression). Shaler and co-workers (Mott et al. 1995; Shaler et al. 1996; Mott et al. 1996) presented exquisite experimental fineness in tensile testing of individual wood fibers within ESEM. Authors described in detail the tensile stage and a Digital Image Conversion (DIC) technique for mapping of micro-strain distribution on digitally captured images. Sippola and Frühmann (2001), Schiekel et al. (2002), and Frühmann et al. (2003) observed *in situ* the crack propagation in tensile or compression micro-specimens, and associated the stress-strain curves with corresponding fractographic evidence. Schiekel et al. (2002) followed the recommendations by Cameron and McDonald (1994) on the procedure of chamber evacuation so that a wood specimen retains its original moisture content.

Further possible applications embrace the observation of other continuous reactions (e.g. hardening of the adhesive, finish curing, crystal growth, surface wetting) that could be studied directly, as they happen. Optical light microscope can be fitted into the chamber of the ESEM to provide simultaneous optical and electron images. Micro-manipulator can be used to scratch the surface of the sample. That should enable the study of the wear resistance, depth-profile analysis of the processes at the surfaces, or separation of specific particles or fibers for subsequent research e.g. with TEM (Transmission Electron Microscope) or AFM (Atomic Force Microscope) (Kaegi and Holzer 2003).

Disadvantages of ESEM.—Lower contrast and lower effective magnifications on wood (up to 8000×) in comparison with conventional field-emission SEM instruments (up to 50.000×) are the major shortcomings of the ESEM. Furthermore, beam damage may happen to uncoated wood and similar samples (Danilatos 1986), especially in wet exposures (Sheenan and Scriven 1991; Forsberg and Lepoutre 1994), during prolonged image acquisition (Kifetew and Sandberg 2000) and at higher magnifications (Mott et al. 1995). These shortcomings of the ESEM can be reduced according to the following general recommendations:

- keep accelerating voltage under 10 kV, preferably under 7 kV
- reduce beam current under 100 pA using a small spot
- decrease duration of beam exposure.

Additionally, beam damage and charging are increased at short working distances, slow scanning rates, and at high magnifications.

Modes of operation in ESEM

In ESEM an operator may choose between a conventional (high vacuum) and three different *environmental* modes of operation (Table 2). The main distinguishing features are the pressure ranges and the associated detector settings. Usually it is the condition of the sample that defines the appropriate mode of operation. Imaging conditions are also dependent on vapor pressure within the chamber. Therefore the physical state of the sample, the conditions within the chamber, and the imaging parameters interact in a complex way, demanding the optimization of all the influential factors. Consequently, before starting an ESEM experiment, the microscope settings have to be prepared.

High vacuum (HV) mode.—In this operational mode ESEM is used as a conventional SEM where high vacuum is maintained within the chamber. Wood specimens must be compatible to the high vacuum (dry) and their surface must be conductive (e.g. coated with gold or platinum).

Low vacuum (Low vac or LV) mode.—The sample is not subjected to a high vacuum, but to low values of water vapor pressure (usually <2 torr), so that biological specimens containing water, such as wood, need not necessarily be dried and sputtered. According to our own experience, vapor pressures above 1.5 torr significantly impair the signal and the quality of the image. The hardware configuration in the low vacuum mode includes a *large field detector* (LFD) and a PLA-bullet with a relatively large aperture (1000 μm). The latter enables a larger field of view (field sizes greater than 2 mm) compared to the *Wet* mode, where smaller apertures have to be used. Therefore the LV mode is advantageous for general surveying of the object at lower magnifications (e.g. 50×). For wood samples with less than 15% moisture content (MC), it is assumed that the wood retains its constant moisture content during the work in LV mode, and hence drying artifacts can be omitted in contrast to high vacuum imaging.

It was established in numerous experiments that the best contrast for wood in the low vacuum mode is achieved at pressures that are ca. 0.5 torr lower than the pressure of maximum brightness. The optimization of the image is obtained by finding the best combination of the vapor pressure and working distance (WD, Fig. 2). The ideal settings of the vapor pressure and working distance are different for every specimen and must be optimized accordingly.

TABLE 2. *Operational modes in the ESEM and their essential parameters.*

Mode of operation	Pressure range	Electron detectors
High vacuum (HV)	10^{-6} mbar/Torr	ET (Everhard-Thornley) SE-detector (BSE)
Low vacuum (LV)	~0.8 Torr	LFD (large field) detector (+BSE)
Low vacuum short distance (LVSD)	~1.8 Torr	LFD detector with ESD cap for short BGPL
Wet (*ESEM*)	>1 Torr	ESD operates better at 1–3 Torr
	up to 10 Torr	GSED operators better at 3–6 Torr

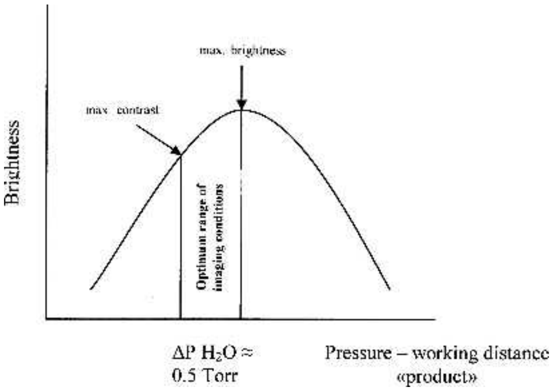

FIG. 2. Schematic presentation of the effect on imaging quality of the product of vapor pressure in the chamber and working distance.

The third parameter in low vacuum imaging to be taken into account is accelerating voltage, which usually ranges between 5 and 10 kV. The higher the voltage, the smaller the spot size; therefore higher resolution can be achieved. However, higher beam voltage also leads to deeper penetration of the beam into the material. Accordingly, with higher voltage, beam damage will increase and resolution may even decrease.

For a good focal depth, which is important for the investigation of porous structures such as wood, it is advantageous to use larger working distances (>10 mm). However, scattering of the primary beam increases with larger working distance. Use of higher voltages to reduce this scattering is very limited because of a risk of beam damage. 10 kV is considered as an optimum accelerating voltage for imaging at working distances of approximately 15 mm. In summary, for a magnification range of 5–10 kX, the voltage must be reduced to approximately 5 kV, and additionally the pressure must be increased (1–1.5 torr) and BGPL reduced (7–12 mm) in order to avoid beam damage and charging.

Low vacuum "short distance" (LVSD) mode.—The LVSD operational mode is essentially the same as the *low vac* (LV) mode, but the LVSD assembly offers the working conditions of higher gas pressures (generally 1.1 to 1.8 torr) and smaller effective working distances. In this mode the beam gas path length (BGPL) at the same working distance is reduced, because the beam passes 5-mm span through an additional conical aperture before it enters the chamber. Its narrower diameter (500 μm) in comparison with the LV aperture reduces the viewing area. *Low vacuum short distance* mode is advantageous to *low vacuum* mode in terms of higher vapor pressures and, consequently, a reduced effect of charging. This is particularly beneficial for higher magnifications than in LV mode on non-conductive specimens such as wood. However, as comparative investigations have shown, some focal depth is sacrificed probably due to a larger scattering effect at higher pressures.

WET mode.—The term "environmental" does not represent ambient pressures (approx. 1000 mbar), but rather pressure and temperature conditions close to the triple point of water. In the *Wet* mode a high relative humidity (up to 100%) can be achieved within the microscope under operating and imaging conditions close to the triple point (0°C/4.5 torr). A special accessory for the beam nozzle, the so-called "wet" aperture bullet, enables the use of vapor pressures up to 10 torr. This bullet is combined with the Gaseous SE Detector (GSED), which records the purest SE signal of all environmental detectors, thereby yielding the most accurate information about the surface texture and topographic properties of the sample. This mode is used for observation of wet or very moist objects. The specimens can be cooled using a cooling stage on Peltier principle. At pressures higher than 4.5 torr, the dehydration of cooled samples can be avoided. In general, optimized conditions are given at pressures between 2.3 to 6 torr in combination with relatively high accelerating voltages (7 to 20 kV) and working distances between 7 and 13 mm. For most wood objects, however, the experiments in the Wet mode have yielded poorer resolution and worse image quality than in LV and LVSD modes.

MATERIAL AND METHODS

This study has been performed using a PHILIPS ESEM (XL30-FEG). Softwood specimens have been used for investigation, selected as fractured or microtomed fragments of solid wood. Only cross-sections have been investigated, as was the case in former structural and fractographic SEM research (Turkulin and Sell 1997, 2002).

Solid samples were prepared from spruce (*Picea abies* Karst.) sticks 10 × 10 mm in cross-section. Surfaces for observation were either microtomed or obtained by tensile fracture.

"Dry microtomed" surfaces were prepared by microtoming the conditioned wood (ca. 12% moisture content) in tangential direction on a mechanical microtome. "Wet microtoming" (after immersion in a mixture of equal volumes of water and ethanol for 30 min), was attempted to reduce the knife damage during microtoming. Some of these specimens were used wet; others were dried naturally to obtain different moisture contents. "Moist" specimens were conditioned for 3 h at 20°C and 50% relative humidity. "Dry" samples were dried overnight in vacuum dryer at 20°C, and finally conditioned for 2 h in room conditions to approximate moisture content of 12%.

Fractured specimens of solid wood were prepared by loading the wood sticks in 3-point static bending. The zones near the tension-failed surfaces were separated and studied. All ESEM specimens were fastened to the mounting blocks using conductive carbon adhesive in such a way that adhesive extended for about 1 mm up the vertical sides of the sample. This was observed to improve conductivity and reduce the charging effects (Turkulin and Sell 1997).

RESULTS AND DISCUSSION

Comments on the ESEM operating routines for wood

Accelerating voltage.—The best imaging results and least charging and damage of the specimen were obtained using low accelerating voltages, mostly within the range of 4.5 to 7 kV (Figs. 3 and 4).

In *low vacuum* or in *Wet* modes, the vapor pressure was utilized to suppress surface charging and edge effects. The conditions on three images on Fig. 3 differ only regarding the vapor

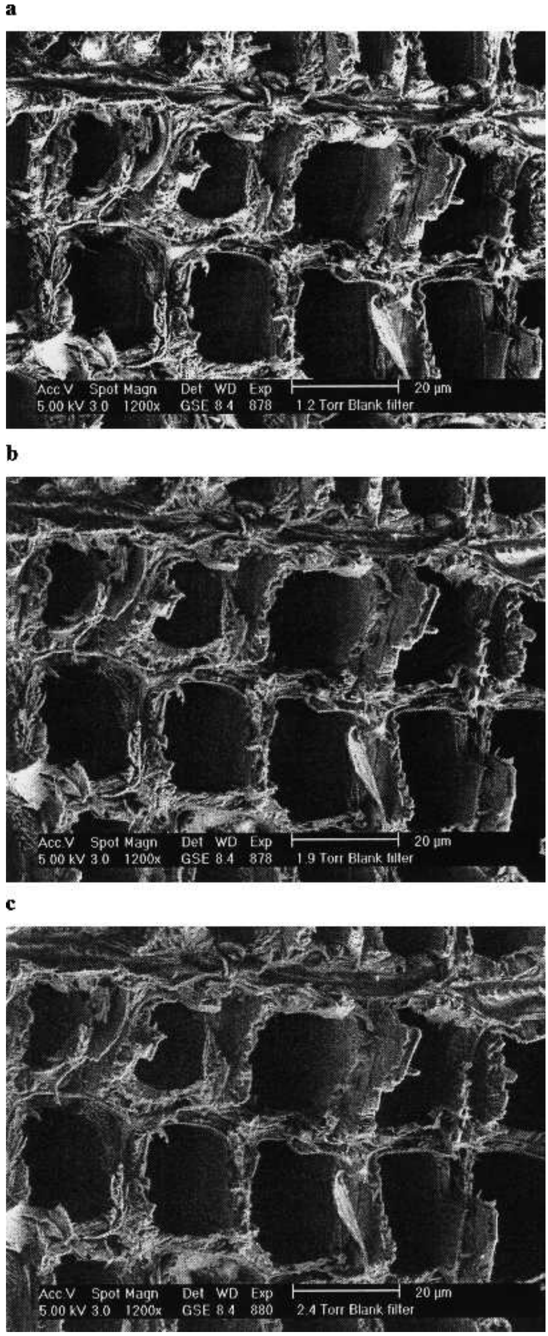

FIG. 3. (a, b, and c) Fractured transverse surface of spruce latewood. *Low vacuum short distance* mode. Acc. voltage 5 kV, magnification 1200×. Characteristic ductile mode of tension-loaded fracture surface is discernible. On these and following micrographs, the AccV denotes the accelerating voltage, GSE the type of detector, WD the working distance. The gas pressure in the chamber is presented in torr.

pressure. Increased vapor pressure has reduced the charging effects, but also rendered the image “pale” due to low signal to noise ratio related to the skirt effect. Additionally, focusing and astigmatism corrections became rather difficult.

Comparison between the images on Fig. 4a (5kV) and 4b (7kV) shows how the different steps in changing the working conditions need not result in image improvement. Initially, the increase in voltage from 5 kV (Fig. 4a) to 7 kV increased the SE signal intensity. However, this emphasized the edge effect, which was attempted to be controlled by the increase in pressure. However, higher pressure resulted in reduction in sharpness, and the overall result of the change of both working parameters on image 4 b is poorer.

In *Wet* mode (work with wet specimens), the voltage must be higher than in *low vacuum* mode, usually between 7 and 15 kV, in order to reduce the negative impact of beam scattering.

a **b**

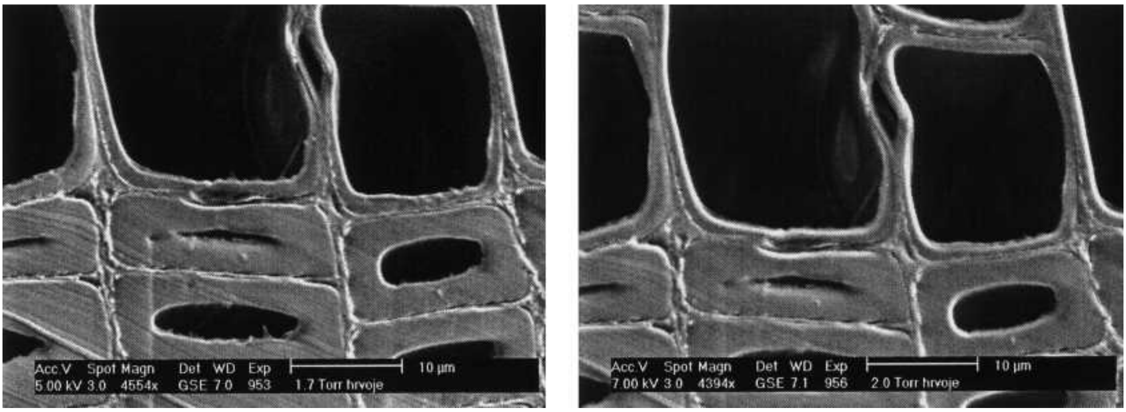

FIG. 4. Transverse surface of spruce obtained by wet microtoming. *Low vac short distance* mode. Acc. voltage 5 kV on Fig. 4a (left), 7 kV on Fig. 4b (right), magnification ca 4500×. Combined increase in acc. voltage and vapor pressure resulted in worsened image quality on Fig. 4b.

a **b**

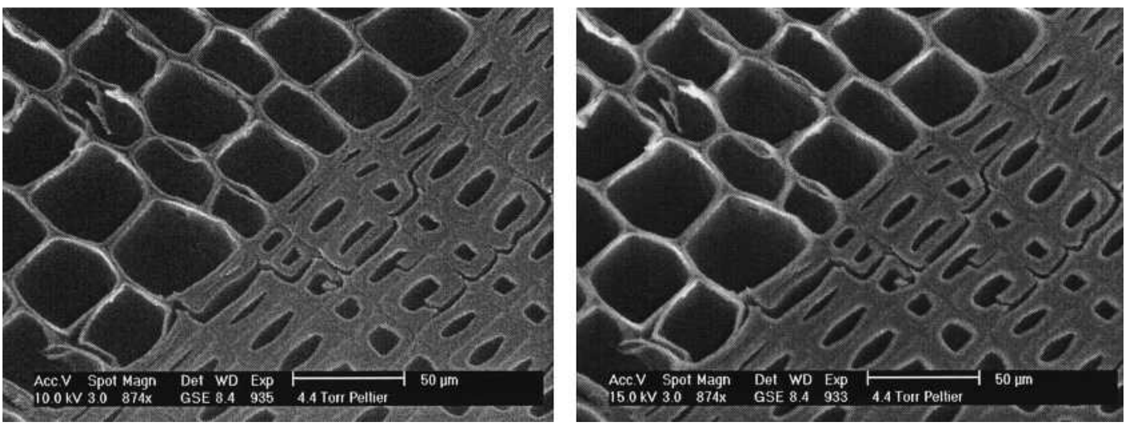

FIG. 5. Transverse surface of spruce, microtomed in wet (saturated) condition. *Wet* (*ESEM)* mode. Acc. voltage 10 kV on Fig. 5a (left), 15 kV on 5b (right), magnification ca 930×. Lower acc. voltage in acceptable range results in better image quality (Fig. 5a).

On the other hand, the general rule also applies here, that the lower the voltage, the lower the effects of beam penetration and beam damage (Fig. 5 a and b). With “dryer” specimens, the 10 kV voltage gives greater edge effect and consequently worse resolution than lower voltages, so further reduction to 7 kV is justified (compare Figs. 6a and 6b).

Vapor pressure and working distance.—In *low vacuum* mode, the often observed edge effect (charging at sharp edges, see Fig. 7a) can be reduced by increased vapor pressure. However, if the increase in pressure is too large, it leads to the obvious loss of contrast and resolution, which can be compensated for by reduction in working distance. Good examples of elimination of the edge effect by changing the pressure while keeping the pressure – WD “product” nearly constant, are pairs of images on Fig. 7. However, the increase in pressure dominates the modifica-

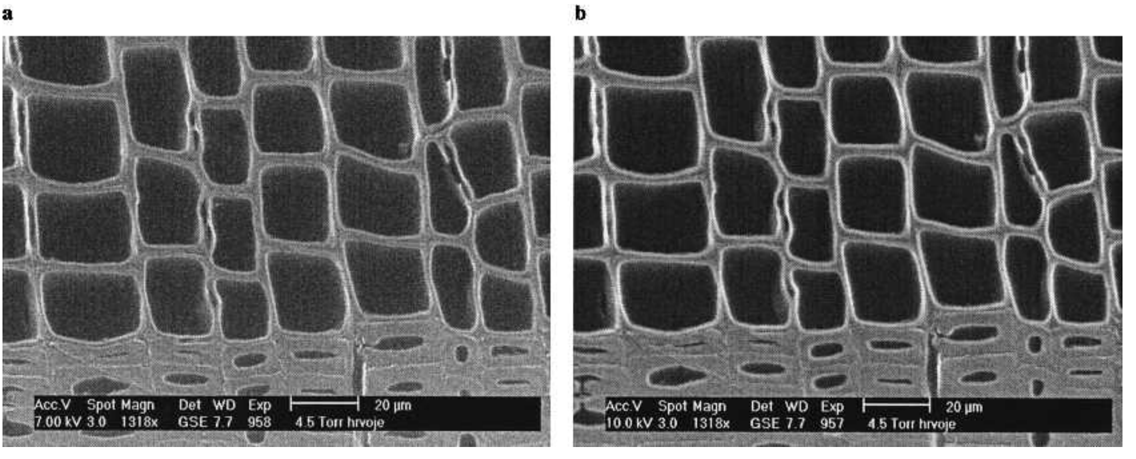

FIG. 6. Transverse surface of spruce, microtomed in wet (saturated) condition. *Wet* (*ESEM*) mode. Acc. voltage 7 kV on Fig. 6a (left), 10 kV on 6b (right), magnification ca 960×. Lower acc. voltage in acceptable range results in better image quality (Fig. 6a), even at cost of reduced contrast.

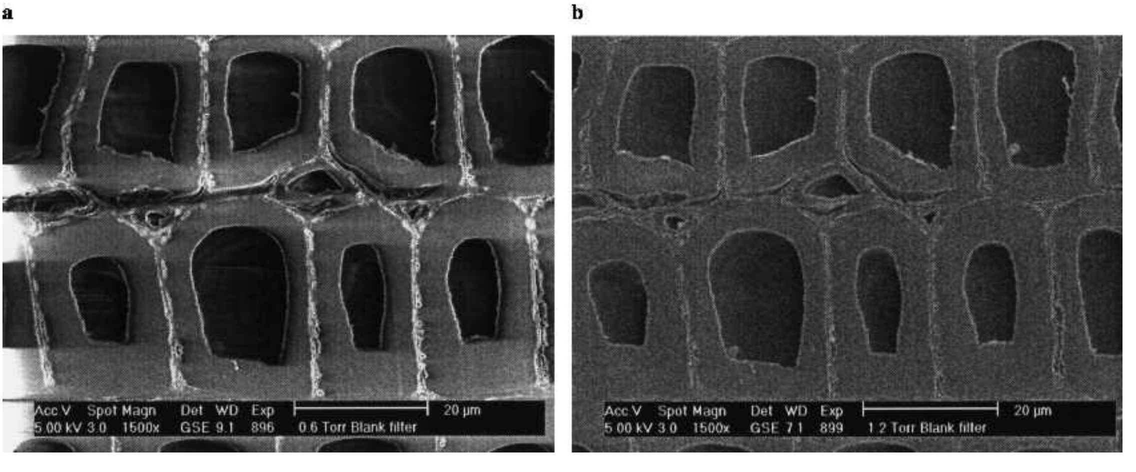

FIG. 7. Uncoated, spruce latewood, microtomed in dry condition. *Low vacuum mode*. Depth sharpness and resolution are relatively poor. Charge on Fig. 7a (left) is subsequently eliminated by increasing the pressure from 0.6 torr to 0.9 torr (Fig. 7b, right), but the brightness and contrast are sacrificed. Magnifications 1500×.

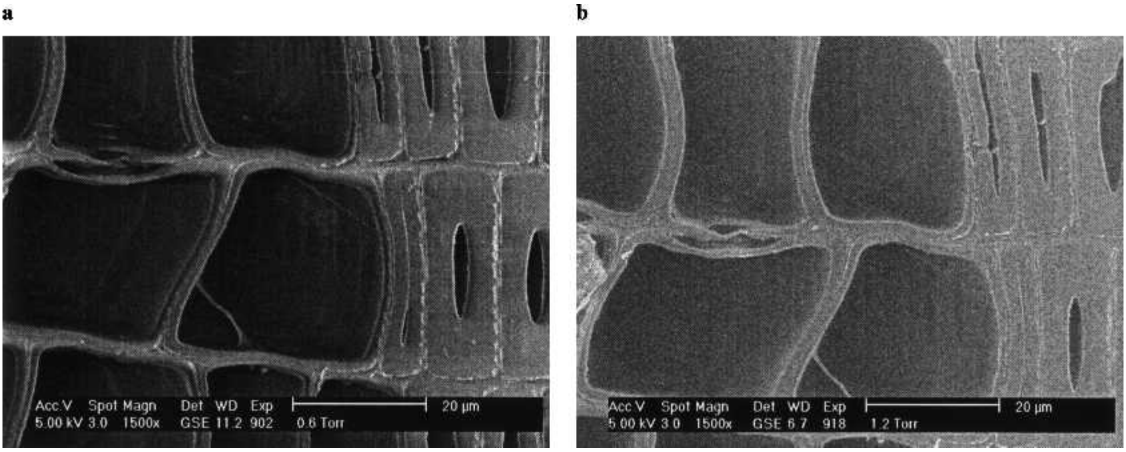

FIG. 8. (a and b) Uncoated spruce, microtomed in wet (saturated) condition. *Low vacuum* mode. Increase in pressure dominates the loss of contrast (Fig. 8b) regardless of the reduction in working distance. Left: Working distance 11.2 mm, pressure 0.6 torr. Right: Working distance 6.7 mm, pressure 1.2 torr.

tion in imaging conditions, and the loss of contrast can not be fully compensated by the reduction in working distance (Fig. 8). In *low vacuum short distance* mode, the principle of image optimization is the same, but the range of practically applicable pressures is higher (1.2 torr to 1.7 torr). The best results were obtained with WD values below 8 mm.

Operational and imaging interactions.—The work on wood in conventional high vacuum SEM is simply done by optimizing the single imaging parameters that hardly interact with each other (such as focus, contrast, astigmatism etc.). In ESEM the optimum experimental conditions for the specimen (with particular physical characteristics and material properties) usually do not result in ideal imaging conditions. In addition to that, different parameters interact with each other in a complex way (Fig. 9). In order that the range of all possible ESEM operational conditions are simplified for the operator in wood research, Table 3 brings a classification of the ESEM modes and useful ranges of selected parameters. The values of operational parameters are based on experience from numerous experiments that are presented in a more extensive report (Turkulin et al. 2004). These optimized values should be taken as general guidance only, and further experimentation with

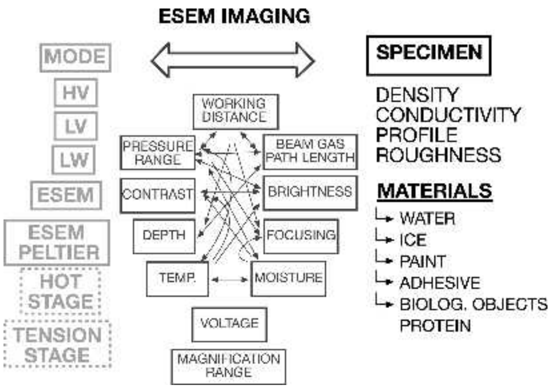

FIG. 9. Schematic presentation of the influential operating parameters and conditions involved in Environmental (ESEM) imaging of wood.

each typical specimen, and with those not yet analyzed in environmental modes, is recommended.

SUMMARY AND CONCLUSIONS

Environmental aspects of ESEM microscopy, i.e. maintaining a humid atmosphere within the specimen chamber, enable the observation of wood in its natural form, without conventional SEM preparation steps. This eliminates the occurrence of typical SEM artifacts on delicate

TABLE 3. *Operational modes and selected working conditions for wood using ESEM.*

Object	Operational mode	Acc. voltage kV	Magnification	Working distance (WD) mm	Vapor pressure Torr	Temp./rel. humidity °C/%	Comments, characteristic samples
All wood objects (MC = moisture content)	**HV** High vacuum (~10 nm Pt sputtering)	5 (up to 10 for greater WD)	50–50 000×	6–12 10–20	—	Ambient RH 0%	Smaller WD for even surfaces and large magnifications, greater WD for smaller magnifications and better focal depth. Beware of coating deposit!
All, especially profiled, rough wood objects MC ≤15% General view	**LV** Low vacuum LV bullet + LFD (Large Field Detector)	5	50–5 000×	>7 (7–12)	0.6–1.5 (start at 1.0)	Ambient RH < 50%	The lowest possible pressure to be chosen (ca 0.5 torr lower than press. at maximum brightness) Large field of view, good survey of the sample. Potential for edge effects!
All, especially textured, fractured objects (close up)	**LVSD** Low vacuum short dist. LV bullet + Std. ESD cap + LFD	5	270–10 000×	7–9 (BGPL = 2–4)	1.1–1.8 (start at 1.2 close to object)	Ambient	The lowest possible WD and pressure to be chosen. Narrow field, good resolution and contrast. Beware of drying defects!
Wet, MC ≥ 15% Moist, saturated wood, living organisms	**WET** Wet bullet + GSED (Gaseous SE Detector)	7 (5–15)	270–2 000×	7–13	1.8–6 (start at 4)	Ambient RH > 50%	Poor resolution, difficult work. General observation of moist, wet or saturated wood. The greater MC, the greater the pressure. Beware of drying defects!
Wet wood + condensation, freezing, melting, drying	**WET** Wet bullet + GSED + cold stage or + heating stage	10 (7–20)	270–5 000×	7–13	2.3–6 (start at 4)	−5 °C– +55 °C RH up to 100%	Conditions as in *Wet* mode, poorer resolution, difficult work. Lower pressures for unsaturated samples. Higher pressures for condensation and wetting of the sample.

wood samples, such as excessive shrinking and collapsing during vacuum drying, or build-up of metal deposits during sputtering. Wet, oily, resinous, or evaporating wood objects may be analyzed in their genuine condition. Further advantages include observations in a wide range of temperatures (−15° to 1000°C), conduction of dynamic processes such as sample drying or condensation of water, freezing and thawing of the specimen during observation, mechanical testing, and so on.

The imaging quality (especially contrasting) of ESEM is inferior to that of SEM, and wood specimens are liable to beam damage. Precautionary steps include the possible reduction of the accelerating voltage, beam current (spot size) and magnification, duration of exposures, and highest possible range of scanning rate and working distance.

The work on the ESEM can be performed through several operational modes that offer various sets of environmental and imaging conditions. These embrace the conventional *high vacuum* (HV) mode and three *environmental* modes: *low vacuum* (LV), *low vacuum short distance* (LVSD), and *Wet* mode. The required conditions of the specimens determine the range of vapor pressure and hence the choice of the mode.

The process of acquiring an image of wood in ESEM is more complex than in SEM, demanding the optimization of a number of interacting parameters. These include the physical conditions of the specimen, conditions of the chamber environment and electronic parameters of the formation and optimization of the image. Generally, the accelerating voltage should be kept as low as possible in all modes. The values of vapor pressure and working distance must be iteratively adjusted until optimum imaging conditions are achieved.

This article presents general interactions between influential parameters for the ESEM work with wood. The guidance for selection of the modes and ranges of useful operating conditions, will permit the easier, better, wider use of this powerful tool in future wood research.

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