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Applying methods of hard tissues preparation for wood anatomy: Imaging polished samples embedded in polymethylmethacrylate

Alberto Arzac[a,b,*], José M. López-Cepero[a,c], Elena A. Babushkina[d], Santiago Gomez[c]

[a] Siberian Federal University, 79 Svobodny pr., 660041 Krasnoyarsk, Russia
[b] V. N. Sukachev Institute of Forest, Siberian Branch of the Russian Academy of Sciences, Akademgorodok 50/28, 660036 Krasnoyarsk, Russia
[c] Department of Pathological Anatomy, Faculty of Medicine, University of Cadiz, Plaza Falla 9, 11003 Cadiz, Spain
[d] Khakass Technical Institute, Siberian Federal University, 27 Shchetinkina St., 655017 Abakan, Russia

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ABSTRACT

Cambial activity records short and long-term environmental signals in xylem anatomy, creating a permanent archive. Quantitative wood anatomy deciphers the relationship between cell structure and function in a spatiotemporal context. Obtaining high-resolution images of wood anatomical preparations is a critical stage in the process of decoding this information. Damage to cellular structures when sectioning by microtome is one of the main problems in the preparation of high-quality micro-sections. Cell damage leads to the occurrence of artifacts – most often related to broken cell walls – hindering the performance of image recognition programs, and increasing the time spent on the manual editing of images. In this work, we propose an alternative method to microtomy, based on embedding-polishing protocols established for hard tissue preparation. Wood samples are embedded in a transparent and non-reactive resin as polymethylmethacrylate (PMM) that is subsequently ground and polished. Being able to acquire images from the stained or unstained polished surfaces of the PMM-blocks and sections (thinner than 100 μm) by using a wide range of optical methods such as reflected polarizing microscopy, epifluorescence microscopy, bright-field microscopy with diffuse illumination and circularly polarizing microscopy. This embedding method improves the mechanical integrity and quality of wood anatomical preparations, eliminating the problem of broken cell walls. Furthermore, this technique allows the preparation and analysis of large tissue surfaces.

1. Introduction

Cambial activity records short- and long-term environmental signals in xylem anatomy, encoding information at inter- and intra-annual resolutions on the adaptation of plants to their environment (Arzac et al., 2018a, 2018b; Olano et al., 2013, 2012; Vaganov et al., 2011). Wood analysis using quantitative wood anatomy (QWA) techniques has significant potential to decrypt this information and reconstruct spatio-temporal environmental series based on the structural and functional characteristics of cells (Fonti et al., 2010).

Quantitative wood anatomy has been a research topic for a long time (see Speer, 2010); however its advance was hindered by the strenuous effort needed to obtain high sample sizes. The rapid evolution of digital imaging technology and image recognition programs (e.g., ROXAS, von Arx and Carrer, 2014) has changed this panorama and has accelerated the progress of QWA in recent times. The potential of QWA extends to the study of many parameters in the xylem, such as cell number (vessels and tracheids), cell size (e.g., lumen diameter and cell wall thickness), ray parenchyma features, and spatial arrangement of cells within the tree ring. Nevertheless, the accuracy and speed of QWA analysis are closely linked to the quality of wood anatomical preparations required for anatomical high-resolution images acquisition (von Arx et al., 2016).

Sectioning for wood anatomy analysis is commonly based on microtomy protocols (Gärtner and Schweingruber, 2013; Schweingruber and Poschlod, 2005), where the stability of the blade during cutting is crucial in order to obtain high-quality micro-sections of 10–15 μm thickness. However, when the blades cut structures with different density (as with lumen or wall dominated areas in conifers), it may occasionally fracture the cell wall (mainly in earlywood cells), creating artifacts that make difficult the automatic cell recognition, thereby increasing the time spent on manual editing. Different embedding techniques such as corn/rice starch (Schneider and Gärtner, 2013) or paraffin (Rossi et al., 2006) used before sectioning fills cell cavities and

* Corresponding author at: Siberian Federal University, 79 Svobodny pr., 660041 Krasnoyarsk, Russia.
E-mail address: aarzak@sfu-kras.ru (A. Arzac).

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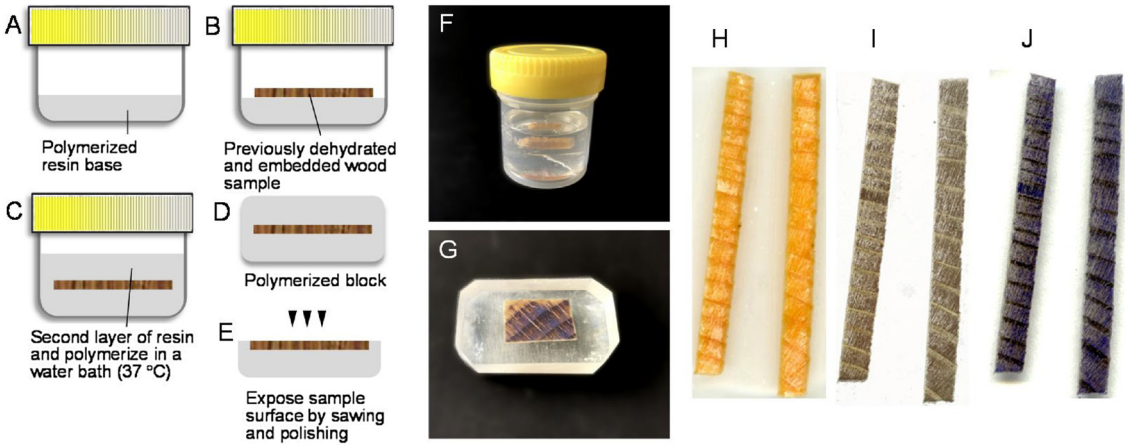

Fig. 1. Scheme for the preparation of the PMM-block (A–C); PPM-block surfaced by sawing or grinding and (D and E); polymerized PMM-block with the infiltrated sample in a polypropylene container together with polyethylene screw cap (F); trimmed PMM-block with the sample surface exposed and polished (G); sections of PMM-block glued to an acrylic slide (3mm-thick) (H); ground & polished sections (< 100 µm thick), unstained (I) and stained (J). Tissues in figures H–J are 45 × 4 mm.

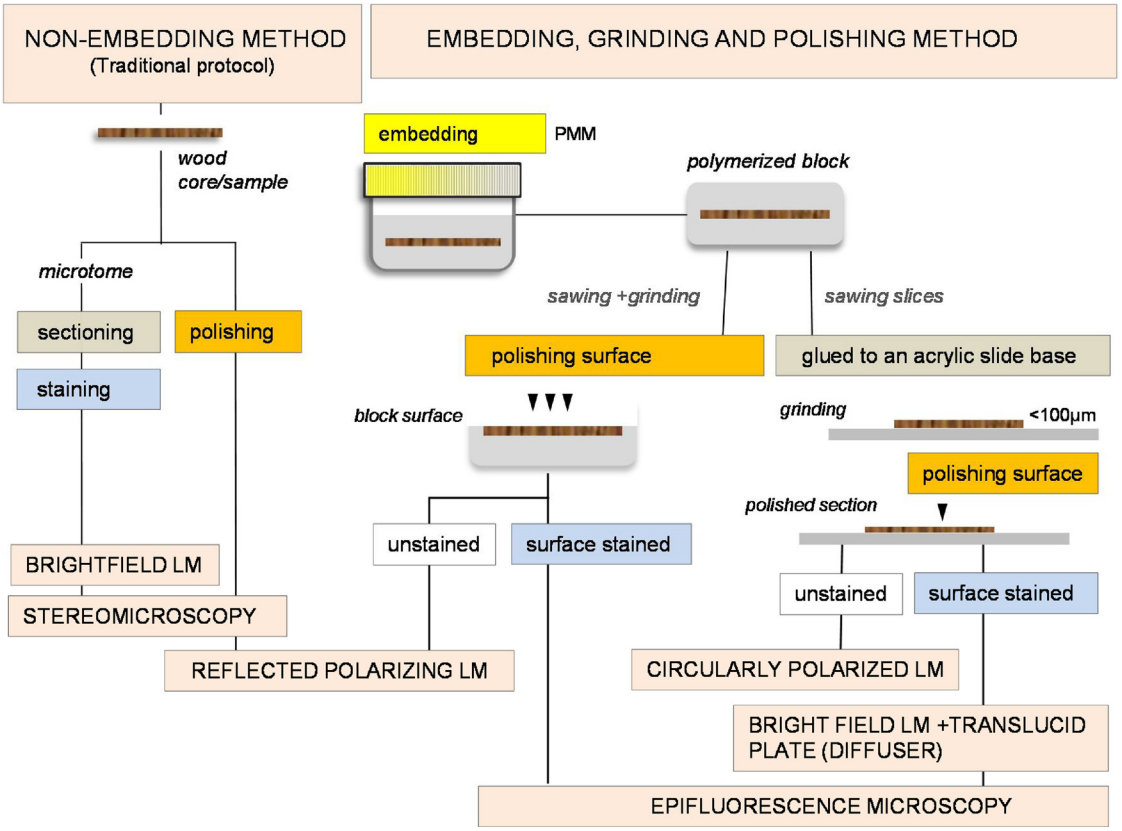

Fig. 2. Flowchart of preparation methods and optical microscopy strategies for imaging block surfaces and sections. The "non-embedding method" refers to the traditionally used protocol in the preparation of micro-sections for QWA, whereas the "embedding, grinding and polishing method" refers to the process described in detail this paper.

increases their resistance, improving cell structure stabilization and considerably decreasing the occurrence of these artifacts, leading to higher quality micro-sections. Although these methods help to improve the quality of micro-sections, the existence of differences between the density of the embedding substance and the wood material, the cutting speed and the clearance angle of the blade might still contribute to the occurrence of artifacts.

In this work, we propose an alternative to wood micro-sectioning based on the translation of techniques developed for hard tissue preparations to obtain a high-quality wood anatomical image. This technique is based upon the embedding of the wood sample in a transparent and non-reactive resin (polymethylmethacrylate, PMM), to then be ground and polished to a highly smooth surface (Bromage et al., 2018; Schenk et al., 1984) thereby allowing the observation by different illumination methods (e.g., reflected light, transmitted diffuse light and polarized light). Although the embedding of hard tissues has been used since the mid of the twentieth century (An et al., 2003; Schenk et al., 1984), as a technique for the study of different tissues (e.g., bones, teeth, fossils, seeds and paleobotanic material), it shares similar protocols with metallography techniques (Benedict, 2015; Jones and Rowe,