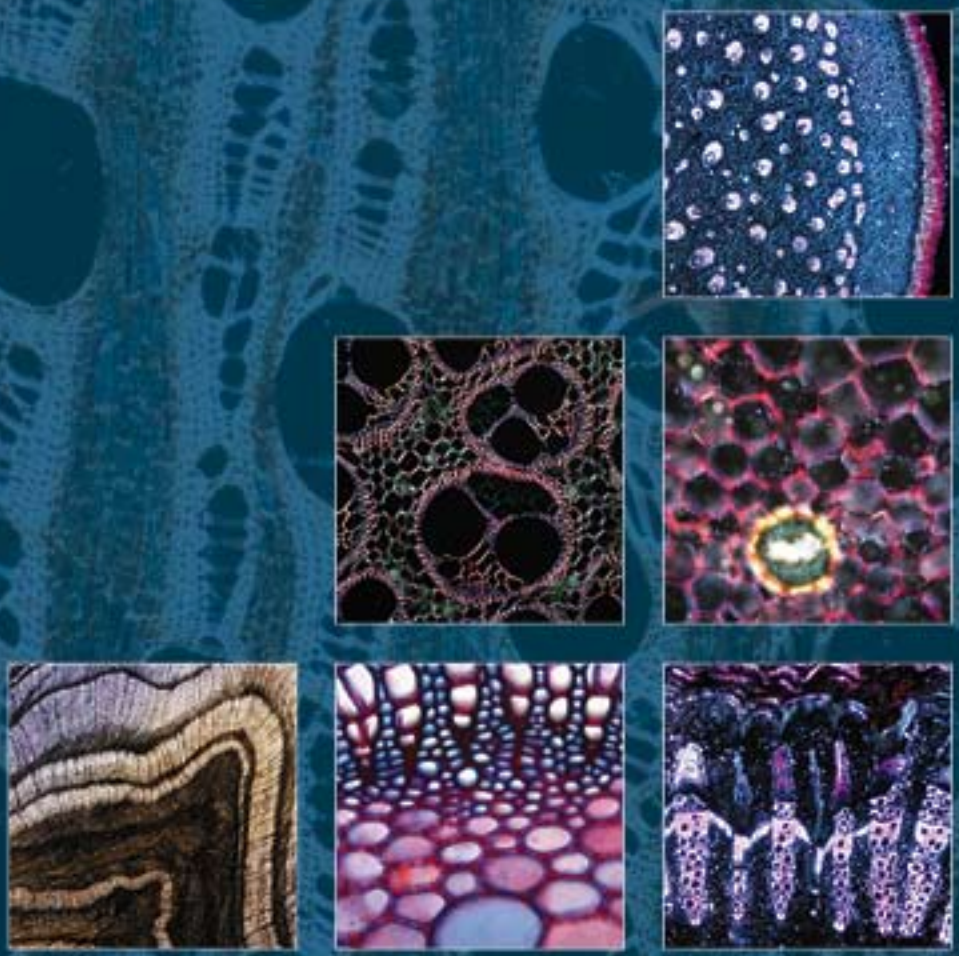
Microscopic Preparation Techniques for Plant Stem Analysis



Holger Gärtner
Fritz H. Schweingruber

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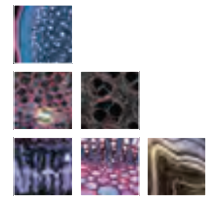

Holger Gärtner
Fritz H. Schweingruber

Design & layout **Annett Börner**

Dr. Holger Gärtner
Prof. Fritz H. Schweingruber

Swiss Federal Research Institute WSL
Landscape Dynamics / Dendroecology
Zürcherstrasse 111
8903 Birmensdorf
Switzerland

E-Mail: holger.gaertner@wsl.ch

Design & layout: Annett Börner, Adelaide
www.dn.com.au/annett-boerner.html

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Eifelweg 37
D-53424 Remagen-Oberwinter

Tel.: 0049-(0)2228-493
Fax: 0049-(0)3212-1024877
E-Mail: nkessel@web.de

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Contents

Introduction

If we had written this booklet 20 years ago, the title would have been different "Microscopic preparation techniques for wood analysis". However, in the last years it became obvious that there is a need in dendrochronology, as well as in the science of plant anatomy (Schweingruber et al. 2011, 2012), to study anatomical stem structures in more detail. Many textbooks describe preparation techniques useful in all field of biology and botany, e.g. Kremer (2002), Mulisch & Welsch (2010), Purvis et al. (1964), Rudzin (1999); or special techniques for wood anatomy e.g. Chaffe (2002), Ives (2001), Hoadley (1990). However, not a single text concentrates speci - cally on xylem and phloem of plant stems, branches and roots.

Based on our experience in our laboratories (Swiss Federal Research Institute WSL, Birmensdorf, Switzerland), and during more than 20 international anatomical courses with participants from all over the world, we have concluded that a "cook book" for simple microscopic plant stem analysis is useful for beginners. The main goal of this booklet is to provide instructions for producing high quality micro sections using simple techniques in an efficie way. Therefore we focus on processes that do not demand embedding plant tissues before sectioning. The main message of this booklet is how to use traditional and new microtomes with disposable cutter blades and simultaneous multiple staining techniques. Having worked with students and experienced scientists with dozens of differen mother tongues and sometimes with limited knowledge of the English language, we have come to the conclusion that pictures can tell better technical stories, rather than long-written instructions.

In the last few years we have learned that new sliding microtomes, new cutting techniques and better staining methods allowed also the preparation of bark and very small soft stems. Since we recognized that secondary stem thickening occurs in all conifers and most dicotyledonous plants and that lignificatio occurs in most terrestrial plants we overcome the boundaries between herb and tree, and between woody and herbaceous plants. Therefore, we analyze the xylem, phloem, cortex and phellem of plant stems in general.

1. Sampling material and sampling strategies

Wood and bark in stems, branches and roots of trees, shrubs and herbs contain information about their genetic origin, the formation time and environmental conditions.

For all tree-ring related studies, the sampling strategy is the most important part when initiating a project. 'Tree'-ring related and wood anatomical studies are not limited to trees, and not even to areas where trees are able to grow. Beyond the limits of tree growth other woody plants such as shrubs, dwarf shrubs or even herbs can be found, also forming annual rings. Therefore, these organisms can potentially be used as proxies for reconstructing past environmental conditions.

Furthermore, research strategies and thus sampling strategies are not restricted to the stem of the woody plants. Whether one is working with trees, shrubs, dwarf shrubs or herbs, all parts of these plants (roots, stems and branches, or even bark) potentially carry environmental information, which can be analyzed in detail. This fact has to be respected before thinking about possible sampling strategies, independent of whether studying macro- or microscopically the annual rings of woody plants.

1.1 Sampling in various biomes

All terrestrial and lacustrine biomes contain important plant material, which can be analyzed in relation to taxonomy, morphology and environmental conditions (Walter & Breckle 1989) (Figs. 1.1–1.6).

Fig. 1.1. High Arctic: mainly dwarf shrubs and long living perennial herbs (hemicryptophytes) growing under dry and moist conditions as well on bolder fields on permafrost. Mosses, sedges and grasses dominate wet plains and lakeshores.

Fig. 1.2. Hot desert: shrubs, dwarf shrubs and hemicryptophytes are scattered on sand dunes and rock fields. Annual plants germinate and exist only during rainy seasons.

Fig. 1.3. Boreal forest: conifers, dwarf shrubs and mosses dominate circumpolar boreal forests on all sites.

Fig. 1.4. Forests in temperate zones: trees, shrubs, and hemicryptophytes dominate most sites.

Fig. 1.5. Tropical forests: trees and lianas dominate tropical rain forests.

Fig. 1.6. Wet sites: mainly sedges, grasses and hydrophytes grow in, and on, the border of seas, lakes and ponds.

1.2 Sampling different life- and growth forms

All terrestrial and lacustrine plant associations contain important plant material, which can be analyzed to reconstruct environmental conditions. Consequently, trees, shrubs, dwarf shrubs, palms, lianas, succulents, annual and perennial herbs, grasses, mosses, lichens and algae can be of interest for dendroecological and/or wood anatomical studies (Figs. 1.7–1.20).

Fig. 1.7. Tree, conifer

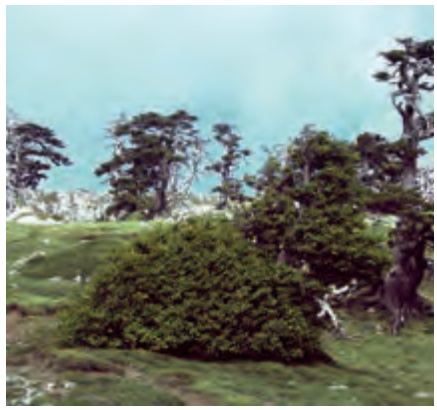


Fig. 1.8. Trees, different species and growth forms

Fig. 1.9. Palm

Fig. 1.10. Liana

Fig. 1.11. Succulent

Fig. 1.12. Dwarf shrub, prostrate

Fig. 1.13. Dwarf shrub, parasite on tree

Fig. 1.14. Dicotyledonous annual plant (therophyte)

Fig. 1.15. Monocotyledonous perennial plant (hemicryptophyte)

Fig. 1.16. Water plant (hydrophyte)

Fig. 1.17. Fern

Fig. 1.18. Moss

Fig. 1.19. Lichen

Fig. 1.20. Algae

1.3 Sampling different parts of plants

For anatomical analyses, the xylem and phloem of stems, branches, roots (root collar), rhizomes of dicots and monocots, needles, leaves and below-ground stems can be used (Figs. 1.21–1.39).

Fig. 1.21. Cross section of a deciduous tree: oak stem (Quercus petraea) with heartwood (1) and sapwood (2).

Fig. 1.22. Cross section of a conifer: spruce (Picea abies), eccentric stem with compression wood (1).

Fig. 1.23. Cross section of a liana (stem): alpine virgin's bower (Clematis alpina), assimilating xylem with very large dilated rays (1).

Fig. 1.24. Cross section of a succulent (stem): Giant cereus (Cereus giganteus), with small but not continuous xylem.

Fig. 1.25. Bark: coniferous tree, Mountain pine (Pinus montana).

Fig. 1.26. Bark: deciduous tree, birch (Betula pendula).

Fig. 1.27. Exposed roots of a deciduous tree (Fraxinus excelsior).

Fig. 1.28. Exposed roots of a conifer (Picea abies).

Fig. 1.29. Stem of a dicotyledonous tree (Convolvulus arborea) with successive cambia.

Fig. 1.30. Stem of a palm with single vascular bundles.

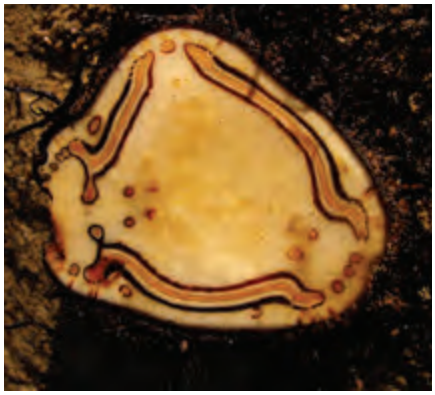


Fig. 1.31. Stem of a tree-fern (Cyathea arborea) with elongated closed vascular bundles.

Fig. 1.32. Stem base of a herbaceous fern (Struthiopteris onoclea), central stem and peripheral leaf-bases.

Fig. 1.33. Long shoots of a conifer (Pinus montana).

Fig. 1.34. Long shoot of a deciduous tree (Betula pendula).

Fig. 1.35. Short shoots of a conifer (Larix decidua).

Fig. 1.36. Seedlings of a deciduous tree (Castanea sativa).

Fig. 1.37. Polar root of an annual plant (Plantago maritime).

Fig. 1.38. Rhizome of a dicotyledonous plant (Viola riviniana).

Fig. 1.39. Rhizome of a monocotyledonous plant (Festuca sp.).

1.4 Sampling dead wood

The xylem of dead material can contain useful information when analyzed microscopically, i.e., well preserved wooden constructions and artifacts, charcoal, archeological wet wood, petrifie wood, stems infected by fungi, stems with scars (Figs. 1.40–1.48).

Fig. 1.40. Posts in the Lagoon of Venice

Fig. 1.41. Artifact, violin

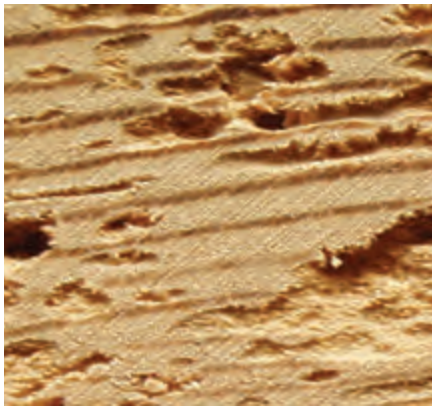


Fig. 1.42. Wood decomposed by insects

Fig. 1.43. Wood decomposed by fungi

Fig. 1.44. Driftwood

Fig. 1.45. Charcoal

Fig. 1.46. Degraded wet wood from a Neolithic lake dwelling in Switzerland

Fig. 1.47. Petrified wood

Fig. 1.48. Mineralized wood on a sword

2. Collection and preservation

2.1 Tools

Herbs can be excavated using common garden tools and parts of plants can be cut with knives or lopping shears. Twigs and small stems can be cut with scissors (Figs. 2.1–2.3). Dead wood (logs, driftwood, etc.) can be cut with handsaws and/or chain saws (Figs. 2.3 and 2.4). For stems of living trees collect samples with well-sharpened punchers (Fig. 2.6) and/or increment corers with diameters of 5 mm or 10 mm (Figs. 2.5 and 2.7). ***Important Note:*** Only very sharp borers and punchers yield useful samples for microscopic slides (Figs. 2.8 and 2.9). Samples taken with dull borers are mechanically stressed, frequently irregularly split and therefore difficu to use for preparing micro sections (Fig. 2.10).

Fig. 2.1. Garden pick

Fig. 2.2. Pocket knife and paper knife (NT)

Fig. 2.3. Lopping shear and handsaw

Fig. 2.4. Chain saw

Fig. 2.5. Increment borer with handles and extractors (Pressler Bohrer) with di erent diameters (left one with 10 mm, right with 5 mm and different length).

Fig. 2.6. Puncher for micro-cores (Type: Trephor; Rossi et al. 2006)

Fig. 2.7. Borer heads (threaded auger) of increment borers. The front part (cutting edge) needs to be sharpened frequently.

Fig. 2.8. Sharpening borers by hand. The cutting edge is sharpened using a quadrangular oil-sandstone.

Fig. 2.9. Sharpening borers by hand. The inner part of the cutting edge needs to be sharpened using a conical sandstone.

2.1.1 The importance of sharpening increment borers

The need to sharpen increment borers is frequently discussed (Bauck & Brown 1955, Jozsa 1988, Grissino-Mayer 2003). Nevertheless, most borers used in the fiel are not properly sharpened and rarely anyone takes sharpening tools out to the fiel to sharpen the borer as soon as the extracted cores are no longer absolutely smooth.

The use of a dull borer results in cores showing uneven surfaces due to compression forces exerted to the core while turning the bit. This is caused by a blunt cutting edge not really cutting into the wood, but being pressed in while turning. For common ring-width measurements, these compressed cores can be prepared and analyzed without problems. In some cases the cores need to be broken because they are twisted. If cores are twisted, this causes problems for density measurements, where the angle of the tracheids needs to be absolutely upright.

In wood anatomy, increment cores had been of no interest for a long time. This changed as soon as the research topics turned in the direction of an ecologically-based wood anatomy and related time-series analyses. In recent years, we developed special holders for increment cores (see section 3.6 – Sectioning with microtomes) enabling to cut micro sections from core pieces up to a length of 6 cm.

For these special purposes, compressed cores cannot be used!

If the cutting edge of the corer is not sharp, the cutting edge is pressed into the wood and the core is at least partly squeezed and twisted every time the borer is turned. These compression forces cause microscopic cracks within the structure of the annual rings, most frequently along the weakest area, the ring boundaries and/or the rays. As a result, the micro sections fall apart (Fig. 2.10) and it is impossible to prepare a continuous section.

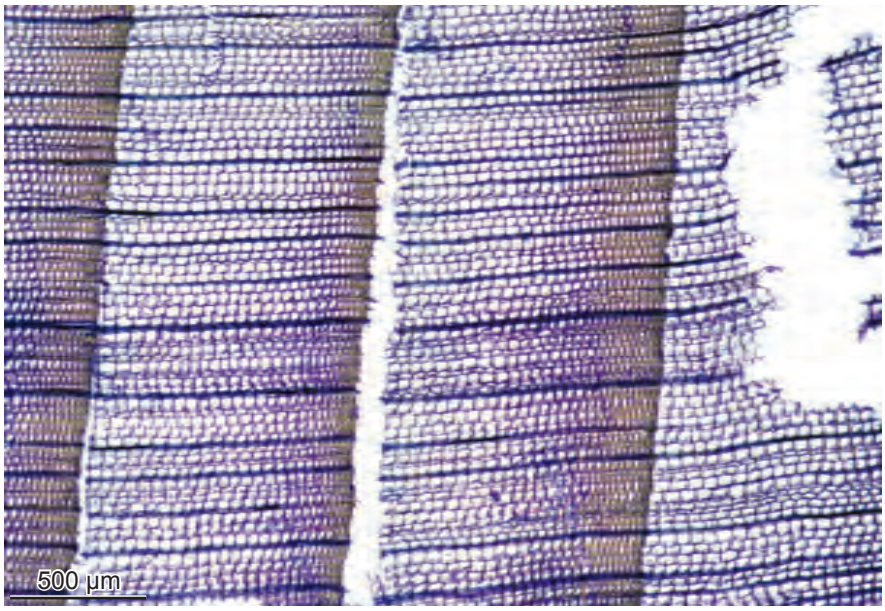


Fig. 2.10. Tangential cracks on a sectioned sample due to dull borer tip.

2.2 Preserving samples for structural analysis

When sampling herbs or bark for microscopic preparation, only fresh material is suitable for high quality slides. After sampling, put the plant, or parts of it, in a re-sealable plastic bag and add a few drops of 40% ethanol. Use very soft pencils for labeling the bags because ethanol dissolves pen ink and ink of permanent markers (Fig. 2.11). Plastic is permeable to ethanol, therefore store bags in closable plastic boxes (e.g., Tupperware: Figs. 2.12 and 2.13). If the boxes are perfectly closed, the material will not dry out for at least one year. ***Important Note:*** if you need to analyze starch grains, the material should only be preserved in water; ethanol will decompose starch grains.

Fig. 2.11. Re-sealable plastic bag labeled with pencil, containing samples and some drops of ethanol.

Fig. 2.12. Plastic box with bagged plant material.

Fig. 2.13. Closed plastic box (airtight) ready for storage of transportation containing ethanol soaked plant material.

2.3 Labeling collected samples

All scientifi results depend on careful labeling. Ecological and dendroclimatological analysis demand more than just a species name. The site characteristics presented in the example below, are of importance. Additional information might be useful, but details are always depending on the aim of your study. If you intend to collect much material, print them on self-adhesive labels (Fig. 2.14)

Example label	
Species:	Fagus sylvatica L.
Life form:	Tree
Collected part of plant:	increment core of the stem at breast height down slope
Plant height:	25 m (height includes flower stalks for herbs)
Short site description:	e.g., exposition, hydrological conditions, influence of wind, light conditions (shadow, competition, browsing)
Geographic location:	Birmensdorf, Kanton Zürich, Switzerland
Altitude above sea level:	460 m
Coordinates:	At least in degrees. Navigation tools (GPS) indicates them much more precise.
Collection date:	Day/Month/Year; season or however you think date should be recorded
Name of collector:	...
Notice about photographs:	...

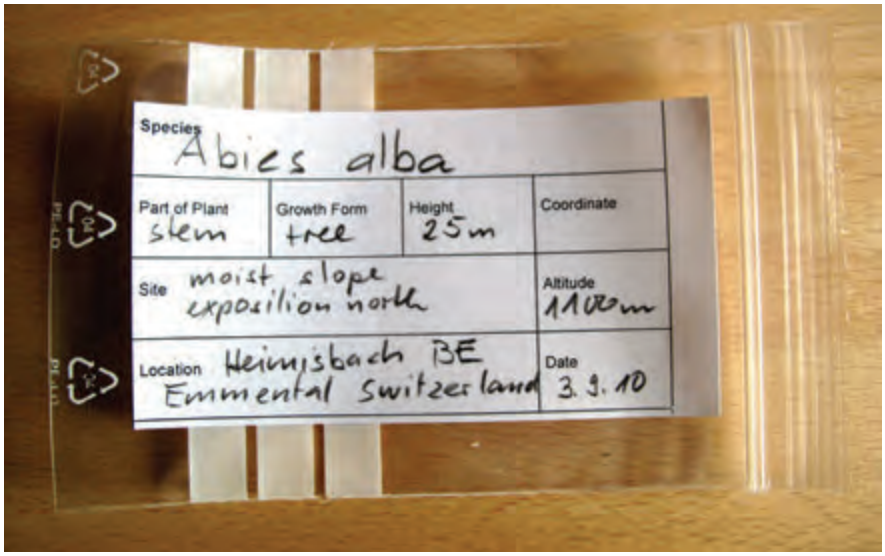


Fig. 2.14. Two di erent types of labels. The upper one is computer generated (advantage: data additionally stored in the computer), the lower one is filled in by hand.

3. Sectioning and maceration

3.1 Stabilization of the material

Fixing the sample tightly in the microtome is a basic requirement for cutting micro sections. The sample has to be "squeezed" between the clamps of the holder. This can be a problem when working with soft material and hollow stems. Soft material cannot be fixe in the holder without being compressed and deformed, so it needs stabilization. The most suitable material for stabilizing soft tissue in the microtome sample holder is homogeneous cork. By using, for example, a cutter knife you can cut the cork to any form and size needed (Figs. 3.1–3.4). Also recommended, but normally less suited, are carrots, the pith of elder stems or common plastic foam (Styrofoam) (Fig. 3.5).

Fig. 3.1. Cutting cork with a cutter knife.

Fig. 3.2. Cutting a groove according to the diameter of the object.

Fig. 3.3. Cork with a flower stalk in the groove.

Fig. 3.4. Cork with opposite grooves with an inserted plant stem, ready for placing in a microtome clamp.

Fig. 3.5. Examples of materials that can be used to fix soft material: carrot, cork, elder pith (often too soft for hollow stems), plastic foam of various consistencies.

3.2 Preparing stem disks for sectioning: sawing, splitting, and boiling

Parts of wooden stems have to be modifie into small blocks, which are suitable to be clamped in microtome holders. It is important that the sidewalls are parallel to each other, if your sample is wedge-shaped it will not be stable enough in the clamp. ***Important Note:*** If your sample is not very stable in the microtome holder, your cuts will not be of good quality because the sample is somehow displaced while cutting, resulting in unevenly shaped thin sections. In extreme cases, the sample will even become stuck in the blade.

When cutting micro sections, the orientation of the sample, and therefore the cutting direction (Fig. 3.6) is of importance. Especially when focusing on the growth development of the plant, a transversal (= cross) section is needed. This direction is the one most frequently used in tree-ring related studies.

Sections across the stems have to be sawed (Fig. 3.7). Make sure that the cut is perpendicular to the fibers Fibers in twisted stems are not parallel to the stem axis. Sections parallel to the fib rs (radial and tangential) must be split (Fig. 3.8). In doing so, the fibe (or tracheid) direction becomes visible and the sample can be correctly oriented in the sample holder of a microtome (Fig. 3.9). The microtome blade must precisely follow the split plane on the wooden block in case of cutting radial or tangential sections. If the sample is split in such a way that it is not precisely parallel to the rays, then they appear short and do not show all of the details. See section 5 on p. 67 for more information.

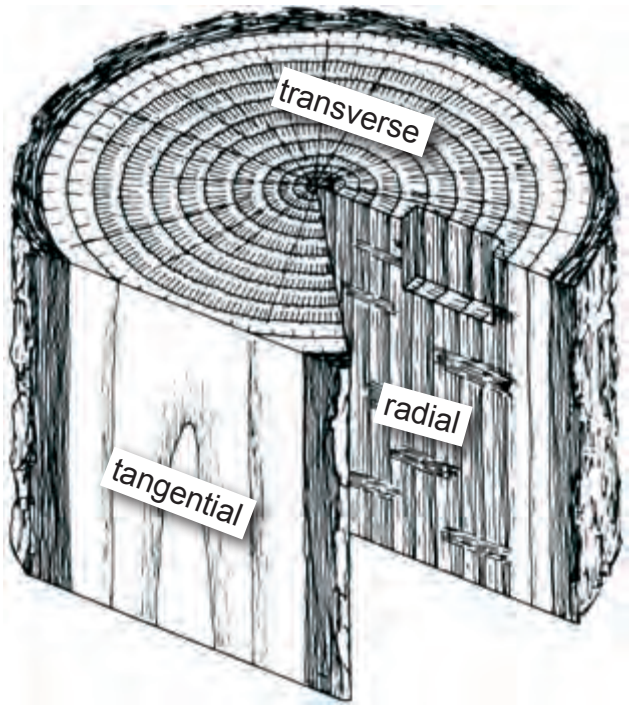


Fig. 3.6. Visualization of the three cutting directions (transversal, tangential and radial) used in wood anatomy (modified after: Schweingruber 1990, p. 13).

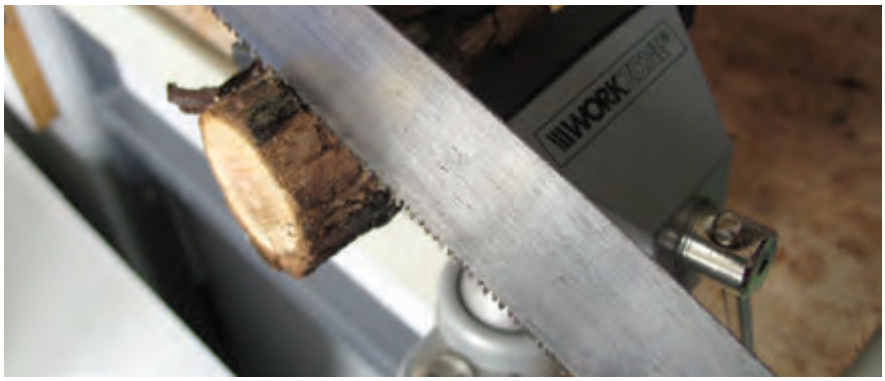


Fig. 3.7. Sectioning of a stem in a bench by a fine-toothed saw.

Fig. 3.8. Splitting a sample parallel to the fibers.

Fig. 3.9. Splitting a block with parallel sides. Only one side must be perfectly radial.

Dry hardwoods, especially tropical woods, must be softened before cutting. This can be achieved either within several minutes or several hours depending on the density of the material (Fig. 3.10). Extremely dense wood (e.g., ebony wood) can be softened in a vapor pressure pan (Fig. 3.11). Dense wood can also be softened by soaking small samples in a mixture of 96% ethanol, glycerol, and water (1:1:1) for several weeks. Before soaking or boiling, label the samples with a soft pencil, or wrap the sample in a labeled, heat resistant textile.

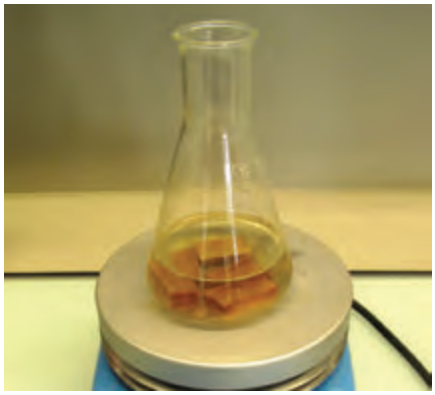


Fig. 3.10. Boiling hard woods in an Erlenmeyer flask on a hot plate.

Fig. 3.11. Very dense wood can be softened by boiling it in a pressure cooker.

3.3 Sectioning by hand

Preparing sections by hand is unlikely to result in high quality slides. For a quick look, parts of plants can be clamped between holders of elder pith or carrot, and then cut (Figs. 3.12–3.15).

Handmade sections of sub-fossil or waterlogged wood are well suited for species identification ***Important Note:*** Only the thinnest section (e.g., 10–20 microns) is suitable for microscopic inspection.

New razor blades have to be pulled slowly in a very steep angle across the object. For example, cut the object like you would cut a salami, not like you would cut butter. Never push blades across the objects.

Fig. 3.12. Correct guidance of the blade for a non-permanent slide of living material.

Fig. 3.13. Incorrect guidance of the blade for a non-permanent slide of living material.

Fig. 3.14. Correct guidance of the blade for a permanent slide of sub-fossil material.

Fig. 3.15. Cross-cut of a waterlogged, soft sub-fossil oak sample.

3.4 Splitting and sectioning charcoal or mineralized wood

When a small sample of charcoal has to be identifie it is best to simply break it (Fig. 3.16). The structure of the cross sectional plane is perfect for episcopic observation. For radial and tangential sections, the sample can be split using a knife (Fig. 3.17).

This same procedure is also useful for mineralized wood, which often occurs along metallic archeological artifacts, e.g., scabbards. Anatomical details in the transverse and longitudinal planes become distinct if all dust particles are blown out with compressed air (Figs. 3.18 and 3.19)

Fig. 3.16. Breaking charcoal across the fiber between fingers.

Fig. 3.17. Splitting charcoal parallel to the fiber with a knife.

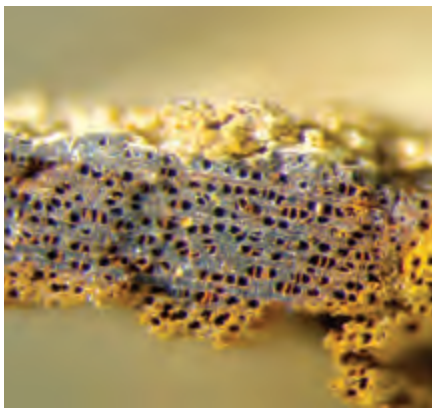


Fig. 3.18. A broken piece of mineralized wood, transverse surface of Alnus sp. Photo: Willy Tegel.

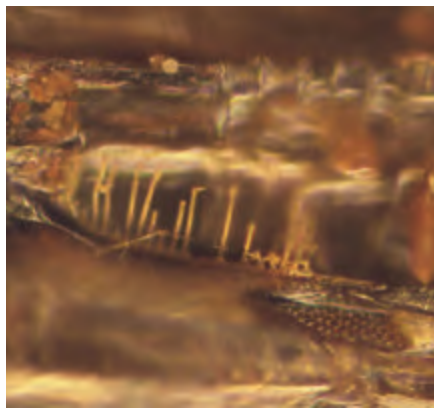


Fig. 3.19. A split piece of mineralized wood, radial surface of Alnus sp. with scalariform perforations. Photo: Willy Tegel.

To prepare microscopic cross sections which are useful for photographic presentation, apply a drop of a mixture of a two-component adhesive, e.g., Araldit, on the transverse side of a broken charcoal surface (Fig. 3.20). The liquid fill all cavities and polymerizes within 30 minutes, after which the surface can be planed with a normal microtome (Fig. 3.21).

In the next step, a small piece of transparent self-adhesive tape (e.g., Scotch tape) is attached to the top surface (Fig. 3.22). The microtome knife is pulled through the charcoal at a sharp angle and a thickness setting of 20–30 microns. The section adheres to the tape and can be mounted onto a slide (Fig. 3.22). At this point, the section is ready for microscopic observations (Fig. 3.23). By using a mounting medium, e.g. Canada balsam, the slide remains permanent, but the adhesive tape has to be kept intact to preserve the integrity of the charcoal section. This procedure stabilizes the fragile carbonized structures. This procedure does not work for longitudinal sections because the fragile carbonized structures split within the adhesive (Fig. 3.24) (Schweingruber 2012).

Fig. 3.20. Two-component adhesive.

Fig. 3.21. Flat transverse cut through a piece of charcoal, which is stabilized with a two-component adhesive.

Fig. 3.22. Charcoal stabilized with a two-component adhesive, covered with an adhesive tape in a microtome holder. The knife cuts the charcoal and the section remains intact by adhering to the transparent tape.

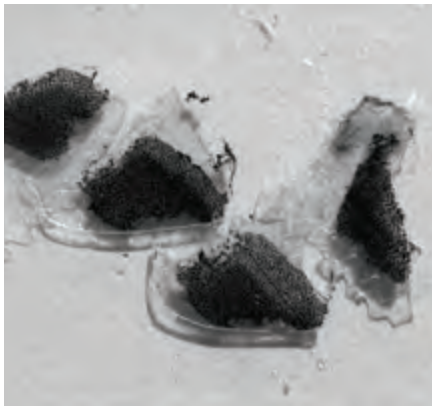


Fig. 3.23. Microscopic cross-sections adhering to adhesive tape on a slide.

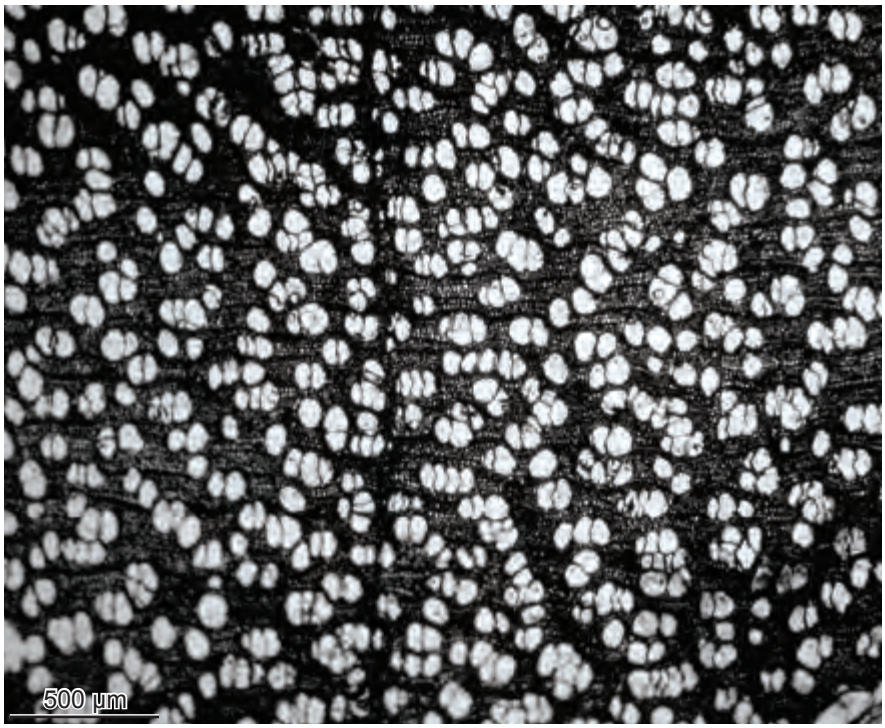


Fig. 3.24. Microscopic cross section of a piece of charcoal of Betula sp.

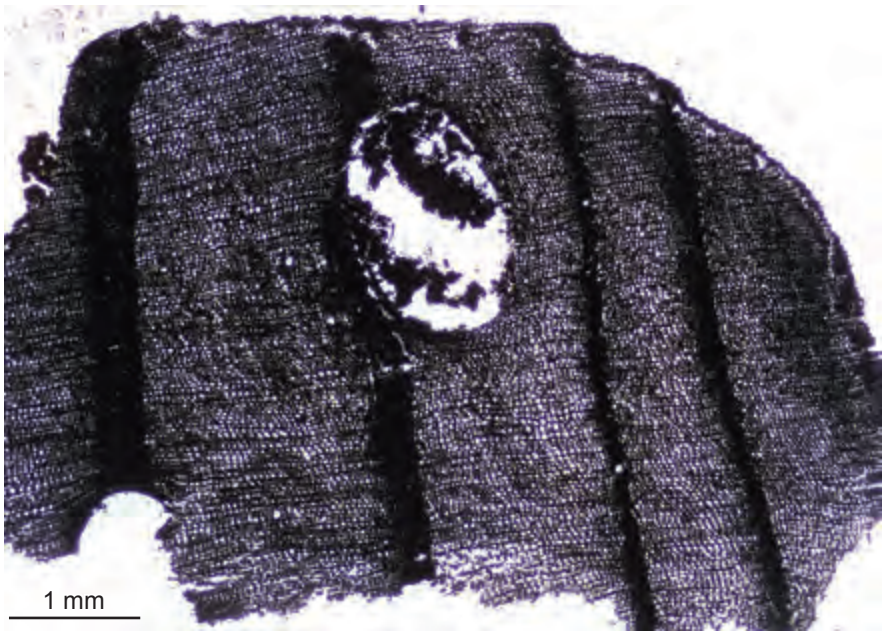


Fig. 3.25. Cross-section of a Neolithic, carbonized piece of Abies alba with an insect gallery.

3.5 Preparation of surfaces for macroscopic observation

Fresh samples (stems, root, branches) are cut with sharp paper knives or razor blades. Stick the 1 cm-long pieces of your sample into grafting wax or modeling clay and let them dry for a few hours. The dry samples and charcoals can be stabilized on glass slides or in petri dishes (Fig. 3.26).

Samples can be observed under the binocular microscope (Fig. 3.27). Objects in petri dishes can be closed, labeled and stored after analysis (Fig. 3.28). Charcoals with more or less flat broken surfaces can also be stabilized in grafting wax (Fig. 3.29).

Fig. 3.26. Stems in grafting wax in a Petri dish.

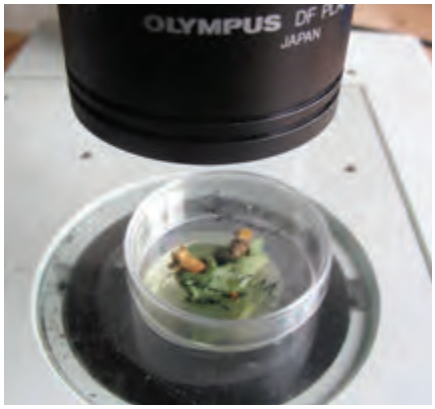


Fig. 3.27. Herb samples below a binocular microscope for counting rings.

Fig. 3.28. Samples with labels, ready for storage. Sample details can be written on the cover of the Petri dish.

Fig. 3.29. Charcoal stabilized in grafting wax.

3.5.1 Making annual rings visible by cutting and sanding

For identifying rings on various kinds of samples (i.e., cores, discs or other pieces of trees, or even entire stems, branches or roots of shrubs or herbs) it is, in most cases, sufficie to cut a plane surface using a sharp paper knife or a razor blade in a correct manner (Figs. 3.30–3.34; see section 3.3).

Fig. 3.30. Correct sectioning: Always cut at an angle to the sample, not across it.

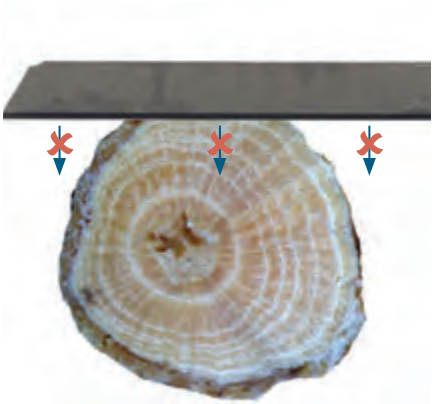


Fig. 3.31. Incorrect sectioning: Never use a knife like a planer.

Fig. 3.32. Perfectly sectioned surface of a small dwarf shrub (Acinos alpinus).

Fig. 3.33. Perfectly sectioned surface of a rhizome of a shrub (Rubus idaeus).

By polishing surfaces with belt sanders (Figs. 3.34 and 3.35), contrasts between the cell lumen and cell wall become more distinct. Sanded surfaces are of diffe - ent quality (Figs. 3.36–3.38). All of them look good at small magnifications but at higher magnification we recognize the dislocated dust particles in all cell lumina. Only very fine-g ained surfaces (400–600 grit sandpaper) yield useful pictures. The contrast of the sample can be enhanced by blowing the dust out of the sample with pressurized air, and fillin the lumina with chalk.

Important Note: Sanded cores cannot be used for isotopic analysis because dust particles are dislocated.

Fig. 3.34. Big belt sander for treating large surfaces.

Fig. 3.35. Small belt sander for polishing small surfaces, i.e., increment cores.

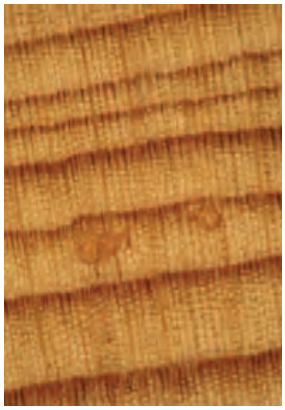


Fig. 3.36. Optimal planed surface of a pine, all lumina are filled with dust and cell walls are clearly visible.

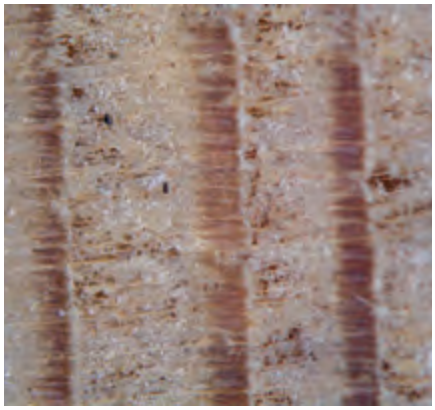


Fig. 3.37. Badly polished surface of a fi , cell walls cannot be observed.

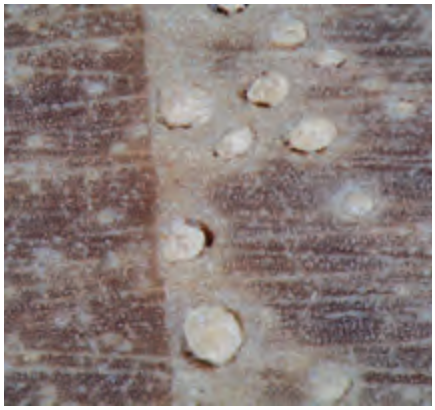


Fig. 3.38. Badly planed surface of an ash, not all lumina are perfectly filled with dust and scratches left by the sandpaper obscure the structure.

3.5.2 Observation surfaces by enhancing contrast

If the wood you are working with is light-colored, with little contrast between annual rings (e.g., diffuse-porou species), the vessels and annual rings can be made more distinct by fillin the cell lumina of cut surfaces with chalk. ***Important Note:*** This is only possible if the surface is cut; it is not possible to apply chalk on sanded surfaces! If your surface is cut, take a piece of white chalk, cover the surface of the section with the chalk and then use your finge to rub the surface, thus pressing the chalk into the cells (Figs. 3.39–3.43).

If the wood is very light-colored, a two-step procedure will further enhance the contrast, increasing the visibility of the ring structures. First, on the cut surface of the sample apply a stain by using a dark (green or black) felt marker (e.g., a thick Edding or other brands). Simply paint the cut surface and wait until the stain is dry. Second, cover the surface with white chalk and rub it into the cells as described above. It is important to wait until the stain is dry, otherwise the chalk absorbs the stain and the result is just a blurred surface with more or less no contrast.

There is another possibility to make rings of extremely diffuse- orous species visible. After staining the cut surface with a dark felt marker, wait a few minutes to let it dry and then cut approximately 0.2–0.5 mm from the dark stained surface. Due to their differen capillarity, earlywood and latewood become distinct, because the stain penetrates less into the latewood than into the earlywood (Fig. 3.44) (Iseli & Schweingruber 1989).

Fig. 3.39. Applying chalk on a cut surface.

Fig. 3.40. Rubbing chalk into the cell lumina.

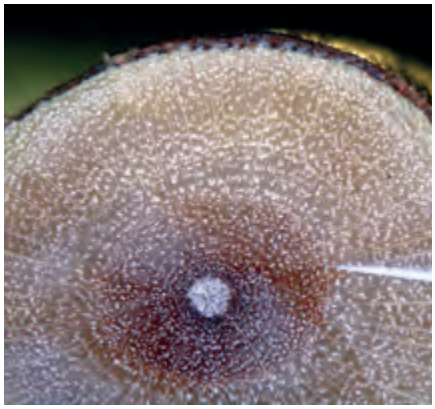


Fig. 3.41. Enhanced contrast by rubbing chalk dust in cell lumina of a species with small vessels (Rubus idaeus).

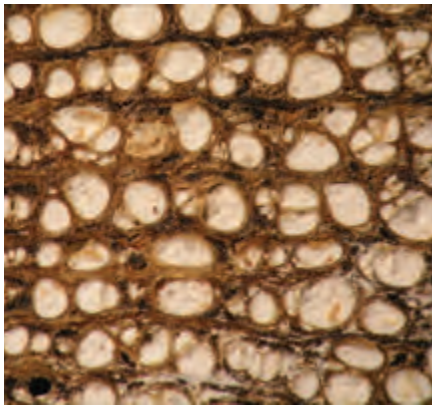


Fig. 3.42. Enhanced contrast by rubbing chalk dust in cell lumina of a species with large vessels (Pueraria lobata).

Fig. 3.43. Enhanced contrast by painting the surface with a marker and rubbing chalk dust in cell lumina (Rubus idaeus).

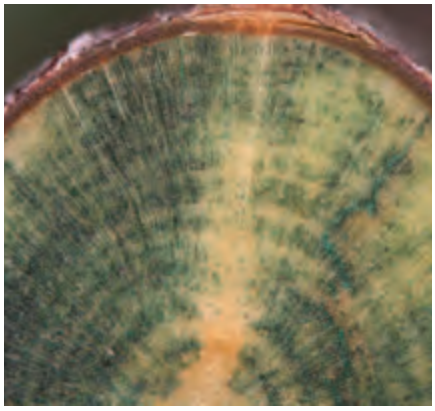


Fig. 3.44. Enhanced contrast between earlywood and latewood by painting the surface with a marker and cutting the surface until ring structures appear (Vaccinium myrtillus).

3.6 Sectioning with microtomes

For a more detailed analysis of wood anatomical features, samples have to be prepared for microscopy and successive image analysis (Schweingruber et al. 2006). As transmission and scanning electron microscopy is mostly used to analyze specifi features as cell wall structures, perforation plates or pittings in high magni - cation (< 1000x) (Carlquist 2001), the most common method for wood anatomical analysis in plant sciences is transmitted light microscopy. For this, micro sections have to be prepared using microtomes, which can also be used for charcoal analysis (see section 3.4).

It is important to note that microtome sections of fresh as well as of degraded plant material can yield high-quality permanent slides. This is especially true when using one of the three types of microtomes presented in this book, which were most recently developed by us and which are commercially available (Fig. 3.45).

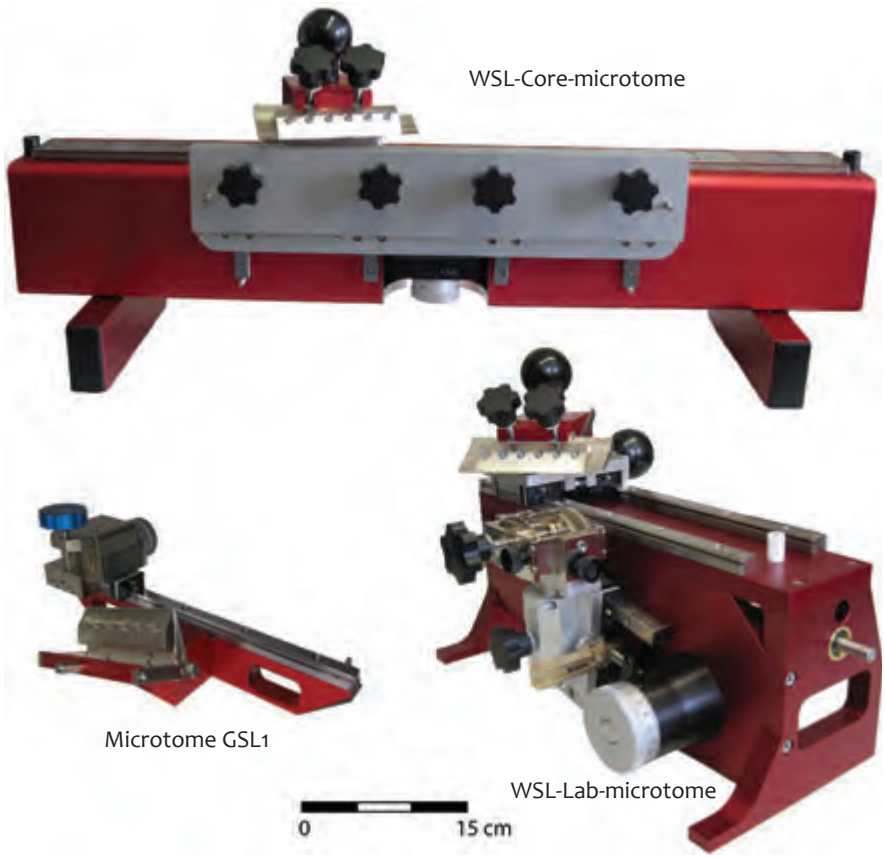


Fig. 3.45. Three new types of microtomes, developed for wood anatomical purposes: microtome type GLS1, the WSL-Lab-microtome (modified Reichert-type) and the WSL-Core-microtome. Design and Production: Sandro Lucchinetti, Schenkung Dapples, Zürich.

3.6.1 Microtome knifes

While further developing our microtomes, we found an efficie replacement for the commonly used microtome knifes, as well as for the rather expensive removable microtome blades.

For all our microtomes we developed a special removable blade holder, enabling to use common NT-Cutter blades (A-type, 0.38) known as paper knifes (Figs. 3.46–3.48). These blades are cheap, available in any office-supp shop, and they provide a comparable quality for cutting micro sections than the really expensive special removable blades for microtomes (Fujii 2003). The stability of the blade while cutting is guaranteed by the fixatio plate slightly overlapping the main holder supporting the blade from the top against the forces occurring during the cutting procedure (Fig. 3.46).

Commercial microtome knives and corresponding sharpening machines are also available, but are only used in special cases (Figs. 3.49 and 3.50).

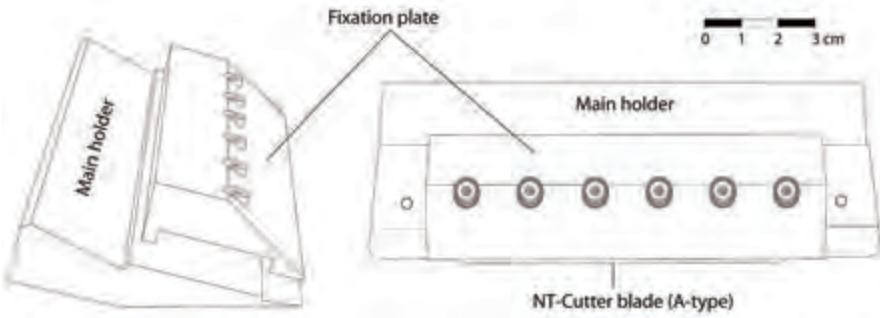


Fig. 3.46. Schematic view of the holder for removable blades (Gärtner & Nievergelt 2010, p. 88).

Fig. 3.47. Disposal blade (NT-cutter).

Fig. 3.48. Removable blade holder.

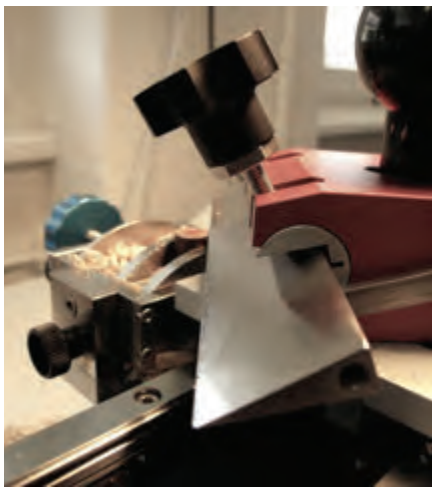


Fig. 3.49. Commercial microtome knife with two plane surfaces. Such types are useful for sectioning very dense wood.

3.6.2 Microtome types

Until recently, when using common microtomes (Fig. 3.51), the main problem was that these sections are actually restricted to rather small specimen in the range of about 0.5 x 2 cm. For an expanded time series analysis, especially when working on increment cores, much larger sections would be required to reduce the amount of sample preparation to an affordabl minimum.

Our microtomes are all based on a fixed-sledg guidance with no internal play. This detail guarantees a high stability of the microtome while cutting, and therefore enables cutting of bigger samples. In addition, specialized holders were developed, fittin into the microtome clamps, which enable working with increment cores of 5 mm and 10 mm diameter (Figs. 3.52 and 3.53), and also with micro-core samples (e.g., collected with a puncher; compare section 2.1, Fig. 2.5) (Figs. 3.54 and 3.55). The maximum length of cores is limited to 6 cm.

Fig. 3.50. Commercial sharpening machine.

Fig. 3.51. The microtome, type Reichert, is well designed, but is no longer produced.

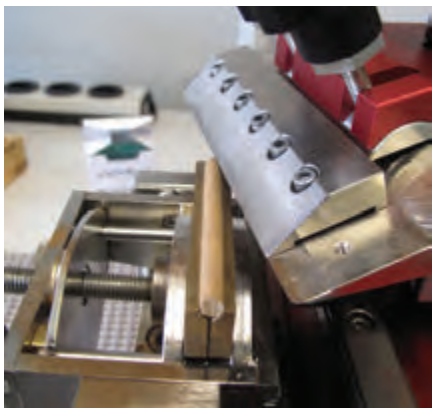


Fig. 3.52. Holder for cores with 5 mm diameter.

Fig. 3.53. Holder for cores with 10 mm diameter.

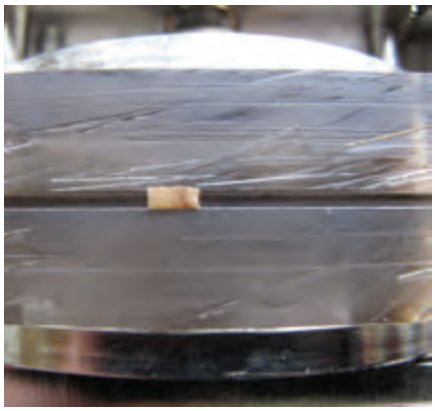


Fig. 3.54. Holder for micro-core samples with 2 mm diameter.

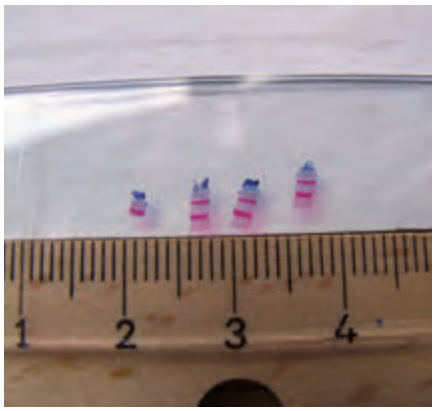


Fig. 3.55. Stained sections from puncher samples.

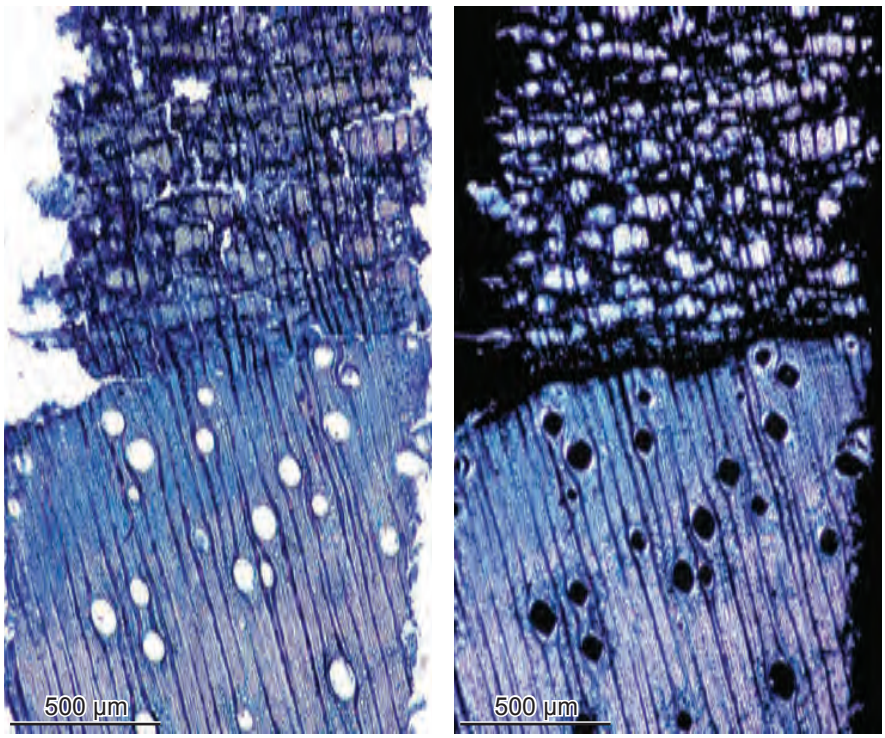


Fig. 3.56. Micro sections made from Pucher cores from Eucalyptus sp., normal light and polarized light.

3.6.2.1 WSL-Core-microtome

The core-microtome (weight: 14 kg; length: 80 cm) is designed to prepare planed surfaces on increment cores up to a length of 40 cm (Gärtner & Nievergelt 2010). Compared to sanding, the procedure commonly used to prepare surfaces on cores, the cells of the annual rings remain open and are not fille with dust. The cell walls are smooth after cutting, and thus clearly visible. This microtome was not designed for producing micro sections, though it is perfectly suited to cut perfect surfaces from increment cores. ***Important Note:*** The core-microtome is suitable for the preparation of samples to be used in isotopic analysis.

◂ *Fig. 3.57. Core-microtome, side view. Developed by: Arthur Kölliker; Design and Production: Sandro Lucchinetti, Schenkung Dapples, Zürich.*

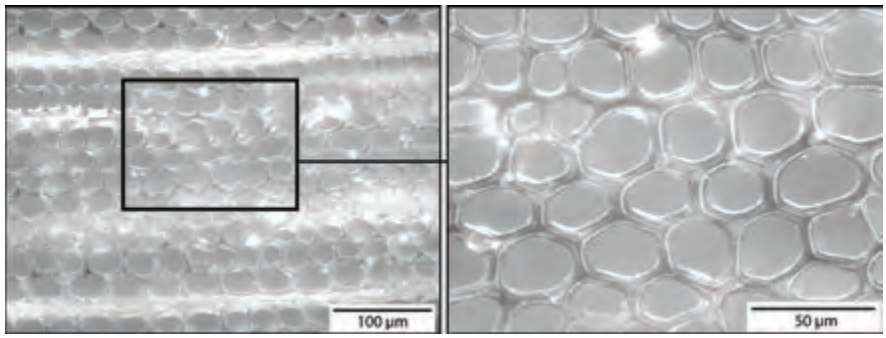


Fig. 3.58. Photos taken of the earlywood portion of a Larix decidua ring at two magnifications (cell walls not stained). White areas on the images are caused by reflections of water on the core surface, because the images were taken right after cutting (modified after: Gärtner & Nievergelt 2010).

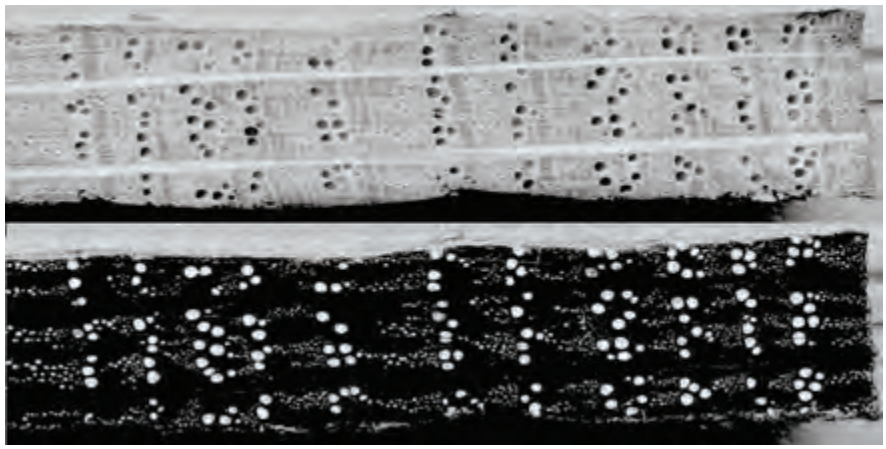


Fig. 3.59. Scanned images of a Quercus petraea core. The upper image shows the core after cutting without further preparation. In the lower image, the core was stained with a black felt marker and then the cells were filled with white chalk to enhance the contrast for image analysis (modified after: Gärtner & Nievergelt 2010).

3.6.2.2 Microtome type GLS1

Functional microtomes, designed to cut micro sections of wooden specimen, are commonly bulky and heavy. In contrast, the design of the GSL1 combines maximum stability, minimal weight and reduced size for a fully functional, small and lightweight microtome (30 x 14 x 12 cm, 3.6 kg). This special design allows using the device not only in a laboratory, it can easily be transported to remote research areas, enabling to cut micro sections right after sampling (Gärtner et al. in review). The fact that the clamp of the sample holder is fixe tightly on the sledge enables cutting all kind of wooden specimen, ranging from soft balsa wood to hard ebony wood. The fixe position of the clamp is also a little disadvantage, because wooden blocks have to be perfectly oriented before cutting, a fina tuning of the sample orientation is not possible. With this microtome design, the sample is pulled over the blade, which is mounted in a fixe oblique position and the carrier with the clamp runs along one rail (Fig. 3.60).

Fig. 3.60. Microtome GSL1. Design: Sandro Lucchinetti; Production: Schenkung Dapples, Zürich.

Fig. 3.61. Cutting a wooden block using the GSL1.

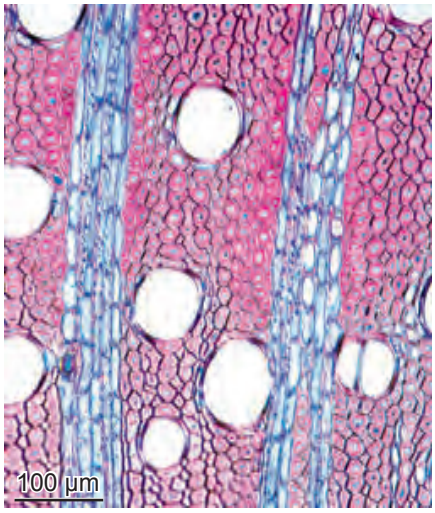


Fig. 3.62. Section of dense material: Rhizophora mangle, cross section, cut with the GSL1-microtome.

Fig. 3.63. Core holder for the microtome GSL1.

Fig. 3.64. Micro-core holder mounted on the microtome clamp.

3.6.2.3 WSL-Lab-microtome (modified Reichert-type)

The lab microtome (Figs. 3.65 and 3.66) was developed based on the design of an old Reichert microtome (see Fig. 3.51), which is no longer available on the market. But it is not just a copy of the old device, it differ in some essential details. The plate to x the knife holder on is xed on two sledges which are guided by two rails. These parallel sledge guidance rails have no internal play, so the only possible movement is forward and backward. There is no movement possible in any other direction. This guarantees maximum stability while cutting, the knife cannot be lifted upwards when the sample is very dense . For this, the lab-microtome is suited for cutting all types of plant tissues ranging from soft herb stems or bark, to very dense tropical wood. The stability of the entire construction even enables cutting so called "Nördlinger" sections (Bubner 2008) (Fig. 3.67).

The knife (or removable blade holder) is mounted to a holder, which is adjustable in the vertical and horizontal planes. The sample holder is manually tightened and adjustable. Fine adjustments of the clamp allow for the correction of the sample orientation for all sample types, e.g., wooden blocks, cores, cork-mounted soft tissues, or very small, bent shoots. The microtome is also equipped with a semi-automated system that lifts the sample in a constant manner, where the uplift of the sample is adjustable between 5 and 30 µm.

◂ *Fig. 3.65. WSL-Lab-microtome (modified Reichert microtome). Design: Sandro Lucchinetti; Production: Schenkung Dapples, Zürich.*

▴ *Fig. 3.66. Back side of the lab-microtome showing the semi-automatic sample transport construction.*

Fig. 3.67. Original of a "Nördlinger" section. Hermann Nördlinger, Professor of Forestry in Hohenheim, Germany, in 1852–1888, made thin sections from 1100 tree species for macroscopic observations (Bubner 2008). He distributed them commercially in volumes containing 50 species each.

Fig. 3.68. Two examples for "Nördlinger" sections cut with the Lab-microtome. The sections can be dried after cutting between two felt layers. Because of their thickness (60 µm) they are stable enough to directly analyze their structure.

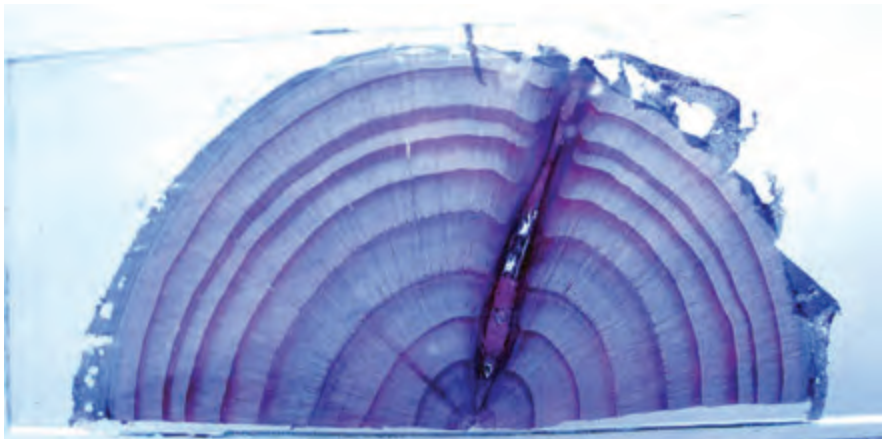


Fig. 3.69. Very large stained thin section of a larch (50 mm in diameter).

3.6.3 Sectioning with microtomes and observation without staining

For all specimen it has to be guaranteed that the sample is correctly oriented in the sample holder. Depending on the purpose, you can cut transversal (cross), radial or tangential sections. For cross sections, the sample has to be positioned with the fibe direction at 90 degrees relative to the blade. For radial and tangential sections, the fibe direction has to be parallel to the blade, whereas for radial sections the cut has to be done parallel to the rays, directed towards the pith (or center of growth).

3.6.3.1 Material of normal stiffness

Clamp the sample very tightly in the sample holder and cut the surface to a fla plane before you intend to take a micro section, using water on a painting brush to keep the surface wet. Without adding water to the surface, the cell walls tend to break while cutting because they are more or less brittle. When adding water to the top of the sample, the cell walls get moist and therefore more flexible

When the surface is plane and all saw marks are gone, bring the blade in a position where it is definitel sharp or change the blade (Figs. 3.70 and 3.71). Now, hold the brush on top of the wet object and pull the blade slowly in a steep angle (pulling angle) under the brush (Fig. 3.72). By using water even on top of the blade, the section glides and does not roll-up on the blade, and can be transported from the brush to a glass slide (Fig. 3.73). Water or glycerol are perfect gliding liquids for sections, although on soft tissues, sectioning requires the use of ethanol 96% or 99% as a gliding solution, which helps stabilizing the tissue.

Fig. 3.70. Position of the pulling angle of the blade in relation to the object. The optimum angle of the knife is around 45 degrees, but depending on the species of your sample and the desired quality of your section, this angle should be adjusted until an optimal section is achieved.

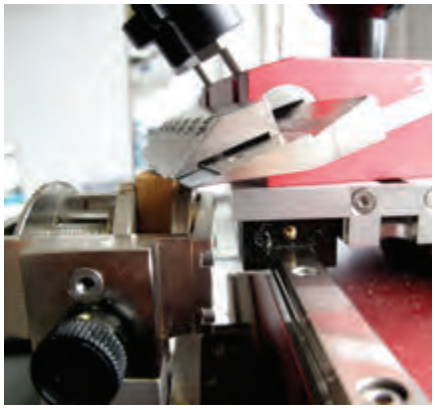


Fig. 3.71. Position of the angle of inclination of the blade in relation to the object. For hard woods, e.g., tropical woods the incline should be fairly steep. In soft material, e.g., archeological wet wood, the incline should be as flat as possible.

Fig. 3.72. Keeping the section flat with a painting brush on the blade.

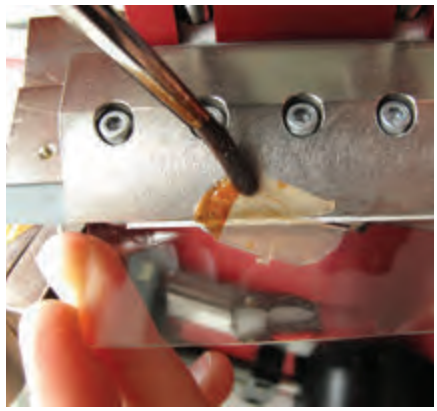


Fig. 3.73. 'Swimming' or gliding a section from the blade onto the glass slide.

If the section rolls while cutting, you can prevent this to a certain degree by placing the brush on top of the section to keep it flat If the sample rolls anyway or even after cutting, put a needle or the tip of a pair of tweezers into the roll and massage the section carefully onto your finge tip to unroll it and harden it with a drop of ethanol. If the section remains flat place it from the finge tip directly onto the slide (Figs. 3.74a–d). Do not stain the section when it is rolled, as the result would be irregular stain-patterns.

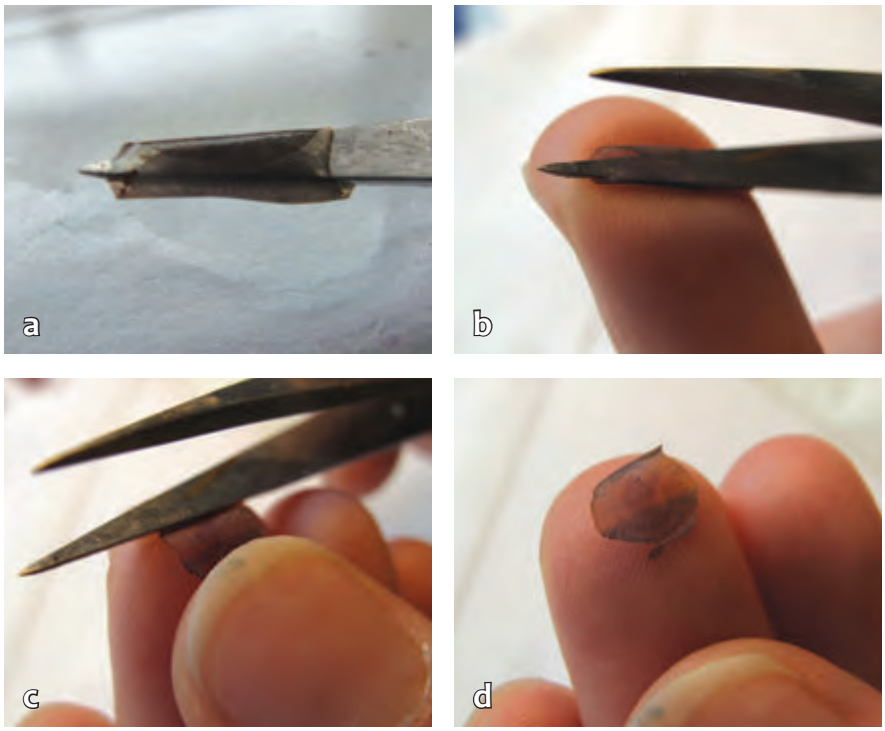


Fig. 3.74. a) The tip of a pair of tweezers must be placed in the center of the rolled section. b) and c) Massage the rolled section on the finger tip over the conical tweezer tip. Stabilize the section in a flat position on the finger tip by putting a drop of absolute ethanol on it. d) Put the flat section onto the slide and start the staining process.

3.6.3.2 Sectioning conifers

Perfect sectioning of conifers is a challenge because secondary walls are only lightly bonded with primary walls especially in earlywood tracheids. Because of this, it is absolutely necessary to use a sharp blade to avoid splitting o the primary walls and bending the cell lumen area inwards (Fig. 3.75). The break-away of the secondary walls can also be avoided by using the full length of the blade for sectioning.

In addition to the above-mentioned procedure, cell lumina can be fille with a non-Newtonian fluid The principle of a non-Newtonian flui is that it changes its consistency from a viscose flui to a solid as soon as it is placed under mechanical pressure. Loïc Schneider, of the WSL Dendroecology group, created such a flui by adding some drops of water and glycerol to corn-starch (Schneider & Gärtner, in press). The consistency of the mass is right if the paste "crumbles" when stirred and flow when at rest. Then it should be liquid enough to just flo into the cells when applied to the surface of the sample. When the blade presses against the surface the flui acts as a solid and therefore stabilizes the cells (Fig. 3.76).

If you intend to electronically measure cell wall and cell lumina dimensions, this simple procedure saves a lot of laborious effor to digitally correct the images.

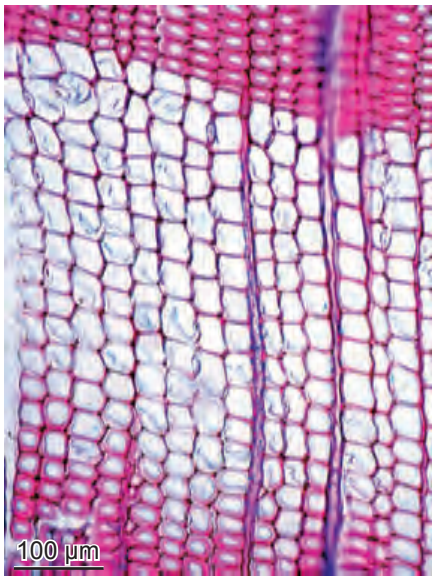


Fig. 3.75. Problematic section: pulled-out secondary walls in the earlywood cells of a conifer. Pseudotsuga menziesii. Section courtesy by Jodi Axelson.

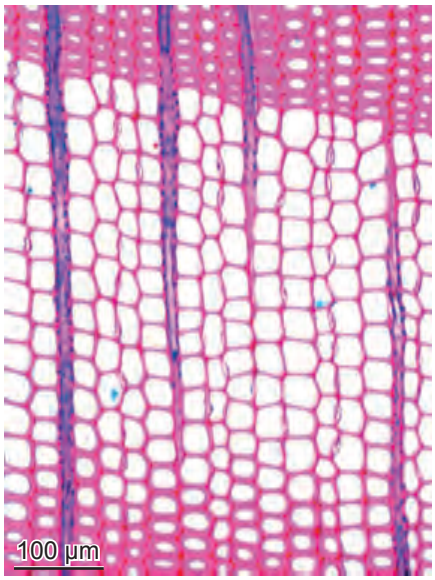


Fig. 3.76. Perfect section: Secondary walls in the earlywood are connected with the primary walls. Pseudotsuga menziesii. Section courtesy by Jodi Axelson.

3.6.3.3 Sectioning of very soft material

Sections of very soft tissues often get compressed at the very slight flang between the blade and the holder. If the blade is not fully inserted into the holder, the gliding space is larger and the section glides without any mechanical resistance onto the blade (Fig. 3.77).

When a high-quality section is ready, you should immediately label the slide either with a soft pencil on a self-adhesive label or with a permanent fin marker directly on the glass (Figs. 3.78 and 3.79). ***Important Note:*** Labels written with ball pens and/or permanent fin markers will dissolve in ethanol.

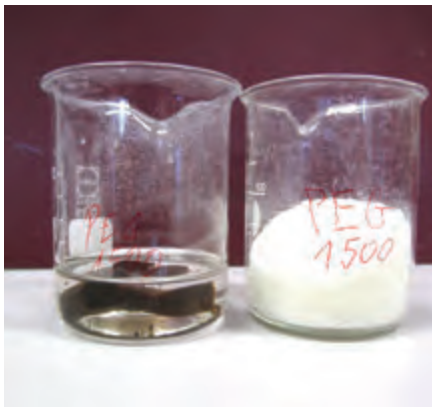
Fig. 3.77. Protrude disposable blade fixed in the blade holder. Soft sections slip onto the flat surface of the blade.

Fig. 3.78. Temporary labeled slides with a permanent marker, be aware that it dissolves in ethanol or Eau de Javelle.

Fig. 3.79. Temporarily labeled slides with a soft pencil on self-adhesive paper labels, ball point ink dissolves in ethanol and xylene.

3.6.3.4 Stabilization and sectioning of waterlogged archeological wet wood

Handmade sections using razor blades are useful for wood identificatio (see section 3.3). For studying large surfaces, or decomposed samples, e.g., structures with degraded cell walls, the material can be soaked with polyethylene glycol 1500 (PEG = Carbowax). The Carbowax flake must be fluidize at temperatures of 60°C. Then, anatomically prepared blocks with a size of approximately 1 cm^3 are placed in the viscous liquid of the Carbowax (Fig. 3.80). The material has to be saturated for at least 24 hours at 60°C in an oven. At temperatures of 20°C the saturated blocks are stable enough to be sectioned with the microtome. Since Carbowax is water-soluble, use glycerol or ethanol instead of water for sectioning. Once a successful section is made, follow all the steps described in section 4. If the sections are thin enough, anatomical characteristics can be recognized in detail (Figs. 3.81–3.84). ***Important note:*** For these types of samples, the pulling and inclination angles of the blade must be minimal.



◂ *Fig. 3.80. Polyethylene glycol 1500. Commercial flakes at room temperature (right). Heated PEG solution at 60°C containing wet wood samples (left).*

▾ *Figs. 3.81–3.84. Sections of waterlogged archeological soft wet wood preserved with polyethylene glycol (Carbowax) and stained with Astrablue/Safranin, Alnus sp.*

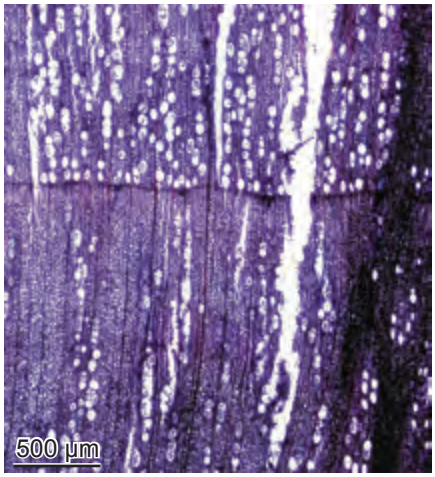


Fig. 3.81. Cross section of a large radially split section.

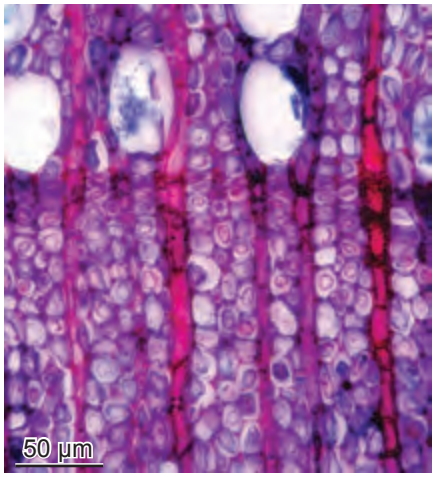


Fig. 3.82. Cross section of an annual ring boundary. Secondary walls of fibers are degraded. Preserved are primary and tertiary walls.

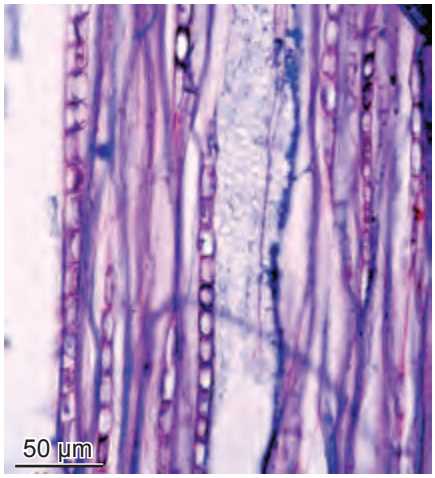


Fig. 3.83. Tangential section. Preserved are the rays, and almost completely degraded are the vessel-walls.

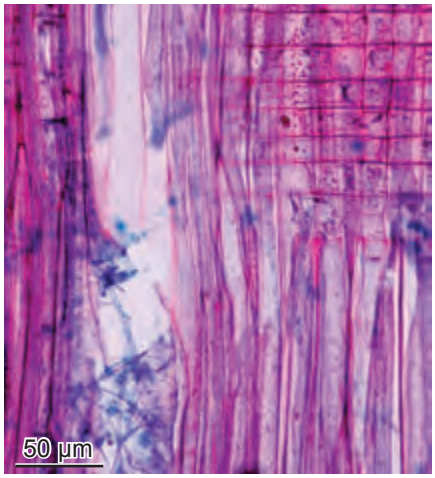


Fig. 3.84. Radial section. Preserved are the rays (red) and degradation products in ray cells as well as hyphae of fungi in the vessel (blue) and tertiary walls of fibers (bluish).

3.6.3.5 Observation of soft herb stems

If you are simply interested in determining the age of the section, and in recognizing the annual ring boundaries, staining is not mandatory. Without staining, anatomical details can be recognized by closing the condenser (Figs. 3.85 and 3.86), or using polarized light (Figs. 3.87 and 3.88).

▾ *Figs. 3.85–3.88. Unstained sections of very soft herb stems.*

Fig. 3.85. Daucus carota, 2-year-old semi-ring porous polar root, normal light transmission.

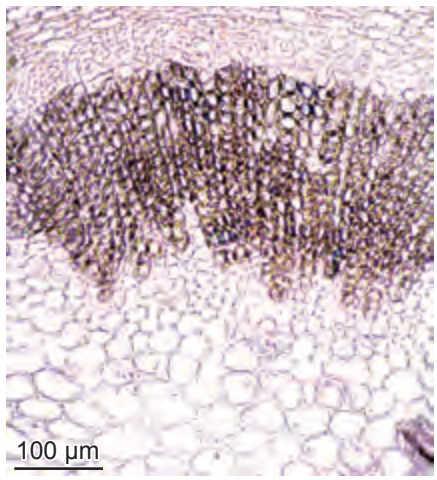


Fig. 3.86. Bellis perennis, 2-year-old vessel-less rhizome, normal light transmission.

Fig. 3.87. Centaurea jacea, 4-year-old polar root, polarized light.

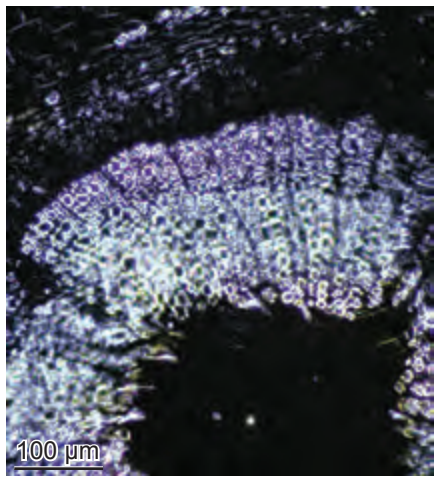


Fig. 3.88. Medicago lupulina, 2-year-old polar root, polarized light.

3.7 Storing glycerol-preserved sections

If the sections don't have to be stained, they can be covered with glycerol (glycerin) and a cover glass, which prevents the sections from drying out (Fig. 3.89). Such preparations can be stored for months in commercial slide holders (Figs. 3.90 and 3.91). The holders must be stored horizontally, otherwise the cover glasses move sideways.

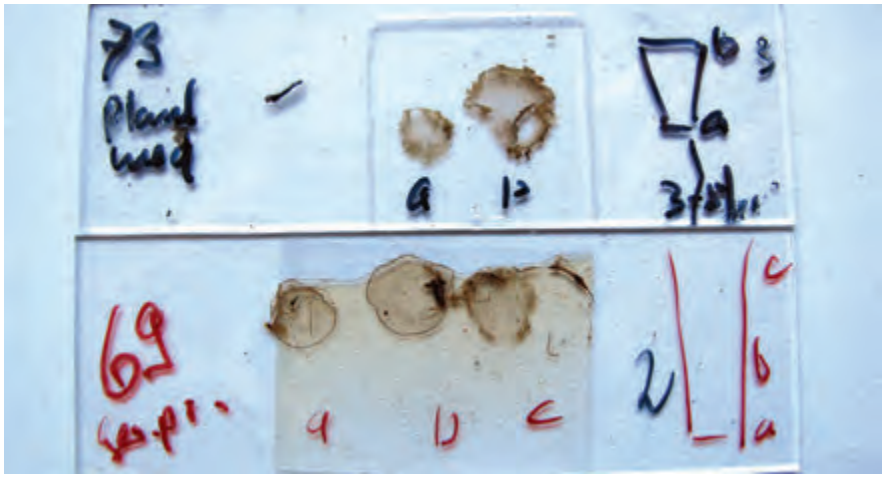


Fig. 3.89. Two slides with sections of herbs, preserved in glycerol since one year.

Fig. 3.90. Slide holder with unstained sections and temporarily labeled slides.

Fig. 3.91. Stack of slide holders in the horizontal position.

3.8 Maceration and measuring axial cell dimensions

Normally, the length of vessels or ray cells can be measured on thin radial sections (Fig. 3.92). By observing sections you precisely know in which intra-annual position the measured vessels occur, i.e., in the earlywood or latewood. Maceration is necessary for measuring fibe length. For ecological studies it is therefore important to isolate a slice within the annual ring (e.g., in the earlywood or the latewood). For technical analysis, e.g., for pulp studies, the samples must represent a mean of the whole wooden block.

After comparing differen maceration methods proposed in the literature, we recommend the following method.

Dispensing the mazeration solution:
1 part hydrogen peroxide (H_2O_2) + 1 part concentrated acetic acid + 1 part water

Fill small Erlenmeyer flask with 50 ml of the maceration solution and add several wood pieces not larger then a match in diameter. After boiling the material (Fig. 3.93) for 3–6 hours, the wooden pieces are completely bleached (delignified and the middle lamella of the fiber are dissolved (Figs. 3.94–3.97). Take small pieces with a tweezer and put them onto a water-permeable, non-staining tissue and wash the sample with water by using a pipette. When the solution does not smell anymore, tease the sample until the fiber are visible. Now put a drop of Astrablue on the tissue with the fibers Dehydration and embedding is described in sections 4.9 and 4.10.

With Eau de Javelle (bleach) it is possible to macerate fiber-nest of wasps (Fig. 3.98). The nest-tissue decays after soaking for a few minutes with bleach. After rinsing the fibe solution with water, it can be dehydrated and stained with Astrablue.

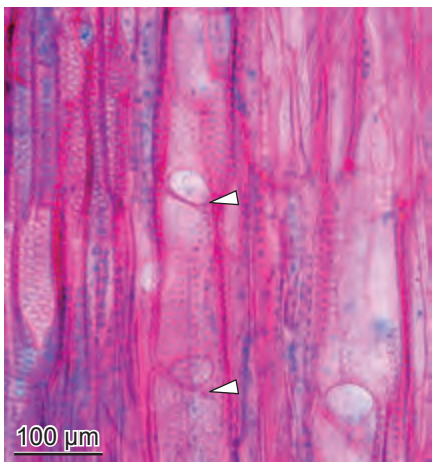


Fig. 3.92. Aspect of vessel length (indicated by the two arrows) in a radial section of a Mediterranean dwarf shrub. Pallenis spinosa.

Fig. 3.93. Mazeration of wood samples in an Erlenmeyer flask, containing a solution of hydrogen peroxide, acetic acid and water, on a heater.

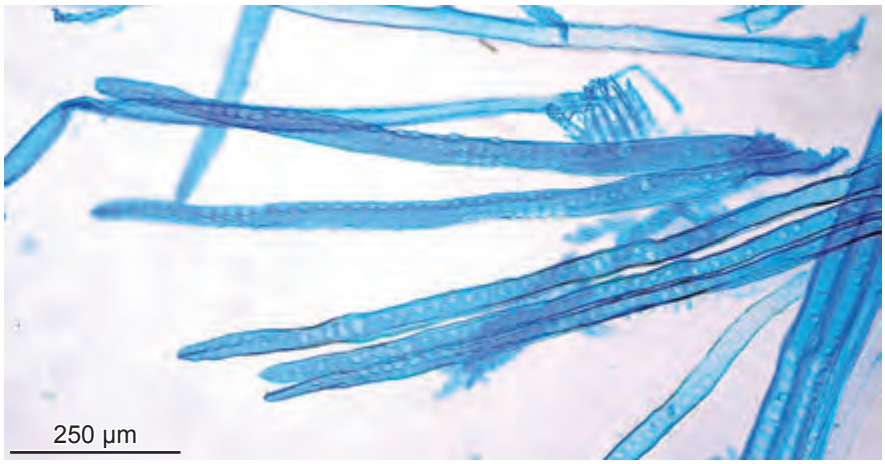


Fig. 3.94. Macerated long tracheids of a conifer, Pinus cembra.

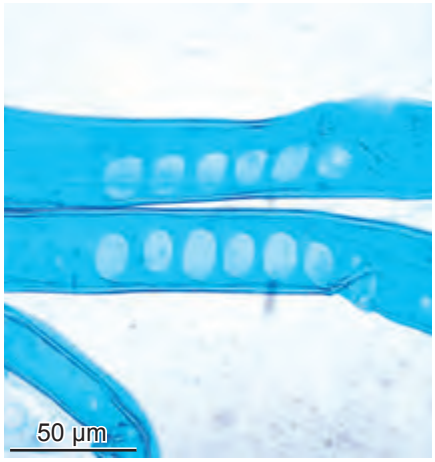


Fig. 3.95. Macerated tracheids of Pinus cembra with fenestrate ray-tracheid pits and bordered pits.

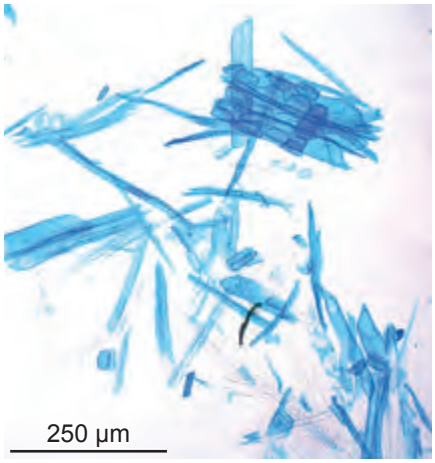


Fig. 3.96. Macerated short fibers, vessels and ray cells of a broadleaf dwarf shrub, Empetrum nigrum.

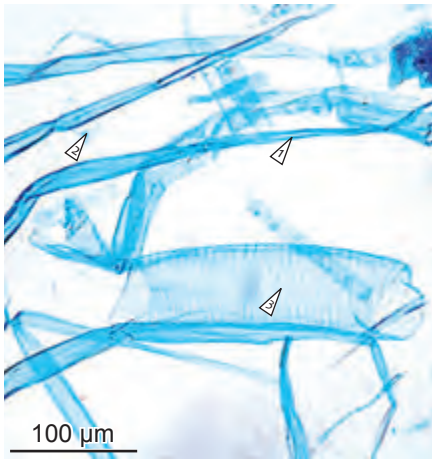


Fig. 3.97. Macerated fibers (1), rays (2) and vessels with helical thickenings (3) of a tree, Tilia sp.

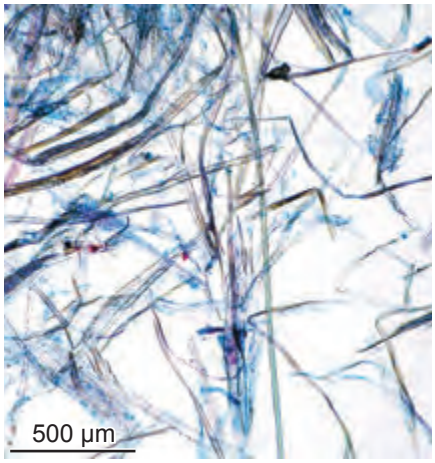


Fig. 3.98. Macerated fibers of a wasp-nest.

3.9 Preparation of textile fibers

Fibers of all textiles, e.g., clothes, lines, ropes and artificia tissues, are already macerated. Their structure can be analyzed by just picking some fiber out of the tissue using, e.g., a pair of tweezers. After dehydration on a glass slide and embedding in Canada balsam as described in section 4.8, most structural details are visible in polarized light. Therefore, staining of the fiber is not necessary. The following fi - ures illustrate images taken using polarized light from unstained fibers extracted from textiles and ropes (Figs. 3.99–3.102).

Fig. 3.99. Isolated Nylon fibers from a shirt.

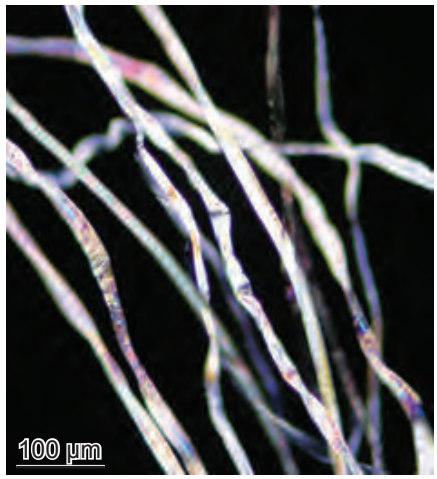


Fig. 3.100. Isolated cotton fiber from a shirt.

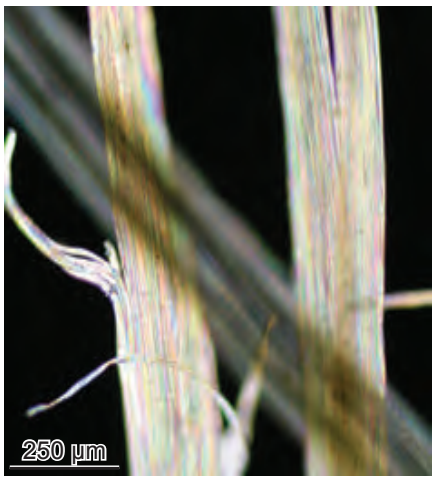


Fig. 3.101. Bundle of fibers in a string of flax of Central Europe.

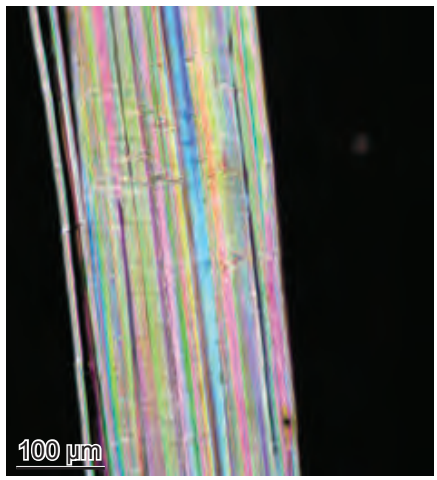


Fig. 3.102. Bundle of fibers in a string of agave from Mexico.

3.10 Preparation of technically composed wood

Plywood is the result of a technical procedure, where several veneers are glued together in opposite ber directions (Figs. 3.103 and 3.104). The main goal of this and most other arti cially recombined wood parts or components is the elimination of anisotropy.

Even plywood can be sectioned with microtomes and stained like common wood samples. Microscopic slides allow the identi cation of the tree species used because the structure is clearly visible. Furthermore, the penetration of the glue into the wood structure at the contact surfaces becomes visible (Figs. 3.105 and 3.106).

Sectioning particleboards without embedding succeeds only in the direction of the longitudinal axis of bers and chips. The staining process is the same as for normal wood.

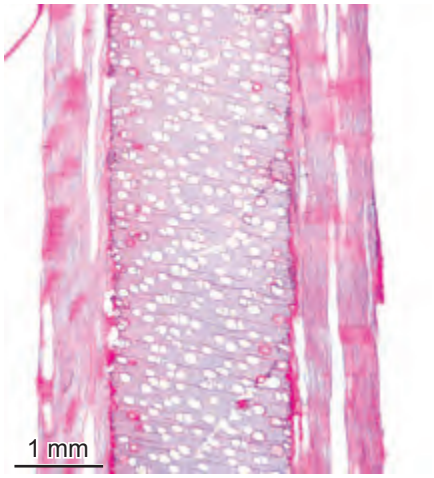


Fig. 3.103. Cottonwood-Plywood consisting of 3 layers, stained with Safranin only.

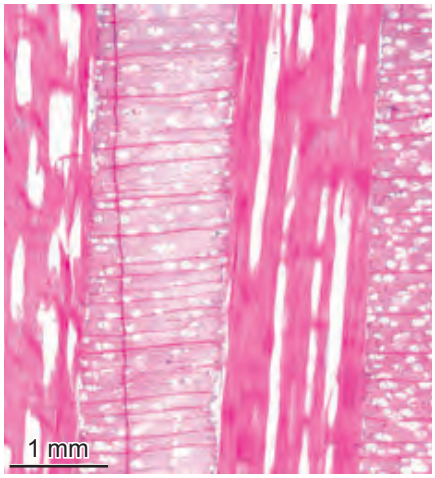


Fig. 3.104. Birch-Plywood consisting of 5 layers, sectioned in opposite directions and stained with Safranin only.

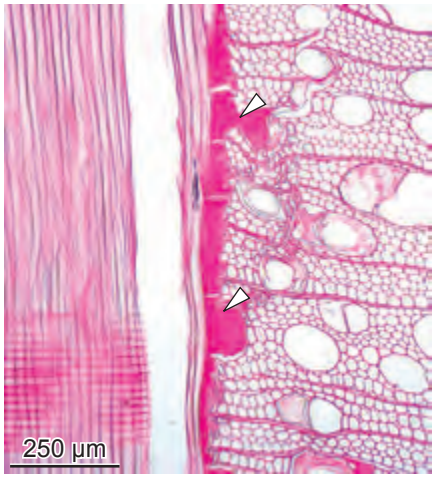


Fig. 3.105. Glued joint of the Cottonwood-Plywood where a huge amount of glue has been used (irregular, intensely red areas, see arrows).

Fig. 3.106. Glued joint of the Birch-Plywood with a minimal use of glue (arrows).

Fiber boards consist of chemically macerated wood and mechanically destroyed fiber (Figs. 3.107–3.112). Wooden hardboard plates consist of common wood bits and pieces, which have been compressed under high pressure (Fig. 3.113). Sectioning these types of artificia wood is possible in all directions.

Fig. 3.107. Particle boards consist of thin, axially sectioned wood chips, which are oriented in all directions. Section stained with Safranin only.

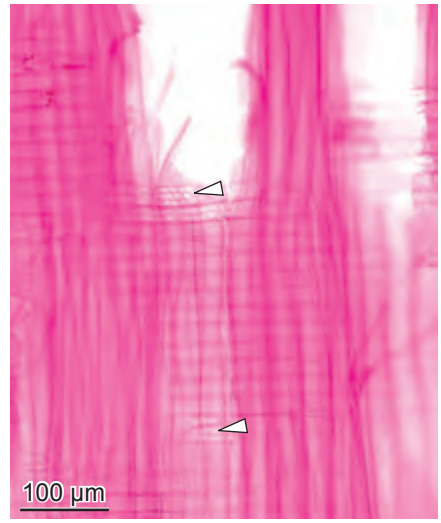


Fig. 3.108. Radial chip of a cottonwood stem. Microscopic anatomical features are well preserved and can be used for wood identification. Reticulate pits (arrow) in the homogeneous ray are an indication for Populus sp.

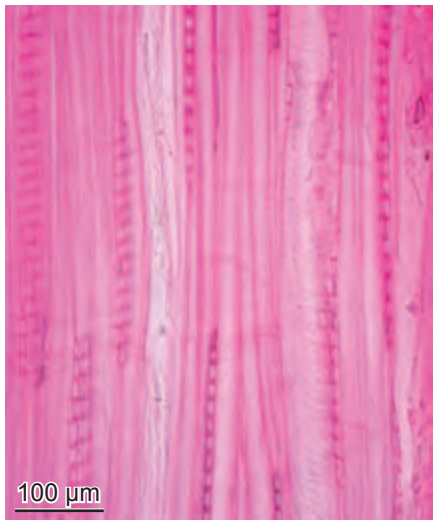


Fig. 3.109. Tangential chip of cottonwood stem.

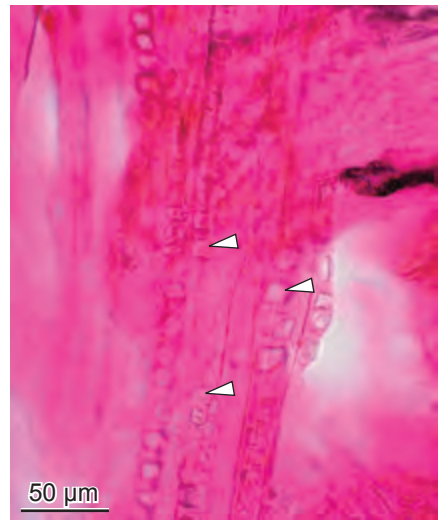


Fig. 3.110. Foreign wood type with crystals (arrows) in a cottonwood chip board.

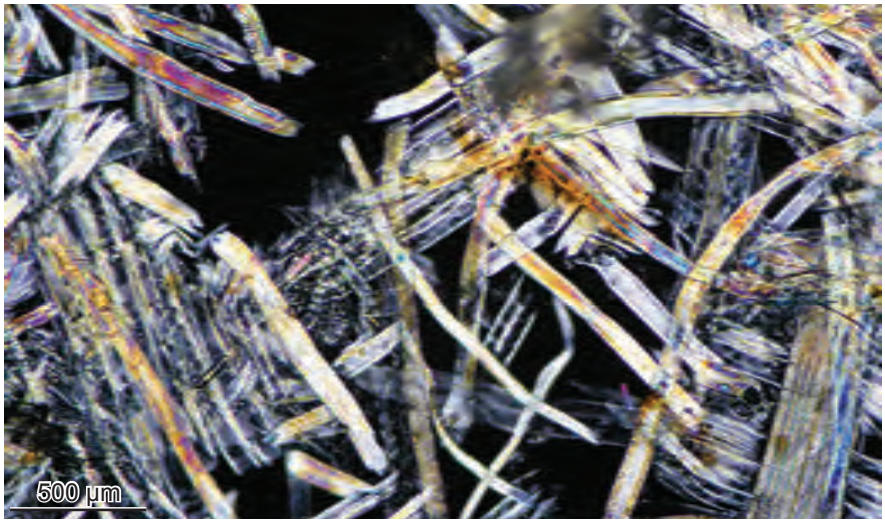


Fig. 3.111. Isolated and broken fibers are characteristic for pressed fiber boards. Polarized light.

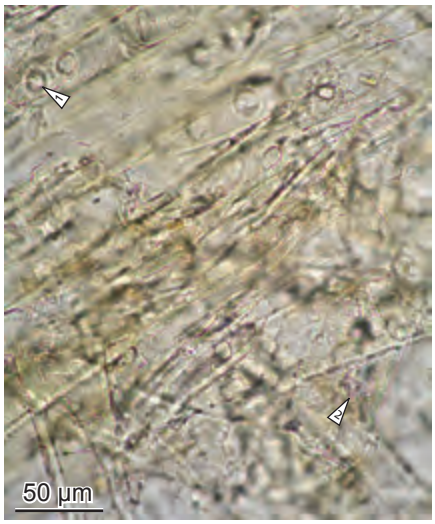


Fig. 3.112. Fiber board with decomposed pits (1) in conifer tracheids and glue (2).

Fig. 3.113. Wooden hardboard is characterized by bent rays and deformed vessels and fibers. Populus sp.

4. Fixation, bleaching, staining, embedding, cleaning, labeling and storing

4.1 Effect of staining, and safety instructions

Samples have to be stained, dehydrated and embedded in order to be permanent. Staining enhances the contrast of cell-wall structures and cell contents for observations and/or image analyses. Depending on the dye used, differen cell types and cell contents can be distinguished.

Preparing staining solutions

- When preparing the liquid dyes, always follow the safety instructions. Powders are poisonous!
- For preparing the solutions needed for staining, you need a balance, plastic or, if possible, glass bottles with a minimum capacity of about 200 ml, inhalation protection, and gloves.
- Before you start the procedure, make sure to cover the table around the balance and below the dye-powder boxes with some paper tissue. It is also best to wear old clothes, gloves and goggles. If you only loose a single grain of the powder, it might stain everything it gets into contact with, so be careful. Moreover, it is severely irritating to the skin if it gets in direct contact.
- Before starting the preparation process, bring the bottles, chemicals (Fig. 4.1), and instruments into a logical order (Fig. 4.2).

Fig. 4.1. Holder with chemicals for preparation of permanent slides. It contains dyes, ethanol and xylene and corresponding pipettes.

Fig. 4.2. Preparation tools: gloves, paint brushes, tweezers, pipettes, needle, paper knife, marker, slides and cover glasses.

The entire preparation process (staining and dehydration) can be done on the glass slide finall used for the permanent slide (Fig. 4.3). Use a pipette fille with the chemicals needed for the respective process (i.e., one for the dye and another for ethanol 75%) to begin the dehydration process and to rinse the section (Figs. 4.3–4.5). Collect all liquids coming o the slide in a waste box, preferably of glass, as some chemicals, i.e., xylene, dissolves some types of plastics (Fig. 4.6)! If you have to work many hours with chemicals it is best to do so under an extractor hood or near an open window (Fig. 4.7).

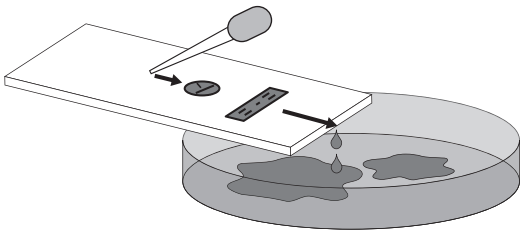


Fig. 4.3. Schematic view of the staining/cleaning process. Use a pipette for the chemicals and run the liquids over the section. For removing the surplus stain you can also place the pipette on top of the section and pump the ethanol through. By doing so you ensure that the dye is also removed from very small cell lumina.

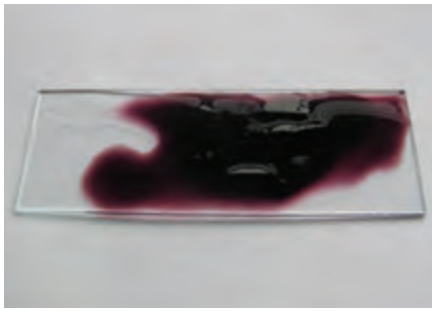


Fig. 4.4. Dye on a slide with micro sections.

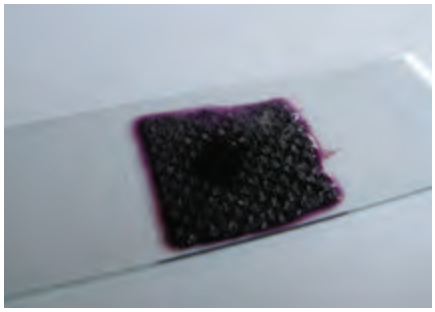


Fig. 4.5. Dye on a slide with a piece of tissue and very small micro sections.

Fig. 4.6. Waste box that is resistant to Eau de Javelle, ethanol and xylene.

Fig. 4.7. Extractor hood.

4.2 Fixation of cell contents

For cytological studies, plant tissues must be soaked with a fix tion solution (Fig. 4.8). This procedure is necessary because without fixatio the cell contents including the nuclei degrade in the ethanol when dehydrating the samples after staining. There are several possible procedures:

Fresh material should be preserved in FAA solution or ethanol 40%. FAA solution and ethanol preserve cell contents but cannot be stained with Astrablue/Safranin.

Dispensing the FAA solution:
5 parts ethanol 96% + 1 part acetic acid + 1 part formaldehyde + 3 parts distilled water

If nuclei and protoplasts are to be visualized by dyes, they must be preserved through an additional fixatio (Nawashin solution) (Fig. 4.8). The two possible procedures are:

- The samples have to be soaked for 3–6 days (Fig. 4.9). Afterwards they must be stored in a glass or plastic bottle containing 40–60% ethanol .
- Micro sections of fresh material, or samples preserved in FAA or ethanol, have to be covered for a few minutes with Nawashin solution. Staining is possible after carefully rinsing them with water (Fig. 4.10).

Dispensing the Nawashin solution (Purvis et al. 1964):
10 parts of 1% chromic acid + 4 parts 4% formaldehyde + 1 part acetic acid

Fig. 4.8. Nawashin solution in a bottle and small glass containers with ethanol 75%, for permanent sample storing.

Fig. 4.9. Fixation of cores with the Nawashin solution in test tubes.

Fig. 4.10. Fixation of micro sections with the Nawashin solution on the slide.

4.3 Bleaching: Destruction of cell contents and dark-stained cell wall components

If unstained sections are brownish, this coloration indicates that the wooden sample contains phenols (Fig. 4.11). Although the presence of phenols is an indication for plant physiological processes (e.g., heartwood formation, compartmentalization (Shigo 1984)), they should be removed before staining the section if one is only interested in the structure of the cells. This is because phenols, like lignin, stain red in Safranin and for this blur the structure of the cell walls.

To dissolve the phenols, put a few drops of Eau de Javelle (bleach) on the slide until the sections appear light (for about 5 minutes) (Fig. 4.11, right). Eau de Javelle available for laboratories is a 13% solution. Common bleach as it is available at retail contains around 5% of Javelle. When using this, you should cover the samples a few minutes longer. Before staining, rinse the slide well with water until you can no longer smell the bleach.

The application of Eau de Javelle to micro sections before staining in general has a positive effec for image analyses, because bleaching also enhances cell wall contrasts (Fig. 4.12).

Eau de Javelle (bleach) is composed of:
sodium hypochlorite ($2\ NaOH + Cl_2$) / potassium hypochlorite ($2KOH + Cl_2$)

Fig. 4.11. Left: Unbleached micro section (Vitis vinifera). Many cell walls contain brownish substances, i.e., phenols. Right: Bleached micro section (Vitis vinifera). Cell walls appear light and transparent.

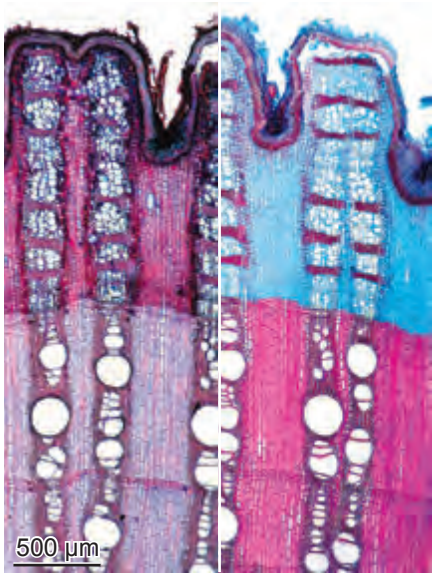


Fig. 4.12. Left: Unbleached micro section (Vitis vinifera) after staining with Safranin/Astrablue. Blue and red colors are not well di erentiated. Right: Bleached micro section (Vitis vinifera) after staining with Safranin/Astrablue. Blue and red colors are well differentiated.

4.4 Staining cell walls

Safranin/Astrablue stains mainly cell walls. Basically, Safranin stains red and creates a very good contrast to further analyze the cell structures using various image analyses programs. Many other dyes are recommended in various textbooks (e.g., Rudzin 1999, Mulisch and Welsch 2010), but in our opinion, Safranin/Astrablue creates the best contrasts between differen cell-wall types (see section 4.2). Safranin stains lignifie cell structures red, and Astrablue stains unlignifi d structures blue (Figs. 4.13 and 4.14). Safranin/Astrablue also stains gelatinous fib rs and makes various chemical compositions visible (Fig. 4.15).

When mixing both solutions (not the powder!) use a ratio of 1:1. There is no need to stain using one dye after the other, instead they can be mixed, staining differen structures in the section simultaneously.

Dispensing the Safranin solution:

0.8 g Safranin powder in 100 ml distilled water

» Never use common tap water for this procedure. Close the bottle tight and shake it until the solution is dark red and no clumps are visible.

» When shaking the solution, you create a certain amount of foam. This foam does not affec the stain and it will disappear after a few hours.

Dispensing the Astrablue solution:

0.5 g Astrablue powder in 100 ml distilled water + 2 ml acetic acid

Without the acetic acid the dye will not remain stable.

» Never use common tap water for this procedure. Close the bottle tight and shake it until the solution is dark blue and no clumps are visible. The Astrablue powder requires a more intense and longer shaking than Safranin to remove all the clumps.

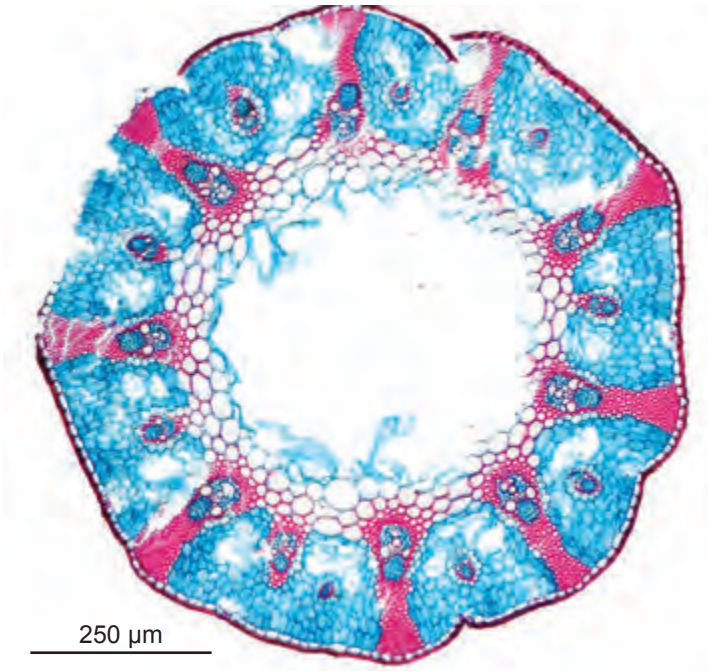


Fig. 4.13. Safranin/Astrablue stained flower stalk of a Gramineae. Lignified (red) are the radially oriented beams of fibers, the vessel cell walls and the external walls of the epidermis. Unlignified (blue) are most parts of the cortex and the phloem within the vascular bundles.

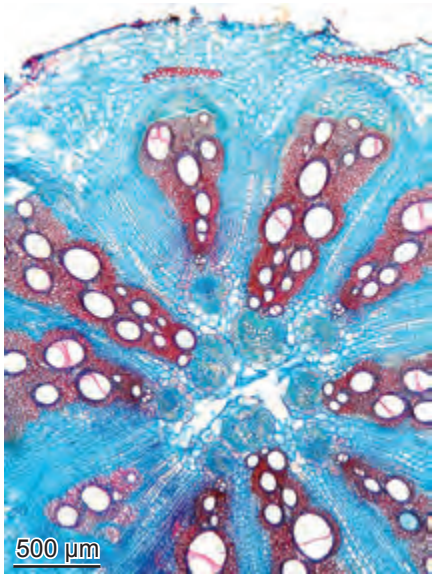


Fig. 4.14. Safranin/Astrablue stained stem of an annual climbing Cucurbitaceae. Lignifie (red) are the xylem of vascular bundles and fibers in the cortex. Unlignified (blue) are the rays and major parts of the bark.

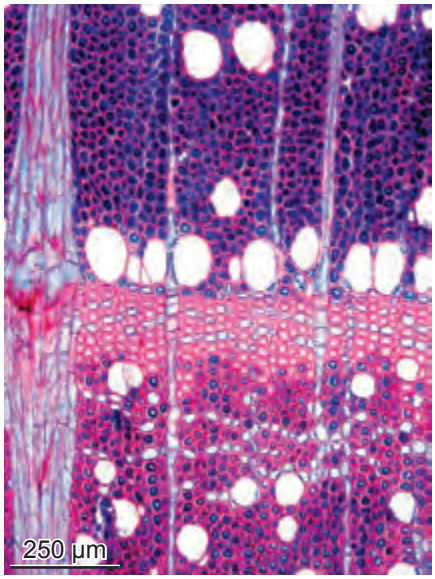


Fig. 4.15. Safranin/Astrablue stained xylem with tension wood of a tree (beech). Red stained are the primary walls of fibers and the secondary walls of fibers in the latewood zone. Dark blue stained are the gelatinous fibers and the axial parenchyma cells.

4.5 Staining cell contents

4.5.1 Nuclei and protoplasts

Picric-Anilinblue is an excellent dye to visualize organic cell contents. Protoplasts, nuclei and even chromosomes appear dark blue and cell walls remain yellow (Fig. 4.16). With the detection of living and dead cells, heartwood and sapwood boundaries become clear, as nuclei are only present in rays of the sapwood, never in heartwood.

This staining can be recommended for studying xylogenetic processes and the detection of sapwood/heartwood boundaries.

Staining procedure: Put some drops of Picric-Anilinblue on sections on a slide. Heat them for a few seconds to approximately 80°C (Fig. 4.17). Clean the section with water and dehydrate it with ethanol 96%, ethanol absolute and xylene. Embed the section in Canada balsam.

Dispensing the Picric-Anilinblue solution:

1 part saturated Anilinblue + 4 parts saturated picric acid (trinitrophenol), dissolved in 95% ethanol

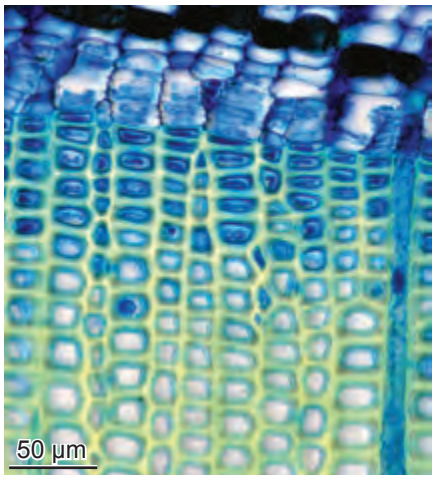


Fig. 4.16. Picric-Anilinblue stained xylem/cambium/phloem zone of a conifer (Abies alba), cored at the end of the growing season. Blue stained are the protoplasts including nuclei and the unlignifi d cell walls. Yellow stained are the lignified cell walls.

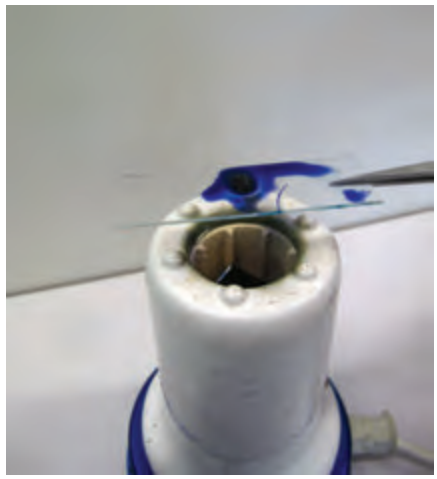


Fig. 4.17. Heater for warming the Picric-Anilinblue solution on slides.

4.5.2 Starch grains

Starch grains indicate where carbohydrates are stored within the tissue (Eades 1937).

Lugol's solution (Lugol'sche Lösung = iodine and potassium iodide solution) has a brownish color (Figs. 4.18 and 4.19).

Put a drop of Lugol's solution on the section on a slide. After a few minutes, starch grains appear brown to violet and lignifie cell walls yellowish. The staining effect disappear after a few hours. Making permanent slides is not possible. Preserve the sections for observations in glycerol.

Starch grains are also visible in polarized light. They appear as light circles with dark Maltesian crosses (Fig. 4.20).

Dispensing Lugol's solution:

10 g of potassium iodide + 5 g of iodine in 100 ml distilled water

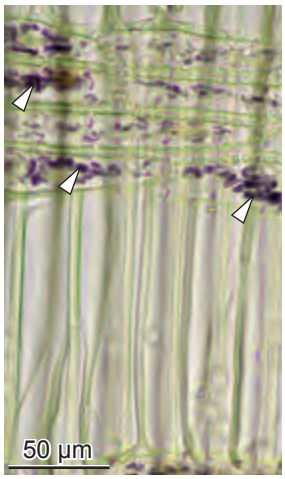


Fig. 4.18. Dark stained starch grains (arrows) with the Lugol's solution in rays of a conifer (Abies alba).

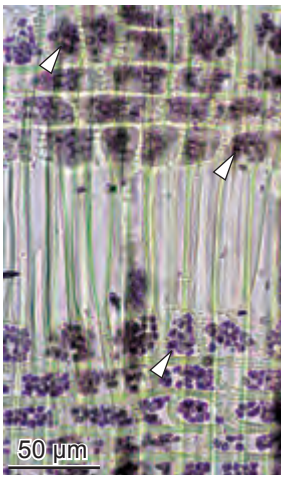


Fig. 4.19. Dark stained starch grains (arrows) with the Lugol's solution in rays of a broadleaf shrub (Corylus avellana).

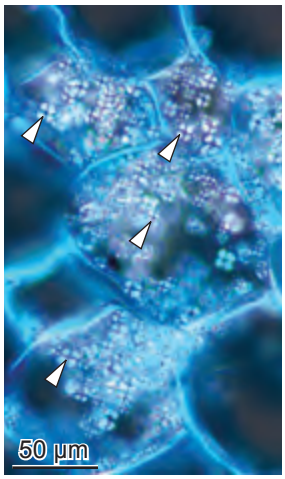


Fig. 4.20. Unstained starch grains (arrows) reflec ed in polarized light.

4.6 Staining cell walls and cell contents

For studying the timing of xylogenetic processes it is necessary to recognize cell contents as well as the lignificat on of cell walls. A combination of Astrablue/Safranin and Picric-Anilinblue dyes yields excellent results. Protoplasts appear blue and nuclei dark blue, unlignifie cell walls light blue and lignifie cell walls red (Figs. 4.21 and 4.22).

Stain the section on the slide with Astrablue/Safranin for 3–4 minutes, rinse the dye with water and then put some drops of Picric-Anilinblue on the section and heat it until the slide gets hot (approximately 80°C). Now rinse out the dyes with water, dehydrate, and embed in Canada balsam.

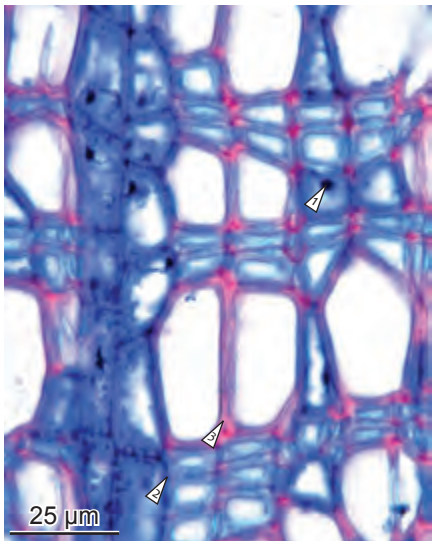


Fig. 4.21. Astrablue/Safranin and Picric-Anilinblue stained cross section of the xylem of a dwarf shrub. Dark blue stained are nuclei (1), light blue secondary walls of fibers (2), and red parts of primary cell walls of fibers and vessels (3).

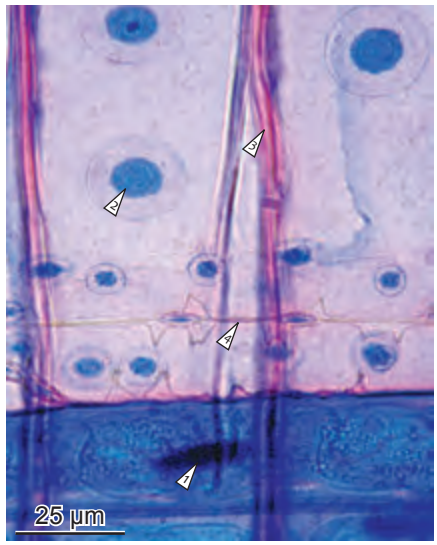


Fig. 4.22. Astrablue/Safranin and Picric-Anilinblue stained radial section of a conifer (Pinus sylvestris). Dark blue stained are nuclei (1), light blue tori of pits (2), and reddish are cell walls of axial tracheids (3) and ray-tracheids (4).

4.7 Staining hyphae, bacteria and decomposed cell walls

Recognition of hyphae is important for the interpretation of wood degradation. Well approved is Picric-Anilinblue for staining hyphae. Picric-Aniliblue stains hyphae and bacteria dark blue, lignifie cell walls yellowish and de-lignifie cell walls blue in various intensities (Figs. 4.23–4.26).

Dispensing of the solution of Picric-Anilinblue is described in section 4.5.1. Staining procedure: Put some drops of Picric-Anilinblue on sections on a slide. Heat them for a few seconds to approximately 80°C. Clean the section with water and dehydrate it with ethanol 96%, ethanol absolute and xylene. Embed the section in Canada balsam.

Staining with Astrablue/Safranin and Picric-Anilinblue creates contrasts, which show cell wall degradations and hyphae (Fig. 4.27).

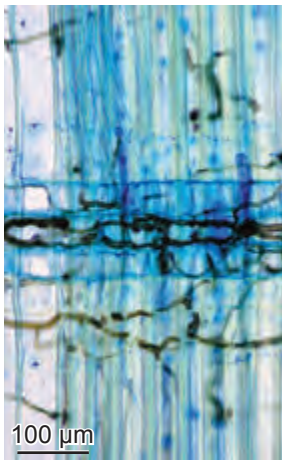


Fig. 4.23. Dark blue stained hyphae with Picric-Anilinblue.

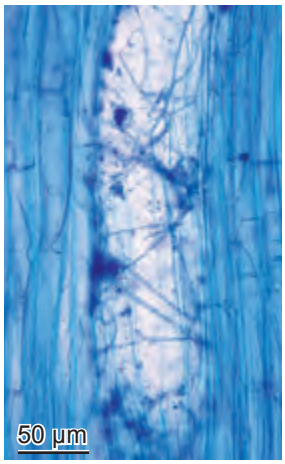


Fig. 4.24. Blue stained hyphae in a vessel of a de-lignified wet wood.

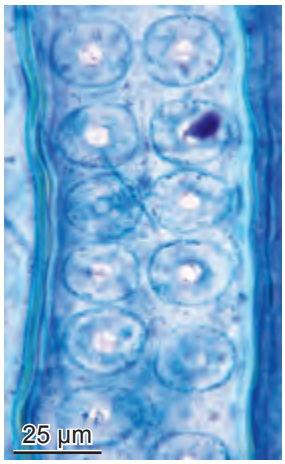


Fig. 4.25. Blue stained bacteria in tracheid pits of a drift wood conifer, Larix sp.

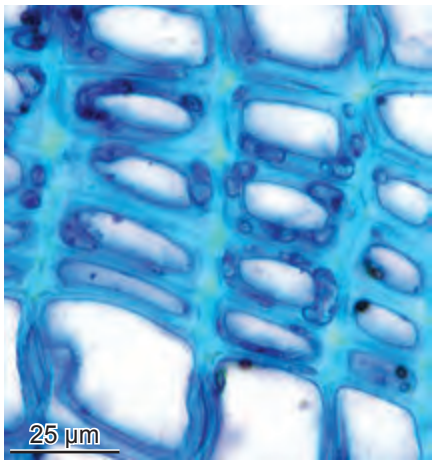


Fig. 4.26. Blue stained red rot caverns in latewood tracheids. Lignified parts appear yellow.

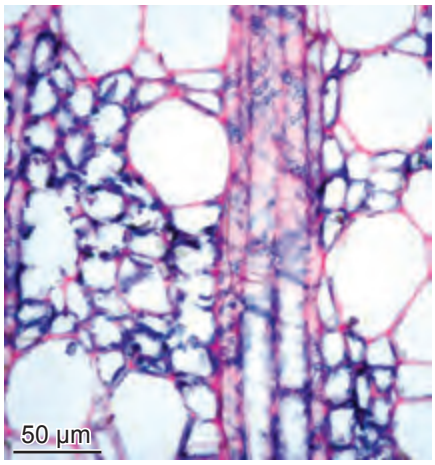


Fig. 4.27. Secondary walls of fibers in the xylem of a broadleaf tree. White rot fungi decomposed and de-lignified mainly the secondary walls of fibers. Hyphae grow spiral-like in the rays.

4.8 Dehydration

Dehydration is necessary to prepare permanent slides for the following reason: The refraction index of water (n = 1.33) is lower than that of microscopic cover glass (n = 1.523). The finalize section will at the end be embedded in Canada balsam, a resin having more or less the same refraction index (n = 1.51–1.54) as the glass of the micro slides and the cover glass.

When the samples have been covered by the stain for 3–5 minutes, they are ready to be processed. Remember: all further steps occur on the slide (Figs. 4.28 and 4.29). First, use water to wash away the surplus stain until the water running over the sample is more or less clear. The use of water for the firs basic cleaning helps save ethanol.

Now you can start the dehydration process by using ethanol 75%. When this runs clear o the sample use ethanol 96%. To really remove the stain from the cell lumina and inter cellular spaces you can place the pipette directly onto the sample and pump the ethanol through the sample. Do this until the ethanol runs more or less clear. If the sections are rolled, it is possible to unroll them (see section 3.6.2). To finaliz the dehydration process, rinse the sample with anhydrous ethanol.

Dispensing anhydrous ethanol:

95 ml ethanol 96% + 5 ml 2,2 dimethoxypropane (acetone dimethyl acetal)

Finally, prepare the section for embedding. Rinse the section with some drops of xylene or Histoclear. If the liquid runs clear, you can continue embedding the sections in Canada balsam. If the liquid gets milky, repeat the dehydrating process with anhydrous ethanol, and finall rinse with xylene again.

Fig. 4.28. Dehydration of a section of wood.

Fig. 4.29. Dehydration of tiny sections of grasses on a tissue.

4.9 Embedding micro sections

Canada balsam and Euparal have excellent optical qualities but are slightly acidic. Therefore, some stains for cell contents bleach out after several years. Furthermore, Euparal has a slight shrinking tendency. In our opinion, Canada balsam is well suited for all stains presented here. Colors remain stable over decades and shrinking does not occur. The only little drawback, despite the fact that xylene has to be used to prepare the section before embedding in Canada balsam, is the slight oxidation (yellowish to brownish color) of the balsam at the edges of the cover glass after several years. However, this does not disturb the optical clarity. A very nice example for the durability of micro sections embedded in Canada balsam is a micro slide prepared by Nördlinger in 1878 (Fig. 4.30). For all other embedding resins, we have no long-term experience.

The preparation of large sections is differen from preparing small sections. For a large section (i) put a small drop of Canada balsam on top of the section, (ii) place the edge of the cover glass on one side of the object without touching it and (iii) fold down the cover glass slowly to fully cover the object. For small sections prepared on a tissue, put a drop of Canada balsam on the glass and turn the tissue with the sections towards the Canada balsam (Figs. 4.31 and 4.32). The small sections stick in the Canada balsam and the tissue can be taken away. The sections are now ready to be covered with the cover glass (Fig. 4.33).

When the cover glass is placed on top of the sections, you should start moving the tweezers on top of the cover glass while exerting a little pressure to it. In doing so, the section will be flattene below the glass, excessive Canada balsam will be pressed out and you avoid the enclosure of air bubbles (Figs. 4.34 and 4.35).

Fig. 4.30. A 135-year-old micro slide of an unstained stem of a silver fir [Abies pectinata (syn.: Abies alba)]. The section is preserved in Canada balsam. The section is transparent, only the borders around the cover glass appear brownish.

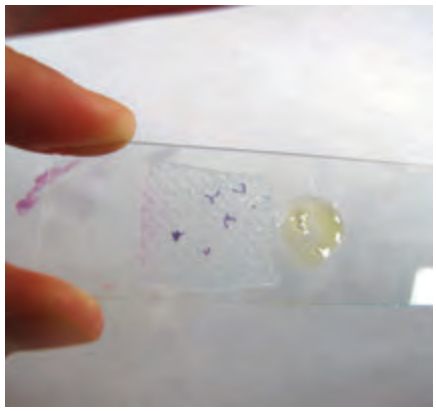


Fig. 4.31. Tissue with small sections next to the glass with a drop of Canada balsam.

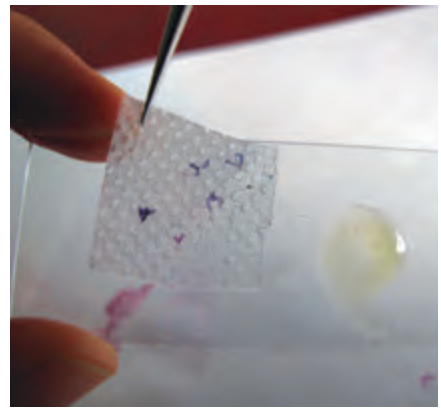


Fig. 4.32. Turning the tissue with the sections towards the Canada balsam.

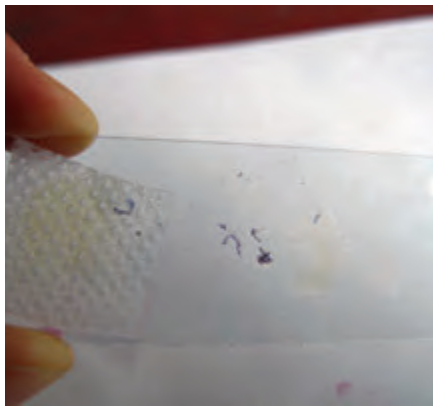


Fig. 4.33. The small sections are trapped in the viscous Canada balsam.

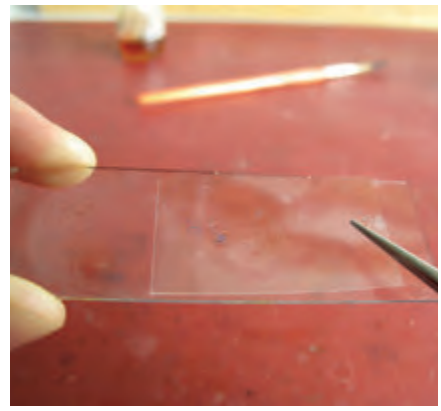


Fig. 4.34. Covering the section with the cover glass. Enclosing air bubbles can be avoided by putting the glass down from one side to the other.

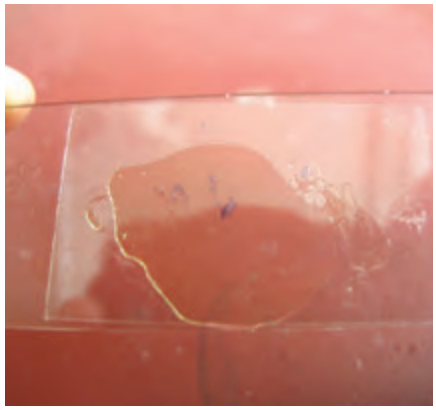


Fig. 4.35. Covered section. The Canada balsam spreads itself over the whole surface of the glass.

4.10 Drying and stabilizing

Slides are placed between two heat-resistant plastic strips on an iron plate. Magnets on the plastic surface press the sections fla (Figs. 4.36–4.38). Without the plastic strips, dried Canada balsam would stick to the plate and it would not be possible to remove the slide without destroying it. If you have no magnets available, use any small, but heavy objects (i.e., box with glass slides) to place on top. The magnet (or weight) is needed to prevent the cover glass from being lifted during the drying process. The slide is dried out after a few days to weeks at room temperature or after 12–24 hours in an oven with a temperature of 60°C (140°F) (Fig. 4.39).

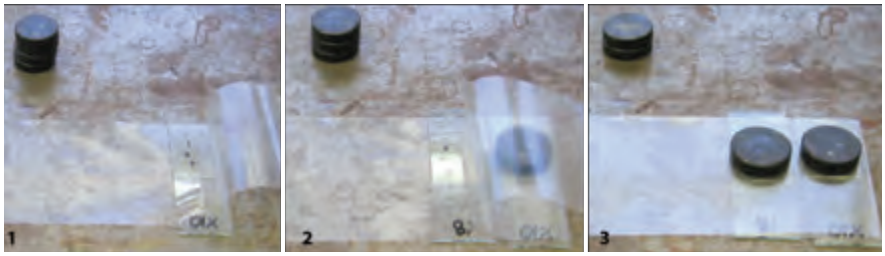


Fig. 4.36. Place the embedded sample (slide) on top of a heat resistant plastic strip on a metal plate (1). Place a second strip on top and fix it with a magnet (or other weight) (2). Repeat the procedure for all other slides (3). Always have a (heat resistant) plastic strip below and above the slide!

Fig. 4.37. Slides on an iron plate between plastic strips, and weighed down with magnets.

Fig. 4.38. Serial production of sections ready to be dried.

Fig. 4.39. Serial production of sections in an oven.

4.11 Removing from plastic strips and cleaning slides

When removing the slides from the oven, make sure that you do not touch the magnets or slides before they are cold. As long as the slides are warm, the Canada balsam is still liquid and the samples will be ruined when removing the plastic strips or even the magnets.

As soon as the slides are cold you can start to remove the magnets very carefully. For best results press down on the covered slide with two fing rs and slowly pull o the magnet. Then take the upper plastic strip covering the slides and slowly pull it o laterally. Make sure to not pull it upwards (Fig. 4.40).

After removing the slides from the plastic strips, the glass needs to be cleaned, i.e., the Canada balsam which squeezed out on top and below the slides during the drying process in the oven needs to be removed.

For removing the Canada balsam, never use chemicals. The easiest and fastest way to clean the slides is using a razor blade or a paper knife (Fig. 4.41). Place the blade fla on the slide and just scrape the dried balsam o the glass.

Fig. 4.40. Cold micro slides after removing the magnets (upper image). Note that the Canada balsam has spilt over the slides. Always pull o the plastic strip sideways in order to not break the slides (lower image).

Fig. 4.41. Cleaning dry slides with a paper knife.

4.12 Labeling

This is a very important step in preparing micro sections in order to avoid mix-ups of your samples. The (basic) labeling should already be done after cutting the section and placing it on the glass slide. But be aware that during the staining and dehydration process there is a risk that the label will be dissolved. Make sure that you place the writing on one side of the glass so that you can hold the slide with your fingers on this side during the staining to embedding process. As soon as the slide is finalized, i.e. the slide is cleaned, you should decide what long-term labeling your slides require. Modern, self-adhesive labels will last for decades on the slide (Fig. 4.42).

Fig. 4.42. Various types of labeling.

4.13 Operating a line for staining, dehydrating and embedding

In most cases you do not stain just one sample. Although this is possible, the more common situation is that you have to process many samples, which are covered by glycerol, in a row. To avoid waiting for each single sample covered by the dye, you can open up a line. This can be done as follows:

- You take the **first slide**, remove the glycerol from the sample by rinsing it with water, cover the section with the stain and place it on the desk.
- Then take a **second slide**, remove the glycerol and also cover this section with the stain and place it next to the first slide.
- Wait for another 1–2 minutes, then take the **first slide** and start the preparation process (washing off the stain, dehydrating, rinsing with xylene, embedding) and place the slide between the plastic strips and load it with a magnet or any other weight.
- Then take a **new (third) slide**, remove the glycerol, cover it with the stain and place it next to the second slide, which is now covered by the stain for about 4–5 minutes.
- Take the **second slide** and start again the dehydration and embedding process and finish it between the plastic strips.
- Take a **new slide**, cover it with the stain, place it on the desk, and continue with the slide already covered by the stain …. **and so on.**

4.14 Repairing cracked (embedded) slides

Sometimes it can happen that you drop a slide, or you accidently break one for another reason. In most cases, these cracked slides can be repaired. Take the broken slide and place it in xylene for about 10 hours. This dissolves the Canada balsam and you can take out the micro section and embed it onto a new glass slide (Figs. 4.43 and 4.44).

Important Note: Remember to soak the sample in a glass container as xylene can dissolve plastics.

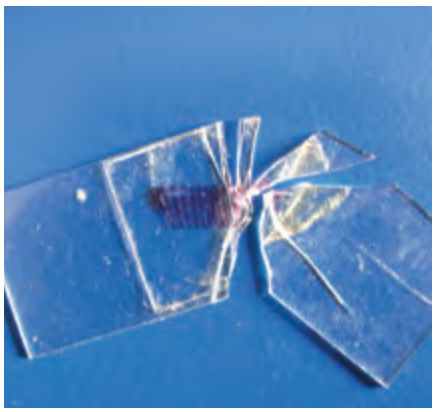


Fig. 4.43. Cracked slide.

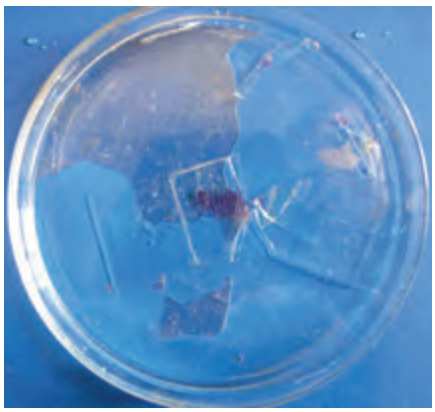


Fig. 4.44. Cracked slide in a petri dish containing xylene. The glass it has to be covered because the xylene evaporates quickly.

4.15 Final storage

There are differ nt possibilities to finall store the embedded slides. They can be stored in prefabricated aluminum plates within special shelves (Figs. 4.45 and 4.46), or, more commonly, in special slide boxes (Figs. 4.47 and 4.48) that can be stacked on any shelf (Fig. 4.49).

Fig. 4.45. Storing slides in prefabricated aluminum plates.

Fig. 4.46. Final storage of slides on the aluminum plates in special shelves. Changing the arrangement is always possible.

Fig. 4.47. Storing slides in prefabricated plastic boxes.

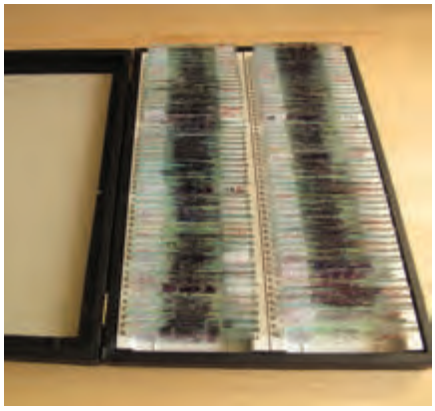


Fig. 4.48. Storing slides in prefabricated cardboard boxes.

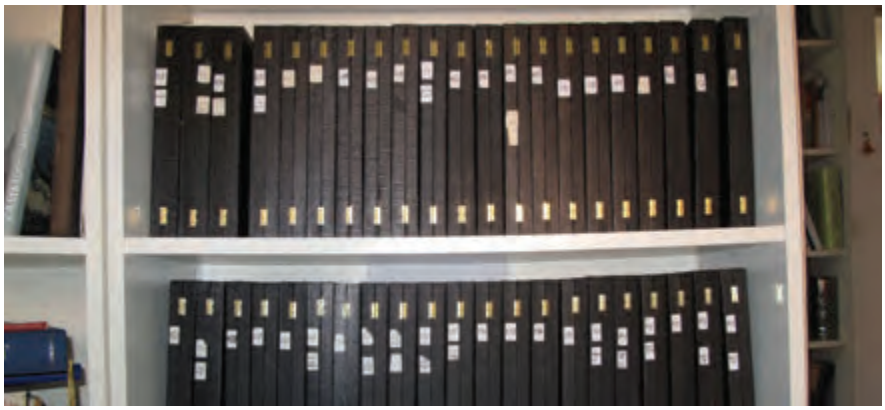


Fig. 4.49. Storing the slides in boxes protects them from dust. Changing the arrangement is hardly possible. A digital classification system is needed to find slides.

4.16 Digital classification of slides

When working with wood anatomical slides it is important to make a classificatio of all characteristics visible on the section. Basically, there is a need to document the following things:

» number of slide,
» species,
» family,
» collected part of plant,
» life form,
» plant height,
» site characteristics,
» vegetation zone,
» location,
» altitude,
» geographical coordinates,
» collection date,

and possibly information on

» sample provenance,
» special features,
» distribution, etc…

Recommended for anatomical classification are

» the IAWA system for softwoods (conifers) (Richter et al. 2004),
» the IAWA system for hardwoods (Wheeler et al. 1989), or
» the system for dicotyledonous herbs, shrubs and trees (Schweingruber et al. 2011).

4.17 Processing time

It is difficu to give any exact information about the time needed to process one sample. The following times are estimates based on own experience over a longer time.

The processing times for Astrablue/Safranin stained permanent slides for an experienced person are:

Cutting:

Cross sections of branches:	approx. 20–30 sections / hour
Cross, tangential and radial sections:	approx. 8 samples (3 sections each) / hour
Cross sections of difficu samples:	approx. 5–10 sections / hour

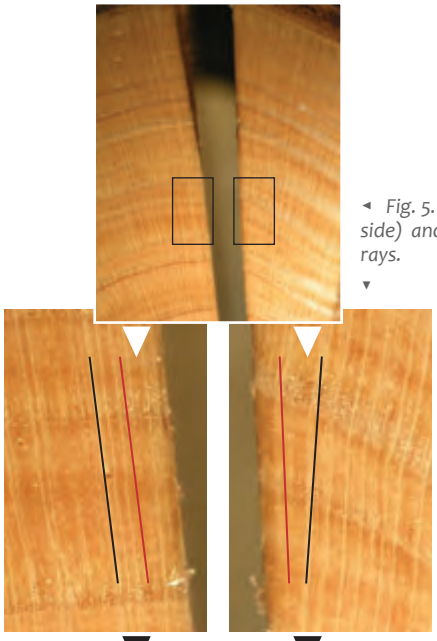
Staining and embedding:

When working in a line as described in section 4.13: 10 slides / hour

Fixation and additional staining with Picric-Anilinblue takes 2–3 minutes longer per slide.

5. Preparation errors

Cutting radial sections requires a very precise orientation of the sample in relation to the cutting direction (see section 3.2). A good orientation can be achieved when concentrating on the ray orientation of the sample. The rays have to be placed parallel to the cutting direction (Fig. 5.1). As a result the rays in the micro section will be quite long and clearly visible (Fig. 5.2). In case the ray orientation is not parallel, but oblique to the cutting direction, the rays in the micro section appear short (Fig 5.3) and specifi features will not be visible in most parts.



◂ Fig. 5.1. Splitting parallel (left side) and oblique (right side) to rays.

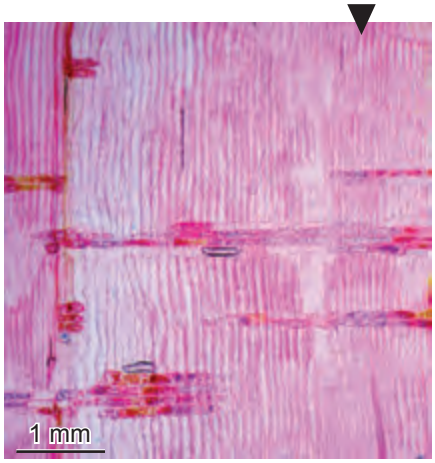


Fig. 5.2. Perfect radial split reveals long sections of rays.

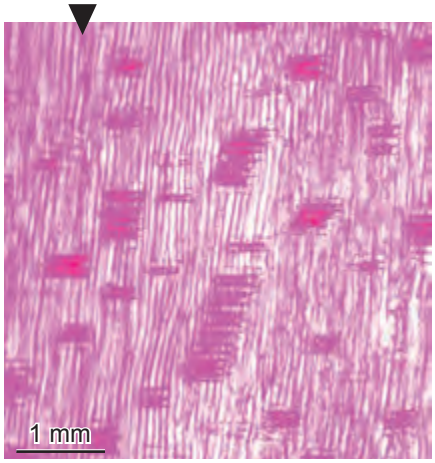


Fig. 5.3. Oblique splits reveals only short sections of rays. The rays also appear short when rays are radially bent within the stem.

Straight lines or even grooves on surfaces and sections are a result of notches in the blade (Figs. 5.4 and 5.5). If such traces appear on surfaces, change the blade or move the blade in the holder. Inconsistent thickness of sections is a consequence of not perfectly stabilized blades (Fig. 5.6), or potentially the wood being too hard for the blade. Irregularly-formed secondary walls in the earlywood of conifers indicate a dull blade or a badly guided blade (Fig. 5.7).

Trapped air bubbles in the slides appear dark and are extremely disturbing (Figs. 5.8–5.10). Large air bubbles can be massaged out from under the slide cover before the slide is dried. Slight warming of a dried slide may expand them so that they disappear.

Some irregularities can be cleaned in the pictures with Adobe Photoshop, or other image processing software.

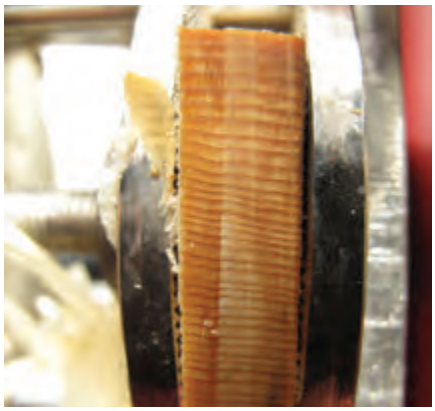


Fig. 5.4. Traces on the surface of the sample are the result of blades with notches. Change the blade.

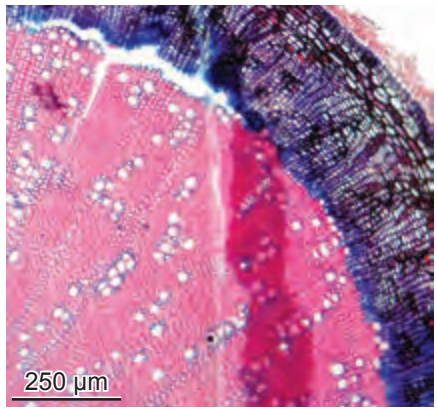


Fig. 5.5. Traces on the slide of are a result of a blade with notches. The damage is irreparable.

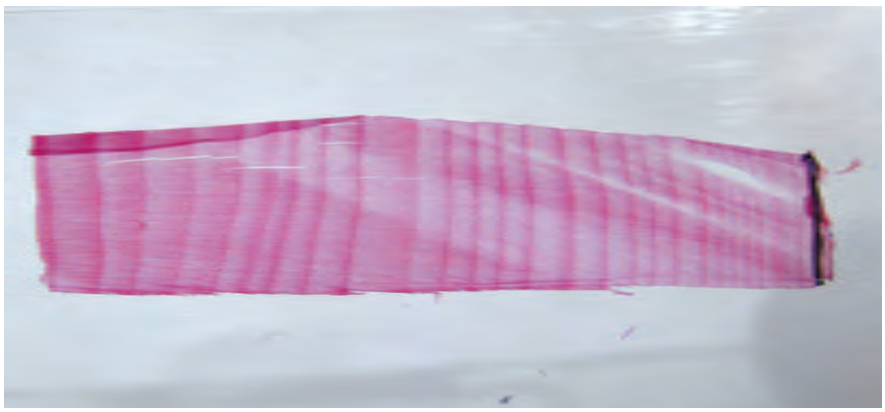


Fig. 5.6. Slides with inconsistent thickness are a result of blades too loosely fixed in the knife holder of the microtome.

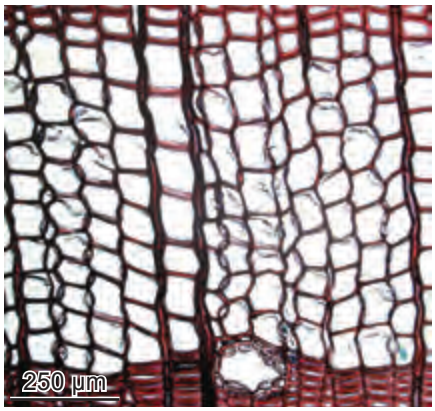


Fig. 5.7. Irregularly-formed secondary walls in the earlywood of a conifer as a result of a dull knife. The secondary wall is split off the primary wall. The damage is irreparable (see section 3.6.2). To avoid, use a new blade and non-Newtonian fluid.

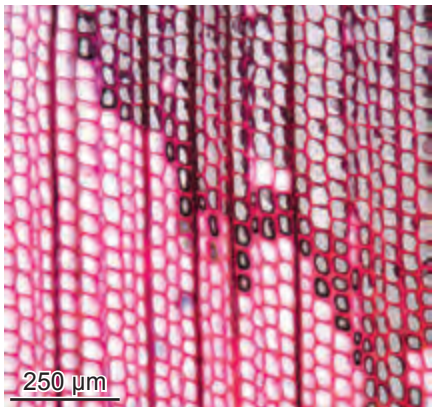


Fig. 5.8. Air in a large part of the slide. Put the slide in xylene for a day and cover the section again with sufficient Canada balsam.

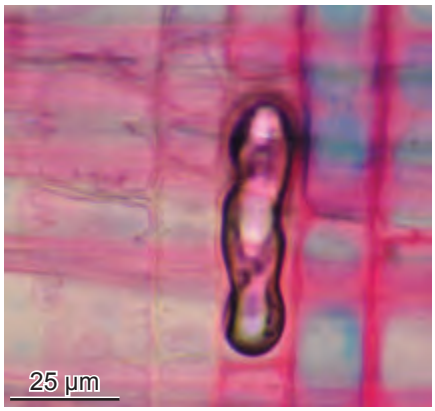


Fig. 5.9. Air bubble in a ray cell. Heat the slide for a few seconds until the air bubbles escape and press the cover glass with a magnet on an iron plate until the slide is cold (see section 4.11).

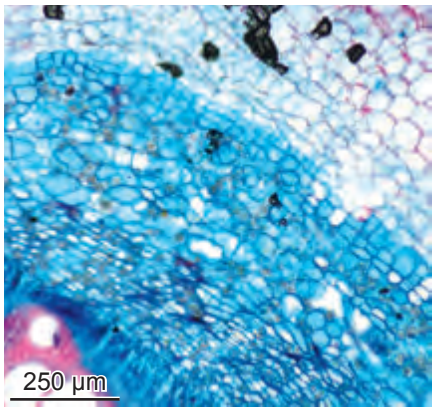


Fig. 5.10. Air bubbles in cork cells. Thee bubbles remain in the cells even after heating.

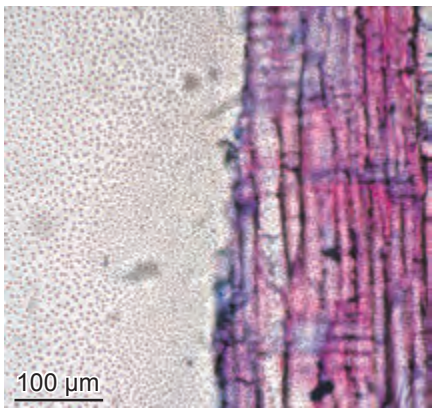


Fig. 5.11. Small dots (water droplets) are a result of insufficien dehydration. Put the slide in xylene for a day, go back to the dehydration process to ethanol 96%, ethanol absolute and xylene. If the xylene runs clear, water is no longer present.

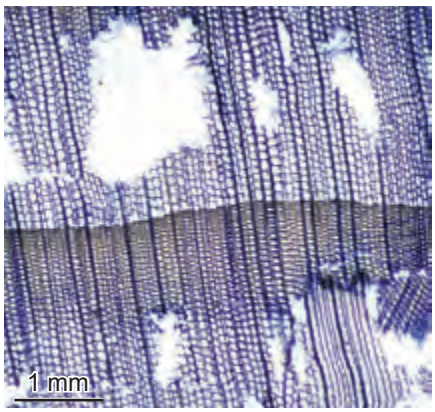


Fig. 5.12. Holes can be old saw marks.

6. Microscopic observation

6.1 Polarized light

New student microscopes with magnification of 40x, 100x, 200x, 400x (and 1000x) can be used for most stem anatomical studies. Extremely informative is the use of polarizing filters No matter which type of microscope you use, the filter are always placed above and below the slide. You can switch between normal and polarizing light by rotating one of the filter (Figs. 6.1–6.4).

Polarized light can be used to differentiat types of cell walls (S1, S2, S3) based on the orientation of their micro fibrils All cell walls consist of more or less net-like, unstructured micro fibrils primary (S1) and tertiary (S3) cell walls, as well as cell walls of parenchyma cells disappear in polarized light. On the contrary, secondary cell walls (S2), consisting of more structured cell walls, more or less oriented in the same direction, light up in polarized light (Figs. 6.5–6.8).

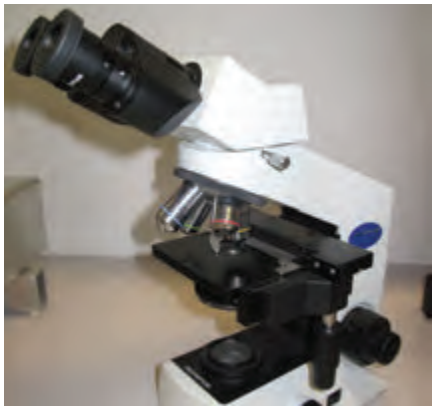


Fig. 6.1. Student microscope with a removable top part.

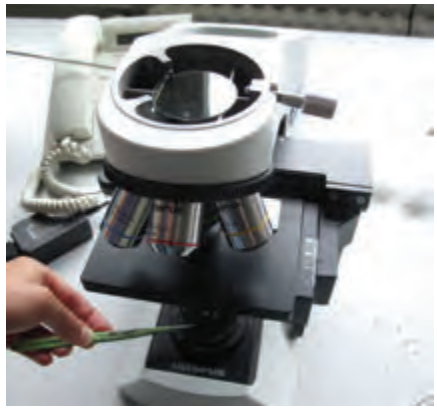


Fig. 6.2. Placing polarizing filters on top of the light source and between the objectives and the oculars. If the top part of the microscope is not removable, place the filter directly on the slide.

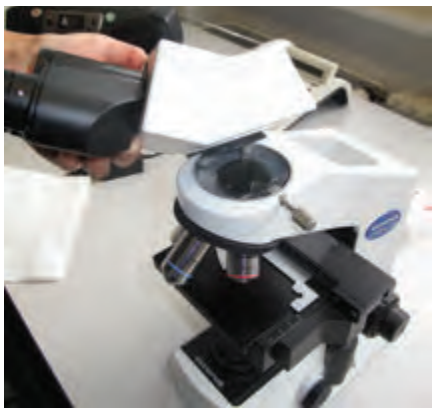


Fig. 6.3. Covering the polarizing filter with the top piece of the microscope.

Fig. 6.4. Rotating the polarizing filter on the light source.

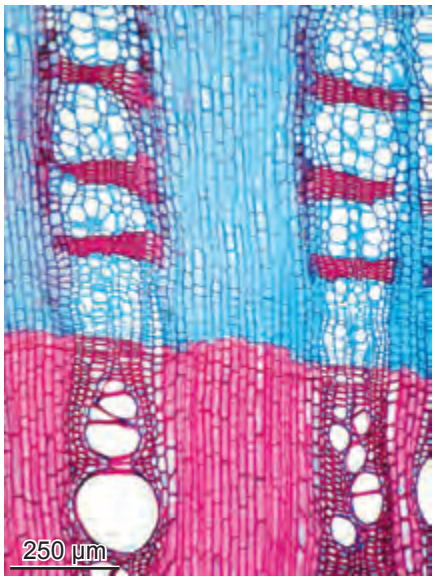


Fig. 6.5. E ect of normal light in a liana stem (Vitis vinifera): Lignified parts are red, unlignified parts are blue.

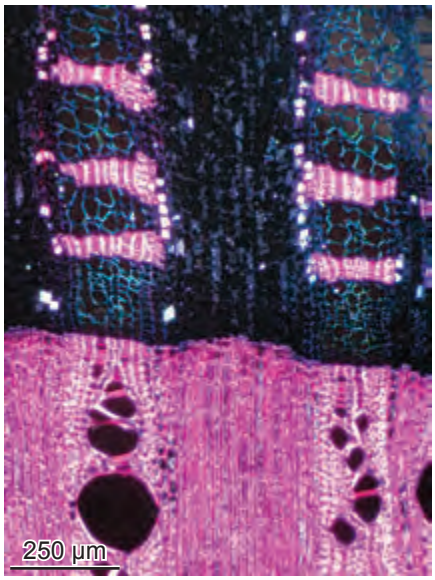


Fig. 6.6. E ect of polarized light in a liana stem (Vitis vinifera): Lignified cells are red, unlignified cells are bluish or disappear. Crystals are white.

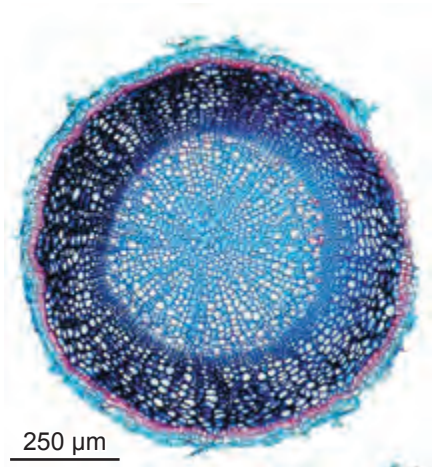


Fig. 6.7. E ect of normal light in a stem of a tiny herb (Cerastium semidecandrum): Lignified parts are red, unlignified parts are blue.

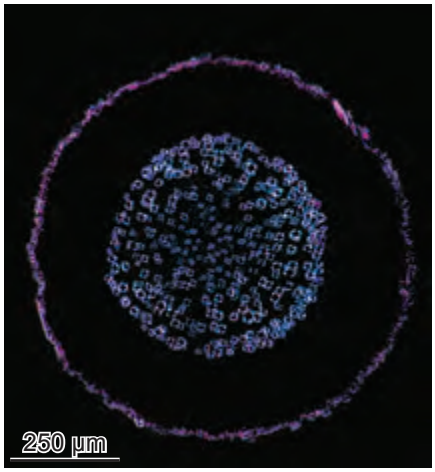


Fig. 6.8. E ect of polarized light in a stem of a tiny herb (Cerastium semidecandrum): Cells with secondary walls in the xylem appear light. Peripheric cortex cells have lignified secondary walls and appear red.

6.2 Measuring cell dimensions

Measuring cell dimensions, such as cell diameters or vessel lengths, is possible with a scale integrated in the ocular of the microscope. With this scale, you can directly see and document the relative dimensions of the cell types and document it. For measuring cell dimensions in millimeters or microns, the scale in the ocular has to be calibrated with a stage micrometer.

Place the scale under the microscope and compare the dimension of the eyepiece micrometer, and calibrate it for all magnification available (Figs. 6.9 and 6.10). Cell dimensions in photographs can be calibrated with photographs of the stage micrometer.

Modern microphotographic systems are equipped with automatic measuring systems.

Fig. 6.9. Stage micrometer with a resolution of 10 microns.

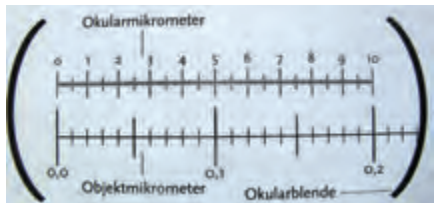


Fig. 6.10. Comparing the scale of the eyepiece micrometer with the metric scale of the stage micrometer The bent lines represent the border of the field of view in the microscope.

6.3 Photography

All companies dealing with microscopes provide sophisticated photographic systems. Since all of them have their own specificatio we will not describe them here. Please contact the company. If you want to document some observations without any intention of publishing it, it is possible to take a photograph with any digital camera through the eyepiece of the microscope.

References

Bauck R, Brown R (1955) Sharpening an increment borer. Minnesota Forestry Notes 39. 2pp.

Bubner B (2008) The wood cross sections of Hermann Nördlinger (1818–1897). IAWA Journal 29: 439–457.

Cha ey NJ (2002) Wood formation in trees. Cell and molecular biology techniques. Taylor and Francis. London.

Carlquist SJ (2001) Comparative Wood Anatomy: Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood. Springer Series in Wood Science. Springer Verlag, Berlin, 448 pp.

Eades HW (1937) Iodine as an indicator of sapwood and heartwood. Forestry Chronicle 13: 470–477.

Fujii T (2003) Application of the "NT-Cutter" knife blade to microtome sectioning of wood. IAWA Journal 24: 241–245.

Gärtner H, Nievergelt D (2010) The core-microtome. A new tool for surface preparation on cores and time series analysis of varying cell parameters. Dendrochronologia 28: 85–92.

Gärtner H, Lucchinetti S, Schweingruber FH (in review) New perspectives for wood anatomical analysis in Dendrosciences: The GSL1-microtome. Dendrochronologia.

Grissino-Mayer HD (2003) A manual and tutorial for the proper use of an increment borer. Tree-Ring Research 590: 63–79.

Hoadley RB (1990) Identifying wood. Accurate results with simple tools. The Taunton Press. Newtown CT, USA.

Iseli M, Schweingruber FH (1989) Sichtbarmachen von Jahrringen für dendrochronologische Untersuchungen. Dendrochronologia 7: 145–157.

Ives E (2001) A guide to wood microtomy; making quality microslides of wood sections. Ernie Ives Sproghton, Ipswich, Suffolk, England.

Jozsa L (1988) Increment core sampling techniques for high quality cores. Forintek Canada Corp. Special publication No. SP-30. ISSN #0824-2199.

Kremer BP (2002) Das grosse Kosmos-Buch der Mikroskopie. Kosmos Verlag, Stuttgart.

Mulisch M, Welsch U Eds. (2010) Romeis, mikroskopische Technik. 18. Auflag , Spektrum Akademischer Verlag, Heidelberg.

Purvis MJ, Collier DC, Walls D (1964) Laboratory techniques in Botany. Butterworth London.

Richter HG, Grosser D, Heinz I, Gasson PE (2004) IAWA list of microscopic features for softwood identi cation. IAWA Journal 25: 1–70.

Rossi S, Anfodillo T, Menardi R (2006) Trephor: A new tool for sampling microcores from tree stems. IAWA Journal 27: 89–97.

Rudzin SE (1999) Plant microtechnique and microscopy. Oxford University Press. New York, Oxford.

Schneider L, Gärtner H (in press) The advantage of using non-Newtonian fluid to prepare micro sections. Dendrochronologia. doi: 10.1016/j.dendro.2013.04.002

Schweingruber FH (1990) Mikroskopische Holzanatomie / Anatomie microscopique du bois / Microscopic Wood Anatomy. Swiss Federal Institute of Forestry Research, Birmensdorf.

Schweingruber FH (2012) Microtome sections of charcoal. IAWA Journal 33: 327–328.

Schweingruber FH, Börner A, Schulze E-D (2006) Atlas of woody plant stems. Evolution, Structure, and Environmental Modi cations. Springer Verlag, Heidelberg, 229 pp.

Schweingruber FH, Börner A, Schulze E-D (2011) Atlas of stem anatomy in herbs, shrubs and trees. Vol. 1. Springer Verlag Heidelberg, Dordrecht, London, New York.

Schweingruber FH, Börner A, Schulze E-D (2012) Atlas of stem anatomy in herbs, shrubs and trees. Vol. 2. Springer Verlag Heidelberg, Dordrecht, London, New York.

Shigo AL (1984) Compartmentalization: A conceptual framework for understanding how trees grow and defend themselves. Annual Review of Phytopathology 22: 189–214.

Walter H, Breckle SW (1989) Ecological systems of the Geobiosphere. Springer Verlag, Heidelberg, Dordrecht, London, New York.

Wheeler EA, Baas P, Gasson PE (1989) IAWA list of microscopic features for hardwood identi cation. IAWA Bull. n.s. 10: 219–332.

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