Microscopic
Preparation Techniques
for Plant Stem Analysis

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

Originalausgabe
© 2013 Verlag Dr. Kessel
Eifelweg 37
D-53424 Remagen-Oberwinter

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

Homepage:
www.forstbuch.de
www.verlagkessel.de

ISBN: 978-3-941300-76-7

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 fields 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. Chaffey (2002), Ives (2001), Hoadley (1990). However, not a single text concentrates specifically 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 efficient 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 different 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 al conifers and most dicotyledonous plants and that lignification 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 root of a deciduous tree (Fraxinus excelsior).

Fig. 1.28. Exposed root 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, petrified 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

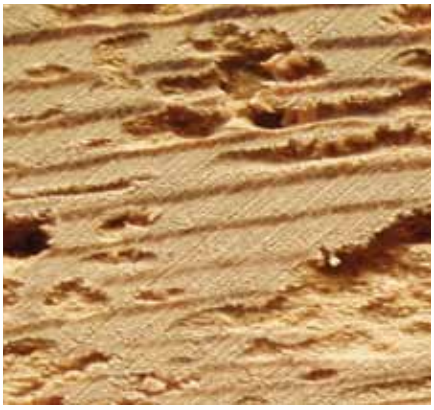

Fig. 1.42. Wood decomposed by insects

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 difficult 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 different 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 field are not properly sharpened and rarely anyone takes sharpening tools out to the field 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.

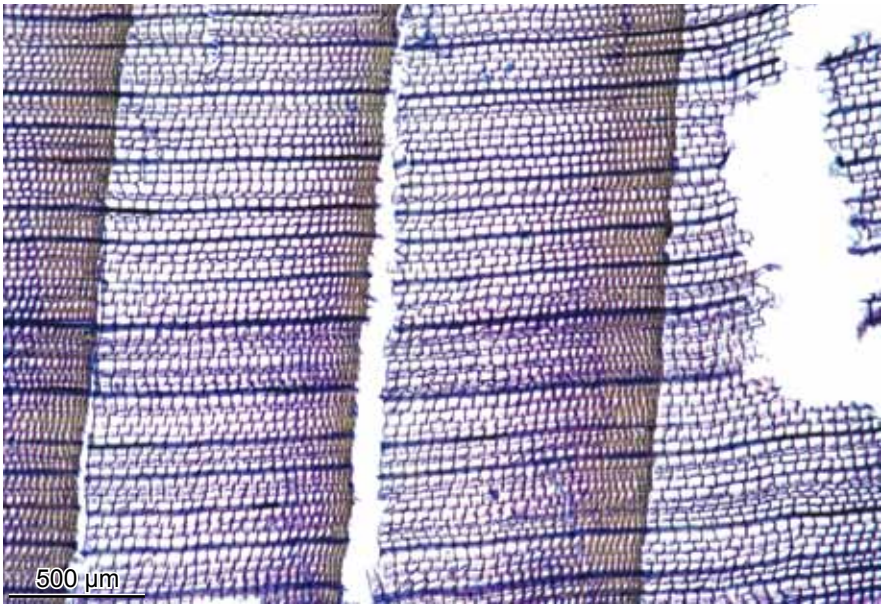

Fig. 2.10. Tangential cracks on a sectioned sample due to dull borer tip.

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 scientific 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:	...

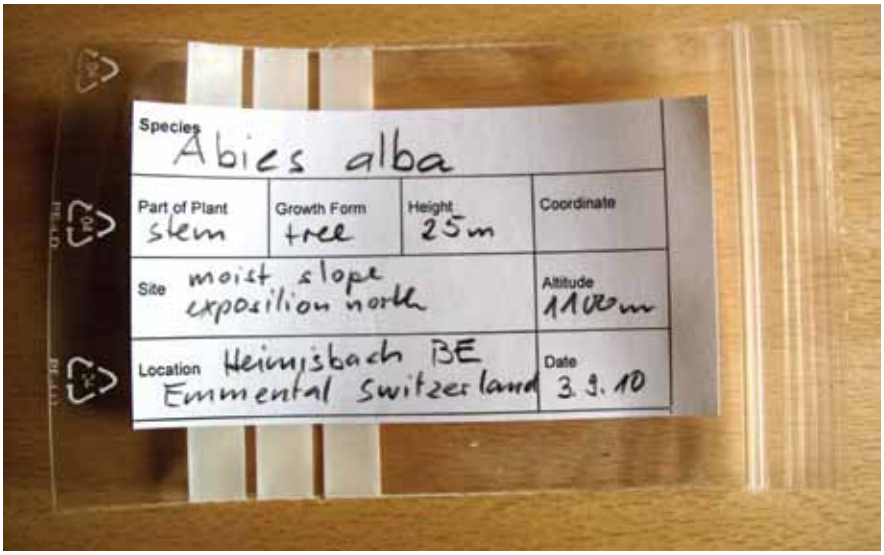

Fig. 2.14. Two different types of labels. The upper one is computer generated (advantage: data additionally stored in the computer), the lower one is filled in by hand.

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 fixed 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 modified 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 fibers (radial and tangential) must be split (Fig. 3.8). In doing so, the fiber (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.

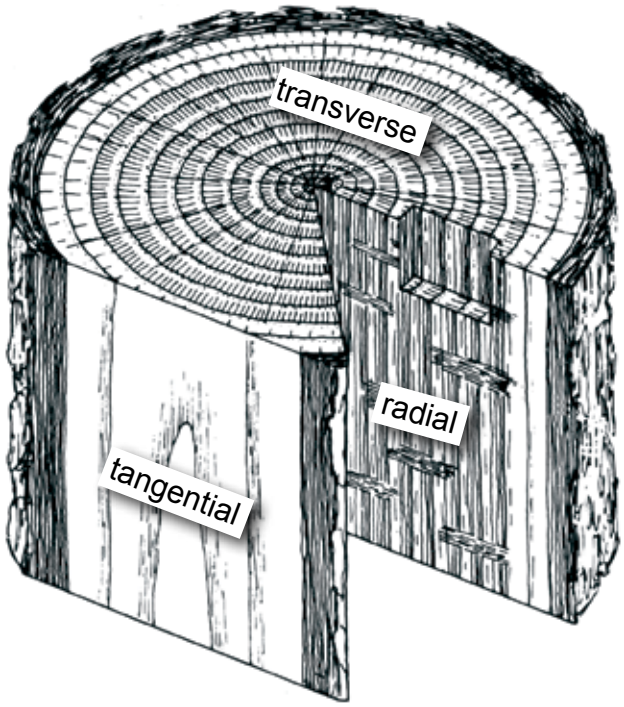

Fig. 3.6. Visualization of the three cutting directions (transversal, tangential and radial) used in wood anatomy (modified after: Schweingruber 1990, p. 13).

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.