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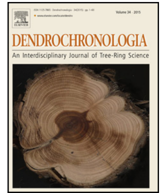

TECHNICAL NOTE

Preparing micro sections of entire (dry) conifer increment cores for wood anatomical time-series analyses

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ABSTRACT

The type of samples most commonly used in dendro sciences are increment cores of conifers. These cores allow for an easy determination and measurement of ring-width variations over long time periods. For wood anatomical analyses, the cores have to be split into pieces to enable the preparation of high quality micro sections for detailed measurements of cell properties. A major drawback of this procedure is the fact that it is labor intensive and time consuming. We present a new technique enabling the preparation of micro sections of entire increment cores up to a length of 40 cm. For that purpose we combined standard wood-anatomical techniques with the application of Mowiol glue and common Tesa tape. We tested the introduced method on increment cores of *Larix decidua* Mill. sampled years ago for ring-width analyses to reanalyze them on a microscopic level. The ability to cut these long sections will tremendously reduce the time needed to prepare micro sections. This is of special interest for wood anatomical image analyses of cores used before to create long ring-width chronologies for any kind of environmental reconstructions.
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Introduction

Integrating wood anatomy in tree-ring sciences is recently gaining in importance regarding the effect of environmental change on the growth and development of woody plants (Rossi et al., 2013; Ziaco et al., 2014). The potential of analyzing cell variations within the annual rings of trees or even shrubs and herbs allows for intra-annual reconstructions of past environmental conditions (Büntgen et al., 2014; Myers-Smith et al., 2015).

The development of new techniques to produce micro sections of parts of increment cores up to a length of 6 cm (Gärtner et al., 2014) was a step toward the integration of wood anatomy in time-series analysis. Besides the visibility of single cells, micro sections of increment cores easily allow to detect very narrow rings in conifers, in extreme cases consisting of two or three cell rows with a very indistinct row of latewood cells (Fig. 1).

These narrow rings frequently occur in trees cored on extreme sites as e.g., the altitudinal or latitudinal tree line (Pritzkow et al., 2014) or also in trees affected by insect outbreaks (Axelson et al., 2014). Potentially, micro sectioning further solves another problem in dendroecological studies: the ring structure of many deciduous species is hardly visible macroscopically on sanded or even on cut core surfaces. This is one of the main reasons why many of these species are not used for dendroecological reconstructions. The possibility of taking micro sections from entire tree cores in combination with a short processing time could overcome this difficulty.

While the approach of cutting increment cores up to a length of 6 cm opened new perspectives (Gärtner et al., 2014), the amount of work needed to prepare a single core is still high compared to simple surface preparation for macroscopic analyses. Furthermore, there are still several drawbacks concerning the potential to reanalyze core samples taken for e.g., climate or geomorphic reconstructions several years ago (e.g., Gärtner et al., 2003). To analyze the ring-width variations on these increment cores they were commonly glued on wooden mounts and, after their measurement, stored under dry conditions to prevent them from fungal infestation and decay. As a consequence, these increment cores are mostly non-detachable from the mounts without breaking into pieces.

Until today, detailed wood anatomical reconstructions of environmental conditions at a site of interest which was already analyzed in the past, require to revisit the sampling location and the repetition of the whole sampling procedure. Although resampling would allow an update and extension of the existing datasets, it is often impossible to repeat the sampling in the same area due to e.g., financial and political restrictions, wild fires, or silvicultural measures.

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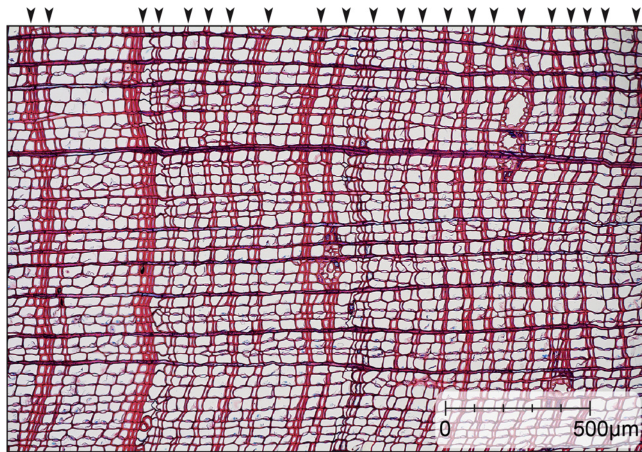

Fig. 1. Detailed image of a micro section cut from an increment core (*Larix decidua* Mill.). Boundaries of all rings visible in the image are indicated by black arrows on top.

Hence, the main goal of this contribution is to present a new method, which enables the preparation of micro sections of whole increment cores of conifers sampled many years ago. This would allow the application of time-series analyses based on wood anatomical properties in future.

Material

A basic requirement to cut off micro sections of entire increment cores is a core microtome (Gärtner and Nievergelt, 2010) or a comparable cutting device which enables the fixation of entire cores for sectioning, respectively.

In our study we used cores of *Larix deciuda* Mill. taken at a sporadic permafrost site on a north facing slope in the Bever Valley/eastern Swiss Alps in October 2006. The 5 mm cores, varying in length between 30 and 40 cm, were glued on wooden mounts directly after coring and sanded for subsequent ring-width measurements. After their analysis the cores were stored in a dry storage room. Since we were interested to know if it is possible to use core samples stored over a longer period to reanalyze them wood anatomically, we used these cores to test the procedure.

When cutting-off sections of these cores with the required thickness of about 15–20 μm, they always broke into several pieces. Consequently, a stabilizing medium was needed. To avoid the breakage of charcoal, Schweingruber (2012) used a tape to fix the samples when cutting-off micro sections. Based on this experience the application of a stabilizing tape to avoid the breakage of the cores was tested. In contrast to the method applied by Schweingruber (2012) the intention was to prepare permanent micro sections without fixing the tape together with the section. Hence, a tape is needed which could fix the later thin section for cutting and, more important, to release the section from the tape after the cutting procedure for the final staining and dehydration process. By consequence, several types of tape were tested. As a result, the common transparent tape (tesafilm® standard, 33 m: 19 mm) consisting of PP-foil and solvent-free adhesive was found to match best regarding the required properties.

To minimize cutting artifacts as for instance broken or distorted cell walls, a non-Newtonian fluid consisting of cornstarch, water and glycerol in the ratio of 10:8:7 was prepared (Schneider and Gärtner, 2013). To further prevent the entire thin section from breakage during the cutting procedure, the core had to be stabilized with a special glue. The glue also ensured that the tesafilm® standard was not directly taped on the section only but also to a solvable medium in-between. Because the glue needs to be water soluble, Mowiol 4-88, a polyvinyl alcohol often used as a stabilizing colloid for embedding preserved histological specimens was chosen (Fukumoto and Fujimoto, 2002). To prepare the Mowiol for the cutting procedure, Mowiol 4-88 granulate was dissolved in water by using a ratio of 1:5.

Beyond the common utensils as brushes, tweezers and lab needles, special glass slides and cover glasses having a size of approximately 35 mm × 400 mm × 2 mm (depending on the length of the increment core) are needed to embed the thin sections later on. Since glass slides of such a dimension are not available at common laboratory equipment companies, a common high quality glass for picture frames with a thickness of 2 mm was used, which is also a quite cheap solution. This glass is available in every common hardware store and is quite easy to cut to the required dimension. For covering the sections the biggest cover glass commercially available (to our knowledge 75 mm × 25 mm; thickness: 0.1 mm) is recommended. These cover glasses can be placed in a row on top of the section, until the entire section is covered. There are special companies producing cover glasses of various sizes on demand. But because this is not a routine size, prices are a matter of negotiation.

For the staining process dyes as e.g., Safranin and Astra blue are needed as well as Ethanol (75% and 96%) for the dehydration of the sections. To embed the sections Xylol and Canada balsam were used (Gärtner and Schweingruber, 2013). If one wants to avoid the usage of Xylol, other embedding substances as Euparal (Ramanna, 1973) are also applicable.

Cutting procedure

Before starting the cutting procedure, the above mentioned material should be prepared and placed next to the microtome or other sectioning device. The dry mounted core has to be fixed tight in the core holder of the microtome having the outermost ring facing toward the blade. In case the core is not fixed on a mount, it needs to be oriented having the tracheid direction upright (which should already be the case when mounted) to guarantee an accurate cross section perpendicular to the growing axis of the stem (Gärtner and Schweingruber, 2013; Gärtner et al., 2015).

Basically, removable blades can be used to cut-off sections of common conifer samples (Fujii, 2003; Gärtner and Nievergelt, 2010; Gärtner et al., 2014). Depending on the density of the sample e.g., if the core shows a high content of compression wood, a common microtome knife might be necessary for cutting. Before starting the cutting procedure a brush can be used to moisten the surface of the core. When dry, the cell walls of wooden samples show a brittle behavior and tend to break while cutting. As soon as the water is soaked in the surface of the core, it can be cut down until a constant plane surface is achieved. The moistening of the surface needs to be repeated whenever necessary to avoid the dry cutting of the sample. When a plane surface is cut, the knife needs to be pushed back behind the core. The position of the core and the blade should not be changed anymore before the core is further prepared for sectioning.

To prevent the occurrence of artifacts, a specific stabilization of the core is achieved by adding a cornstarch solution to the surface of the core with a brush (Fig. 2a) to stabilize the cells (Schneider and Gärtner, 2013). The brush can be used to spread the fluid over the cut surface of the core. The exertion of pressure needs to be avoided since it would harden the fluid directly which prevents the solution from penetrating into the cells. Hence, it is recommended to wait for a few seconds after the solution is spread to ensure that the liquid entirely fills up the cell lumina. Thereafter, the excessive

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