

## CONSTRUCTION OF A PRECISION HAND MICROTOME FROM AN INEXPENSIVE MICROMETER

Author: Turner, Glenn W. Source: Madroño, 70(2) : 97-103 Published By: California Botanical Society URL: https://doi.org/10.3120/0024-9637-70.2.97

The BioOne Digital Library (<u>https://bioone.org/</u>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<u>https://bioone.org/subscribe</u>), the BioOne Complete Archive (<u>https://bioone.org/archive</u>), and the BioOne eBooks program offerings ESA eBook Collection (<u>https://bioone.org/esa-ebooks</u>) and CSIRO Publishing BioSelect Collection (<u>https://bioone.org/csiro-ebooks</u>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <u>www.bioone.org/terms-of-use</u>.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# CONSTRUCTION OF A PRECISION HAND MICROTOME FROM AN INEXPENSIVE MICROMETER

GLENN W. TURNER

## Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-7411 gturner@wsu.edu

## ABSTRACT

I present here instructions for the construction and use of a simple hand microtome made from a small brass tube, a steel washer, and a micrometer. The micrometer spindle becomes the specimen advance rod to smoothly push specimens through the tube to its open end, where the washer acts as a cutting guide for razor blade sectioning of specimens. Specimens are held firmly within the tube, wedged between pieces of firm polystyrene foam or a similar material. I present micrographs showing a sampling of plant specimens sectioned using this microtome and briefly discuss the historical origin of its design.

Key Words: Freehand sections, hand microtome, hand sectioning, microscopy of unfixed plant tissues, microtomy, Ranvier microtome, Ross microtome.

Freehand sections are often used to investigate the anatomy of fresh plant tissues. They can be produced rapidly without tissue fixation or embedding (Ruzin 1999). Simple hand microtomes can assist freehand sectioning and improve the quality of the sections.

During my dissertation research in plant anatomy at the University of California at Davis, I made several hand microtomes built from inexpensive micrometers. The micrometer's spindle becomes the specimen advance rod of the microtome. The differential screw mechanism built into the micrometer design ensures smooth, precise advancement of the specimen during sectioning. A small tube provides a chamber where the specimen is held, wedged firmly between two pieces of stiff polystyrene foam (PSF) "styrofoam." A steel washer at the end of the tube serves as a cutting guide. With very little practice, an inexperienced operator can use one of these microtomes to quickly and reliably produce thin freehand sections of plant leaves, stems, and fine roots. Over the years, I found these microtomes useful for research and teaching. In this paper, I describe the construction and use of a hand microtome and present images of sections made with it.

## MATERIALS AND METHODS

## Materials

- An inexpensive outside micrometer, or a micrometer head. I used a General No. 102 outside micrometer (General Tools and Instruments, Secaucus, NJ) purchased from Amazon.com for \$15. Often, low-cost micrometer heads alone (consisting of only the spindle rod, thimble, and barrel) are available for a similar price. They can be used directly without modification.
- A small, rigid-walled, brass tube. I used a 35 mm long hose coupling with an internal diameter of 9 mm (Fig. 1A), but any stiff tube with similar dimensions

should also work. In the past, I have often used small brass pipe fittings.

- A steel washer with an inside diameter slightly larger than the internal diameter of the tube. I used a washer with an outer diameter of 44 mm and an internal diameter of 11 mm (Fig. 1A).
- Epoxy putty. I used PC-Metal<sup>TM</sup> epoxy putty (Protective Coating Co., Allentown, PA). This epoxy resin cures to become a very hard material.
- A hacksaw to remove the upper portion of the micrometer.
- A vise to clamp the micrometer.
- Fine sandpaper. I used 320-grit and 1500-grit sandpaper.
- Firm polystyrene foam (PSF), such as expanded polystyrene foam, extruded polystyrene foam, or similar materials, to hold specimens within the microtome while sectioning. I used scrap Styrofoam<sup>TM</sup> UtilityFit<sup>TM</sup> 15 PSI extruded polystyrene foam insulation (Dow Chemical Co., Midland, MI). This material provided excellent firmness for sectioning.
- Good-quality, single-edge razor blades. I used Personna GEM<sup>®</sup> single-edge, stainless steel, uncoated blades (Edgewell Personal Care Company, Shelton, CT). I have found that good-quality, single-edged razor blades work better than double-edged blades for the hand microtome. Single-edged blades are more rigid than double-edged blades. While singleedge blades follow the cutting guide, the thinner, double-edged blades tend to bend downward and cut deeper below the exposed surface, often resulting in poor-quality sections.

## Construction of the Microtome

First use fine sandpaper to remove any inwardlyprojecting fragments of metal at the lip of the tube

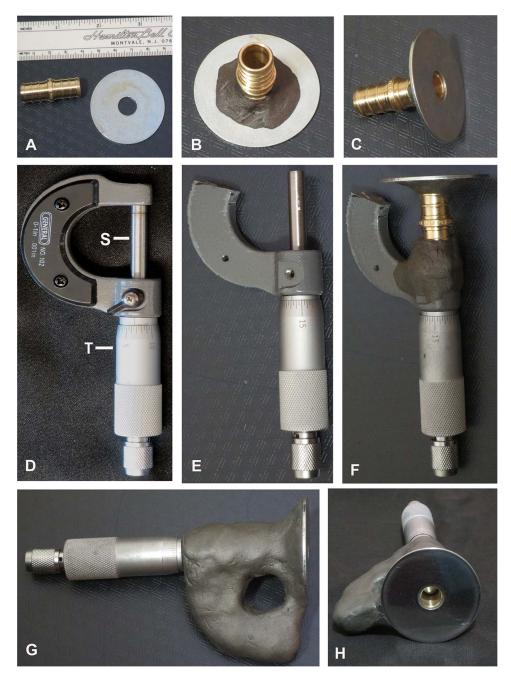


FIG. 1. Construction of the hand microtome. A. The brass tube and steel washer used for the specimen chamber and cutting guide of the microtome. B and C. The washer has been attached to the brass tube with epoxy putty. D. The General #102 micrometer that will become the base of the microtome and provide the differential screw and specimen advance rod; S = Spindle, T = Thimble. E. The micrometer with its upper portion removed. F. The washer and tube assembly centered around the micrometer spindle rod and attached to the micrometer base with epoxy putty. G and H. The finished hand microtome with a polished cutting guide (washer) and a handle fashioned from epoxy putty.

that might affect the movement of the specimen. Clean the tube and washer with soap and water or alcohol to remove surface grease that might hinder the binding of the surfaces to epoxy. Next, attach the tube to the washer with a small ring of epoxy putty (Fig. 1B, C). Be sure that the washer is well-centered and that no part of the washer covers the opening of the tube. Then use a hacksaw to remove the upper portion of the micrometer at the level of the upper extension of the spindle rod (S) (Fig. 1D, E). Next, use a ring of

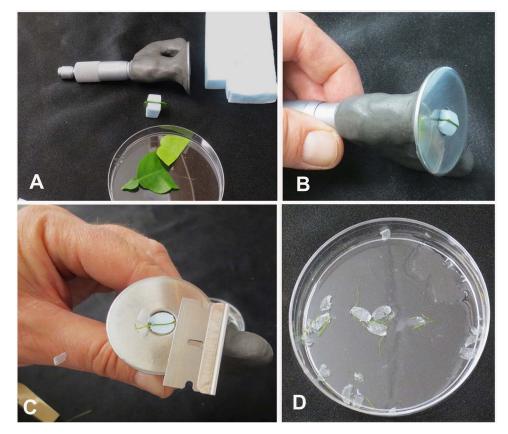


FIG. 2. Sectioning with the hand microtome. A. A sample from a citrus leaf is wedged within a small plug of firm Styrofoam. B. After trimming the excess sample from the sides of the PSF plug, the plug is squeezed to fit within the specimen chamber. C. After advancing the spindle rod until it presses firmly against the PSF plug, the specimen is sectioned with a razor blade. A small, partial, turn of the micrometer screw advances the specimen past the cutting guide. D. Leaf and PSF sections in a Petri dish.

epoxy putty to attach the tube and washer assembly to the micrometer, placing the tube over the spindle and attaching it above the thimble (T) (Fig. 1D, F). Be sure that the micrometer spindle rod is well-centered in the tube. Hold the tube centered while the epoxy sets. I find it helpful to use an epoxy putty with a quick setting time. Use additional epoxy putty to mold a handle around the cut C-shaped portion of the micrometer. This provides extra strength to the microtome's structure and a convenient handle to hold while sectioning (Fig. 1G). Finally, use fine sandpaper to polish the surface of the washer (now the cutting guide) as well as inside the tube (Fig. 1H).

If the diameter of the spindle rod (specimen advance rod) is much smaller than that of the tube's internal diameter, sectioning could be improved by the addition of a small washer, or any flat plug, with an outer diameter close to the inner diameter of the tube to help distribute the pressure on the PSF. The plug could be permanently glued to the end of the specimen advance rod, however, if the rod is not well-centered, the edge of the plug could rub strongly against the edge of the tube when the screw is turned. Alternatively, the plug could be placed loosely on top of the spindle rod prior to inserting the PSF and specimen. Then the plug would still distribute the pressure from the specimen advance rod, but it would not rotate with the rod and would not rub excessively against the tube.

#### Sectioning with the Microtome

Specimens must be firmly held within the microtome while sectioning, or the razor blade will pull the specimen out of alignment while cutting, creating thick and unusable sections. Firm pieces of PSF work well to clamp specimens. Specimens must be squeezed tightly, even if this causes some compression of the specimens.

Sectioning procedure. First, check that the cutting guide is smooth and clean. If it is not, clean and re-polish the cutting guide before use, so that the razor blade can move smoothly across with little resistance. Next, cut a square of firm PSF so that it is larger than the bore of the microtome's specimen chamber (the tube). The PSF square should extend about 2 mm beyond the edge of the chamber on all sides. Slice the PSF square in half. Wet the surfaces of both halves, and sandwich a sample of plant tissue between them (Fig. 2A). Specimens should be well hydrated. Next, carefully compress the

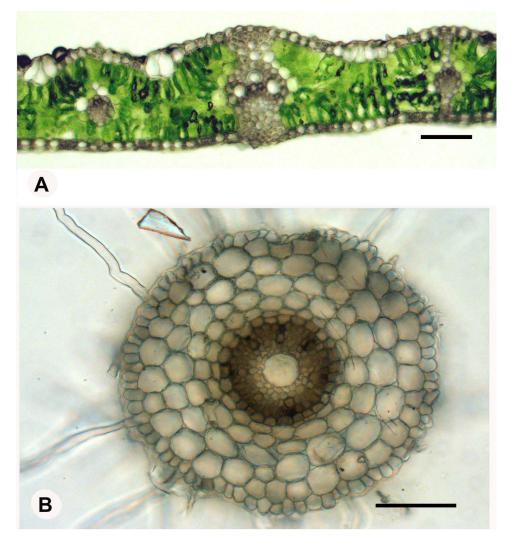


FIG. 3. Unstained, unfixed, plant specimens sectioned with the hand microtome. A. Mature leaf transverse section of *Triticum aestivum* L. (Poaceae). Scale bar = 100  $\mu$ m. B. Transverse section of a young polyarch root of *Triticum aestivum* L. Scale bar = 100  $\mu$ m.

PSF sandwich and insert it within the microtome's specimen chamber. The pressure from the compacted PSF will hold the specimen firmly while it is being sectioned (Fig. 2B). After inserting the specimen, hold a finger over the end of the microtome and advance the specimen advance rod until the sample presses firmly against the finger. Remove your finger from the end of the microtome. Advance the specimen about 1 mm past the cutting guide and slice through the PSF with a single-edged razor blade in order to create a clean face of the PSFheld specimen at the cutting plane of the microtome. Advance the specimen with a small turn (about 1/8 to 1/4 turn) of the specimen advance rod and section the specimen by moving the blade along the cutting guide with a slicing motion (Fig. 2C). Repeat the sectioning process. Cut slowly and carefully, always keeping fingers away from the sharp edges. Collect the sections in a petri dish (Fig. 2D) or place them directly on a slide.

The micrographs (Figs. 3, 4, and 5A, B) show sections of plant specimens cut with the hand microtome described here.

*Photography.* The photographs shown in Figs. 1 and 2 were taken with a Canon PowerShot ELPH 110 HS digital camera. The micrographs of Figs. 3, 4, and 5 were taken with a Leica DMI3000 B inverted microscope using a Leica DFC450C digital camera and Leica Application Suite (LAS X) software.

### DISCUSSION

I presented here the construction and use of a simple hand microtome built over the base of an inexpensive micrometer. It is a variant of a Ranvier microtome. According to Bracegirdle (1978) the design originated

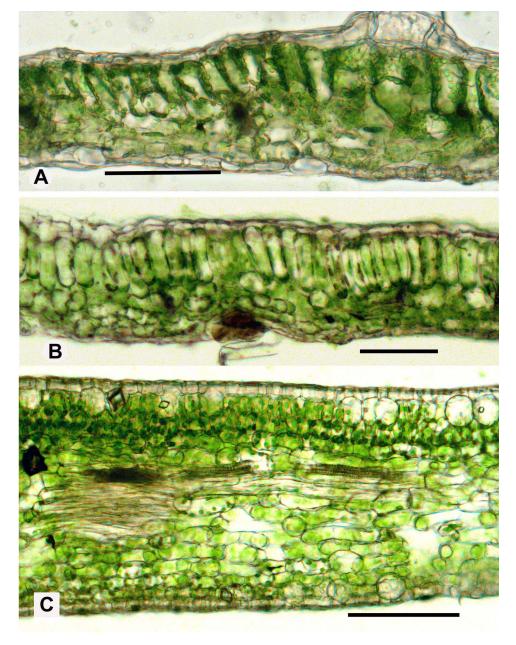


FIG. 4. Unstained, unfixed, leaves sectioned with the hand microtome. A. Transverse section of a mature leaf of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). Scale bar = 100  $\mu$ m. B. Mature leaf transverse section of *Mentha x piperita* L. (Lamiaceae). Scale bar = 100  $\mu$ m. C. Mature leaf transverse section of *Citrus x paradisi* Macfad. (Rutaceae). Scale bar = 100  $\mu$ m.

with H. Schacht, F. Currey, and A. Ross in the early 1850s. In 1851 Hermann Schacht's book on plant microtechnique included a design for a simple hand microtome that consisted of a tube within which samples were wedged between slices of cork. The specimen was simply pushed through the tube from below by the operator's finger, and sectioned with a razor at the end of the tube. Frederick Currey translated Schacht's book into English in 1853 and included changes to the microtome's design. Currey added a simple thumb screw to advance the specimen, with a metal disk at the end of the screw to evenly press against the specimen when the screw is turned (Fig. 5C). Currey noted that Andrew Ross (a prominent optician and manufacturer of microscopes) constructed the microtome for him and that Ross had suggested the changes (Schacht 1853; Bracegirdle 1978). The 1855 edition of Currey's translation illustrated an improved version of the microtome with a cutting guide, consisting of a flat metal disk at the end of the tube. Bracegirdle (1978) suggested that this microtome was

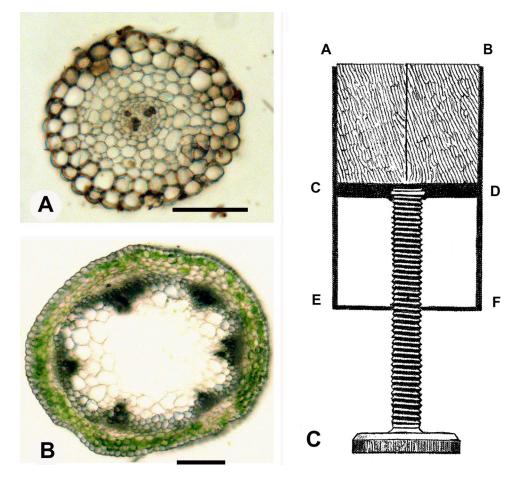


FIG. 5. Sections of fresh plant specimens, and an illustration of the original hand microtome from Schacht (1853). A. A root transverse section of *Dracaena sanderiana* Mast. (Asparagaceae). Scale bar =  $100 \mu m$ . B. Transverse section of a young inflorescence shoot of *Arabidopsis thaliana* (L.) Heynh. Scale bar =  $100 \mu m$ . C. A diagram of the original microtome designed by Andrew Ross, redrawn from Figure 1 in Frederick Currey's 1853 translation of Hermann Schacht's book of plant microtechnique. The box (ABEF) defines a brass tube closed at the end (EF). A split cork (holding a specimen) is shown in the upper portion of the tube pressed by a brass plate (CD) that is advanced by a screw. The following is F. Currey's footnote concerning the figure: "Dr. Schacht recommends a metal tube, open at both ends, and a cork divided into two parts. The disk and the screw were suggested by Mr. Ross, who made for me the instrument here described. It is better that the cork should not be quite divided, as there is then less risk of injuring the object by friction whilst pushing the cork into the tube. The screw being well made, the razor very sharp and flat-sided, and the cork sound and good, wonderfully thin sections may be obtained with the instrument here recommended.—Tr."

likely a commercial development of Ross' design. Subsequently, the same design was popularized by Louis-Antoine Ranvier, and the device soon became widely known as the Ranvier microtome. Commercial versions have been available from the mid-19th century to the present day. Some 19th-century versions had high precision specimen advance screws (including modified micrometer heads) and elaborate specimen holding clamps (Bracegirdle 1978).

The microtome that I describe in this paper differs from the original Ranvier hand microtome by the addition of a differential screw mechanism for the specimen advance rod, derived from the spindle advance of a micrometer. Using a differential screw provides a smoother, more precise movement of the specimen advance rod than would be possible with a simple mechanical screw. A micrometer differential screw mechanism uses two differently threaded regions, on the spindle rod and micrometer barrel, with different thread pitches. When the micrometer thimble is turned, the rod's movement is restricted and is proportional to the difference of the pitches of the two threads. This allows for much finer movement with each thimble rotation than would occur with a simple screw with a single-thread pitch (Schwertz and Burge 2012).

Low-cost outside micrometers are available from several sources in the \$10 to \$20 range. Very well-made micrometers with high precision can cost more than \$200, but the low-cost instruments work well for hand microtomes, and higher precision is unnecessary. The thickness of sections appears to be limited by the softness of unfixed, unembedded, fresh specimens, rather than the fine movements of the specimen advance rod, so greater precision from the more expensive micrometers would not improve sectioning. High precision single-thread screws and bolt sets are also available. Presumably, they would make excellent specimen advance mechanisms for hand microtomes, but they appear to be more expensive than the lowcost micrometers (in the \$30 to \$40 range).

## CONCLUSIONS

The simple, low-cost, hand microtome presented here is a precision instrument capable of producing highquality freehand sections of plant material and other biological specimens, useful for research or teaching.

#### ACKNOWLEDGMENTS

I thank Mr. Stephen Lancaster, Dr. Kay Lancaster, and Dr. Melvin Turner for very helpful discussions. I thank Dr. Andrei Smertenko for use of his microscope.

#### LITERATURE CITED

- BRACEGIRDLE, B. 1978. A history of microtechnique: the evolution of the microtome and the development of tissue preparation. Heinemann Educational Books, London, UK.
- RUZIN, S. E. 1999. Plant microtechnique and microscopy. Oxford University Press, Oxford, UK.
- SCHACHT, H. 1853. The microscope, in its special application to vegetable anatomy and physiology, translated by Frederick Currey. Samuel Highley, London, UK.
- SCHWERTZ, K. AND J. H. BURGE 2012. Field guide to optomechanical design and analysis. Vol. FG26, p. 38 *in* J. E. Greivenkamp (ed.), SPIE Field Guides, SPIE Press. Bellingham, WA.