2 Preparation of Wood and Herb Samples for Microscopic Analysis

Here I present rather simple but effective methods of wood preparation for the purpose of basic structural analyses, generally referring to the description in Schweingruber (1978). Various sources on the subject of microscopic techniques (e.g., Clark 1981; Chaffey 2002; Gerlach 1984; Harms 1965; Schömmer 1949), Schweingruber and Poschlod 2005) may also be consulted.

2.1 Labeling, Transport and Storage

Thick plastic bags are suitable for transportation and final storage. Carefully washed samples can be conserved for longer periods in such bags in 40% ethanol or in any commercial alcohol, even whiskey or vodka. (Thin walled plastic bags should be avoided since they are alcohol-permeable.)

Plants are labeled with a very soft pencil, e.g., Stabilo Aquarellable, on thick paper or plastic sheets.

The following characteristics should be noted:

- Latin name and its author
- Part of the plant, e.g., rhizome, primary root–shoot transition, stem, twig
- Life form and height of the plant, e.g., therophyte, hemicryptophyte, dwarf shrub, shrub, tree
- Growth form, e.g., cushion plant, succulent
- Phenological stage of the plant, stem deformations
- Climatic zone, e.g., subarctic, semiarid
- Site conditions, e.g., dry slope, wet meadow, windy ridge
- Locality, region, country
- Altitude, collection date

2.2 Sample Preparation for Microtome Sectioning

For sectioning with the microtome, the samples are prepared depending on the size of the plants: approximately 1-cm-long cubic sections are cut (transversal) or split (radial and tangential) from stems or other parts of plants. Hand saws without set teeth are preferable (e.g., German Wolf, Japanese ARS or Swiss Felco saws).

Green wood can be cut without prior softening. Splitting off a piece of the wood with bark often prevents extreme bark shrinking. From green wood with intact cambium and bark, and from the taproot of herbaceous plants, entire cross sections can often be obtained by dabbing the surface with absolute alcohol with a fine brush prior to cutting. (Alcohol hardens the tissue immediately.) Samples which are water-saturated and very soft are placed for 24 h in solutions of 30, 60 and 100%t poly(ethylene glycol) 4000 and kept in an oven. Before cutting, we glue a little strip of Scotch tape on the surface. Doing so, the thin section remains complete.

Dry, medium-hard wood samples are softened by lightly boiling them for between 5 and 10 min. As written identification marks (made by pencil or non-waterproof felt-tip pen) often disappear during boiling, the samples may be popped into ladies' stockings, separated from each other by knots, like a row of sausages. The order of the knots must be written down.

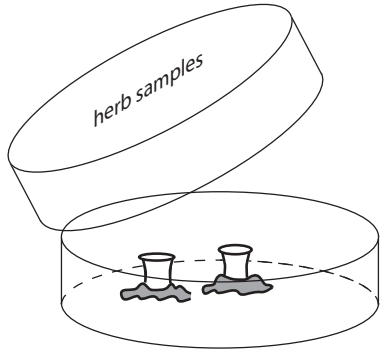
Dry and very hard wood samples can be cut after boiling them for several hours in a pressure cooker. They can be softened by keeping them in a 1:10 solution of glycerine and water for several days.

Dry, soft wood samples, such as Norway spruce with expressed narrow annual rings, should be put into warm water for several minutes prior to cutting.

2.3 Sample Preparation for Episcopic Microscopic Examination

Cross sections of wooden samples are first planed with a knife, a razor blade or they are just polished with sandpaper to prepare them for microscopic examination. The samples are then embedded in grafting wax as shown in Fig. 2.1.

Under the stereoscopic microscope, ring boundaries may be seen clearly without any additional treat-



▲ **Fig. 2.1.** One-centimeter-long root collars embedded in grafting wax. Petri dishes with covers are well suited for storage and transportation

ment. Tree-ring structures in brownish wood may show better contrast by rubbing chalk into the pores. Ring-structure visibility in very bright wood with bigger pores may be improved by staining the surface with a green or red permanent marker and also by rubbing chalk into the pores. Alternatively, ring visibility may be improved by staining the surface with safranin and cutting away the surface for half a millimeter. Improvements of ring boundary visibility are feasible when differential absorption of stain in earlywood and latewood takes place as a result of differing penetration depth below the stem surface (Iseli and Schweingruber 1989).

On the root collars of perennial herbaceous plants, the annual ring boundaries are usually visible on dry surfaces. Rings become occasionally pronounced when damp surfaces are treated with chlorine, zinc, iodine or phloroglucin (Schweingruber and Dietz 2001). When a relatively thick section immersed in water or glycerine is placed on a dark surface, and subjected to bright, reflected light (lamp, sunlight), and observed under a stereoscopic microscope with ×20 to ×40 magnification, the ring boundaries become visible that would be otherwise hard to identify on microsection.

Charcoal samples are analyzed under reflected light using a stereoscopic microscope with magnifications up to ×100 or under an episcopic microscope. All relevant characteristics can be observed on the fractured surface. Small charcoal pieces should be held between thumb and forefinger, and a protruding bit can be broken off sideways with the thumb of the other hand. In this way, a smooth fractured surface and can be examined more easily. Using a scalpel, one can create radial or tangential fractures. Characteristics along longitudinal fractures include wood rays, perforations or spiral thickenings. These can be securely identified with an episcopic microscope or a stereoscopic microscope with high magnification (90:1).

Fractured charcoal surfaces do not photograph well. Good photographs may, however, be obtained after having soaked the pieces in synthetic resin such as Technovit.

Boundaries between heartwood and sapwood of unicolored coniferous specimens can be differentiated macroscopically by the following procedure:

- Dip them into 45% perchloric acid for 1–3 s. After 20 s, the heartwood appears greenish, whereas the sapwood turns light brown.
- Spray the surface with a hydrochloric acid solution containing 0.4% alcoholic solution of 2,4-dinitrophenylhydrazine. The sapwood will adopt a red–orange color (Sandermann et al. 1967).

2.4 Making Thin Sections

Thin sections for microscopic inspection are successfully prepared if the operator has a skilled and very steady hand. For high-quality sections, a sliding microtome is mandatory. The best instrument for the purpose is a classic sliding microtome by Reichert-Jung. Well-sharpened knifes are a prerequisite for good sections (type C). The knife sharpener from Leica produces very sharp blades, but manual sharpening is possible with a cheap sharpening tool from EUROMEX. Disposal blades (available from Leica or paper knife blades NT) can be used for small soft samples.

There are few basic rules with which even beginners may produce good results: set the microtome knife to a section thickness between 10 and 60 µm (ring boundaries may be visible even on thick sections). Place a drop of absolute alcohol on the flat surface of the samples; lightly place a wetted aquarelle brush over the sample and slide the knife across. This procedure prevents the section curling. The knife should be angled in a way that at least one quarter of the blade is used when taking a section, and it also helps if the blade contact point is wetted. The section can be slid off the blade and placed on a wetted microscope slide using a brush. Before the section is covered by a cover slip, add glycerol as a mounting medium for temporal storage for hours or days. Samples need to be checked under the microscope for cracks in the section that might come from nicks in the cutting edge of the microtome blade.

2.5 Staining of Thin Sections for Permanent Slides

Once the section has been placed on a slide, sample preparation procedures can start (Fig. 2.2). Liquids are dropped directly onto the section with pipettes while holding the slide at an oblique angle so that surplus liquid runs off into a container. Prior to staining remaining glycerol needs to be rinsed off with water.

A naturally dark colored sample on a slide may be observed microscopically without previous staining.

There are a number of alternative staining methods (Gerlach 1984). Commonly used products are astra blue (0.5 g astra blue and acetic or tartaric acid (2 g in 100 ml distilled water) and safranin (1 g in 100 ml distilled water). Astra blue is mixed with safranin in a ratio of 1:1 and a drop of the solution is placed on the section for 2–3 min. After this the sample is first washed several times with 95% alcohol until it runs clear, followed by rinsing with absolute alcohol to dehydrate the section. Staining makes unlignified cells appear blue, whereas lignified cells turn red. Gelatinous fibers in tension wood also appear blue as lignification is at its minimum..

As mentioned before, dehydration can be achieved with absolute alcohol. The very hydrophilic absolute alcohol is replaced with 95% alcohol mixed with 5% 2,2-dimethoxypropane (Fluka). The slide is rinsed several times with alcohol and then a drop of xylol is placed on the section to test for the presence of water. Dehydration is incomplete if the xylol turns milky, indicating that additional absolute alcohol washing is required. When xylol runs clear on the slide, a small drop of Canada balsam is placed in the center of the section and a cover slip pressed on top. In my experience Canada balsam is the best and most permanent embedding resin. To prevent the thin section from buckling, which makes examination difficult, the slide with the cover slip is sandwiched between PVC strips with two small magnets placed on either side to keep the sandwich together and air bubbles out during drying in an oven (Fig. 2.3). The oven is set at 60°C for 12 h. After drying, any hard resin remaining outside the cover slip can be scraped off with razor blades.

2.6 Preparation of Impermanent Slides

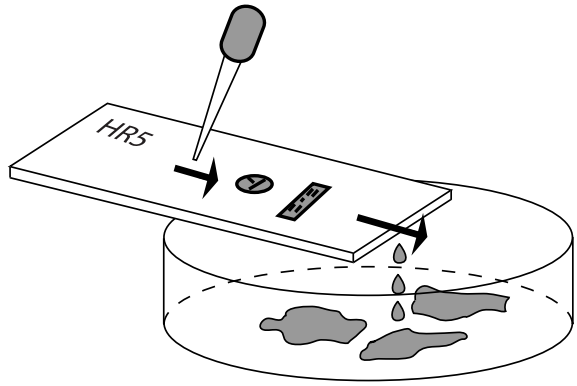
A mixture of 2% phloroglucin, 96% alcohol and 10% hydrochloric acid is dropped onto the section for 20 s. All lignified tissues will appear red.

Species containing a lot of slime (mucilage) or starch are difficult to examine. In such cases the section is first soaked in a drop of bleach (calcium hypochlorite, CaO_2Cl_2 = Eau de Javelle) for 5–10 min to deteriorate remaining protopast, e.g., nuclei and starch. The section is then rinsed with water until the smell of bleach has disappeared, after which it is ready for staining and dehydration.

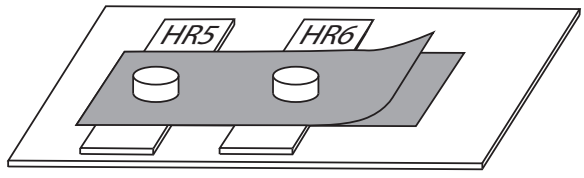
2.7 Microscopic Observation and Photography

Microscopes with objective magnifications of ×2, ×4, ×10, ×20, ×40 and ×90 (for charcoal) are all well suited for observations. Cells with and without secondary walls can be distinguished under polarized light. All cells with secondary walls appear bright, whereas all others remain dark. Ring boundaries appear mostly distinct when polarized light is used.

We use commercial laboratory microscopes with digital equipment. Dark green filters enhance pale tissues for black-and-white photographs.



▲ **Fig. 2.2.** Staining and dehydration procedures



▲ **Fig. 2.3.** Microscope slides between two plastic strips loaded with magnets on an iron plate. From Schweingruber and Poschold 2005

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