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Enhancing Wood Sample Preparation for SEM Imaging: A Detailed Study of Epoxy Resin Impregnation, Cutting, Sanding, and Polishing for Fragile and Heterogeneous Samples

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Abstract

Accurate characterization of wood microstructure requires high-quality sample preparation, especially for degraded, mineralized, or embedded samples. Through a detailed and systematic investigation, we suggest a simple, fast, and cost-effective method for preparing transverse surfaces of wood for scanning electron microscopy. The methodology used is easily accessible and simple. We compare traditional microtome cutting using both disposable blades and a fixed knife with sanding and polishing techniques. The choice of method is determined by the physical condition and structural characteristics of the sample. Disposable blades, when used with continuous moistening, yielded the best results for reference wood, while the fixed knife proved to be more effective for mineralized or epoxy-embedded samples due to their rigidity and resistance to deflection. Maintaining blade sharpness and surface moisture were critical in all cutting techniques. Polishing proved to be a particularly effective technique for the preparation of degraded, mineral-rich, or heterogeneous samples. In combination with epoxy embedding, it offers a practical alternative to microtome cutting, ensuring the protection of fragile structures, the long-term stability of samples, and the possibility of re-polishing. Epoxy impregnation was easy for degraded wood, while sprucewood required vacuum-pressure treatment. Care must be taken when polishing to avoid reaching non-embedded regions beneath the surface.

Key words: epoxy resin impregnation, polishing and sanding, sample preparation, scanning electron microscopy, wood

Introduction

Wood is a complex, natural, and heterogeneous material with an intrinsic variability in its properties (Eder et al., 2021). The anatomical study of wood and the understanding of its structure are crucial for understanding the biology, general behavior, and mechanical properties of wood and the materials or composites made from it (Wu, 1998). There are a few microscopic techniques that are suitable for the study of wood, each with some advantages and disadvantages. Transmission light microscopy (TLM) is currently the most commonly used method for wood observation, and scanning electron microscopy (SEM) is one of the most powerful techniques available to us today for wood observation. The latter can be used to obtain information about morphology, chemical composition, tissue orientation, and some other properties of wood (Merela et al., 2020). One advantage of using SEM for wood observation is relatively simple sample preparation (Kučera, 1981). The thickness of the sample is not critical (Novak, 2018), but the surface to be observed must be of sufficient quality. Merela et al. (2020) confirmed that the best method for preparing the wood surface is to cut a frozen or pre-moistened sample. Most anatomical studies use thin sections (1–25 μm) for TLM (Novak, 2018) or surfaces obtained by conventional methods of cutting either freehand or with a microtome for SEM or other microscopy techniques (i.e., confocal laser scanning microscopy- CSLM) (Merela et al., 2020; Kitin et al., 2021). For wood species with high density and/or even dry wood, it is often challenging to obtain a high-quality surface. Producing a thin transverse section of wood that is of sufficient quality for microscopic observation is very labor-intensive and time-consuming using conventional preparation techniques (Kitin et al., 2021).

Numerous studies have focused on sample preparation using a microtome or razor blade (Exley et al., 1974; Kučera, 1981; Barbosa et al., 2010; Prislan et al., 2014; Merela et al., 2020). It is well known among wood scientists that adequate surface quality can be obtained by cutting either freehand or using various microtomes in combination with different embedding and softening techniques (Kučera, 1981; Merela et al., 2020). Kučera (Kučera, 1981) emphasised that high-quality blades are essential for obtaining clear images at high magnification. He provided a comprehensive description of different microtome blades, with emphasis on glass knives as well as some cutting techniques. Although wood sectioning has a long history, preparation of heterogeneous plant parts without damaging their structure remains a challenge (Barbosa et al., 2010; Merela et al., 2020). When a blade cuts through structures of varying density, artefacts can form that make further analyses difficult

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(Arzac et al., 2018). For example, mineral inclusions in wood are problematic during sectioning. It is recommended to remove them with hydrofluoric acid before sectioning (Jansen et al., 1998), unless the minerals themselves are the focus of the study. As wood is a heterogeneous material, various artefacts are often encountered when cutting microscopic samples, especially when preparing transverse surfaces.

Embedding is usually necessary for sectioning degraded, very soft (i.e., waterlogged), brittle, or very heterogeneous wood, as well as for small samples that require support from an embedding medium to achieve satisfactory results (Wilcox, 1964*a*; Barbosa et al., 2010; Pace, 2019). Various embedding media have been proposed in the past, including paraffin, celloidin, epoxy resin, acrylic resin, polymethylmethacrylate, polyester, and polyethylene glycol. (Wilcox, 1964*b*; Spurr, 1969; Rapp & Behrmann, 1998; Rossi et al., 2006; Hamann et al., 2011; Arzac et al., 2018; Heck et al., 2020; Mozzi et al., 2021; Balzano et al., 2022*a*, 2022*b*). The choice of embedding medium usually depends on personal preference. Any resin or other clear mounting medium can be used as long as it infiltrates any pores, cracks, and other small spaces in the sample and does not affect the structure or optical properties of the sample (Heck et al., 2020). Although some authors report that embedding may be unnecessary or complicated (Celant & Coccolini, 2015), time-consuming (Barbosa et al., 2010), or difficult (Schweingruber, 2012). A common requirement for all embedding procedures is that the embedding medium must penetrate into the sample. Generally, less lignified samples are easier to embed than highly lignified material (Hamann et al., 2011).

Splitting or fracturing wood to obtain surfaces for SEM observation is a common technique for archaeological wood preparation (Gärtner & Schweingruber, 2013; Celant & Coccolini, 2015). Although this method is simple, it results in uneven surfaces, and most cells remain uncut or closed, making it impossible to observe their interior. Additionally, transverse surfaces cannot be prepared by splitting (Novak, 2018; Merela et al., 2020).

Sanding of wood surfaces is practised for dendrochronological observation of macroscopic features (i.e., large pores, tree rings, parenchyma) as it is a fast method, but the potential of using this technique for anatomical observations at the micron level has been largely overlooked (Kitin et al., 2021). The quality of sanded surfaces is not comparable to those prepared using established microtome techniques, as empty spaces (lumina, etc.) fill with dust and other debris as a result of sanding (Novak, 2018; Merela et al., 2020). Wu (1998) found that sanding with coarse sandpaper (grit 800 or coarser) results in poor surface quality, while using finer sandpaper (finer than grit 800) results in an exposed microstructure. To obtain a distinct microstructure, sanding with fine paper should be followed by polishing and cleaning (Wu, 1998).

Polishing of embedded samples for microscopic observation is commonly used in histology, metallography, palaeobotany, and even biology, but is not usually employed for the preparation of wood samples (Akca & Trgo, 2015; Mukhamadiyarov et al., 2016; Arzac et al., 2018). Soil, ash, or fossil samples are typically embedded in resin and then cut/and polished (Ueshima & Sakanakura, 2019; Heck et al., 2020). Polishing of wood samples for SEM observation is described in detail by Wu (1998), Witovisk et al. (2014), Dickson et al. (2017), Arzac et al. (2018), Zelaya-Lainez et al. (2019), and Kitin et al. (2021). Polishing turned out to be particularly useful for the preparation of problematic samples, such as fossilised or calcified wood (Witovisk et al., 2014; Kitin et al., 2021). Imaging resin-embedded and polished samples enables high-quality images as the resin prevents any movement or distortion during the polishing process (Dickson et al., 2017). It has been found that sanding and polishing can reveal the microstructure of wood (Wu, 1998). However, Spiecker et al. (2000) noted that the surface obtained by sanding and polishing may not be suitable for high-resolution imaging. In more recent experiments, researchers (Arzac et al., 2018) have found that high-resolution images can be obtained from polished wood without sectioning. Polishing can be applied to a wide range of wood materials. The size of the sample is not limited by the sectioning equipment, but by the size of the mould that can be polished or the size of a sanding wheel, allowing the preparation of larger samples (Dickson et al., 2017; Arzac et al., 2018).

It has been shown that epoxy resin as an embedding medium typically penetrates sufficiently and also acts as a binder and holds loose or already degraded parts of the sample together during preparation and examination (Gärtner & Schweingruber, 2013; Arzac et al., 2018; Heck et al., 2020). Embedding wood with resin does not typically lead to complete filling of the lumina pores (Zelaya-Lainez et al., 2019). A more efficient epoxy resin impregnation was described by Xia et al. (2019), who used a vacuum to remove air bubbles and achieve better penetration of the epoxy resin into the wood (VARI process). A reduction in porosity from 66 to 33.8% was achieved. The researchers state that small pores ($\leq 50\,\mu$m) were difficult to impregnate. Witovisk et al. (2014) recognized epoxy impregnation as a useful method for preparing samples of fossil wood or permineralized charcoal. In addition, samples prepared in this way are preserved and can be easily stored for further analyses (Witovisk et al., 2014).

In this work, we focus on sanding and polishing the transverse surfaces of epoxy-embedded wood samples that are difficult to cut in a conventional way. Conventional protocols for sampling, preservation, fixation, and staining of thin sections are described in detail elsewhere and are not addressed in this work (Exley et al., 1974; Jansen et al., 1998; Hamann et al., 2011; Gärtner & Schweingruber, 2013; Prislan et al., 2014; Tardif & Conciatori, 2015; Pace, 2019). The focus of this work is on a simple, fast, and accessible sample preparation technique for the transverse surface of mineralized wood, degraded wood, or any type of wood sample where cutting is problematic. While wood anatomists usually require tangential and radial planes in addition to the transverse plane for a complete anatomical description, this study focused exclusively on the transverse surface, as it presents the most complex preparation challenges, particularly concerning the penetration of debris into open lumina. The success demonstrated here suggests that the method should also be applied to the less demanding tangential and radial surfaces. We will compare surfaces prepared by cutting with a sliding microtome using a solid steel knife and disposable blades, as well as by sanding (grit 2500) and polishing (with 0.03 μm diamond paste). We assume that embedding the samples in epoxy resin fills the empty spaces in the wood matrix, preventing dust from penetrating the pores and minimizing the samples' distortion during preparation. This makes the embedded samples robust and durable for long-term storage. We believe that the

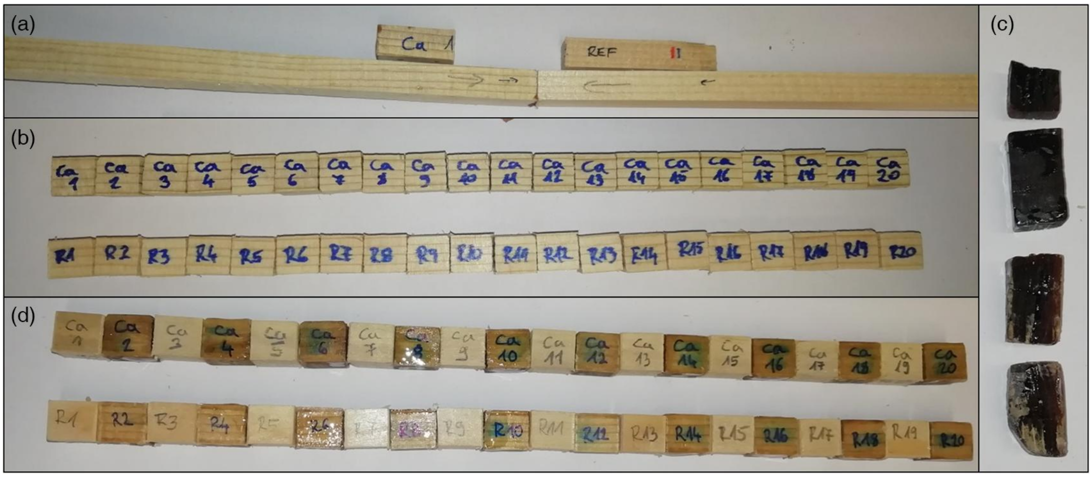

Fig. 1. (**a**) Mineralized (left) and reference (right) sprucewood samples prior to cutting and embedding; (**b**) Cut and marked samples prior to embedding; (**c**) Epoxy-embedded degraded samples of beechwood; (**d**) Non-embedded (odd-numbered) and epoxy-embedded (even-numbered) sprucewood samples.

proposed procedure will be particularly effective for degraded samples. Once polished, the epoxy-embedded samples can be used for subsequent observations or re-polished if damaged. Polishing non-embedded samples could result in voids filled with debris and disruption of the cell walls. Therefore, we assume that polishing embedded samples results in the best surfaces for SEM observation. We also expect that disposable blades work better than a solid steel knife for cutting, but the solid knife produces flatter surfaces.

Materials and Methods

Preparation of Wood Samples

European sprucewood (*Picea abies*) was chosen for the majority of the samples due to its greater heterogeneity compared to certain deciduous trees. We prepared an oriented lath of 500 mm × 10 mm × 10 mm in size, which was cut into two halves (Fig. 1a). Half of the lath was mineralized prior to embedding (the mineralization procedure is described in the next paragraph) (marked Ca), while the other half of the lath was left un-treated and served as a control reference (marked R). Both parts of the lath were further cut into 10 mm × 10 mm × 10 mm cubes and marked accordingly (Fig. 1b). Additionally, severely degraded samples (50 mm × 25 mm × 15 mm) of European beechwood (*Fagus sylvatica*) from previous fungal durability experiments—described in detail elsewhere (Repič et al., 2022)—were prepared as well (Fig. 1c). After the exposure to fungi, all samples were dried for several weeks, cut to a length of 10 mm and embedded in epoxy resin as described above. The samples marked with even numbers and the degraded samples were embedded in epoxy resin (Figs. 1c, 1d). Prior to embedding, the samples were air-dried on a countertop for two weeks. The commercially available epoxy resin Crystalres (Samson Kamnik, Slovenia) was used. Its relatively low viscosity (500 cP) and long curing time (48–72 h) allow it to penetrate deeply into the wood structure. To improve penetration, only 10 mm-long samples were impregnated with the two-step vacuum-pressure impregnation. The samples were placed in a container, separated with a plastic mesh, and covered with epoxy resin. The container was placed in a glass desiccator and exposed to a vacuum (0.3 bar absolute) for 4 h at 22° C, followed by an overnight (approx. 12 h) pressure treatment (10 bar absolute). During this time, the epoxy resin was cured to a thick gel. At this point, the samples were removed from the chamber, and excess epoxy resin on the surface of the samples was wiped off with a cloth and acetone. The impregnated samples were then left at room temperature to fully cure for another week.

Mineralization was achieved by impregnating the wood with a calcium acetoacetate solution, as described in detail elsewhere (Pondelak et al., 2023). The lath was submerged in the solution and placed into a pressure chamber for 4 h. Initially submerged lath was exposed to a vacuum (0.6 bar absolute) for 30 min, followed by a 3-hour pressure treatment (10 bar absolute) and finally a 20-minute vacuum exposure (0.6 bar absolute) to remove excess solution. After impregnation, the lath was dried on the countertop for a week, and then post-treatment was carried out for 72 h in a climatic chamber (Kambič, Slovenia) at an elevated temperature (80° C) and fluctuating relative humidity between 40 and 95% (9 cycles with humidity fluctuations in total). The dry uptake of carbonates into the wood was about 10%.

Cutting on a Microtome

A sliding microtome (Leica SM2010, Germany) was used to cut the transverse surfaces of the samples for microscopic observation. Samples were mounted in such a way that they were cut in a tangential direction and with the blade at an angle of approximately 45° to the cutting direction (Fig. 2). Two different types of commonly used knives/blades were used: (1) fixed solid knife with "D" profile of the blade and (2) low-profile disposable blades (Leica DB80LS). Prior to cutting, the samples were conditioned under laboratory conditions (T = 20° C, RH = 65%) for several weeks. Cutting was either performed dry, without liquid water, or with water applied directly to the surface during cutting. For each surface, a fresh, unused part of the blade was used for the final cut.

Sanding and Polishing

Prior to sanding and polishing, the samples were moulded in the Varidur 20 acrylic mounting system (Buehler, USA). After curing, the samples were taken out of the moulds and sanded with a Beta vector grinder-polisher (Buehler, USA).

Fig. 2. Cutting with a sliding microtome equipped with a low-profile disposable blade.

Sandpapers of granulations P 600, P 1200, P 2500, and P 4000 were used for sanding. Diamond suspensions with grain sizes of 1 μm, 0.25 μm, and 0.03 μm were used for polishing. The load applied to the sample was set to 10 N for sanding and 5 N for polishing. The rotational speed of the sanding/polishing disc was set to 250 rpm. We used a steady water flow for rinsing and cooling the samples during sanding. Sanding and polishing were carried out in stages, sanding from the coarsest to the finest granulation, followed by polishing. Each sanding stage lasted 5 min, and each polishing stage lasted 8 min. For polished samples, the entire process described above was completed, while for sanded samples, the process was completed after sanding with P2500 grit sandpaper. After sanding and prior to polishing, preliminary observations were made using a CARL ZEISS AXIO Imager Z2 m metallographic microscope and AXIO VISION 9/2009 software (ZEISS Group, Germany) to ensure that the surface quality was sufficient for further processing.

Scanning Electron Microscopy (SEM) Observation

All samples were examined with a scanning electron microscope (JEOL IT 500 LV, Akashima, Japan). The samples were attached to the sample stage with a conductive carbon tape. The samples were not coated/sputtered. SEM observations were performed with a backscattered electron detector in a low-vacuum mode. An accelerated voltage of 10 kV was used at a working distance of 10 mm. Micrographs were captured at 200×, 500×, and 1000× magnification. The low-vacuum pressure was carefully adjusted (between 80 and 110 Pa) to optimize image quality and resolution while preventing surface charging. The bright white inclusions and linear features visible in some micrographs are not due to charging artifacts, but rather to include minerals or polishing/grinding traces, respectively.

Results and Discussion

Although wetting of the sample during the cutting is an established practice in the preparation of microscopic wood samples (Merela et al., 2020), we found that this method is also superior to dry cutting for mineralized wood samples and epoxy-embedded samples. Figure 3 shows a comparison between microscopic images of wet and dry-cut mineralized and epoxy-embedded sprucewood samples. As Jansen et al. (1998) found, the addition of water and glycerine makes cutting easier and reduces the risk of errors as the fragile section floats on the liquid and is not dragged against the knife. The cutting force is reduced, and the knife slides more easily through the sample, which also reduces the waving of the blade. With dry cutting, the slices broke during cutting and were difficult to handle, but with the addition of water, the percentage of successfully prepared slices was much higher, and handling the wet slices was easier.

Dry cutting resulted in delamination of the cell wall, which obstructed the view of the inside of the cell (Fig. 3a). With the addition of water during cutting, the delamination is significantly reduced (Fig. 3b), while the quality of the surface is still insufficient for further analyses. By embedding the samples in epoxy resin, the effect of cell wall delamination was practically eliminated (Figs. 3c, 3d). On the other hand, a wavy surface was obtained when the samples embedded in epoxy resin were cut dry (Fig. 3c). This was particularly evident when using the disposable blade. When the sample was cut with the addition of water, the cut was smoother (the surface was much less wavy) (Fig. 3d).

By wetting the sample during cutting, delamination in the cell wall was significantly reduced as the blade was able to slice through the soft tissue more easily. The vibrations of the blade are lower, and the overall movement of the blade is smoother compared to dry cutting. Quite a lot of residue remains on the surface of the dry-cut sample, while the addition of water during cutting leaves the surface more or less clean. We have observed that the thin slices break and crumble easily when cut dry, whilst the slices remain largely undamaged when cut with the addition of water. The addition of water during cutting proves to be the better option in wood research in most cases, but in cases where water could damage or alter the sample under observation, special care must be taken to skip this step or to determine the effect of water on such samples beforehand. From this point onwards, all cutting tests were carried out with the addition of water.

When hard material is introduced (in our cases, epoxy-embedded wood and mineralized wood), the special attention must be given to the cutting technique. Mineral inclusions are the cause of faster dulling of the disposable blades because the cutting edge breaks off (Fig. 4a). Disposable blades are much thinner and have a smaller cutting angle compared to the fixed knife. The cutting edge can break off in a single pass of the knife over a hard inclusion. As can be seen in the micrograph (Fig. 4b), the path of the blade to the mineral inclusion was relatively smooth, and after the blade hit the mineral inclusion, the surface was rough (red arrows), indicating that the blade was blunted on the first pass over the inclusions. As the disposable blades quickly become blunt on mineralized wood, we recommend not using them in such cases, but using a fixed knife instead, which is less sensitive to hard inclusions. While the fixed knife also becomes dull when cutting hard material, its greater rigidity and better resistance to deflection ensure that the cutting edge does not break off or chip, making it significantly less sensitive to hard inclusions and resulting in less pronounced dulling effects.

Comparison between a fixed knife and a disposable blade is clearly presented on the micrographs in Figure 5. The disposable blade tends to produce more artefacts on the surface, such as a roughened surface on mineralized wood embedded in epoxy resin (Fig. 4b) and a wavy or uneven surface (Figs. 5d, 5f).

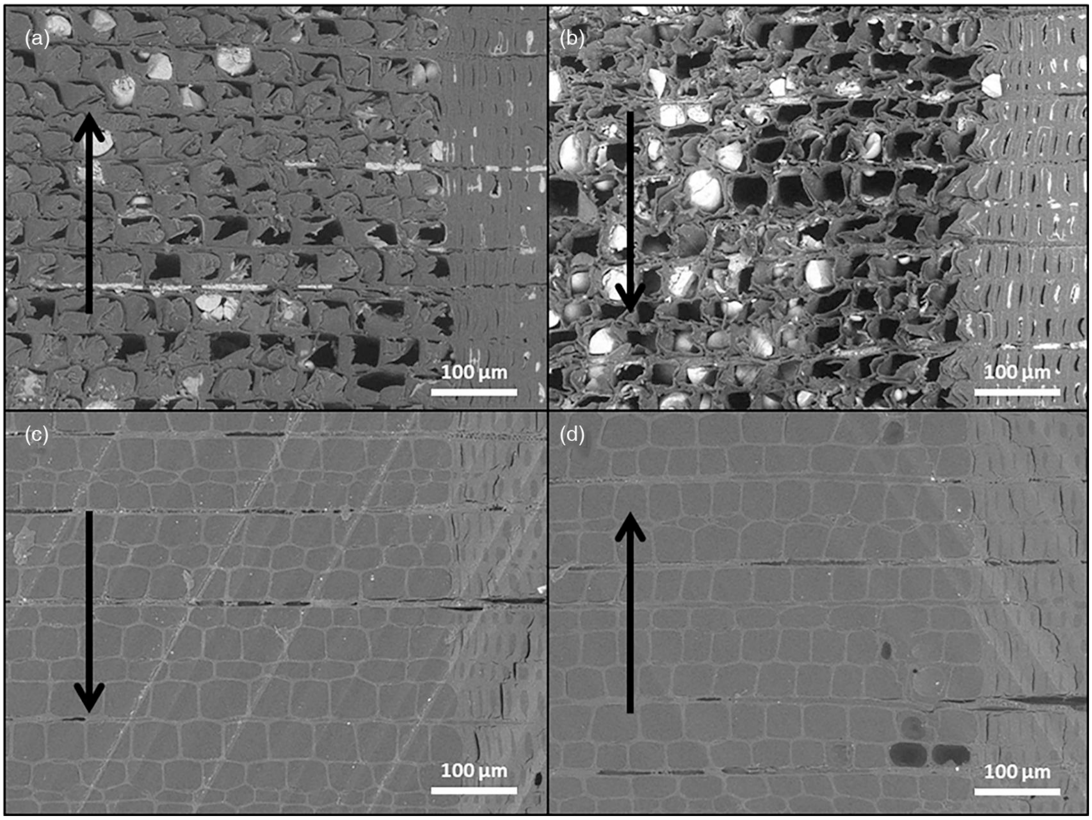

Fig. 3. Comparison between dry cutting (**a, c**) and wet cutting (**b, d**). Micrographs of a mineralized sample cut with a fixed knife (**a, b**) and an epoxy-embedded reference sample cut with a disposable blade (**c, d**). The cutting direction is marked with a black arrow.

Although the fixed knife is not as sharp as the disposable blade, it produces a better cut surface of the mineralized samples, as it is not affected as much by the hard mineral inclusions. Additionally, the fixed knife is more rigid and produces far fewer vibration-related artefacts, such as a wavy or uneven surface. The wavy surface is obvious when observing samples at low magnification, but at higher magnifications (e.g., 1000×), this defect is no longer so obvious. It should be noted that the wavy surface created with a disposable blade was most apparent when cutting epoxy-embedded samples, whereas this effect was much less pronounced with non-embedded samples. This makes sense as the embedded samples are much more difficult to cut compared to the non-embedded samples. The disposable blades are sharper and produce clean cuts in the reference samples (Fig. 5b) compared to the fixed knife (Fig. 5a). It is obvious that the fixed knife did not give satisfactory results as the delamination of the cell wall was quite severe (Fig. 5a). The disposable blade, on the other hand, produced a much higher quality surface with very little delamination and clearly visible structure (Fig. 5b). In the case of reference samples embedded with epoxy resin, both blades produced satisfactory results (Figs. 5c, 5d). The surface produced with the fixed knife is flatter (Fig. 5c) than the wavy surface produced with the disposable blade (Fig. 5d). The same can be observed for mineralized, embedded samples (Figs. 5e, 5f). One can also immediately see the smoother cutting surface produced by the disposable blade (Fig. 5f) compared to the cutting surface of the fixed knife (Fig. 5e). The surface produced by a fixed knife is much rougher, but flatter compared to that produced by the disposable blade. The cut surface of the mineralized samples embedded in epoxy resin is unsatisfactory in either case. The path of the fixed knife is smoother and has less resistance. The disposable blade generally cuts more easily, but in some samples embedded in epoxy resin, it also showed some resistance.

This resistance is related to the deflection of the disposable blade (which is not very rigid) and tends to follow the morphology of the sample. This usually results in an uneven surface and an uneven thickness of the slice. The choice of the blade depends mainly on the material to be processed. The fixed knife is not as sharp, which leads to artefacts such as delamination, torn fibres (instead of cut fibres), cracks in the slice, etc., but on the other hand, the flexible nature of the disposable blade also leads to certain artefacts: mainly wavy and uneven surfaces, in some cases even rapid knife damage. Based on the data from this analysis, it can be said that when cutting fresh or unmodified wood, it is best to use the disposable blade with water added, while for hard materials, very heterogeneous materials, and wood with very hard inclusions (epoxy-embedded wood and/or mineralized wood), a fixed knife works better. In general, the cutting quality of the disposable blade is not surpassed by the cutting quality of the fixed knife, but as it turns out, the quality of the fixed knife cannot be neglected either.

Sanding and polishing are basically just two sides of the same coin. Both techniques differ from cutting mainly in that material is removed from the surface. Cutting can remove a relatively large, single piece of material, whereas sanding and polishing produce a lot of small particles that need to be removed. This creates a problem because the particles produced during sanding and polishing are small enough to penetrate into cavities in the wood (mainly lumina) (Figs. 6a, 6c). It is very difficult to remove the debris from the sample prior to

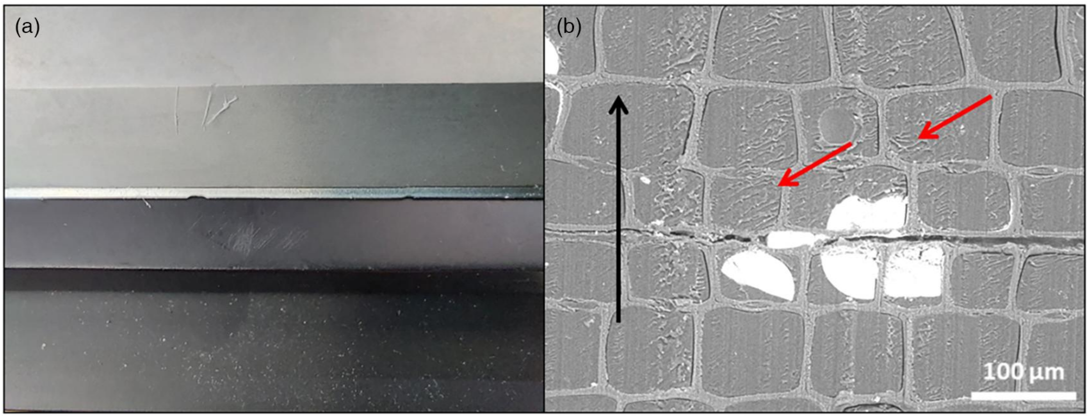

Fig. 4. (**a**) The damage to the disposable blade after cutting the epoxy-embedded mineralized sample; (**b**) Knife dulling effect during cutting of the mineralized sample. The dulling effect is marked by two arrows in the center of the image, while the cutting direction is indicated by the left arrow.

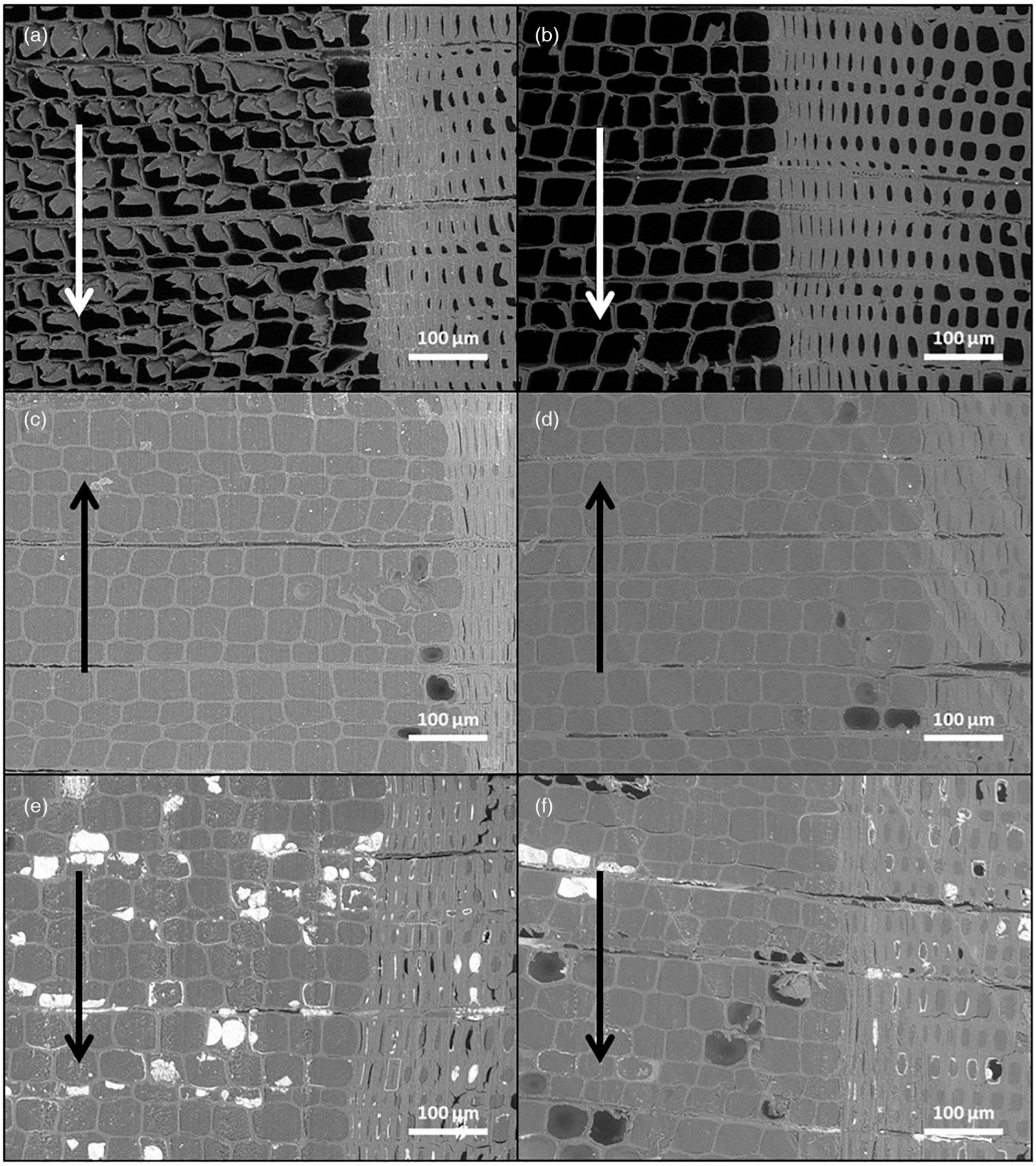

Fig. 5. Comparison between wet cutting with a fixed knife (**a, c, e**) and a disposable blade (**b, d, f**) on: the reference sample (**a, b**), the epoxy-embedded reference sample (**c, d**), and the epoxy-embedded mineralized sample (**e, f**). The cutting direction is marked with a white (**a,b**) or black arrow (**c–f**).

observation, even by blowing with compressed air or washing with isopropanol/water. Because non-embedded materials tend to collect debris in voids, cleaning was difficult. While testing other methods (compressed air blowing, rinsing with a water jet, and vacuuming), we found that adding water during sanding or polishing was necessary and sufficient. Sonication and alternative solvents were excluded to keep the process simple and fast, and to avoid potential artefacts or absorption of foreign substances. For this reason, we embedded our samples in epoxy resin before sanding and polishing. The increased hardness of the samples after epoxy resin impregnation contributes to better sanding and polishing. The comparison of the micrographs of the sanded samples without epoxy resin (Figs. 6a, 6c) and the samples embedded with epoxy resin (Figs. 6b, 6d) shows that the samples should be embedded in order to obtain a usable surface for observation. The surface of the earlywood on the sample without epoxy resin is practically indistinct, while the individual cells in the latewood are visible. The empty spaces within the cells are completely filled with debris. In the mineralized sample (Fig. 6c), it is not possible to distinguish individual mineral inclusions in the image, let alone analyse them.

In the case of sanding, the embedded samples turn out well. The surface prepared by sanding is very even, and in the case of mineralized wood, the inclusions did not affect the dulling of the sandpaper so much. The sandpaper does not tear out the individual inclusions, so in principle they are all in place (which is difficult to say when cutting), and the sandpaper does not dull so quickly. The quality of the sanded surface is therefore predictable.

As can be seen in Figure 7a, there are much debris in the tracheids of the earlywood after polishing, and similar to the sanded sample, the structure of the wood is difficult to recognise. In contrast, the tracheids of the latewood were polished well, their structure is clearly visible, and there are far fewer deposits in the tracheid voids. Significantly better results were obtained when polishing the samples embedded in epoxy resin. In Figure 7b polished sample is shown in which the empty tracheid lumina are filled with epoxy resin, and the fragile structure of the earlywood is supported during polishing. The result is clean samples (no space for debris) with a clearly visible structure.

In the case of the non-embedded mineralized sample, the mineral inclusions are not visible due to the accumulation of debris in the empty spaces of the cells (Fig. 7c). In this respect, polishing and sanding of non-embedded samples result in a similarly poor surface. Figure 7d shows a polished embedded mineralized sample. The quality of the prepared surface, the definition of the anatomical elements, and the clearly visible mineral inclusions in this image indicate the great advantage of polishing the embedded samples compared to other methods. As already mentioned, the epoxy resin acts as a binder and ensures that the inclusions do not move and that the wood structure is strong and rigid. This helps to improve the polishing, and the result is a really high-quality surface suitable for even the most demanding analyses.

Although an air jet (commonly used for removing dust from samples and polishing discs) was initially applied, a substantial amount of dust remained on the surface of the samples. We determined that rinsing with water during sanding was a more effective method for removing dust particles. Samples embedded in epoxy resin are very suitable for this procedure, as they are not as prone to cracking when repeatedly exposed to water and dried. Some cracks formed when the polished samples were rinsed and dried, but they were not as large as reported by Wu (1998). Rinsing with water is impossible when polishing with diamond suspensions. As a result, there are some debris on the surface and empty cells of the non-embedded samples that need to be considered before further analyses.

We have observed that the non-embedded samples quickly absorb water during polishing, which is not a problem in itself, but the diamond suspensions used for fine polishing are also absorbed into the sample. Firstly, this reduces the number of polishing grains, and secondly, all particles that have been absorbed with the water in the sample (including sanding debris and polishing grains) remain in the sample, which must be taken into account. In contrast, no such problems were observed when polishing samples embedded in epoxy resin. Additionally, they run more smoothly on the sanding disc, and there is virtually no absorption of water or particles into the sample. It is worth mentioning that the sanding of the samples embedded in epoxy resin is slightly slower compared to the reference samples, which makes sense as the hardness is also increased. This must be taken into account as these two types of samples are better not to be sanded at the same time, as more material would be removed from the non-embedded sample.

A direct comparison between sanded and polished samples is shown in Figure 8 using an example of reference and mineralized sprucewood, both embedded in epoxy resin. A comparison between sanded and polished samples shows that these samples are quite comparable at lower magnifications (200×) (Figs. 8a, 8b), while at higher magnifications (500×), it becomes clear that polished samples are more suitable for observation and analyses (Figs. 8c–8f). As can be seen in Figure 8a, sanding with P2500 grit sandpaper at 200× magnification gives a high-quality image as no grinding marks are visible on the surface. At 500× or 1000× magnification, the surface of the sanded sample is much coarser (Figs. 8c, 8e) compared to the polished sample, observed at the same magnifications (Figs. 8d, 8f). Traces of abrasive grains can be seen on the surface in all directions, especially on the epoxy resin. This is due to the rotation of the sample and the rotation of the sanding disc during sample preparation, as well as the fact that the cell wall is not as hard as the epoxy resin and can bend a little when the abrasive grain hits it. Therefore, the cutting depth of the individual grains in the cell wall is less than that in the epoxy resin. For many purposes, these traces are not a problem, but we recommend observing polished samples instead of sanded samples for accurate analyses and obtaining correct images. The main advantage of sanding over polishing is the shorter sample preparation time and slightly lower cost. We believe that for most applications it makes sense to polish the samples, but in a few cases (e.g., for ongoing quality control or observation at low magnifications) sanding alone is sufficient.

In some cases, the image of the polished samples still shows traces of abrasive grains (Fig. 8f), which can be attributed to a too short polishing time or too deep grooves after the last sanding before polishing. This can be solved by extending the polishing time or by more frequent intermediate inspections before polishing.

Embedding samples in epoxy resin results in rigid, reusable samples that can be reused multiple times and re-polished if required. However, when observing with the SEM, the epoxy

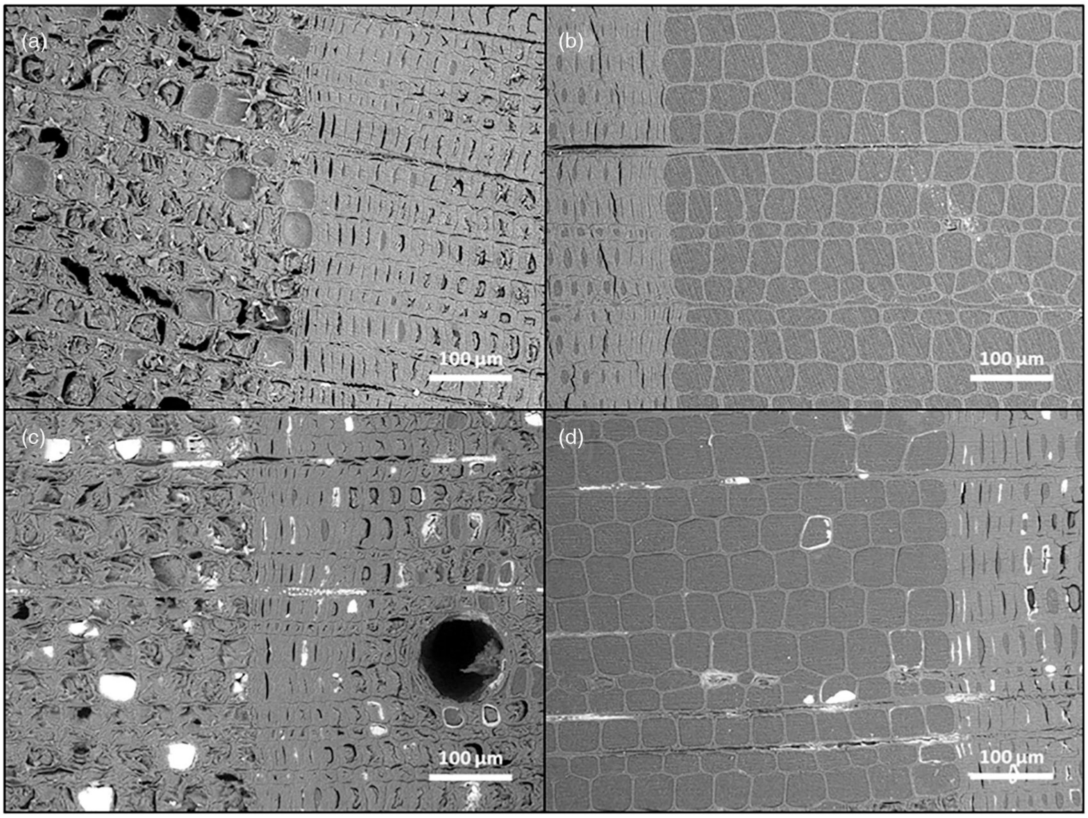

Fig. 6. Micrographs of sanded samples: (**a**) reference sample; (**b**) epoxy-embedded reference sample; (**c**) mineralized sample; (**d**) epoxy-embedded mineralized sample.

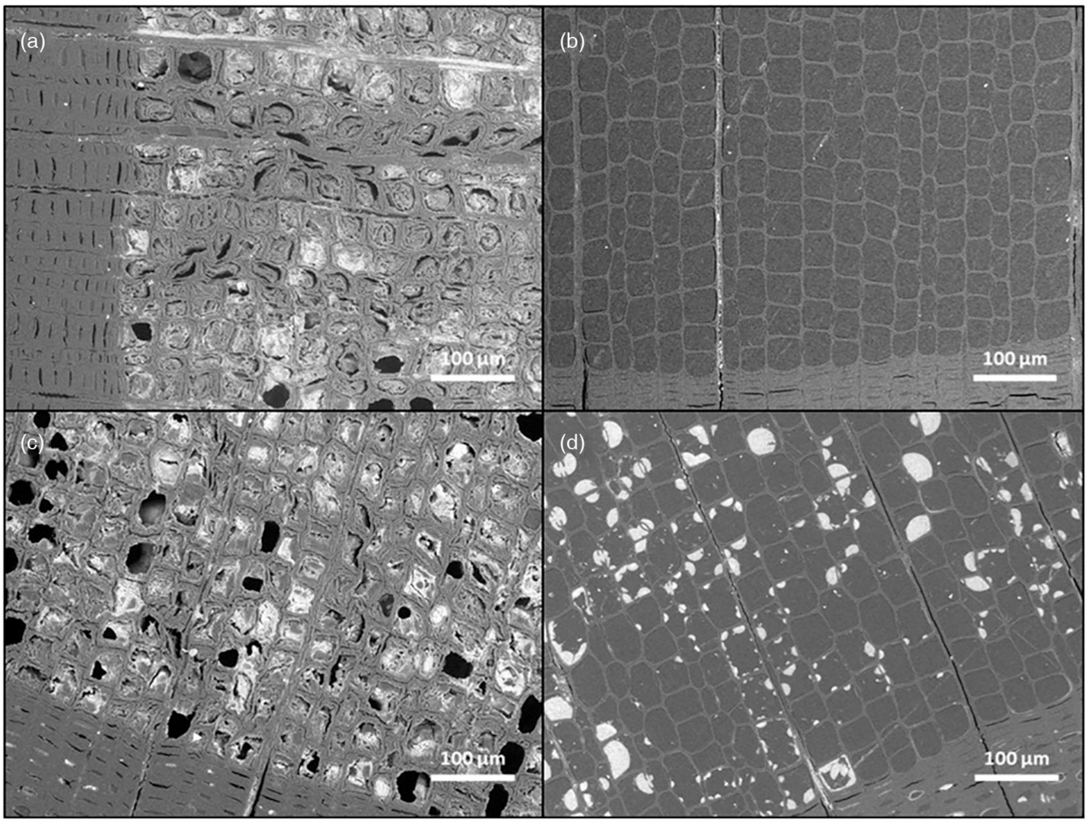

Fig. 7. Micrographs of polished samples: (**a**) reference sample; (**b**) epoxy-embedded reference sample; (**c**) mineralized sample; (**d**) epoxy-embedded mineralized sample.

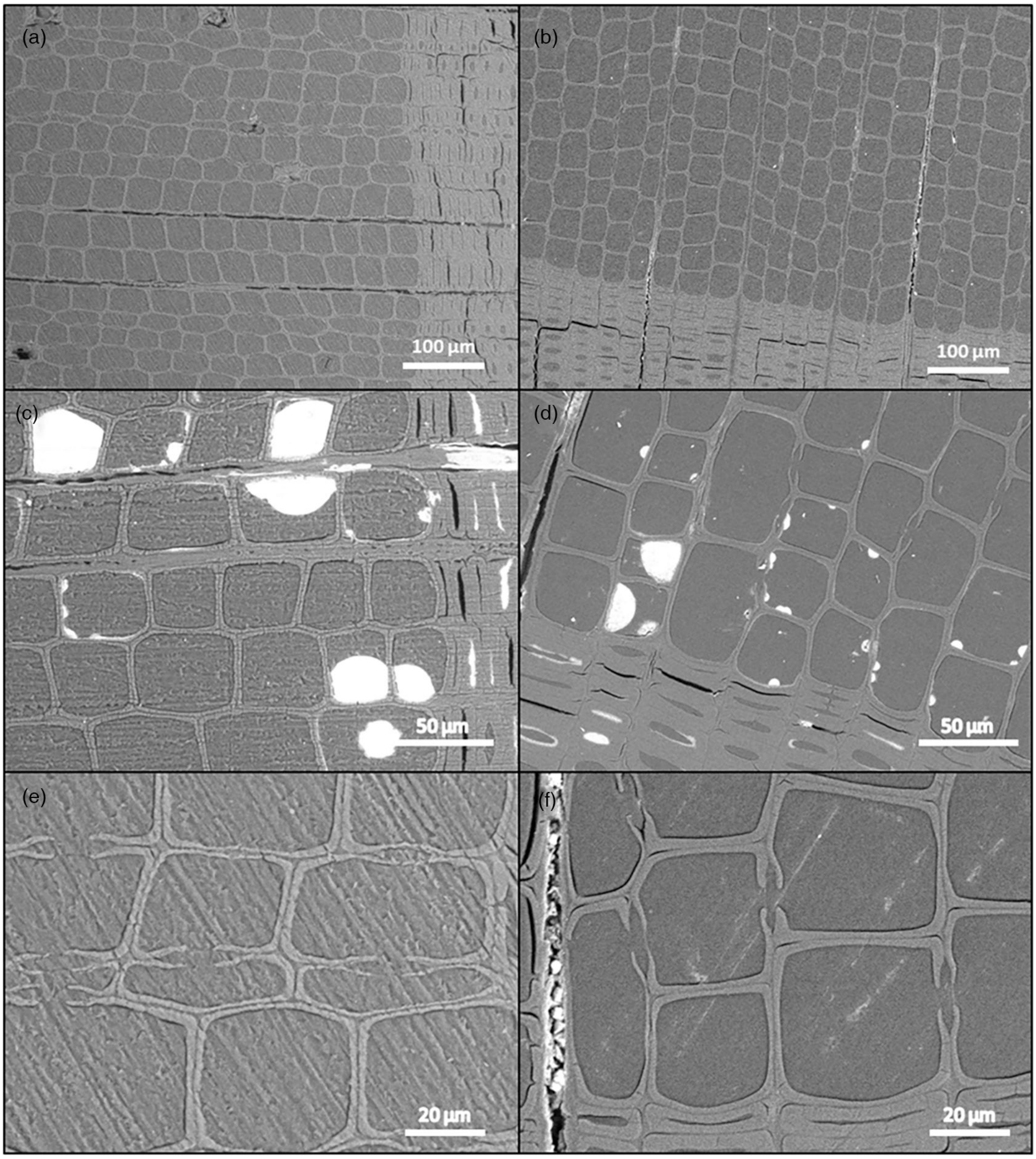

Fig. 8. Comparison between sanded (**a, c, e**) and polished (**b, d, f**) samples. Micrographs of epoxy-embedded reference samples (**a, b, e, f**) and epoxy-embedded mineralized samples (**c, d**).

resin fills the empty spaces inside the wood, making it impossible to see the inside of the cells. Considering that the SEM is known to allow 3D viewing (in depth) to a certain extent, this is quite a disadvantage. On the other hand, the epoxy resin impregnation hardens and stabilises the sample very well, which is particularly useful for fragile historical or degraded samples known for their fragility and, in some cases, rarity. The defects that usually occur during cutting (chipping, delamination of the cell wall, collapse of the structure, etc.) do not occur with this method. At high magnifications, the surface quality of epoxy-embedded and sanded polished samples is still very good (Figs. 8c–8f) and allows very accurate analyses. Samples embedded in epoxy resin are less sensitive to water and less prone to cracking due to drying out under the harsh conditions in the microscope and are generally less sensitive to handling. Without epoxy resin, neither sanding nor polishing makes sense, as the samples are damaged and the debris remains in the voids (Figs. 6a, 6c, 7a, 7c). Very little is visible, and even that is out of place. In general, embedding samples in epoxy resin is very practical in many cases, but if you want to see the sample "in depth", it is no longer appropriate. It is also important to note that the epoxy resin is primarily used here to harden and stabilize the sample to facilitate preparation, and the resulting composite does not reflect the structural integrity or mechanical properties of structural lumber; consequently, care must be taken if interpreting the presented microstructures for structural analysis.

The comparison between cutting and polishing is the most interesting, as both methods can lead to an excellent result, but not neither are suitable for mineralized wood. In Figure 9, a direct comparison of the two methods can be seen. Figures 9a and 9c represent cut non-embedded samples, while Figures 9b and 9d represent polished embedded samples. In the latter, the clear structure of the wood stands out

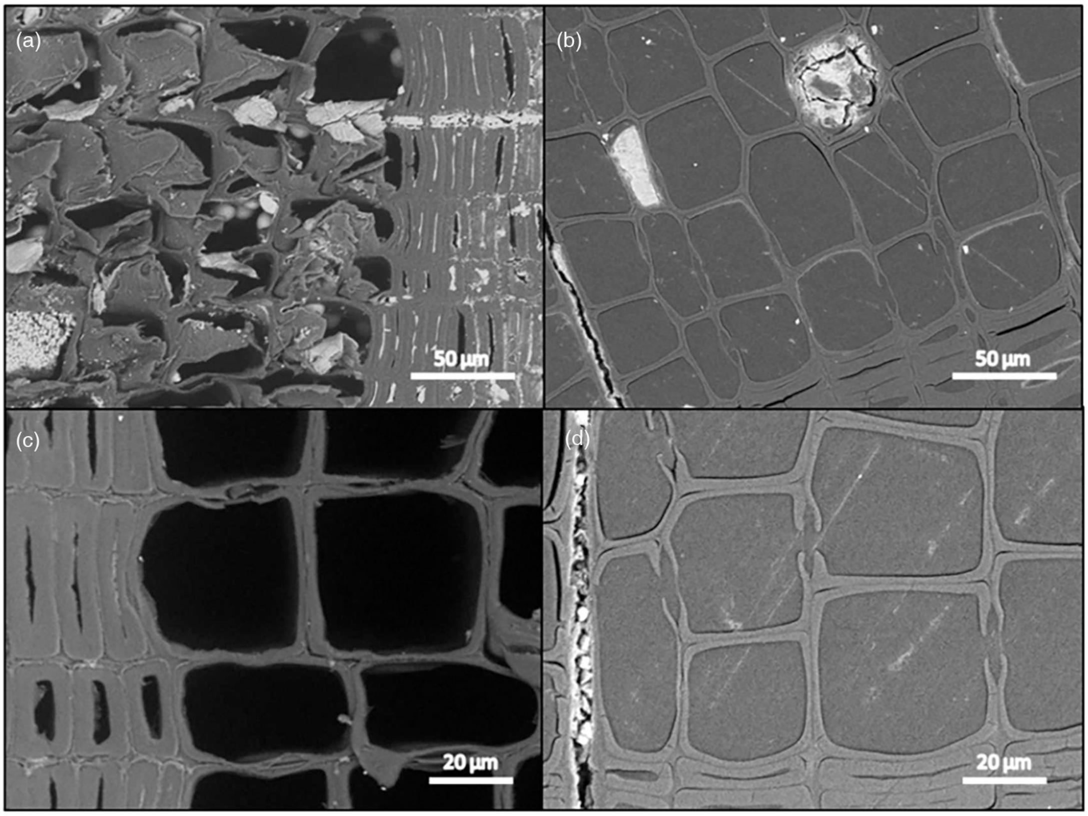

Fig. 9. Comparison between cut non-embedded (**a, c**) and polished epoxy-embedded (**b, d**) samples. Micrographs of the wet cut surface of mineralized (**a**) and reference (**c**) samples, and polished surfaces of epoxy-embedded mineralized (**b**) and reference (**d**) samples.

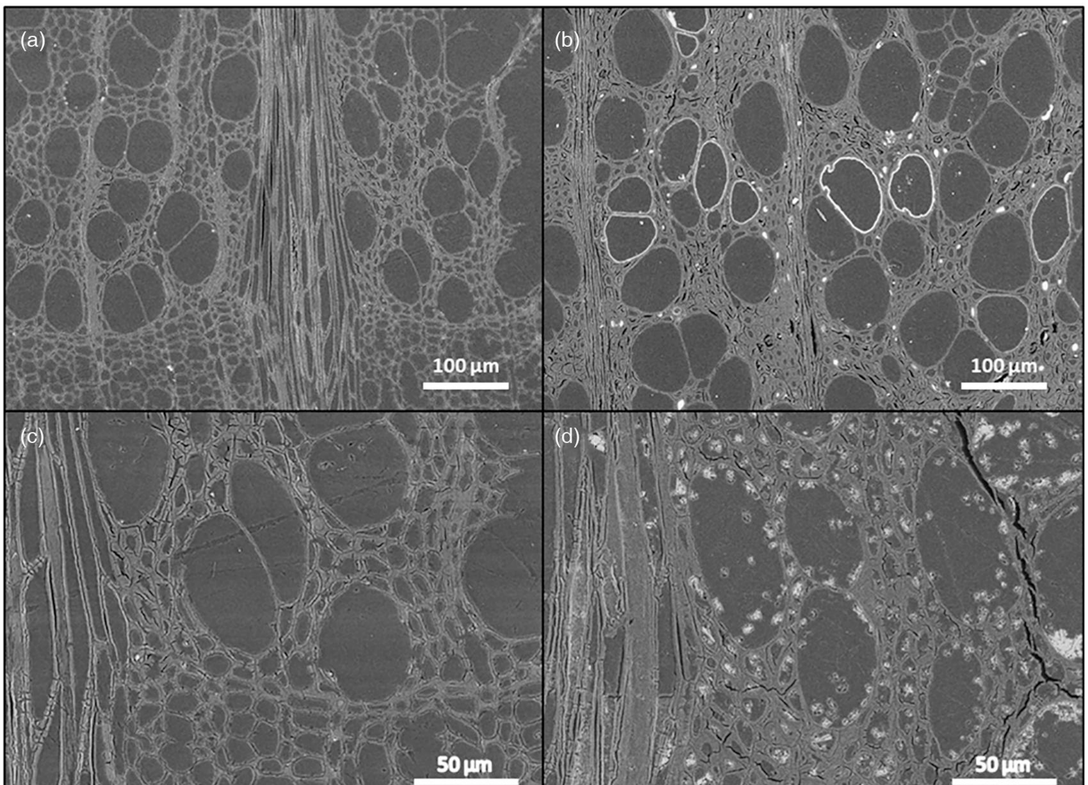

Fig. 10. Micrographs of epoxy-embedded and polished degraded beechwood (Fagus sylvatica) samples at 200× magnification: (**a**) reference, (**b**) mineralized; and at 500× magnification: (**c**) reference, (**d**) mineralized.

the most. In contrast to the cut, non-embedded sample (Fig. 9c), the piths in the cell walls of the earlywood are very clearly visible; in some cases, even the position of the pith membranes (Fig. 9d). This shows that this technique is suitable for observing the pith membranes in cross-section (Figs. 9b, 9d). It should be noted at this point that the aspiration of the piths could also be caused by a rather harsh epoxy impregnation. On the other hand, in the polished samples embedded in epoxy resin, we cannot see what is immediately below the surface (as in the cut sample). In the cut sample, mineral inclusions are visible on the inside of the cell wall just below the cut surface (Fig. 9a), which is not the case with the polished sample (Fig. 9b).

The great advantage of polishing embedded samples before cutting non-embedded samples becomes clear when you compare images of mineralized wood. Since a very hard material is inside a softer "matrix", this poses a problem when cutting. As we have already described, the knife is damaged by the hard inclusion when cutting, resulting in a poorly cut surface. We have found that polishing is the best method for preparing samples of mineralized wood. In this case, we can conclude that epoxy resin embedding with further grinding and polishing, unlike cutting, does not damage the samples, which is consistent with the conclusions of Mukhamadiyarov et al. (2016).

It should be noted that the preparation of a well-cut sample requires specialized equipment and considerable skill and experience. In contrast, the preparation of a polished sample requires only a grinding/polishing machine and minimal training. Even inexperienced researchers can achieve high-quality, polished wood samples with a little training. If the surface quality of the polished sample is not sufficient, the final polishing steps can be simply repeated on the same sample with minimal damage to the sample.

Epoxy-Embedded and Polished Degraded Wood

Due to the aforementioned advantages, the polishing process was chosen for the preparation of highly degraded and mineralized beechwood (*Fagus sylvatica*) samples for SEM analysis. The impregnation of degraded beechwood with epoxy resin proved to be easier than the impregnation of sprucewood, which is to be expected given the higher porosity. Polished samples of degraded wood exhibited high-quality surfaces suitable for microscopic analysis (Fig. 10). The epoxy resin acts as a binder when the wood structure has degraded to such an extent that the sample disintegrates, as can be seen in the top right-hand corner of Figure 10a. The epoxy resin hardens the material and fills the empty spaces (lumina) so that the anatomical elements remain in place during preparation, which makes preparation of the sample much easier. On the other hand, the epoxy resin impregnation process itself is relatively harsh, and care must be taken not to alter or damage the sample. During the impregnation process, loose residues could be pushed around the wood. The effect of impregnation on the integrity of damaged samples was not investigated in this study. Figures 10a and 10c show the extent of cell wall thinning in beechwood as a result of fungal degradation. The sample was degraded to such an extent that the cell wall is no longer present in some places (Figs. 10a, 10c). The mineralized sample is significantly less degraded (Figs. 10b, 10d).

Degraded mineralized wood was considered to be the most difficult of the selected materials to prepare, as it contains very hard mineral inclusions in a relatively soft and highly degraded matrix, which has very poor mechanical properties. This example shows the greatest advantage of the proposed method. We also found that degraded wood embedded in epoxy resin is more susceptible to cracking than sound wood embedded in epoxy resin. The same applies to mineralized wood. A large crack can be seen in Figure 10d, which is due to the fact that the sample was dried after preparation and before SEM observation. This crack could have been avoided, at least to a certain extent, if the sample had been dried more slowly. A clear difference in the morphology of mineral inclusions can be observed between degraded mineralized wood (Figs. 10b, 10d) and non-degraded mineralized wood (Figs. 8c, 8d). The mineral inclusions introduced into the wood undergo morphological changes after being exposed to fungi. This phenomenon has already been observed and has been documented in a published study by Repič et al. (2022).

Conclusions

This study demonstrates the feasibility of preparing high-quality microscopic samples using mechanical preparation methods. Our results show that polishing is a viable alternative to microtome cutting when preparing the samples for SEM. Although the data presented here were generated using professional laboratory grinding and polishing equipment, the underlying methodology is based on simple mechanical principles. The procedure is accessible because it requires only a grinding/polishing machine, suggesting that comparable results could be achieved using basic grinding/polishing devices similar to laboratory equipment. Epoxy impregnation followed by polishing offers an alternative to cutting for preparing degraded or heterogeneous samples. The process is also simple, time-saving, and requires minimal training. We believe that this procedure can assist researchers in dealing with delicate materials and facilitate the preparation of wood samples for microscopic observation. It should be noted that this study focused exclusively on preparing the transverse surface, as it presents the most complex challenges. The method is expected to be readily applicable to the less demanding tangential and radial planes typically required for a full anatomical description.

Both polishing and cutting have produced satisfactory results. The choice of method depends on the needs and requirements of the researcher. If deeper observation is desired or there are concerns about the undesirable effects of epoxy resin impregnation, cutting may be the preferred method. However, for very delicate and fragile samples or those containing a significant amount of minerals or hard inclusions, epoxy resin impregnation with polishing offers an accessible and affordable alternative to cutting that ensures sufficient quality of the prepared surface. As noted by Witovisk et al. (2014), epoxy impregnation with polishing has been confirmed to provide long-term preservation of samples, which can be stored and re-polished and analysed in the future if required.

As far as cutting is concerned, it was found that the best prepared surface of the reference wood is the one cut with a disposable knife and continuous wetting of the sample with water. However, in the case of mineralized wood (wood with many mineral inclusions) and wood embedded in epoxy resin, it was found that better results can be achieved with a fixed knife. A disposable blade cuts very well but tends to dull and break when it encounters hard mineral inclusions, and it flexes and creates a wavy surface when cutting hard, embedded samples, whereas a fixed knife is not as sharp, penetrates the material slightly harder, but is stiffer and creates a flatter surface. In both cases—disposable blades or fixed knives—it is important to keep the blade as sharp as possible and to keep the surface of the sample wet during cutting.

In this study, it was found that the embedding of degraded samples is quite easy, as the epoxy resin easily penetrates them, while the impregnation of the sprucewood is much more difficult, but still possible with vacuum-pressure impregnation. The depth in this case is sufficient to facilitate polishing, but care must be taken not to remove too much material, as there are voids below the penetration depth of the epoxy. If we had to suggest a method for individual samples, we would say that reference wood is best prepared with a disposable blade, for very hard or epoxy-embedded samples, we would choose a fixed knife, while heterogeneous mineralized or degraded wood is best prepared by polishing. The latter is also very suitable as an alternative to cutting embedded samples.

Availability of Data and Materials

No data was used for the research described in the article. Data is available on request to the corresponding author.

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Author Contributions Statement

R.R. and A.S.Š. conceptualization. R.R., M.M., and A.P. methodology. R.R. data curation. R.R., M.M., and A.P. formal analysis. A.S.Š. funding acquisitions and supervision. R.R. writing first draft. M.M. and A.S.Š. review and editing.

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Conflict of Interests

The authors declare that they have no conflict of interest with the contents of this article. All the authors agreed with the publication.

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