IAWA Journal, Vol. 16 (1), 1995: 81–86

A VIDEO TECHNIQUE FOR IMAGING THE THREE-DIMENSIONAL ARCHITECTURE OF WOOD

by

Ann M. Lewis

Department of Forestry and Wildlife Management, University of Massachusetts, Amherst, Massachusetts 01003-4210, USA

SUMMARY

This paper describes a video micrographic technique to image the internal three-dimensional structure of wood. The technique uses high resolution video and an optical disk recorder to give immediate access to noise-free serially-recorded images in both still-frame and motion modes. By using a hydrophilic embedding medium, small woody tissue samples can be successfully embedded, sectioned at thicknesses that are useful for video micrography of the exposed tissue surface, and serially recorded to analyse three-dimensional architecture. The technique can be used by researchers working in wood anatomy, xylem development, and water transport. It also promises to be useful for studying the three-dimensional architecture of small, non-woody structures. Modifications of the technique make it useful for larger woody and non-woody material.

Key words: Serial sections, wood anatomy, wood structure, xylem anatomy, xylem structure, xylem architecture, video analysis.

INTRODUCTION

Researchers use recorded images of serial sections to analyse the structure of xylem, a tissue with complex three-dimensional architecture. Zimmermann and Tomlinson developed and improved upon several techniques for reconstruction of the three-dimensional architecture of xylem during their investigations of the vasculature of monocots and dicots (see Zimmermann 1976 and literature cited therein). There are three basic methods that they devised for vascular reconstruction. The surface technique uses a modified sliding microtome to advance a stem along its vertical axis after a transverse section is planed off and an image of the newly exposed surface is recorded with a ciné camera. The camera is fixed above the sample, and the image may be magnified through a dissecting microscope mounted between the sample and the camera. In the second method, transverse sections permanently mounted on microscope slides are recorded with a ciné camera attached to the dissecting or compound

microscope. Before recording, a drawing tube is used to superimpose the image of a new transverse section onto a sketch of a previously recorded section so that alignment is maintained. The third method uses an optical bridge to join the ciné camera to two microscopes to make a comparison, or shuttle, microscope. This allows recording of serial sections from alternating microscopes after alignment of the superimposed microscope images. In each of the three methods, the completed film is viewed on a film analyser where the axial dimension of the sample is represented by time. A reconstruction of the three-dimensional architecture can be rendered on paper from the film analysis.

Zimmermann, Tomlinson, and coworkers continued to use three-dimensional reconstruction to analyse the vascular structure of the palm, *Rhapis excelsa* (e.g., Zimmermann & Sperry 1983), and Akachuku (1987) used cinematography to measure the variation of *Quercus rubra* vessels along their length. Adams (1977) used Zimmermann and Tomlinson's surface technique to analyse the medullary bundle system of the tree fern, *Cyathea fulva*. Cox and Tomlinson (1985) used a variation of the surface technique when they used a radial arm saw to section rhizomes of another tree fern, *Lophosoria quadripinnata*, for morphological and anatomical analysis. Work on monocot vasculature continued with French and Tomlinson's (1986) comparison of the architecture of compound vascular bundles in Pandanaceae, Cyclanthaceae, and Araceae, and with Scribailo and Tomlinson's (1992) replacement of the ciné camera with a video camera and recorder to analyse the complex pattern of the vascular traces that supply the floral appendages in *Calla palustris*. Meicenheimer and Leonard (1990) investigated development of the veins in leaf primordia of *Linum usitatissimum*. They approached three-dimensional reconstruction in a different manner when they used a Zeiss Videoplan into which enlarged photomicrographs were digitized with a digitizing tablet.

Although the sectioning, recording, and analysing systems have continued to improve, none of these techniques work well with small samples of coniferous wood. My laboratory has tried several methods to analyse the three-dimensional architecture of small woody samples. This paper describes a video micrographic technique that works well with small woody samples and offers several other improvements over past techniques. It also discusses modifications of the technique that should make it more applicable to both larger and softer material.

Serial images of the newly exposed surface of a woody sample are recorded through a microscope onto an optical disk after sequential removal of thin sections with a microtome. The method modifies the surface technique described by Zimmerman (1976) in order to work at high magnifications with woody samples but without the necessity of mounting hundreds of microscope slides as required when using a comparison microscope. The video imaging technique has one major advantage over the cinematographic methods used by Zimmermann and coworkers: the operator can monitor the results as the imaging progresses; there is no wait for film processing. The videographic system is of use to researchers working on xylem development and water transport and is especially useful in the analysis of the three-dimensional architecture of wood.

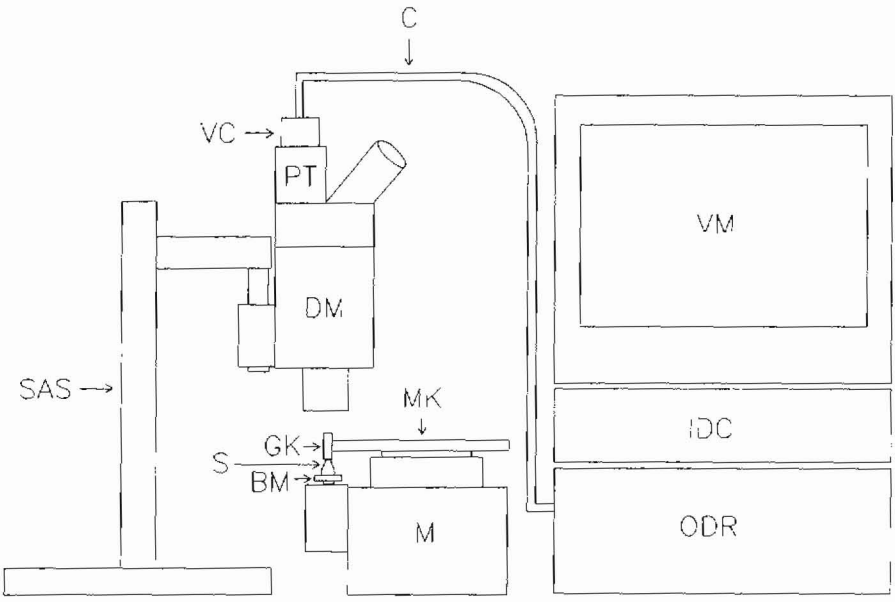

Fig. 1. The video micrographic system (not to scale). – BM = brass mount; C = cable connecting video camera through its main camera box (not shown) to the optical disk recorder; DM = dissecting mieroscope; GK = glass knife; IDC = improved definition converter; M = sliding microtome: MK = microtome knife; ODR = optical disk recorder; PT = phototube; S = sample embedded in plastic resin; SAS = swinging-arm stand; VC = video camera; VM = video monitor. The fiber optics illuminator is not shown.

THE SYSTEM

The videographic system consists of a high resolution video camera suspended over a sample held in a sliding microtome (Fig. 1). A dissecting microscope, placed between the sample and the camera, magnifies the image to the camera. The camera is connected through an optical disk recorder/player and an image enhancement device to a video monitor. The optical disk recorder records the image as taken from the camera. The image enhancer converts the interlaced video image to a non-interlaced one, which makes the displayed image very stable, and uses digital signal processing to reduce interference and improve the quality of the image displayed on the monitor. The following is a list of specific items: an AO sliding microtome (model 860; American Optical Co., Buffalo, New York, USA) with a specimen carrier that moves only in the vertical direction; an Olympus SZH binocular dissecting microscope (Olympus Corporation, Lake Success, New York, USA) with $\times 1$ and $\times 2$ objectives (the objective's working distance must be sufficient to allow the knife to pass between the sample and the objective) on a rotating nosepiece and a phototube that includes a $\times 5$ photo ocular, yielding a maximum magnification of $\times 640$ to the camera; the microscope is supported by a swinging-arm stand (model SMS20-18, Diagnostic Instru-

ments, Inc., Sterling Heights, Michigan, USA); a Toshiba CCD color video camera with 300,000 picture elements (model IK-C30A; Toshiba America Consumer Products, Inc., Wayne, New Jersey, USA) attached to the microscope's phototube; a Panasonic Optical Disk Recorder (model TQ-3031F; Panasonic Industrial Co., Secaucus. New Jersey, USA) with double-sided disks for a total of 108,000 frames in normal resolution; an NEC Image Smart™ Improved Defimition Converter, the image enhancer (model IDC-1000; NEC Home Electronics (USA) Inc., Wood Dale, Illinois. USA); and an NEC Data Smart™ Data Monitor (model DM-2000P; NEC Home Electronics (USA) Inc., Wood Dale, Illinois, USA). A bifurcated fiberoptic lamp (Fiber-Lite model 180, Dolan-Jenner Industries, Woburn, Massachusetts, USA) illuminates the sample. The camera output goes to the optical disk recorder in standard VHS format. The outgoing signal from the optical disk recorder to the image enhancer is sent via a super VHS cable, and the signal from the image enhancer to the monitor is sent via an RGB cable.

The video part of the system may be purchased for under US$ 25,000. The microscope stand costs US$ 1,300; the fiber optics illuminator costs under US$ 800. The microtome (US$ 8,000) and dissecting microscope (US$ 4,500) would be needed for most recording systems.

THE SAMPLE

Small longitudinal wood sections were used in development of the system. The samples had the following dimensions: 4 mm along the tracheid length, 1 mm along the stem radius. and 90 µm thick. To prepare a section for processing, stain it with basic fuchsin or safranin dissolved in water and rinse well. Embed the wood sample in JB-4 Plus® water soluble plastic resin (Polysciences, Inc., Warrington, Pennsylvania. USA). Paraffin embedding is inadequate to contain samples during the final microtoming for video recording – the samples break away from the encasing paraffin and sectioning becomes impossible. JB-4 is hydrophilic and remains somewhat flexible at ambient relative humidities and adheres well to the woody tissue. The flexibility also allows for the removal of thicker sections than other resin embedding media. Trim and mount the embedding blocks containing the samples with Krazy Glue® (Jadow and Sons, Inc., New York, NY. USA) (other cyanoacrylate adhesives may not adhere) onto the head of a brass flathead screw, bolt, or SEM stub, so that the long axis of the sample is perpendicular to the flat surface of the fastener or stub.

Clamp a brass mount with its attached sample in the microtome sample carrier. Adjust the mount so that the transverse surface of the wood is parallel to the cutting plane of the glass knife. The simplest solution for holding the glass knife securely is to mount it with melted paraffin wax to the end of a dulled microtome knife; the microtome's knife clamp holds the dulled knife. The glass knives must be changed often, and I would suggest trying a diamond knife for more longevity. Blacken the sides of the embedding block with a black permanent marker and cover or blacken the horizontal shiny surfaces of the specimen clamp and brass mount to reduce the amount of reflected light hitting the sample and the camera.

PROCESSING

In order to produce a series of images that can be analysed, remove 10 μm sections and record one image of the newly exposed surface of the tissue in the block after removing each section. The sections may be mounted on microscope slides for other analysis if desired. It is easy to toggle between the live image and the last recorded image or several of the recorded images to check on alignment and progress.

Adjust ambient relative humidity to regulate the flexibility of the embedding block. More humidity softens the block and makes sectioning easier by reducing the number of cracks and chips occurring with the removal of sections thicker than those for which the embedding medium was designed. Less humidity hardens the block and insures that it remains in the same place within the video frame. It is imperative to test the complete system (sectioning, recording, and analysing) with expendable material before using irreplaceable samples.

APPLICATIONS

This videographic technique was developed to count the number of intact tracheids within small coniferous wood samples (unpublished data). The technique could easily be modified for larger wood samples by holding unembedded fresh or dried samples directly in the specimen clamp. For very large samples, the microscope can be eliminated and appropriate lenses mounted directly to the video camera, and a specialized clamp can be used to advance long specimens, similar to Zimmermann's (1976) surface technique. Non-woody plant tissues, including leaf vasculature and herbaceous stem segments, could be embedded and analysed using the same methods used for the small wood samples. Many specimens could be cut with a regular microtome knife, but tissue that is soft may be handled most easily after staining and embedding in paraffin. Recording images from the surface exposed by sectioning reduces the stretching and buckling encountered when recording from the sections themselves. With this system, the difficult problem of image alignment when readjusting material in the specimen clamp is solved by realigning the sample with the last recorded image.

This system is more appropriate for some analyses than digitizing techniques where the researcher uses digitizing tools (manual or computer algorithms) to outliue the image for computer storage and reconstructiou (e.g., Meicenheimer & Leonard 1990) because it eliminates the approximation inherent in digitizing and because it is much faster than manually digitizing serial sections for three-dimensional display. Some digitizing software does have the advantage of being able to display a three-dimensional renditiou directly from the digitized transverse-sectional images. The system described here has several advantages over the low to medium cost videotape systems that we previously tried: 1) the resolution is much higher. 2) the images are noise-free even when viewing a single frame in still-frame mode, 3) particular frames can be recalled instantaneously, and 4) there is no degradation of the recording medium as the images are viewed repeatedly or as a still-frame is viewed at length. Of currently avail-

able videotape systems, only television studio quality equipment can provide the above-listed advantages (L. Wolloff, Crimson Tech, Cambridge, Massachusetts, USA, personal communication). This technique and variations on it promise to be very useful in the analysis of plant tissue structure.

ACKNOWLEDGEMENTS

I thank P. B. Tomlinson for allowing me to try his videotape systems. Barbara O'Connell's assistance with sample preparation and refinement of the sectioning method was invaluable. Research was supported by McIntire-Stennis Grant No. 68 of the Massachusetts Agricultural Experiment Station. Preliminary research was funded by the Maria Moors Cabot Foundation for Botanical Research at the Harvard Forest, Petersham, Massachusetts and by the USDA-Forest Service, Northeast Experiment Station, Northern Hardwoods Management Project.

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