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TECHNICAL NOTE

The core-microtome: A new tool for surface preparation on cores and time series analysis of varying cell parameters

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Abstract

A microtome designed for the surface preparation of entire increment cores allows cutting plane surfaces on cores up to a length of 40 cm. Compared to the common sanding procedure, the wood cells of the annual rings remain open, not filled with swarf, and the cell walls are smooth and hence clearly visible. This article aims at describing the functionality of the microtome and the procedures needed for an accurate surface preparation to achieve a good contrast for subsequent image analysis. Possible applications for a more detailed analysis of variations in the tracheid structure of conifers and vessel sizes of oak are presented, which can be included in time series analyses.

Keywords: Dendroecology; Wood anatomy; Increment cores; Preparation techniques

Introduction

Short-term as well as long-term changes of environmental conditions do have a distinct impact on the development of trees and shrubs (Schweingruber et al., 2006). The effects of these changes are archived in the rings of various soft and hard wood species at different structural levels, hence providing invaluable information about past environmental conditions (Schweingruber, 1996). Analyzing annual variations in tree-ring formation is a well-established method to reconstruct past climate conditions by measuring parameters such as ring width (e.g., Esper et al., 2008), wood density (e.g., Bouriaud et al., 2005) or even isotope ratios (e.g., McCarroll and Loader, 2004). Common dendrochronological analysis over long time periods (centennial to millennial) are based on macroscopic analysis, e.g., by measuring ring-width variations or inter-annual density fluctuations. This is also true for isotope analysis. Even though the rings are split in various tangentially oriented thin sections for subsequent isotopic analyses, the detailed anatomical structure which has to be analyzed microscopically is not focused.

In climate impact research specific, process-related modifications of typical ring structures are used to reconstruct the recurrence intervals of geomorphic processes (Gärtner, 2007a). In this context, variations of the common anatomical structure within a ring, as for example, the onset of compression or tension wood cells, are used to date certain environmental impacts on an intra-annual level (Heinrich and Gärtner, 2008). In addition to this, anatomical changes were analyzed in relatively small specimen and for this over short time periods (intra-annual to decadal) enable reconstructing continuous processes as root exposure (Gärtner, 2007b). In dendroecology, wood anatomical techniques are used to analyze short- or mid-term processes as insect

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infestations (Pohl et al., 2006) or changes of hydrological conditions (Ford and Brooks, 2003).

Although many anatomical features related to environmental conditions are known (Schweingruber, 2007), wood anatomical techniques have rarely been applied to long-term time series analyses.

Compared to common ring-width measurements, identifying wood anatomical variations requires more complex preparation techniques. In this regard, detailed procedures of sample cutting, staining or embedding are manifold and they always depend on the aim of the study (Hoadley, 1990; Carlquist, 2001). Traditionally, wood anatomical features or the developmental stages of the xylem are analyzed based on small blocks of wood or micro cores, respectively (Schweingruber, 2007).

Regarding the effects of the predicted global warming, the development of existing and new methods to record and quantify past and recent environmental processes is required (Gärtner, 2007a). Consequently, there is a need for dendrochronologists to develop new techniques for a better visualization of basic anatomical characteristics. Regarding time series analyses, plane surfaces on cores or other samples are required while leaving the cell lumina open and clearly visible for macro- and microscopic analyses of cell characteristics within the annual rings. Several techniques exist, but there is still no satisfying solution for a time and cost efficient preparation of the surfaces.

A step towards the solution of this problem is the invention of a core-microtome presented here. This microtome is designed to cut increment cores resulting in a plane surface with open cell lumina. The design and the handling of this microtome are described along with some basic techniques to prepare the samples to achieve a good contrast for the subsequent image analysis.

Surface preparation on cores

Time series analysis in dendrochronology is mostly based on the measurement of ring-width variations on increment cores. To do so, the visibility of the ring boundaries has to be enhanced and the surface of the entire core should be plane to avoid frequent manual adaptations of the focus while doing the measurement. This is of special importance when using more recent image analyses programs for ring-width measurements such as WinDendro (Regent Inst., 2004). Here, a plane surface is the prerequisite for acquiring accurate scan images of the cores.

Until now, the most time effective procedure doing the sample preparation is sanding the cores perpendicular to the longitudinal axis of the cells using different grains and finally polishing them using fine abrasive paper (Hoadley, 1990). By doing so, the common ring boundaries especially in conifers get clearly visible and, if carefully sanded, even the structure of single cells is visible. Unfortunately, thin cell walls tend to break and split during the sanding procedure and all cell lumina are filled with dust resulting in a reduced visibility especially of extremely narrow or light rings. Consequently, certain sections of the cores need to be additionally cut using razor blades to enhance the visibility of these structures. Beyond controversy, cutting the surface of cores is the best solution to enhance the visibility of the rings and even single cells. Nevertheless, this additional cutting is time consuming. Therefore, a straightforward technique and, of course, an appropriate tool for cutting the entire surface is needed.

Core-microtome

Based on this and on the knowledge gained from cutting micro-sections for anatomical analyses, we developed a new type of microtome enabling to cut

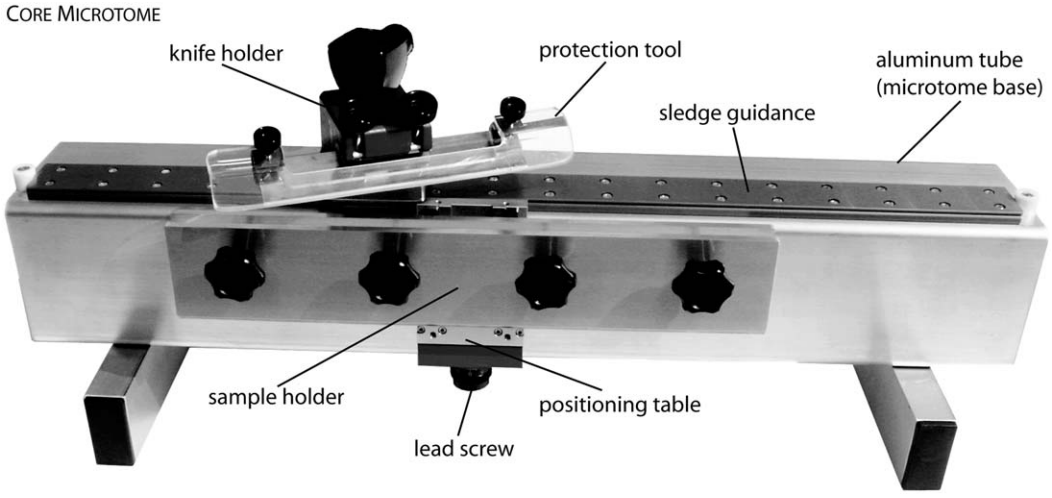

Fig. 1. . The core-microtome and its components as described in the text. The protection tool is designed to be placed on the microtome knife to avoid injuries while handling the microtome.

plane surfaces on entire cores (Fig. 1). The so-called core-microtome consists of two main components: (i) a 40 cm core holder attached to a infinitely adjustable (μm) positioning table and (ii) a 70 cm rigid sledge guidance with a pivot holder for microtome knives (or removable blade holders) mounted on top of the sledge.

The base of the microtome is a simple square-type aluminum tube of 75 cm length and 12 cm × 11 cm height and width, respectively. Sideways, in the center of the tube, a vertically oriented positioning table is fixed, controlled by a precision lead-screw enabling to manually lift the sample holder in a range from one to several micrometers at a time.

The sample holder consists of two aluminum plates (40 cm × 6.5 cm × 0.5 cm) and a Plexiglas spacer of the same length but variable height and thickness (Fig. 2).

One aluminum plate is permanently attached to the positioning table to stabilize the holder and to avoid any lateral movement while cutting. The second plate is attached to the fixed plate by four screws which also hold the spacer in-between. The thickness and the height of the spacer depend on the samples to be cut. For unmounted cores (0.5 or 1 cm) the thickness is 0.4 or 0.8 cm, and the height is adjusted for having the upper side of the spacer 0.25 cm thick, or 0.5 cm below the upper edge of the plates, respectively (Fig. 2a). Consequently, the surface of a 0.5 cm core can be cut down to a width of 0.4 cm and for a 1 cm core down to 0.9 cm. For mounted cores the thickness and height of the spacer can vary depending on the dimensions of the mount (Fig. 2b). Along the lower part of the spacer, four thread rods are screwed in sticking out the Plexiglas for about 0.3 cm on one side. As a result, the second aluminum plate gets slightly inclined and the upper edge clamps the core (or the mount) absolutely tight along its entire length. Before fixing the screws of the plate, the entire core (or the mount) has to be in contact with the upper side of the spacer. Thus, the sample is entirely stabilized and any interfering movements of the sample while cutting are suppressed.

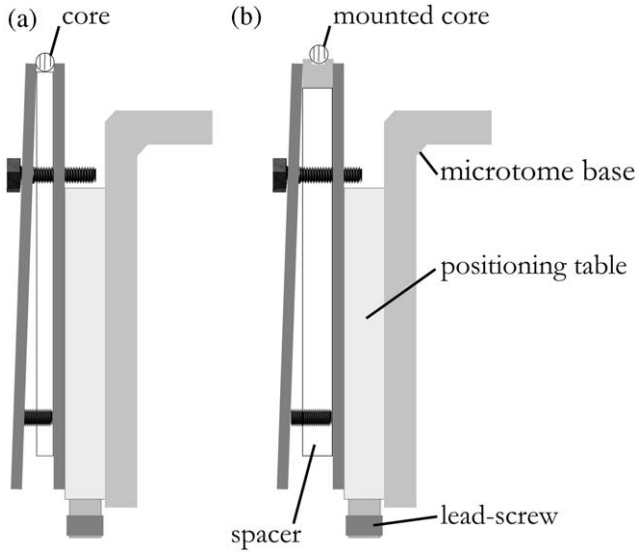

Fig. 2. Schematic (side) view of the sample holder (compare Fig. 1) illustrating the fixation of (a) unmounted and (b) mounted cores by using different sized spacers. The height and the thickness of the spacer for mounted cores depend on the size of the mount.

The blade holder is attached to a thread rod fixed on the sledge of the microtome as it is done on common microtomes. By opening the screw head, the horizontal angle of the holder, and hence also the blade, can be adjusted relative to the core at any position needed. The microtome knife (or the removable blade holder) is placed in an adjustable spacer adapted in the holder. With this, the vertical angle of the knife can also be fixed depending on the properties of the sample. The design of the knife holder was adapted from common microtome constructions to ensure the full flexibility for cutting samples of varying densities as it is needed for cutting micro-sections. With this, an optimal surface preparation can be achieved.

Cutting procedure and surface preparation for image analysis

The advantage of cutting an entire core is not only having a plane surface, but also having the single cells within the rings open (not filled with swarf) and the cell structure clearly visible. If the cores are cut as long as they are fresh, the cell walls are soft enough to cut them without any problems. But in most cases cores are taken days or weeks before they are processed in the laboratory and therefore are dry. If the cores are dry, the cell walls get frangible and tend to break while cutting. Consequently, as soon as the core is fixed in the holder, it needs to be moistened by simply brushing the top surface with water. This is in contrast to the sanding procedure, where the cores have to be dry enough to be sanded without blurring the surface. Generally, the water is soaked in after a few seconds and the core can then be cut down continuously. To achieve good results, cutting down the core should be done in steps, i.e., 10–20 μm.

To avoid the problem of using common microtome knives and especially to avoid the time consuming sharpening procedure, a special removable blade holder was developed for the use of common NT-Cutter blades (Fig. 3). The application of these low cost NT-Cutter blades (A-type) is proven to be feasible even for cutting micro-sections (Fujii, 2003). The stability of the blade while cutting is guaranteed by the fixation plate slightly overlapping the main holder supporting the blade from

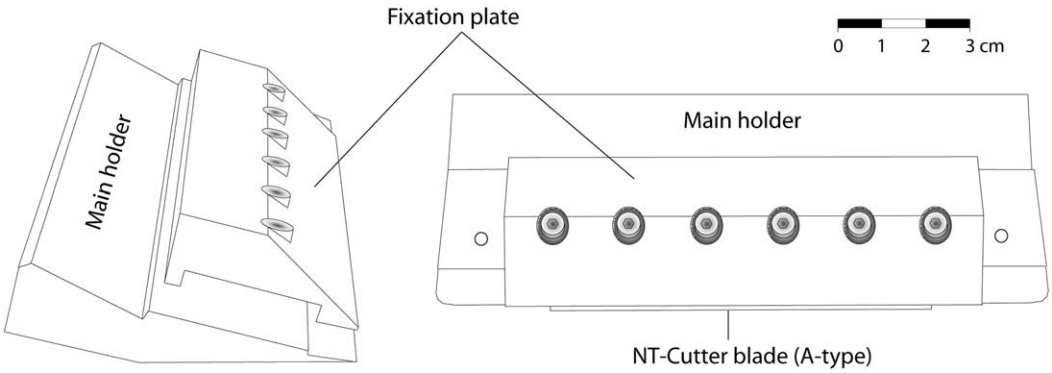

Fig. 3. Schematic view of the removable blade holder designed for the use of NT-Cutter blades. The base (main holder) is angled to guarantee an optimized basic angle of the removable blade relative to the sample surface.

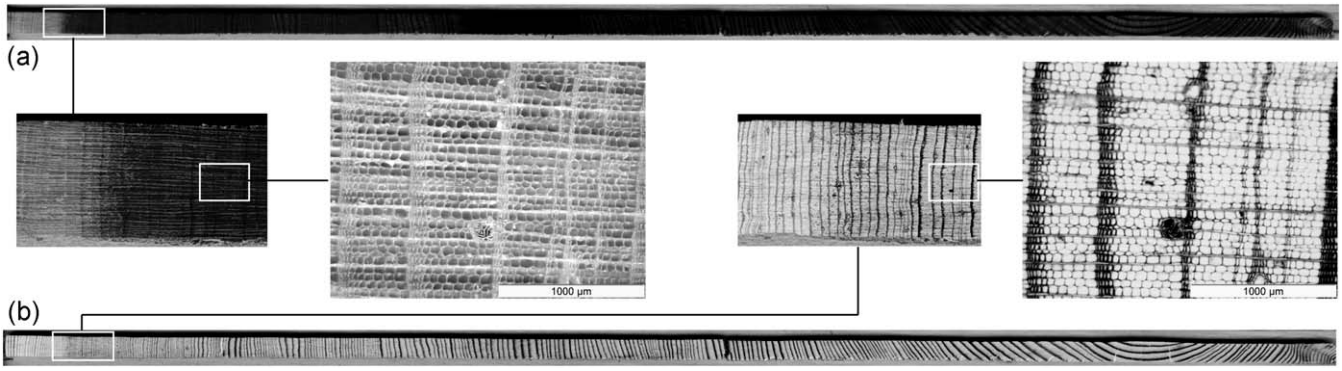

Fig. 4. (a) Scan image of a *Larix decidua* core surface cut by the core-microtome without further preparation. Although the rings are not very distinct at a lower magnification, the high magnification illustrates the visibility of the single cells. (b) The same core as in (a), but here the cells are filled with white chalk powder. This enhances the visibility of the ring structure even at lower magnifications.

the top against the forces occurring during the cutting procedure.

The resulting surface of the core is plane and, if mounted, totally parallel to the lower side of the mount. On the first view, the rings of the core are visible to the naked eye, but not as distinct as they are after sanding (Fig. 4a). This is due to the fact that the cells are not filled with deposits of grinding swarf and for this stay dark. When magnifying the surface, the cell structure of the rings gets clearly visible (Fig. 4a, left microscopic image).

Especially narrow and light rings are easy to detect and the risk to overlook light rings or rings consisting of only two cell rows is minimized. To enhance contrast for analyses on lower but also higher magnification, the surface of the core can be covered with white chalk powder (Fig. 4b). By simply rubbing in the chalk, the cell lumina get filled and the cell walls stay dark. Maximum contrast can be achieved, when the cut surface is first stained with dark ink, e.g., by using a felt marker. As soon as the ink is dry, the white chalk can be rubbed in resulting in a high contrast between cell lumen and cell wall. This simple procedure is not only instrumental in enhancing the visibility of ring boundaries in conifers, but also for analyzing ring boundaries and vessel distribution in hardwoods (Fig. 5).

Possible applications in time series analysis

As shown in Figs. 4 and 5, the cut surface of the cores preserves the structure of the single cells within the annual rings. Cell walls are not frayed and the surface of the entire core is plane. Besides the already mentioned advantages for image analyses (use of flatbed scanners), the cut surface also facilitates the application of high frequency densitometry. Here, a micro-electrode-probe is continuously scanning the surface of a core to define wood density variations based on the ratio of cell wall to the air-filled open cell lumina (Schinker et al., 2003). For this, the probe has to be continuously in contact to the sample surface which requires a plane surface.

The continuously cut surface of the cores also facilitates a more detailed analysis of variations in the

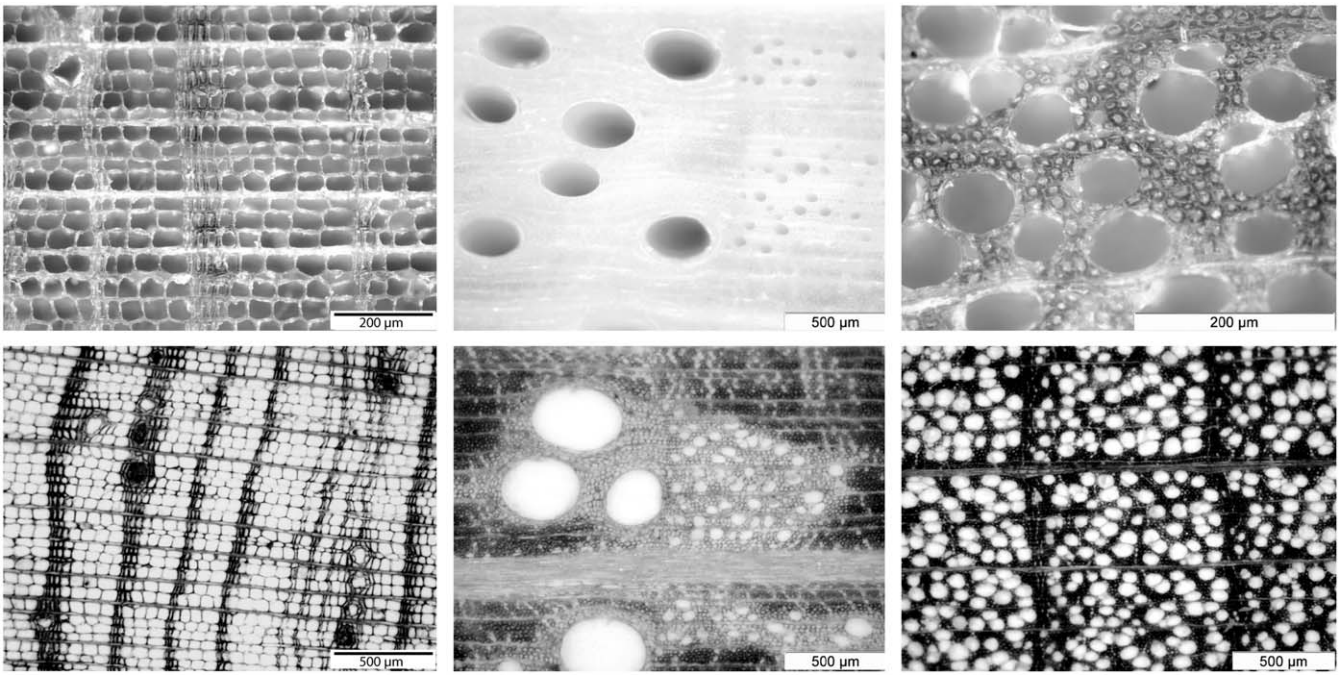

Fig. 5. Micro-photos taken form cores after cutting (upper images) and after filling the cell lumina with white chalk (lower images). Left: *Larix decidua*; center: *Quercus petraea*; right: *Fagus sylvatica*.

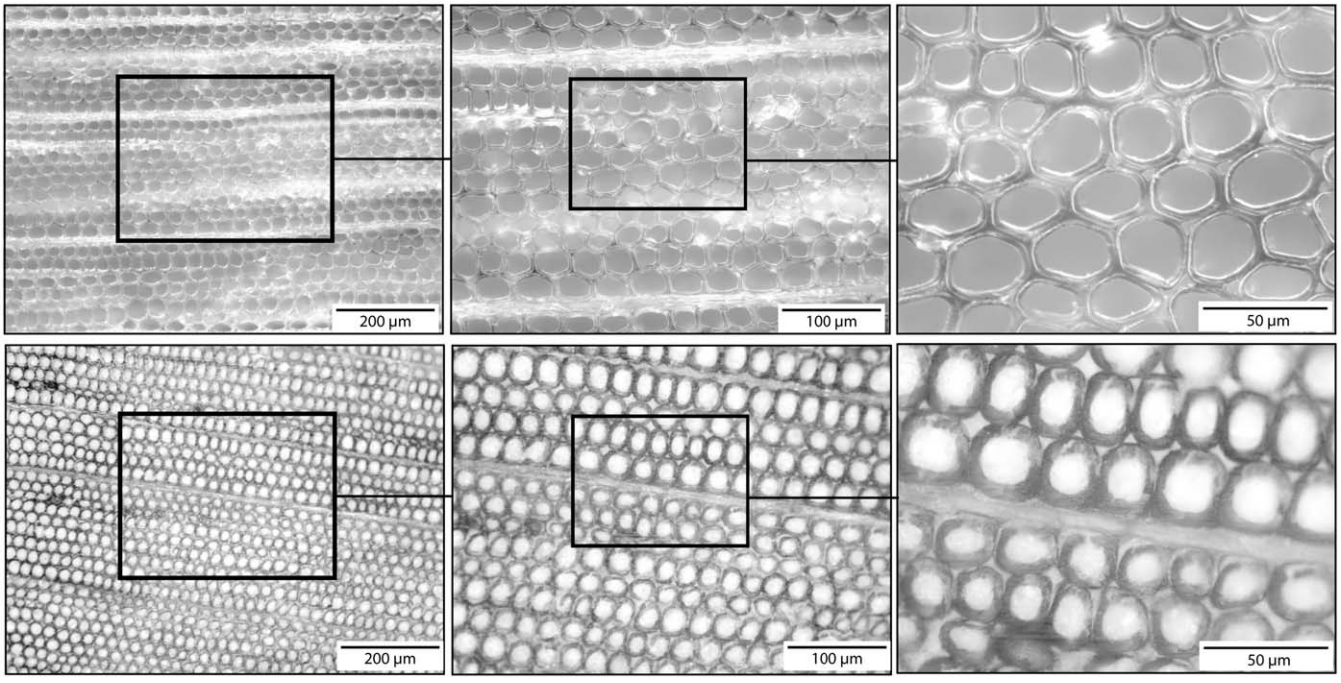

Fig. 6. Micro-photos taken from the earlywood portions of two rings of a *Larix decidua* core at three different magnifications (cell walls not stained). Upper line: the clearly visible cell structure at 500 × magnification (right) indicates that the cells do show a tendency towards compression wood, expressed by a more rounded shape and thicker cell walls but no intercellular spaces. White areas here are caused by reflections of water at moist areas of the core surface, because the images were taken right after cutting. Lower line: earlywood portion of a subsequent ring showing distinct compression wood (rounded shape, thick cell walls and intercellular spaces). The cells are filled with white chalk powder.

tracheid structure of conifers. As shown in Fig. 6, tracheids can be analyzed in high magnification, their lumen size and even cell wall thickness can be measured. Unfortunately, some restrictions still occur concerning an automated measurement of these parameters. When the lumina of the tracheids are not filled with chalk, the contrast between cell lumen and cell wall is not high enough for any image analysis system to automatically detect the single structures (Fig. 6). Consequently, the measurements have to be done manually. When using chalk, at high magnifications (e.g., 500 ×) the border between cell lumen and cell wall is sometimes not distinct enough for an automated border detection. Although this disadvantage is still a problem, other substances to fill cell lumina for creating a high-quality contrast as wax are under consideration and ongoing tests will most likely reveal a better solution in the near future. Nevertheless, the ability for manual measurements of single cell parameters without the need of preparing micro-sections can be helpful, e.g., when randomly analyzing cell sizes within single ring to analyze the effect of cell size variations on earlywood or latewood width (Handa et al., 2006). Moreover, the analysis of intra-annual variations of cells within longer time series as density fluctuations or transformations towards compression wood (Fig. 6) can easily be detected and included in the ongoing analyses.

In contrast to tracheids of conifers, vessels in hardwoods are characterized by their relatively big size. Here the use of white chalk to enhance the contrast for image analysis facilitates the measurement of vessel lumina even at lower magnifications (10–20 ×). It has been shown by Garcia Gonzalez and Fonti (2008), that analyzing vessel sizes by preparing radial sections of stem discs (cleaning vessel lumina with air pressure, staining the xylem with dark ink and filling the vessels with white chalk powder) provides feasible information for analyzing the climatic signal related to these cells. The use of the core-microtome simplifies the procedure and entire cores can be prepared for further analyses. After cutting, the core is stained using a black felt marker and vessels are filled with white chalk powder (Fig. 7).

Then, the cores can be scanned on a flatbed scanner or images can be taken using a stereoscope and a digital camera to do ring width as well as vessel size measurements. The magnification required for a successful measurement depends on the aimed cell structures. For ring porous species as oak, the cores should be scanned at 1200 dpi which is sufficient for analyzing earlywood as well as latewood vessels (Fig. 8). When using a stereoscope and a digital camera, the magnification should be at least 10 × for measuring earlywood vessels and, depending on the resolution of the camera, about 20 × for latewood vessels.

Conclusion

The ongoing development of computers and image analysis systems facilitates the measurement of new parameters in dendrochronology. For this, there is a need for dendrochronologists to further develop their preparation techniques to be able to reveal the information stored in the anatomical structure of tree rings. The invention of the core-microtome is a step into this direction enabling to analyze, for example, vessel size variations in addition to ring width on increment cores without further preparation. Although this tool is not thought to be a surrogate for the existing

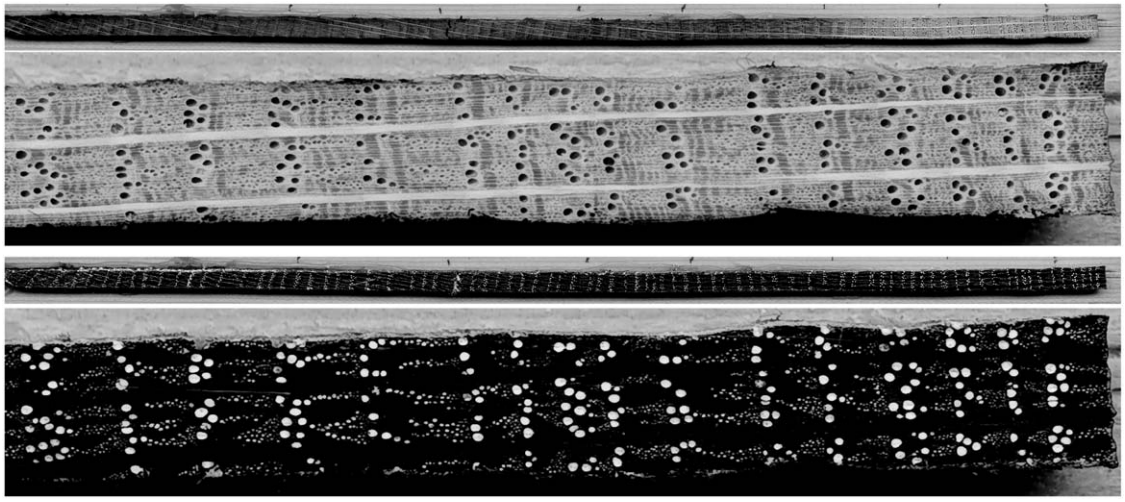

Fig. 7. Scan images of a *Quercus petraea* core at two different magnifications. The upper images show the core after cutting without further preparation. For the lower images, the core was stained with a black felt marker and then the cells were filled with white chalk to enhance the contrast for image analysis (compare Fig. 8).

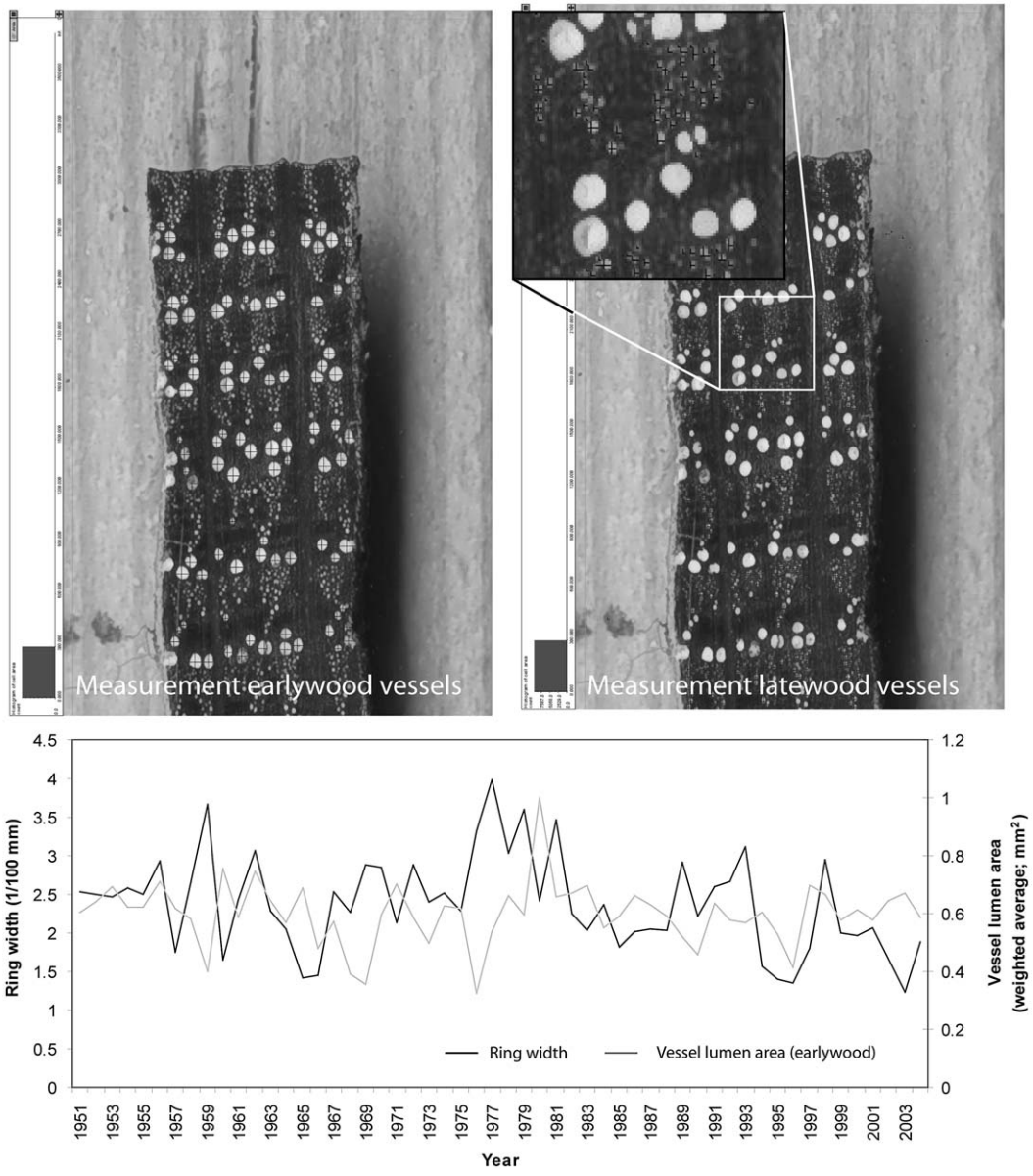

Fig. 8. Example for an automated measurement of earlywood (upper left) and latewood vessels (upper right) based on the scan image of a *Quercus petraea* core (compare Fig. 7). The lower diagram illustrates the resulting mean earlywood vessel size variation along the entire core as well as the respective ring-width variations.

preparation techniques, it expands the traditional method of sanding cores to support a more detailed analysis of long chronologies.

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