The OPN Microtome: An Inexpensive, Open Source Hand-Held Mini Microtome

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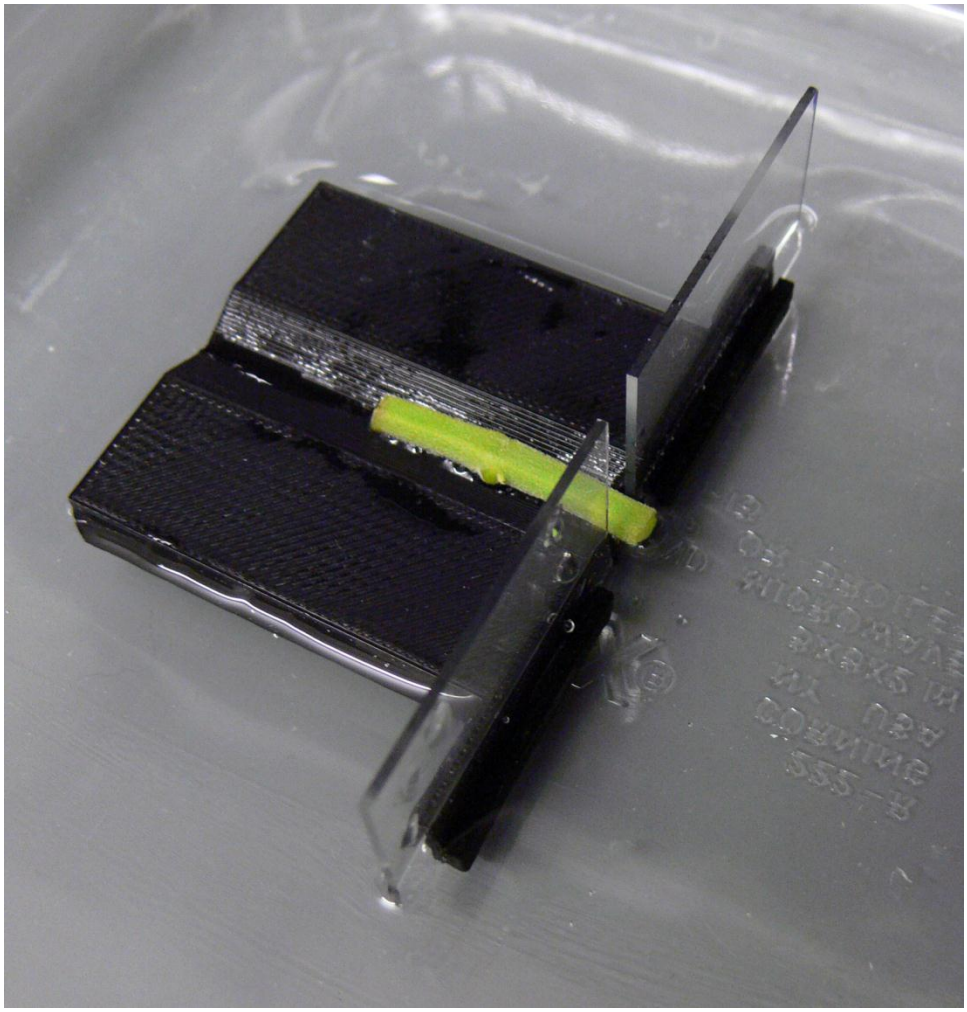

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Introduction

One common way to study plant anatomy is to examine hand sections of plant tissue under the microscope. Because of their rigid cell walls, plants can be sectioned without the use of chemical fixation and plastic or wax imbedding. In addition, when stained, sections made in this manner can reveal significant internal detail of various cellular structures.

The most common way of hand sectioning plant samples is to hold the plant tissue under water and carefully slice a thin section with a sharp razor blade. When performed by an experienced technician, the results can be quite dramatic. One major problem with this "free hand" method, however, is that the technique requires a great deal of practice in order to become proficient at it. Plus, a person's fingers are exposed to a sharp blade without any protection, which can pose a serious safety hazard.

As a result, over the years, there have been a number of innovative ideas for making relatively inexpensive hand-held microtomes as well as for improving the hand-sectioning process itself **(1 - 5)**. For example, one common approach is to use a carrot or potato to hold the plant tissue securely in place while making thin slices with a razor blade **(5 - 7)**.

There are also a number of basic hand-held microtomes for sale online that can be used for hand sectioning, such as the Omano hand-held microtome (and similar models from vendors like Carolina Biological, Home Science Tools, or Spectrum Scientifics) as well as the MT 5500 and MT 5501 cylinder microtomes and the MT 5503 hand-hand microtome by Vincent Leermiddelen Scientific. However, some of these devices can be relatively expensive (e.g., they generally cost between $40 and $225 each at February 2017 prices, not including shipping or taxes), and some models further require wax embedding of the sample that will be sliced.

In an effort to help bring down some of these costs and make the process of hand sectioning plant tissue more widely available to others, this manual describes how to build or 3D print a mini microtome that is relatively inexpensive (roughly $6.50 per instrument if using the website makexyz to 3D print the parts) and which should also improve both the safety and efficiency of making thin hand sections. This manual also describes how to use this mini microtome to prepare samples of different plant tissues for microscopic viewing, so that students can begin exploring plant anatomy as part of a teaching lab or other educational activity.

Finally, in keeping with the names given to many of the other instruments and pieces of equipment designed in our lab, this mini microtome is called "the OPN Microtome" since its plans and parts are intended to be open and accessible for all to use. Ultimately, we hope that the OPN Microtome will be a useful tool that helps both teachers and students study the anatomy and physiology of various plant species in their classrooms and labs.

Constructing the OPN Microtome

There are two ways to make the OPN Microtome. The first approach uses items that should be available in most biology teaching labs (primarily, glass slides and epoxy). The second method requires a 3D printer. However, for readers that do not have access to 3D printing technology, there are a number of websites (e.g., makexyz, Shapeways, and others) that will 3D print and ship any uploaded STL files to you for a fee, which is usually based on the size and volume of the part.

Making an OPN Microtome Using Glass Microscope Slides

To make the version of the OPN Microtome that is built from glass microscope slides, readers will need the following materials:

- 10 glass microscope slides (Fig. 1);
- a set of quick-drying (e.g., 5-minute) epoxy, which includes both a resin and a hardener in separate (or separated) tubes (Fig. 1, upper left);
- several toothpicks or similar items for mixing the epoxy (Fig. 1, lower right);
- a small disposable surface on which to mix the epoxy, such as several Post-It Notes ® (Fig. 1, lower right), a small piece of cardboard, or other similar material;
- a triangular file (or glass cutter) to scribe a line in one of the glass slides (Fig. 2); and
- a standard ruler (Fig. 2).

Step 1. First, use a toothpick (or other similar item) to mix up a small amount of epoxy on a disposable surface, such as several Post-It Notes ® (Fig. 1, lower right) or a piece of cardboard. Then, glue seven microscope slides together, so that they are stacked one on top of the other (Fig. 1, lower left). Use a toothpick to spread a small amount of epoxy around on top of each slide before stacking the next slide on top. Also, it is important to use only a small amount of epoxy, so that the glue does not ooze out of the sides. In addition, make sure to keep the slides and edges flush, so that the sides and ends are smooth and even. This step is necessary because, later, two face plates will be glued to the front of this stack, and an uneven surface there may prevent these pieces from fitting together smoothly. Once the stack of slides has been glued together (Fig. 1, lower left), let the quick-drying epoxy set for at least 15 minutes to ensure a tight bond.

Figure 1. Some of the materials used to make the glass slide version of the OPN Microtome. In all, seven glass slides are glued together (lower left) using quick-drying epoxy. Then, an additional slide is used to make the face plate, and two others are added to create a channel for the samples of plant tissue.

Importantly, as explained in the Hazards section, please do not to touch the epoxy with your bare hands because the compound can irritate your skin and cause possible allergic reactions. Also, do not breathe in the fumes of the epoxy since those vapors can also prove harmful. For these reasons, readers should consider wearing a pair of safety gloves when working with epoxy. Similarly, readers should work in a well-ventilated area (or under a fume hood) in addition to wearing any necessary masks or respirators when using the adhesive compound.

Step 2. Next, to make the face plate for the OPN Microtome, take a new glass microscope slide and identify its center line using a ruler and a permanent or felt-tipped marker (Fig. 2).

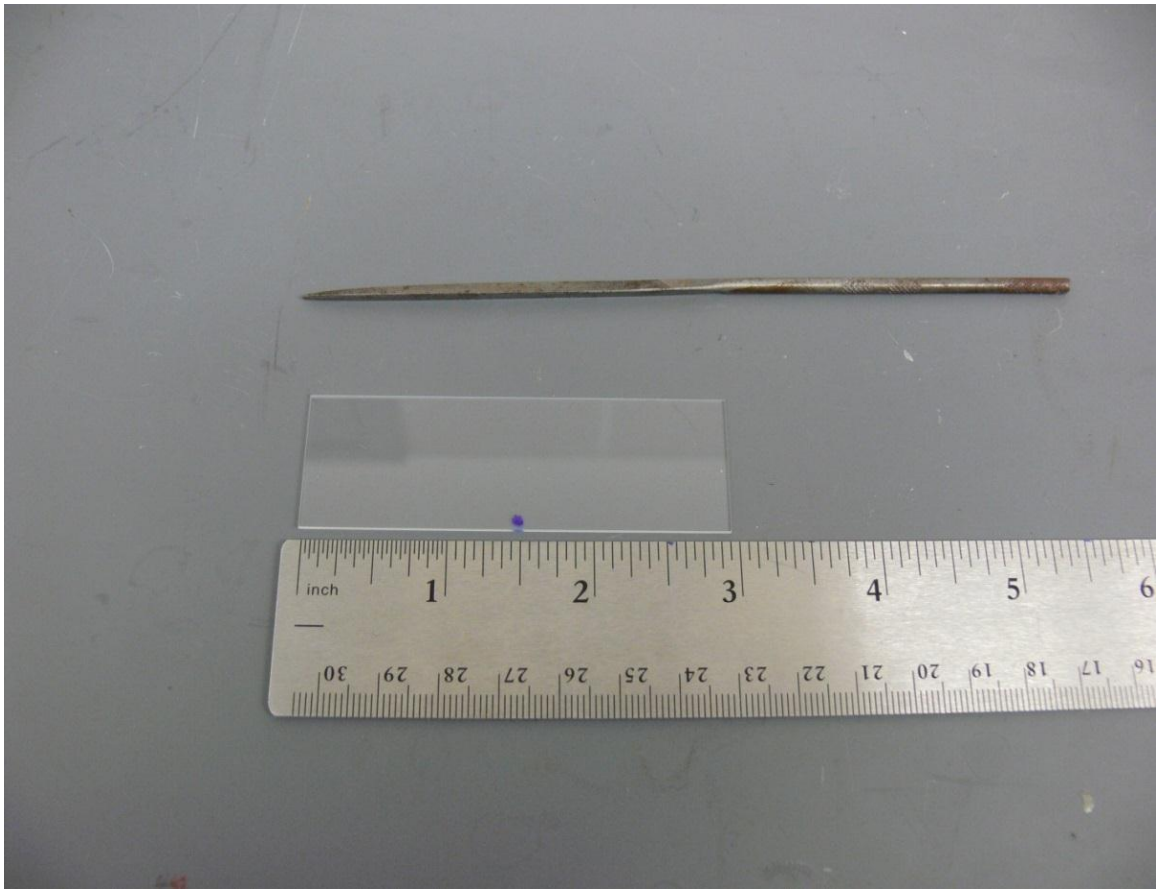

Figure 2. The materials used to scribe a line down the center of a microscope slide. While a narrow tip triangular file works well (top), readers can instead use a hand-held glass cutter. Also, note how the center of the slide was first identified using a felt-tipped marker before the line was etched.

Step 3. Then, hold a straight edge (e.g., a ruler) across the center of the slide, and use the sharp edge of a triangular file (or glass cutter) to scribe a line down the center. Use enough pressure to scratch the glass, but not so much pressure that the slide breaks (Fig. 3).

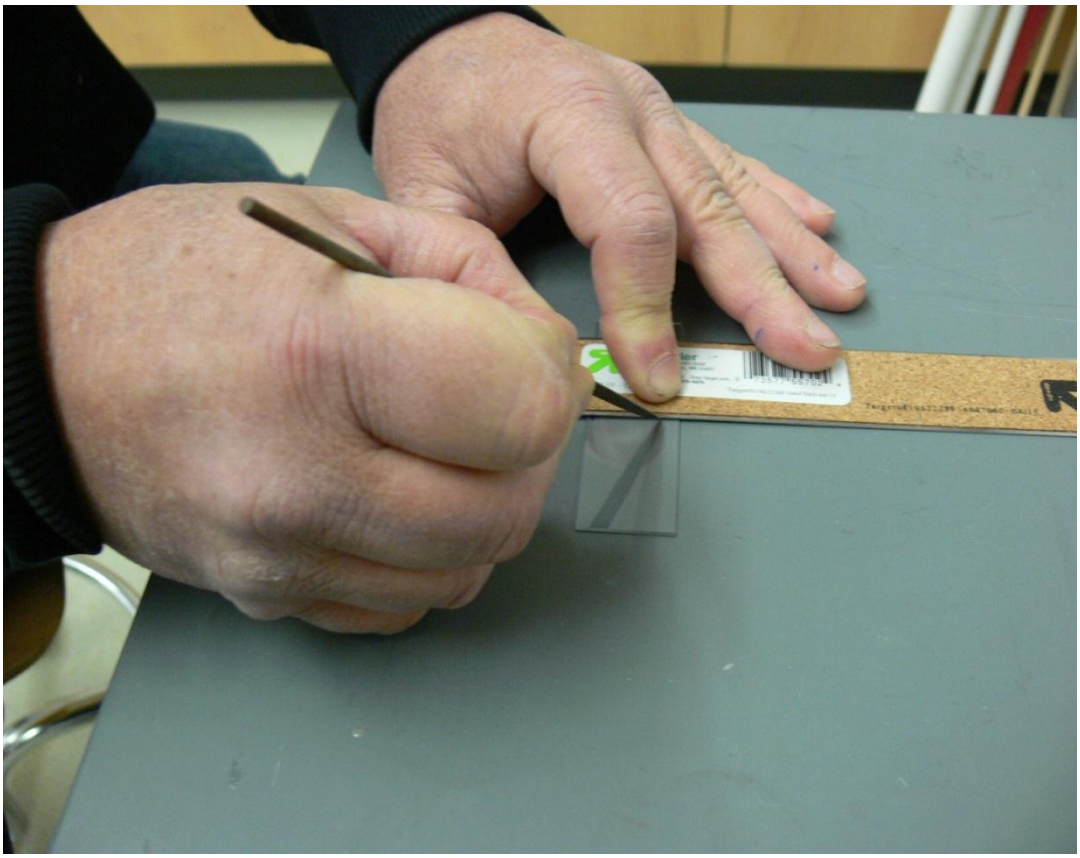

Figure 3. Scribing a line down the center of a glass slide using the pointed end of a triangular file and a straight edge (i.e., ruler). Alternatively, a glass cutter can be used instead of a file.

Importantly, during this process, readers should wear the proper eye protection (e.g., goggles or safety glasses) to protect their eyes from any small glass pieces or particles that might prove harmful. Readers may further want to wear a pair of safety or examination gloves to provide an additional layer of protection against any potential cuts or scratches. In addition, wearing these gloves may make it easier to grip the glass slide.

Step 4. Now, carefully break the slide along the scribed line (Fig. 4). Ultimately, these two glass pieces will form the left and right sides of the face plate for the OPN Microtome. Again, please wear safety glasses or goggles during this process to protect your eyes from any potential harm that could be caused by any small pieces of glass, which might become airborne when the slide is broken in half. Also, readers may want to wear a pair of safety or examination gloves for this step to provide an additional layer of protection from any possible cuts or scratches (and to make it easier to grip the slide).

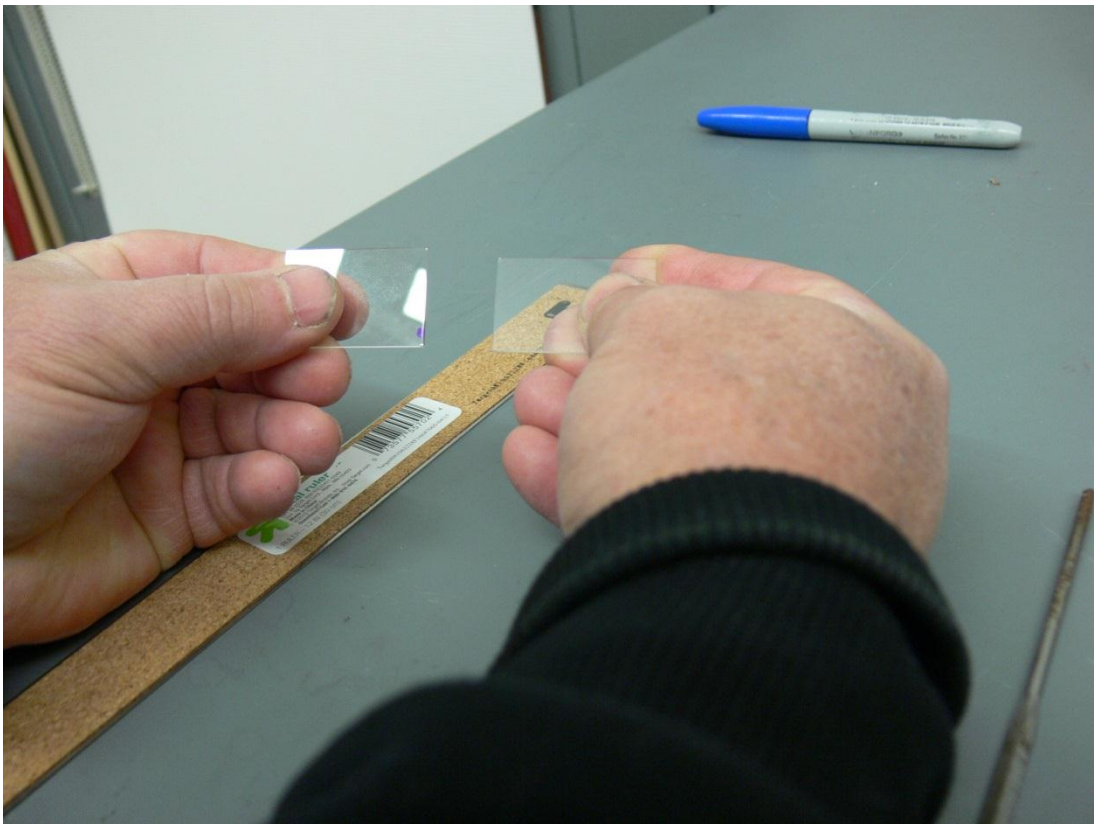

Figure 4. Carefully break the glass slide along the scribed center line to create the left and right pieces of the face plate for the OPN Microtome.

Step 5. Next, use a small amount of epoxy to glue the left and right pieces of the face plate to the front of the slide stack (Fig. 5). The bottom edge of these pieces should be flush with the bottom of the slide stack, so that the assembly sits level when placed on a flat surface. Also, make sure to leave a 0.5-cm gap (approximately) between the pieces to create an opening through which the plant tissue can later be inserted for slicing (Fig. 5, bottom center). In addition, let the assembly sit for at least 15 minutes, so that the quick-drying epoxy can begin to form a tight bond.

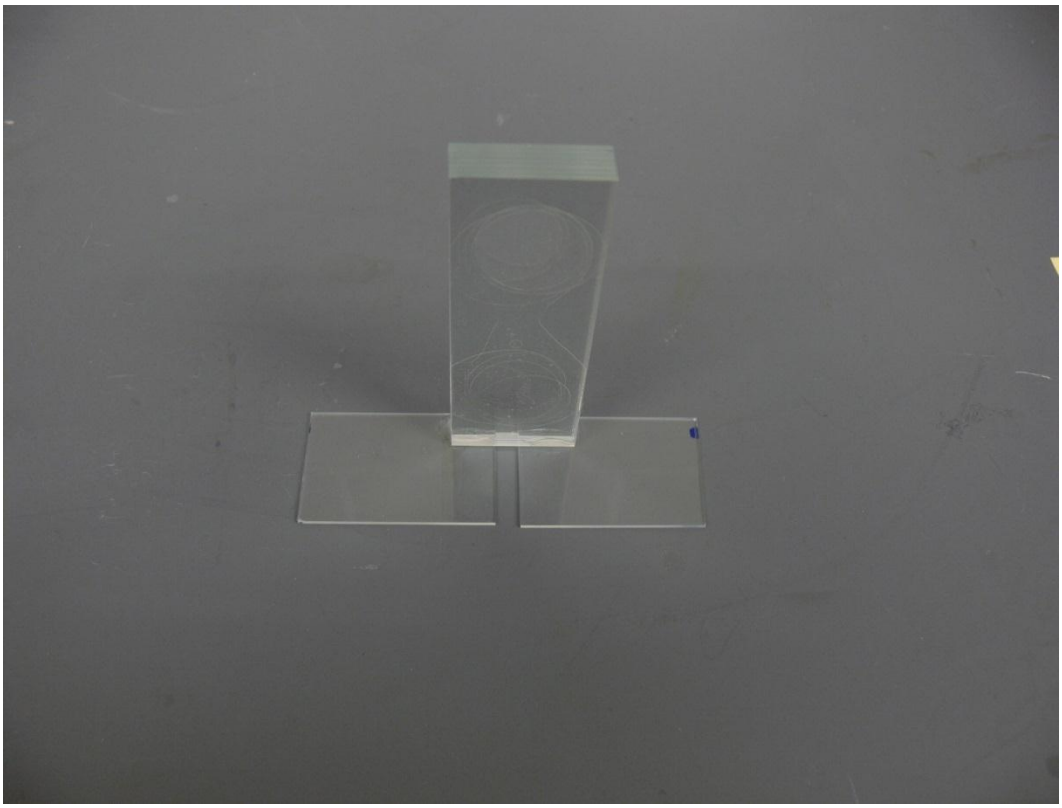

Figure 5. Gluing the left and right pieces of the face plate to the front of the seven-slide stack. Note the 0.5-cm gap between the two pieces (bottom center). Later, the plant tissue will be slid through this opening for slicing.

Step 6. Once the face plate has been fixed to the slide stack, mix up another small amount of epoxy (as described above) and carefully glue two microscope slides to the top of the slide stack, leaving a 0.5-cm wide channel between the two slides (approximately) so that plant tissue can be slid through the opening in the face plate (Fig. 6). Again, use only a small amount of epoxy, so that the liquid compound does not ooze out the sides or into the channel, which may make it more difficult to position the plant tissue there for slicing.

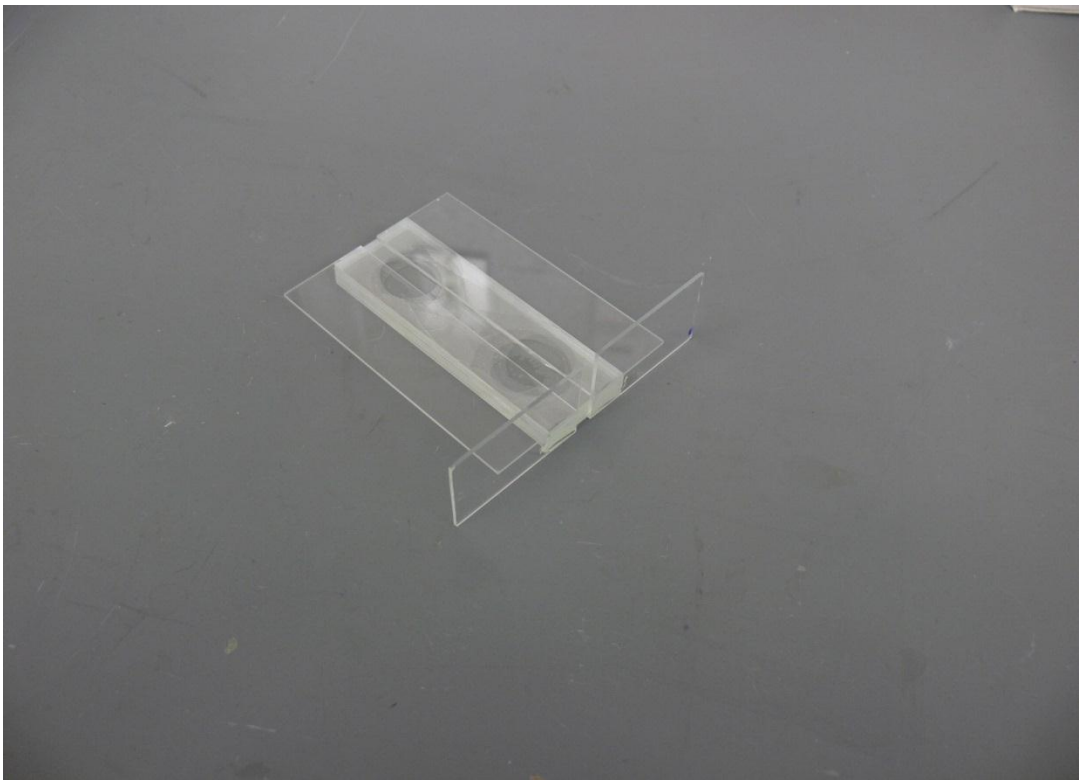

Figure 6. Gluing two additional slides on top of the seven-slide stack to create a 0.5-cm-wide channel, so that plant tissue can be slid through the corresponding opening in the face plate.

Also, given the 0.5-cm-wide channel, the two glass slides placed on top of the OPN Microtome will extend far over each side of the stack (Fig. 6). As a result, readers may need to hold each slide in place for several minutes while the epoxy sets (or come up with their own method to prevent each slide from "drooping" before the epoxy dries). Then, let the OPN Microtome sit overnight to give the epoxy a sufficient enough time to cure. At that point, this version of the OPN microtome will be finished and ready to use.

3D Printing the OPN Microtome

To make the 3D-printed version of the OPN Microtome, readers will need the following materials:

- a standard 3D printer and related filament (e.g., typically, we use ABS filament, but PLA will also suffice);
- the CAD or STL files for the OPN Microtome (S1 and S2, respectively);
- one glass microscope slide;
- a triangular file (or glass cutter) to scribe a line down the center of that slide;
- a standard ruler;
- one set of quick-drying (e.g., 5-minute) epoxy, which includes both a resin and a hardener in separate (or separated) tubes;
- several toothpicks or similar items for mixing and applying the epoxy; and
- a small disposable surface on which to mix the epoxy, such as several Post-It Notes ®, a small piece of cardboard, or other similar material.

Also, for readers who do not have a 3D printer, certain online services (e.g., makexyz, Shapeways, etc.) will 3D print and ship any STL files that you upload for a fee, which is usually based on the size and printing volume of the part. For example, the website http://3dprintingpricecheck.com/ estimates that it would cost approximately $6.50 to print out either version of the OPN Microtome using the makexyz 3D printing service (at February 2017 prices, not including any taxes or shipping). Of course, in the long run, purchasing a relatively inexpensive 3D printer (e.g., $500 or less) and the related spools of filament (about $20 to $25 each online) will likely prove to be the most economical choice, especially if printing many parts over time. Plus, once a school or classroom has a 3D printer, teachers and students can begin designing their own equipment to use in the lab (in addition to using the printer for other projects as well).

Step 1. First, using the STL files included with this manual (S2), 3D print either (or both) model(s) of the OPN Microtome. One version (S2A) has a v-shaped channel (Fig. 7, left) and can be used for hand slicing plant roots and stems. The other version (S2B) has a square channel (Fig. 7, right) and can be used for hand sectioning leaves or other delicate plant tissues. We further include the CAD files for these parts (S1), so that readers can modify these designs to fit their particular needs. However, because these pieces were created using the free version of DesignSpark Mechanical, readers will likely need to use that program to make any modifications to the CAD files. Also, before 3D printing any parts, please make sure to read the Hazards section below in order to learn about some of the safety risks involved in this process.

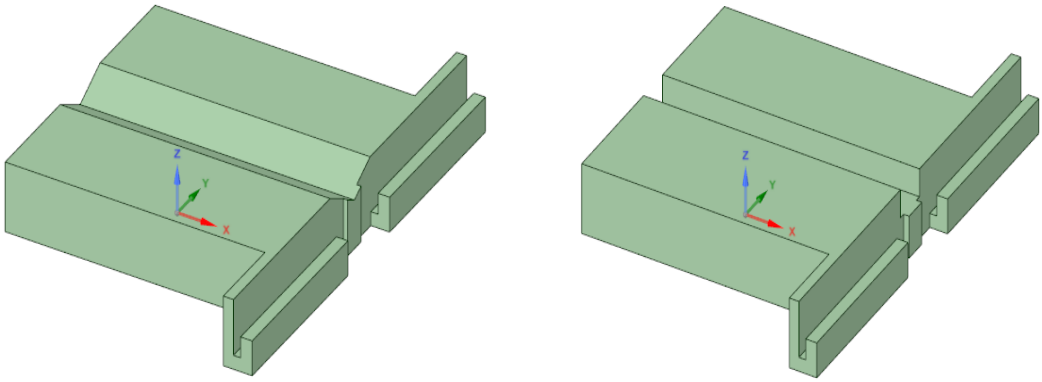

Figure 7. Schematics of the 3D printed version of the OPN Microtome, whose CAD files were created with the free version of DesignSpark Mechanical. The microtome with a v-channel is shown on the left, and the one with a square channel is depicted on the right.

Step 2. As with the OPN Microtome made from glass slides, use the triangular file or glass cutter to scribe a line down the center of the glass slide to make the two pieces of the face plate (Figs. 2 - 4). Then, break the microscope slide along the scribed line, and use a small amount of epoxy to glue the left and right pieces into their respective slots in the front of the OPN Microtome (Fig. 8). In the process, make sure to leave a small (e.g., 0.5-cm wide) gap between the two plates, so that any plant tissue can be slid through the opening for slicing. Also, as explained in the Hazards section below, make sure to follow the proper safety precautions and wear the appropriate protective equipment (e.g., gloves, goggles or safety glasses, masks or respirators as needed, etc.) when working with epoxy and cutting the glass slide.

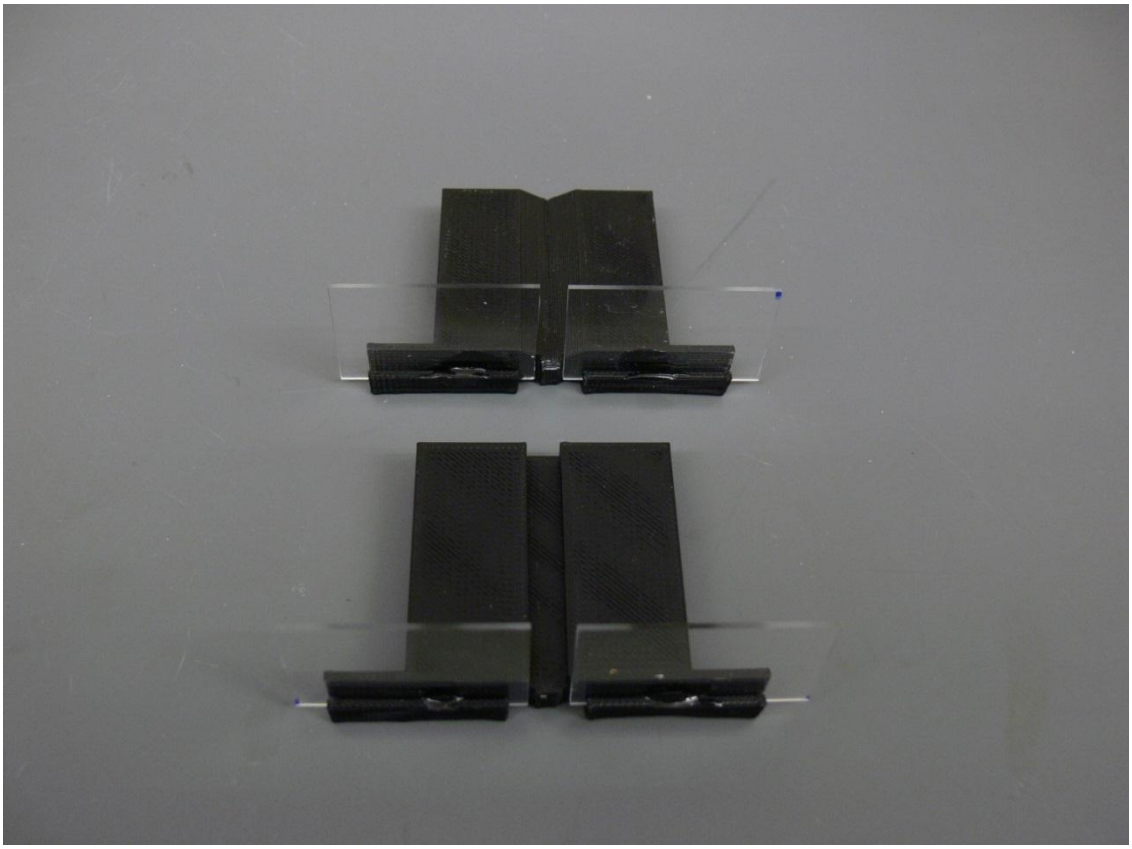

Figure 8. The 3D-printed versions of the OPN Microtome after the pieces of glass slide have been glued in place to create the front face plates. Note that a small gap of roughly 0.5 cm is left between the plates to accommodate the tissue to be sectioned.

Making the Knife for the OPN Microtome

To make the knife for either version of the OPN Microtome, readers will need the following items:

- one set of double-edged razor blades (be extremely careful; they are very sharp);
- a collection of standard glass microscope slides;
- one set of quick-drying (e.g., 5-minute) epoxy, which includes both a resin and a hardener in separate (or separated) tubes;
- several toothpicks or similar items for mixing and applying the epoxy; and
- a small disposable surface on which to mix the epoxy, such as several Post-It Notes ®, a small piece of cardboard, or other similar material.

Step 1. First, lay out all of the materials. Also, readers who are comfortable working with double-sided razor blades can cut the blades in half with a pair of scissors (Fig. 9, right), which will double the number of knifes that can be made.

Figure 9. Some of the materials used to make the knife for the OPN microtome: double-edged razor blades (bottom); standard glass sides (middle); and quick-drying epoxy (top). In addition, readers will need several toothpicks to mix and apply the epoxy as well as a small disposable surface (e.g., several Post-It Notes ® or a piece of cardboard) on which to work.

Step 2. Next, mix up a small amount of epoxy (as described above) and glue each razor blade to a glass slide. Make sure to let the blade hang over the edge of the glass slide by approximately 3 or 4 mm or roughly $^{1}/_{8}$ to $^{3}/_{16}$ of an inch (Fig. 10). Also, explained in the Hazards section below, make sure to follow the proper safety precautions when making these knifes (e.g., use a toothpick or other similar item to mix and apply the epoxy, wear the appropriate gloves and eye protection when doing so, work in a well-ventilated area or under a fume hood, do not breathe in any of the epoxy fumes, wear a mask or respirator as needed, etc.).

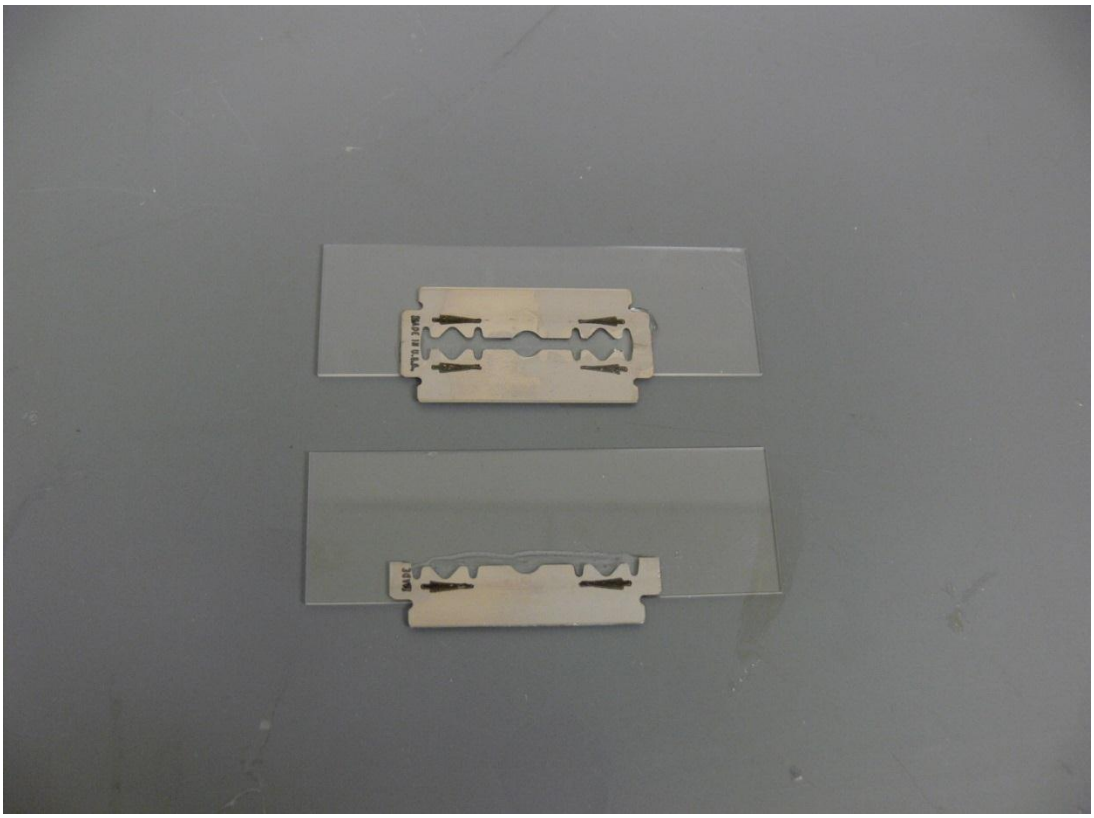

Figure 10. Making the knife for the OPN Microtome by gluing double-edged razor blades onto standard microscope slides (using quick-drying epoxy). Note that the razor blade extends past the edge of the slide by approximately 3 or 4 mm (or roughly $^{1}/_{8}$ to $^{3}/_{16}$ of an inch).

Then, let each knife sit overnight to give the epoxy a sufficient amount of time to cure, so that it can form a strong bond. Once finished, readers can hold onto the glass slide when making their hand sections (instead of grasping the razor blade itself), which should allow for safer and easier cutting of plant tissue (and which may also prove more accurate and consistent than the typical “free hand” method of slicing).

Using the OPN Microtome

Like other OPN instruments available on the OPN Lab "Equipment" web page, the OPN Microtome was designed to be easy to use (and understand). In fact, once the microtome itself and corresponding knife are assembled, slicing plant tissue is relatively straightforward. Nevertheless, for those who are unfamiliar with the process, the pages below explain how to use the OPN Microtome to hand section two types of plant tissue: (i) roots, shoots, and stems; and (ii) leaves and other delicate tissues.

Root, Shoot, and Stem Sections

To make hand sections of any plant roots, shoots, or stems, readers will need the following materials (Fig. 11):

- an OPN Microtome (Fig. 11, bottom left);
- a knife for the OPN Microtome (Fig. 11, bottom middle);
- a fine tweezers (Fig. 11, bottom right);
- a large Pyrex or Petri dish (Fig. 11, upper left);
- a squirt bottle filled with deionized or distilled water (Fig. 11, top left);
- a glass multi-well staining plate (Fig. 11, middle left);
- A 0.1% solution of Toluidine Blue (100mg of Toluidine Blue in 100 mL of deionized or distilled water) in an appropriate container, such as an eye dropper bottle (Fig. 11, top middle);
- one box of standard glass microscope slides (Fig. 11, top right); and
- one box of standard slide cover slips (Fig. 11, middle right).

Figure 11. The materials needed to make thin sections of any plant roots, shoots, or stems using the OPN Microtome.

Step 1. Begin by placing the OPN Microtome in the Pyrex dish. Add tap water until the level reaches the bottom of the channel in the microtome (Fig. 12). Also, while we explain how to hand section a plant stem using the 3D-printed version of the OPN Microtome with a v-shaped channel (S1A, S2A), readers could use the version made from ten glass slides instead (Fig. 6). The v-shaped channel of the 3D-printed version, however, may make it easier to position and control the plant root or stem as it is being sliced.

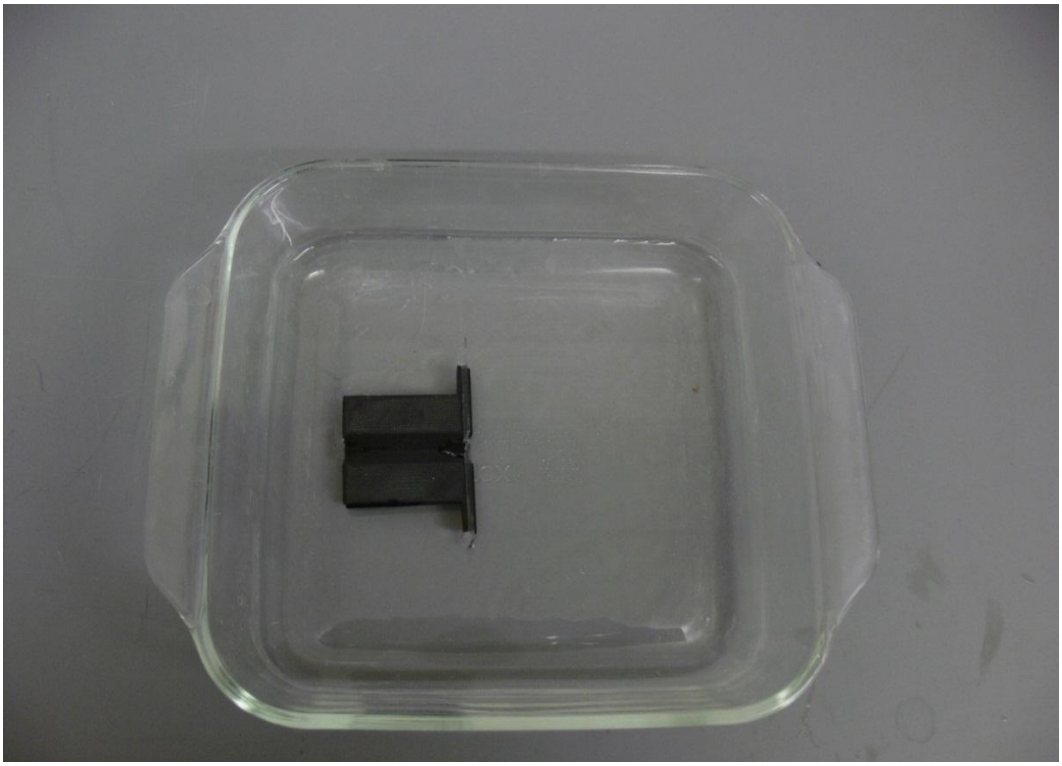

Figure 12. Placing the 3D-printed version of the OPN Microtome in a Pyrex dish, which was then filled with tap water until the level reached the bottom of the v-shaped channel in the microtome.

Step 2. Obtain a piece of the root, shoot, stem, or petiole (i.e., a stem that joins a leaf to a stalk) that will be used for the plant sections (Fig. 13). Readers should note that they may need to use a single-edged razor blade, scissors, or other cutting tool to obtain this sample (Fig. 13).

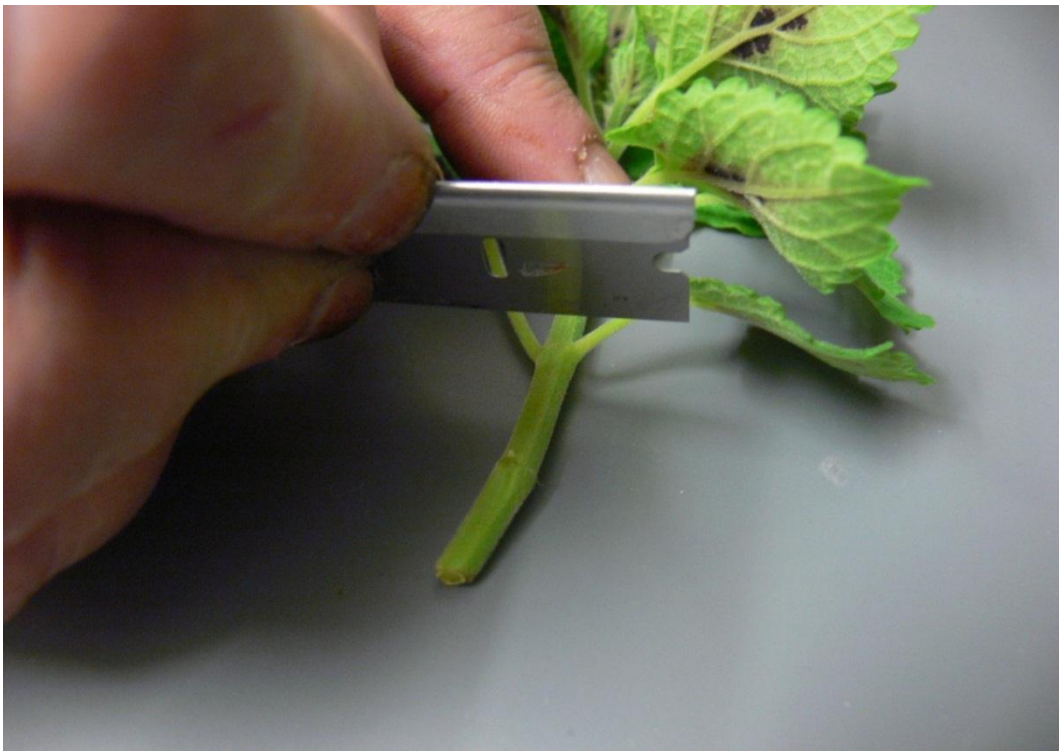

Figure 13. Using a single-edged razor blade to obtain a sample of a plant stem for hand sectioning with the OPN Microtome.

Step 3. Place the stem in the v-shaped channel of the OPN Microtome, and slide the stem forward so that one end extends past the glass slides that serve as the front face plate (Fig. 14). Alternatively, if using the glass version of the OPN Microtome, readers can press the stem up against one of the sides of the square channel or press the stem firmly against the bottom of the channel, which should help keep the stem in place.

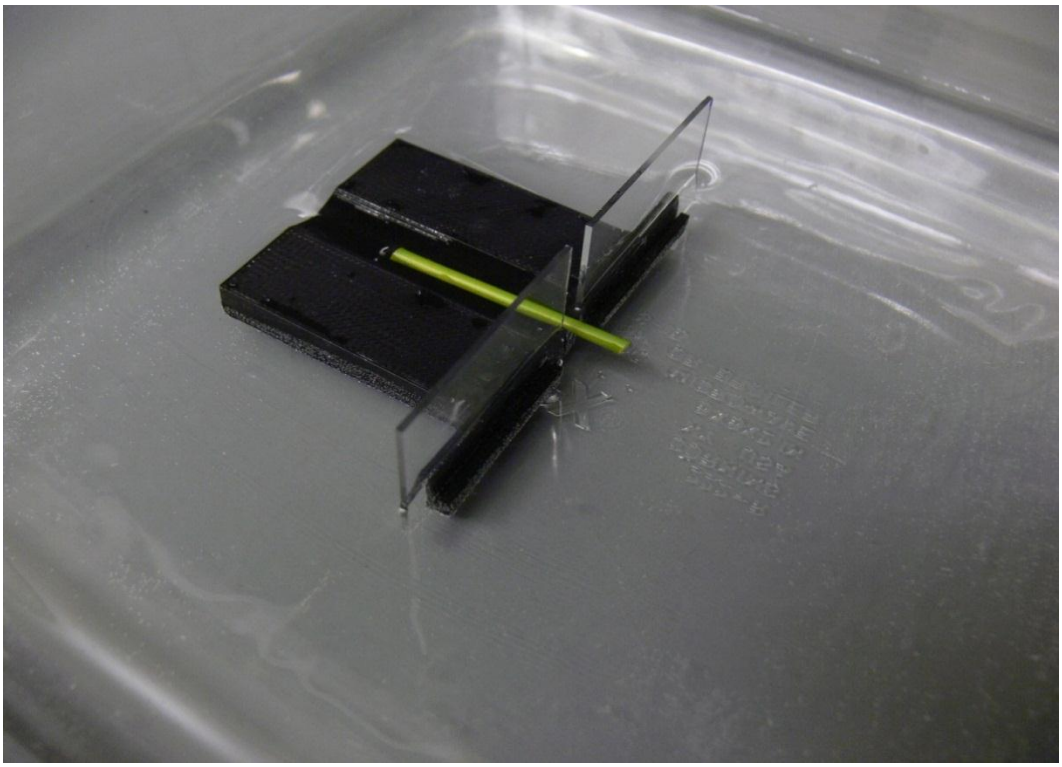

Figure 14. Placing a plant stem in the v-shaped channel of the OPN Microtome and then sliding the stem forward to prepare it for slicing.

Step 4. Make the first cut (to clear off the excess plant tissue) by holding the stem tightly in the OPN Microtome and then using the knife to slice through the sample (Fig. 15). Hold the knife so that the razor blade presses up against the glass face plate (Fig. 15). Also, make sure to hold the knife so that that the razor blade is facing inward (toward the face plate) and not outward (Fig. 15) since this will provide a better and safer cut. Finally, when slicing the plant tissue, use firm, downward strokes. Readers may also want to wear a pair of safety or examination gloves when making these sections simply to provide an additional layer of protection over their bare skin.

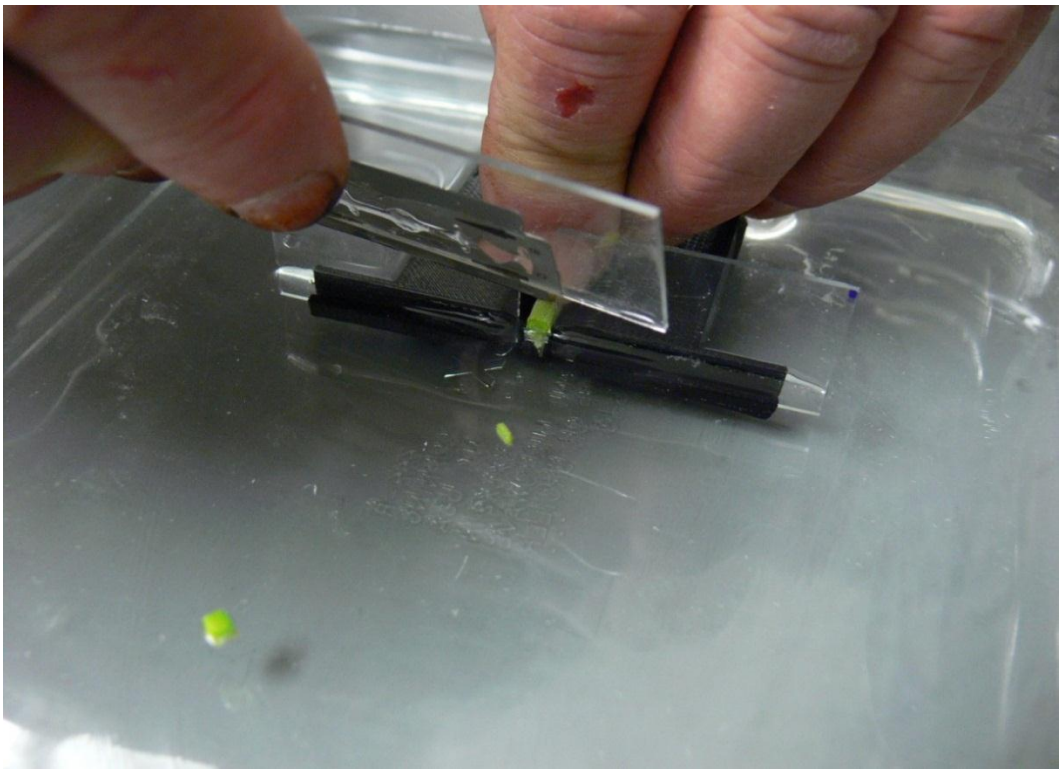

Figure 15. Making the first cut to trim off the excess tissue by holding the plant stem firmly in place and slicing downward with the knife for the OPN Microtome. Also, make sure that the razor blade faces toward the glass face plate for a better and safer cut. For an additional layer of protection over their hands, readers can wear a pair of safety or examination gloves.

Step 5. Next, to obtain thin sections for viewing under the microscope, carefully slide the piece of tissue forward a small amount. Then, slice off the section using the knife for the OPN Microtome (as described above). The section should drift off into the water (Fig. 16). Make sure to practice this technique of sliding the tissue forward ever so slightly in order to obtain very thin sections. Also, for the best results, make sure to use a new knife regularly since slicing the plant tissue will dull the blade.

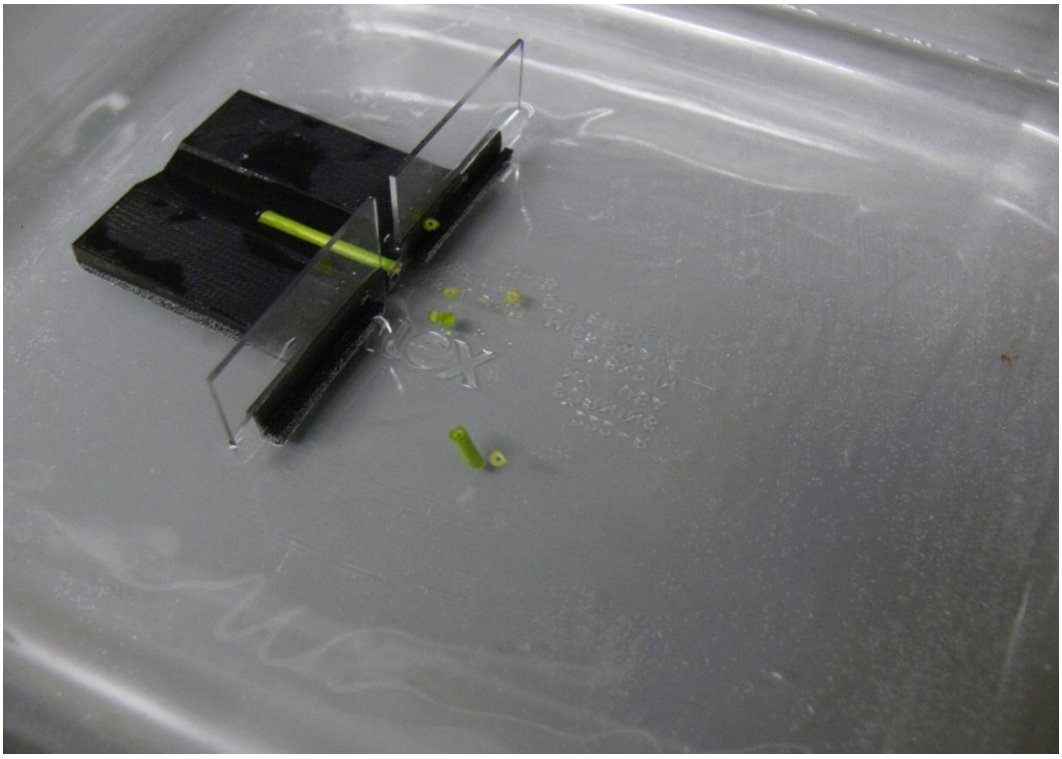

Figure 16. Obtaining thin sections by moving the stem forward a very small amount and slicing off the overhanging tissue with the knife for the OPN Microtome.

Step 6. Once finished with the slices, pick up a thin section of tissue with the tweezers, and place it on a microscope slide (Fig. 17). Then, to view the unstained sample under a microscope, add a drop of water and place a cover slip over the tissue slice (Fig. 17). Finally, place the slide on the stage of a microscope and view the plant section at the desired magnification (Fig. 18).

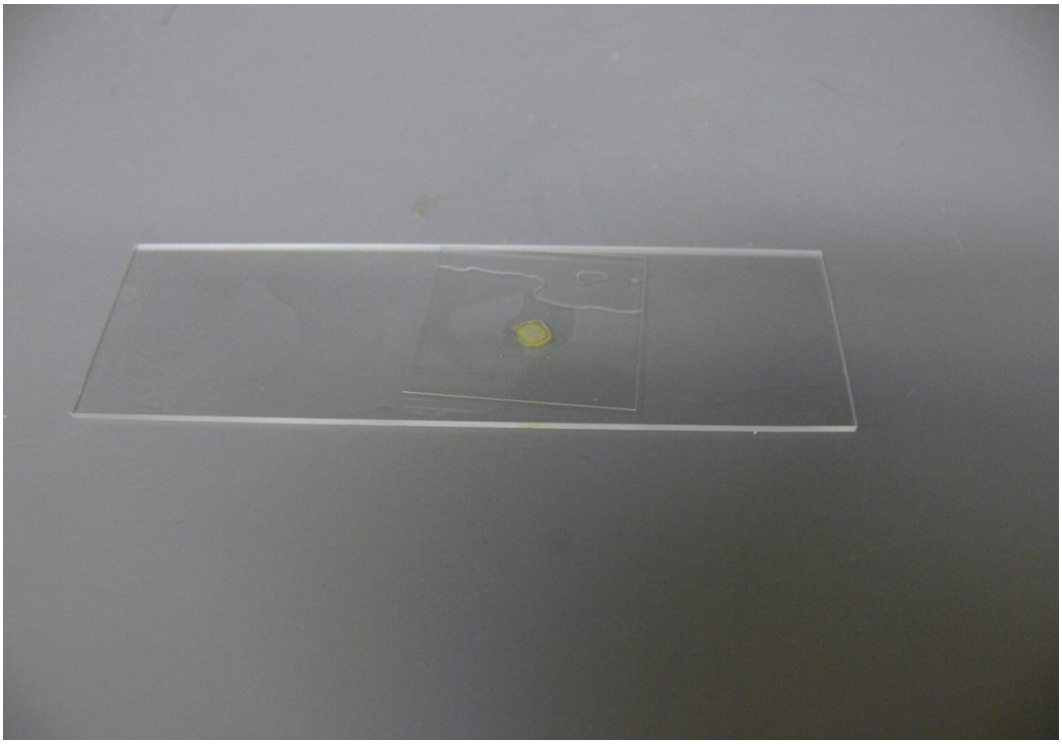

Figure 17. Preparing an unstained section of plant stem for viewing under a microscope by placing the section on a glass slide, adding a drop of water, and covering the sample with a standard glass cover slip.

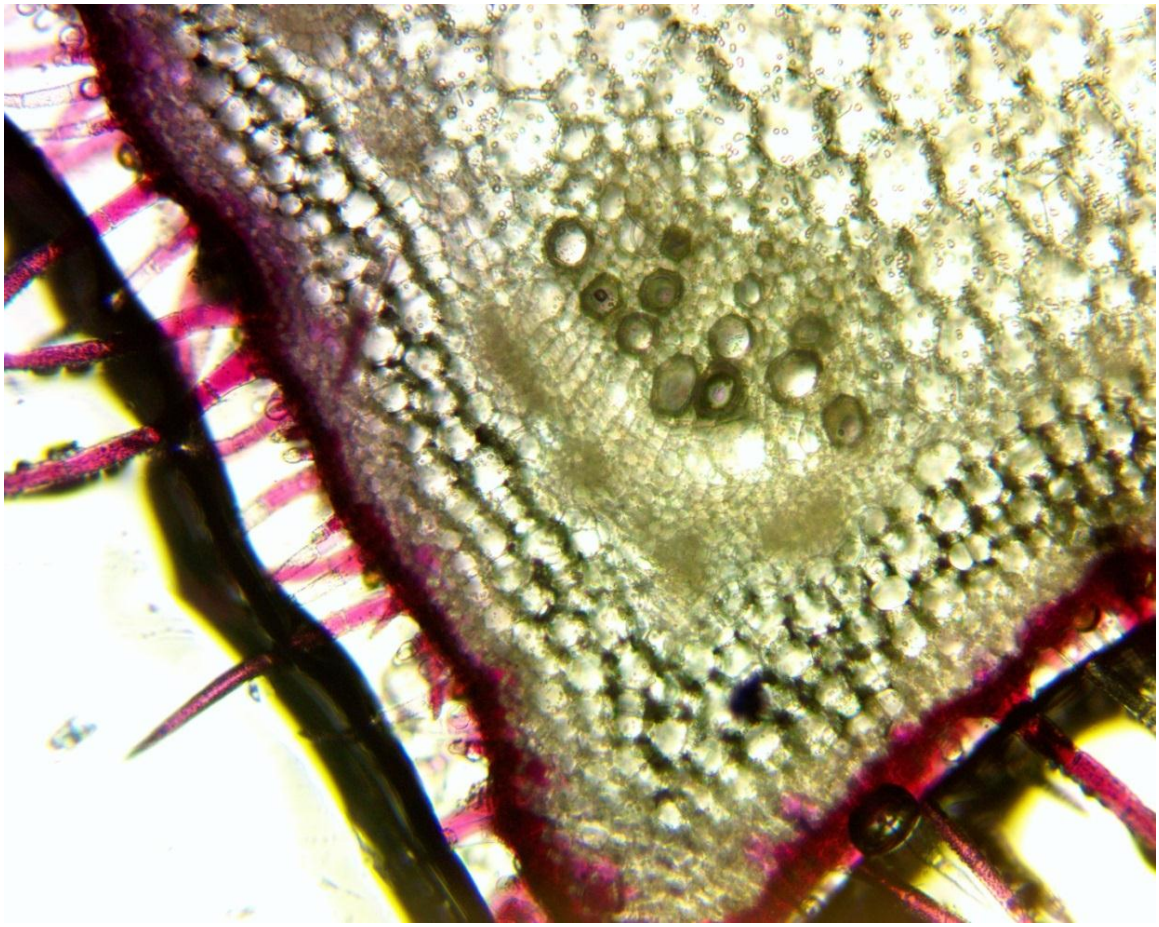

Figure 18. An unstained cross section of a dicot (*Begonia*) stem at 100x total magnification. The image was generated using an OMAX 16-MegaPixel digital microscope camera (and its related TOUP software) when viewing the slide using an Olympus CH-style microscope.

Step 7. To add more contrast and color to the tissue, readers can stain the slice with 5% Toluidine Blue solution (or use other plant stains). If staining a sample with Toluidine Blue, first fill one well in the multi-well plate with a few drops of the 5% Toluidine Blue solution (Fig. 19, left image). Then, fill two other wells with deionized or distilled water (although tap water will also suffice). Next, using the tweezers, pick up a tissue slice and place it in the Toluidine Blue solution for 10 to 60 seconds, depending upon the type of plant tissue involved (Fig. 19, left image). Note that readers will need to conduct some pilot tests here to determine the appropriate amount of time for a given sample. Then, remove the tissue slice from the Toluidine Blue well and place it in each of the successive water wells for 10 seconds at a time (Fig. 19, right image). Finally, mount the section on a slide (as described above), and view the sample under a microscope (Fig. 20).

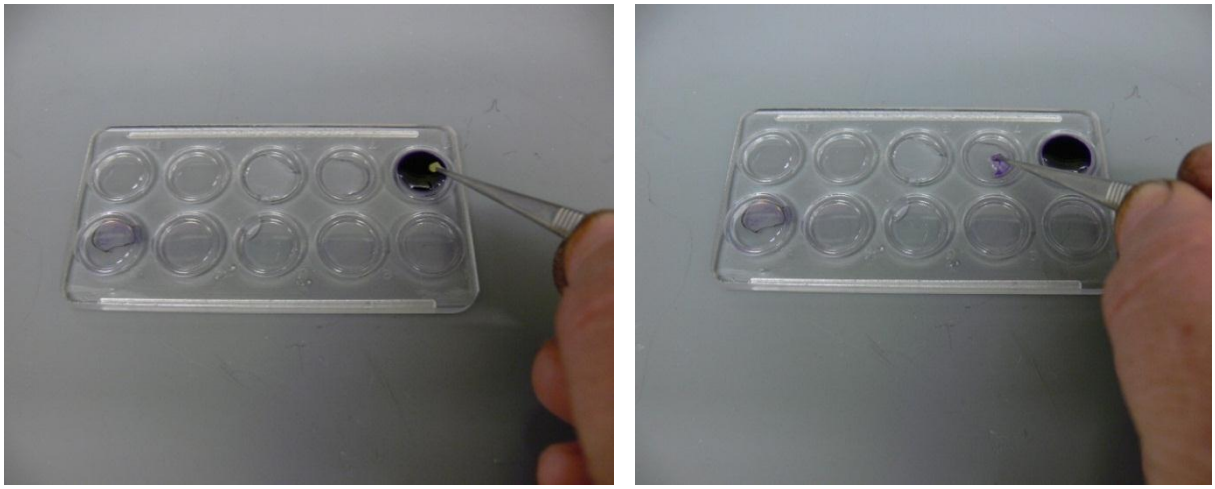

Figure 19. Staining a thin section of plant tissue with 0.1% Toluidine Blue solution (left), and then rinsing the sample in two successive wells filled with deionized or distill water, although tap water will also suffice (right).

Importantly, as explained in the Hazards section below, because Toluidine Blue is a potential skin and eye irritant and since it might cause other serious health problems if absorbed directly into the bloodstream, readers should wear the appropriate protective equipment (e.g., gloves, goggles, lab coats, etc.) when working with the stain.

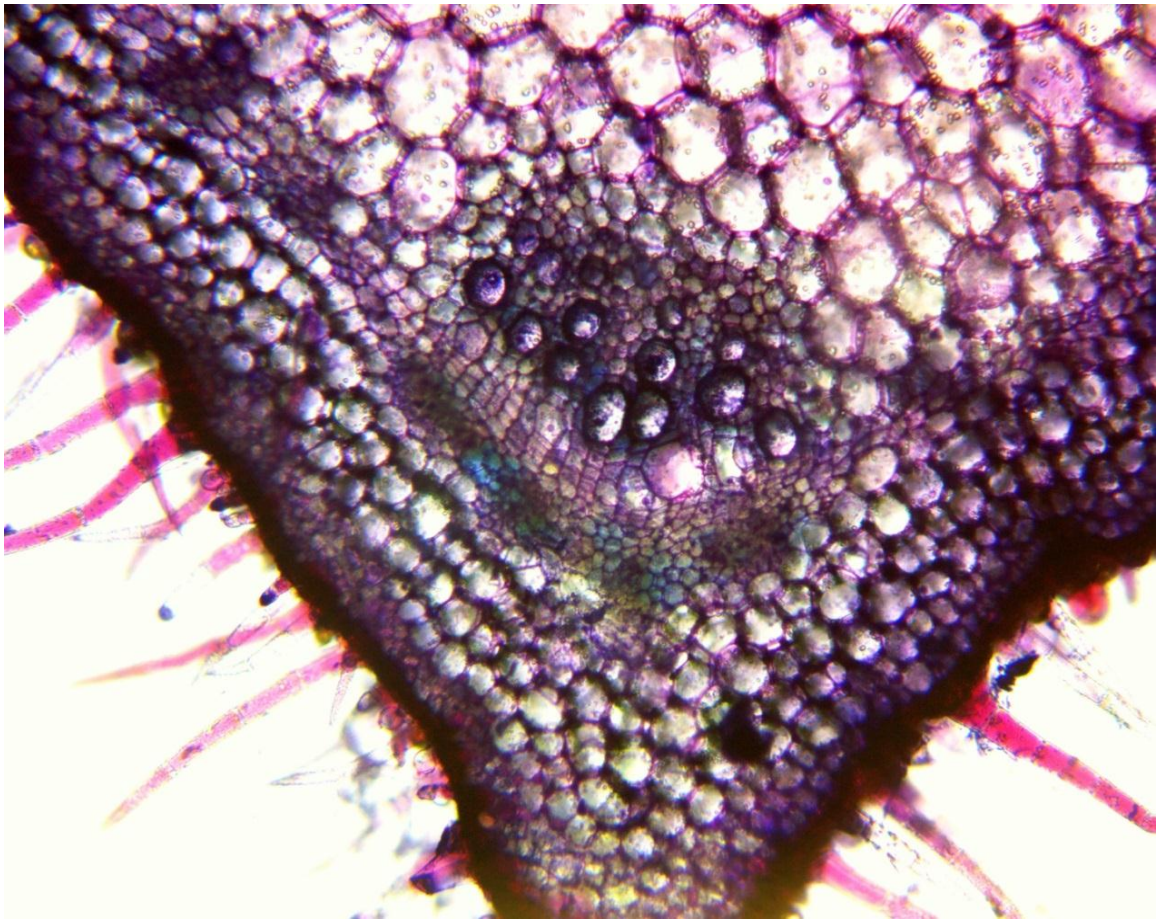

Figure 20. A cross section of a dicot (*Begonia*) stem at 100x total magnification that has been stained with a 0.1% solution of Toluidine Blue. The image was generated using an OMAX 16-MegaPixel digital microscope camera (and its related TOUP software) when viewing the slide using an Olympus CH-style microscope.

Leaf and Delicate Tissue Sections

To make hand sections of leaves or other delicate tissues, readers will need the following items:

- an OPN Microtome with a square channel – either the 3D-printed version (Fig. 21, upper left) or the one made from glass slides;
- a single-edged razor blade (Fig. 21, lower left) for cutting the leaf or other piece of delicate plant tissue to obtain the sample;
- Scotch ® permanent mounting tape (Fig. 21, right) or another similar item to ultimately hold the leaf or other delicate tissue in place as it is being sliced;
- a pair of scissors for cutting the mounting tape; and
- the other materials listed above for making root, shoot, and stem sections, including the knife for the OPN Microtome (Fig. 11).

Figure 21. Some of the additional materials needed to make sections of leaves or other delicate pant tissues using the OPN Microtome. Readers will also need a pair of scissors for cutting the mounting tape and the other items pictured in Figure 11 above.

Step 1. First, for delicate plant tissues (such as leaves or small roots), readers will need to provide additional support for their sections. While we use Scotch ® permanent mounting tape for this purpose, other similar materials should suffice. We then cut two 1-inch long pieces of the mounting tape, which will act like a "sandwich" to hold the plant tissue in place (Fig. 22, lower left). As for the tissue itself, readers should obtain a leaf or other delicate tissue sample (Fig. 22, center), and then slice a small section of the leaf along one of the veins (or other area of interest) using a single-edged razor blade (Fig. 22, lower right).

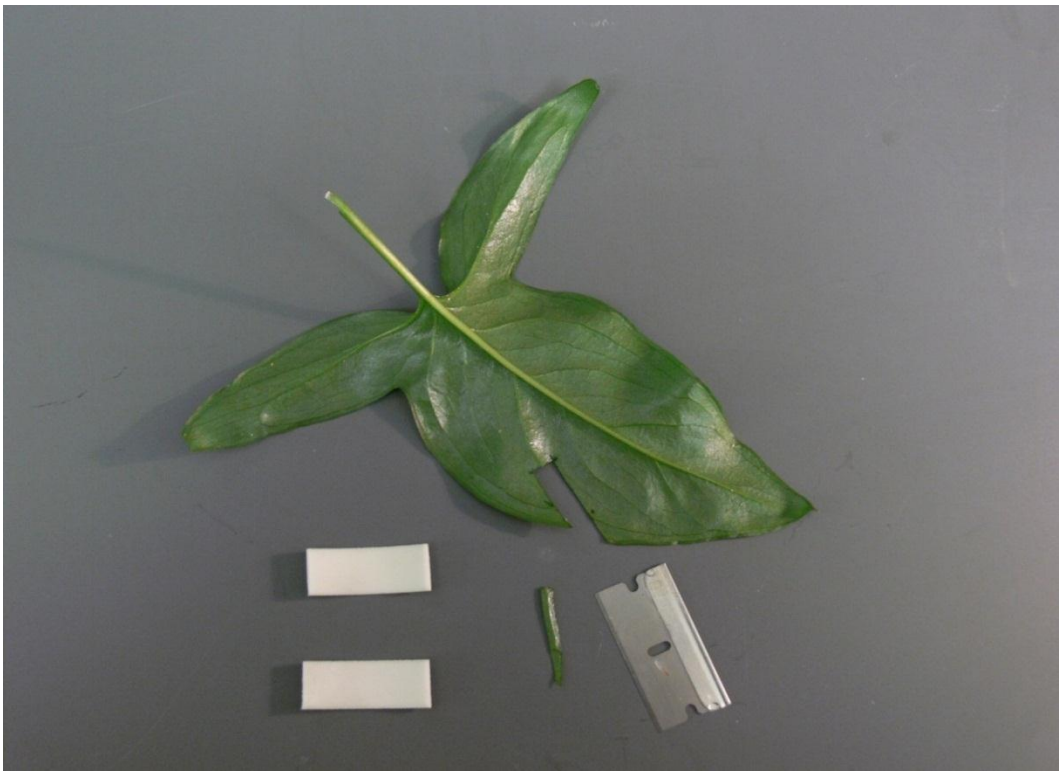

Figure 22. Cutting a thin section of leaf with a single-edge razor blade to obtain a delicate tissue sample. Note how the section included a vein in the leaf to examine that structure under the microscope.

Step 2. Next, place the leaf section in the middle of one of the pieces of mounting tape with the "sticky" side up (Fig. 23). Ultimately, the leaf section will be "sandwiched" between the two pieces of mounting tape to provide support for the delicate section when it is being sliced.

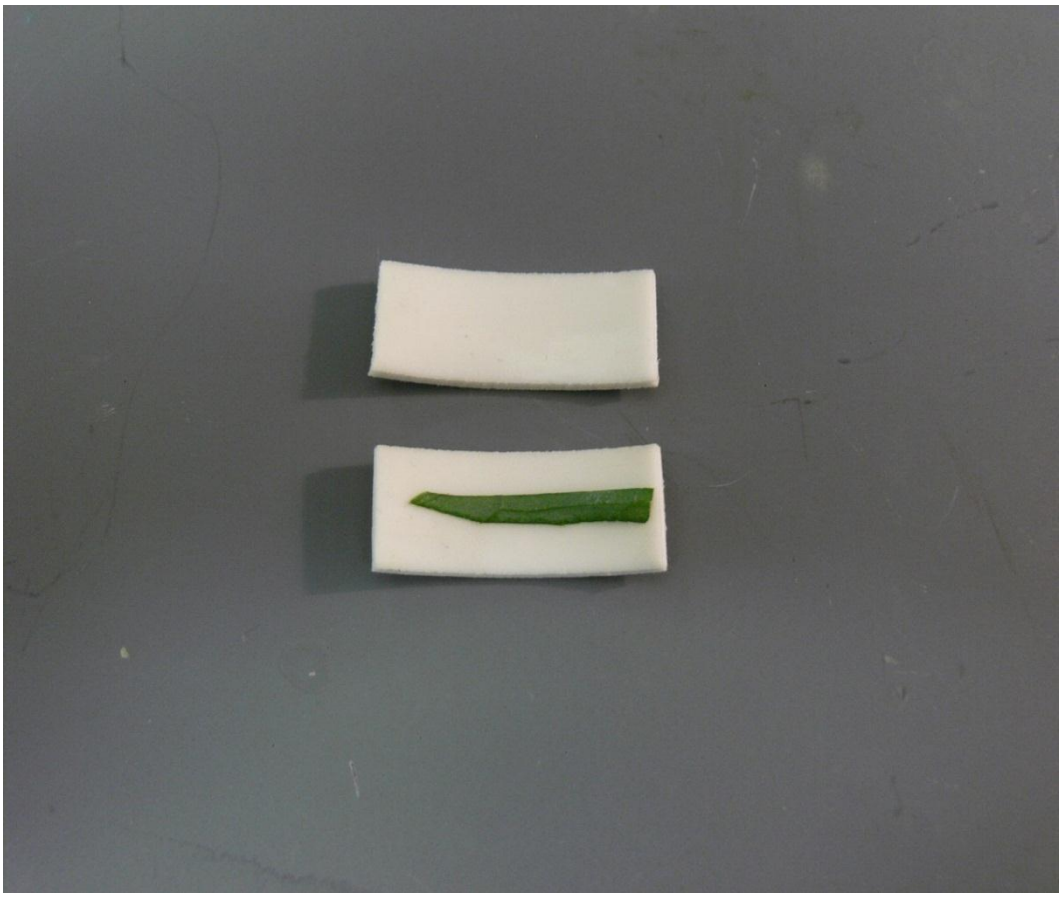

Figure 23. Placing the leaf section on the sticky side of a piece of Scotch ® permanent mounting tape to create a "sandwich" that will support the tissue while it is being sliced.

Step 3. Then, place the other piece of tape on top of the leaf section (sticky side down), and press the two pieces firmly together (Fig. 24). Now, the leaf section should be "sandwiched" between the two pieces of mounting tape (Fig. 24). As explained above, this additional amount of support is necessary to prevent the leaf (or other pieces of delicate plant tissue) from crumpling or tearing when sliced.

Figure 24. Making a "sandwich" out of mounting tape to support the leaf (or other delicate plant tissue) while it is being sliced.

Step 4. Now, trim the sides of the "sandwich" with a scissors, so that the sample will fit nicely into the square channel of the OPN Microtome (Fig. 25). Readers should further note how the tissue does not extend to the edges of the sandwich (Fig. 25, center).

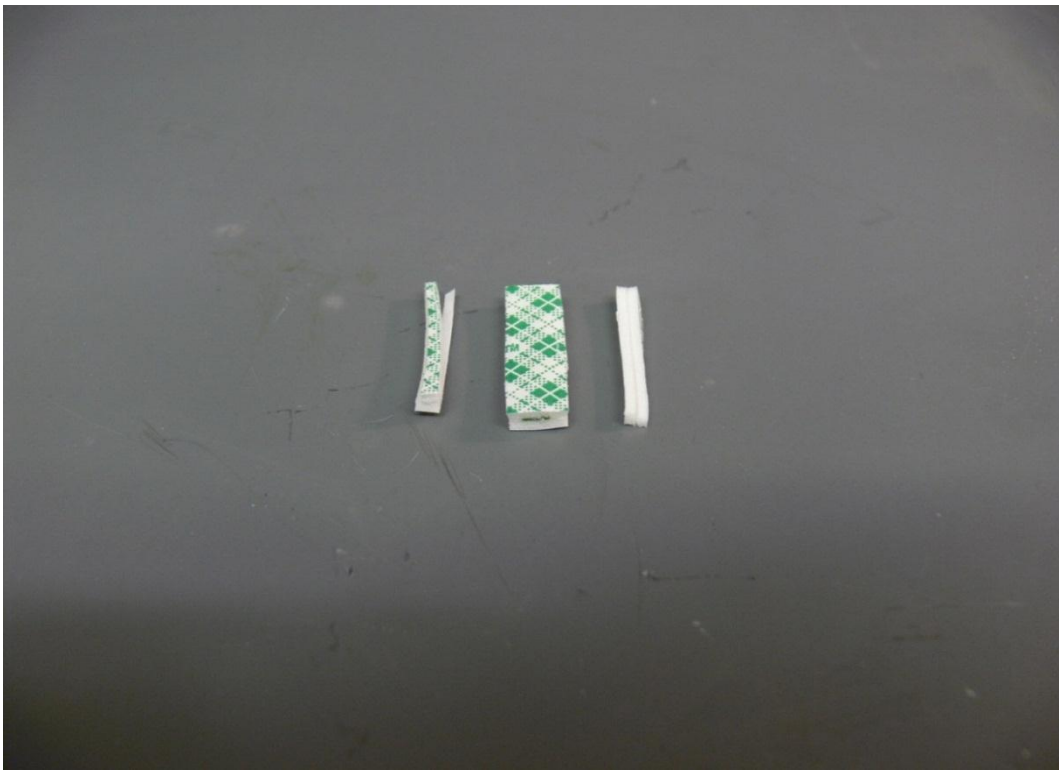

Figure 25. Trimming the leaf "sandwich" with a pair of scissors, so that it will fit into the square channel of the OPN Microtome. Note how the tissue does not extend to the edges of the sandwich (center).

Step 5. Next, peel back the paper on the top and bottom of the tissue "sandwich" roughly ¼ of an inch and cut this paper away (Fig. 26). It is important to remove this backing paper from the mounting tape because the paper itself is difficult to cut through, which can end up damaging the leaf section. Ultimately, cutting off the backing paper in this manner will leave a small amount of the sticky surface from the mounting tape exposed on each side of the tissue "sandwich" (Fig. 26). Also, please do not remove the rest of the backing paper from the tissue sandwich since that will make it more difficult to let go of the mounting tape or adjust your grip when slicing thin sections.

Figure 26. Removing some of the backing paper on the leaf tissue sandwich (roughly ¼ inch from the edge) to make for easier slices.

Step 6. Once those shorter strips of backing paper have been removed, place the tissue "sandwich" in the square channel of the OPN Microtome, which (as described above) should be sitting in a large dish that has been filled with water all the way up to the bottom of the channel (Fig. 27). Make sure that the end of the sandwich with the backing paper removed is facing forward (Fig. 27). Then, carefully slide the sandwich forward and use the knife for the OPN Microtome to slice off a thin section (as described above). By holding onto the backing paper, your fingers should not stick to the sandwich, making it easier to adjust your grip as you make the slices. Also, grasp the tissue sandwich tightly when making the sections and use a sharp knife since the mounting tape can dull a razor blade fairly quickly. Consequently, if planning to make a number of sections or section a number of different samples, readers should have several OPN Microtome knifes available.

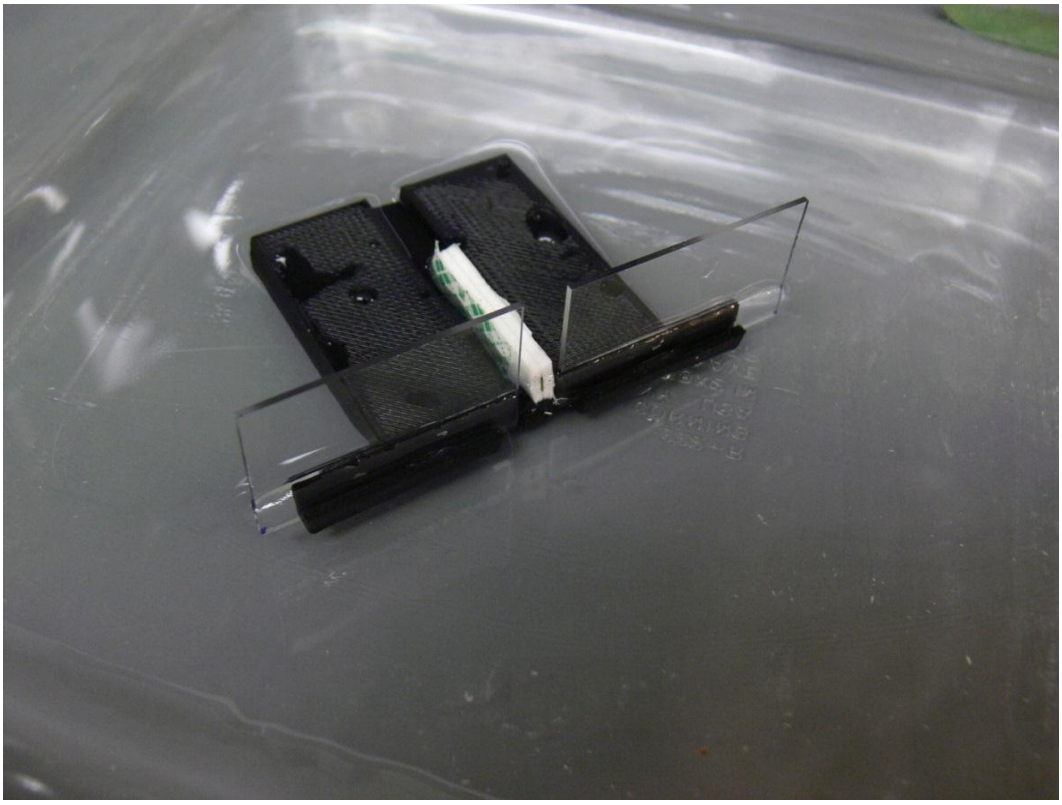

Figure 27. Placing the trimmed tissue sandwich into the square channel of the OPN Microtome to make thin sections. Note that the end of the sandwich with the backing paper removed is facing forward and will be sectioned. Also, an OPN Microtome knife with a sharp razor blade is needed to get thin and clean sections given the presence of the mounting tape. Readers may even need to use several OPN Microtome knives when making their slices since the mounting tape will likely dull the razor blades fairly quickly.

Step 7. After slicing several thin sections, readers can then place the sections onto separate microscope slides (as described above) and view (or take digital photographs) of their tissue samples whether stained or unstained (Figs. 28 and 29).

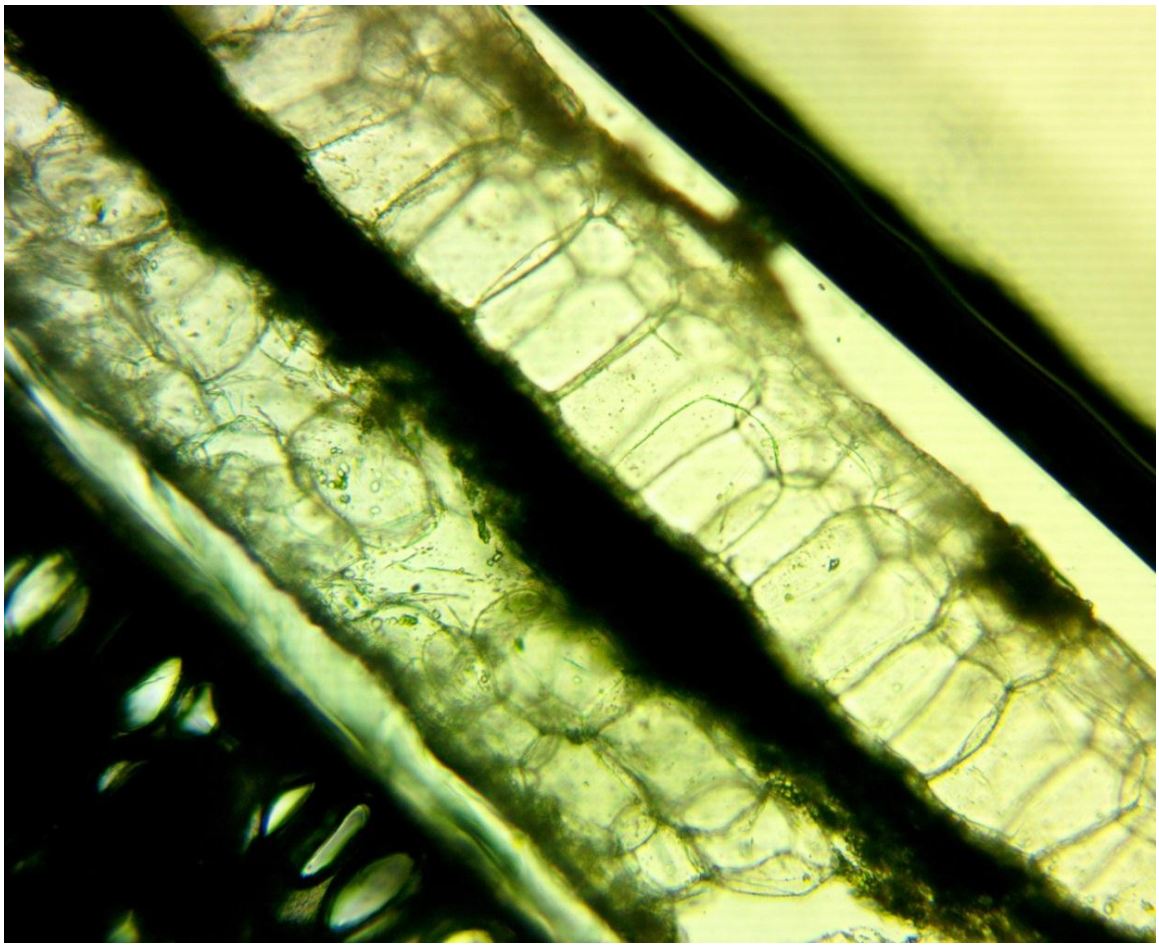

Figure 28. An unstained cross section of a dicot (*Begonia*) leaf cross section at 100x total magnification. The image was generated using an OMAX 16-MegaPixel digital microscope camera (and its related TOUP software) when viewing the slide using an Olympus CH-style microscope.

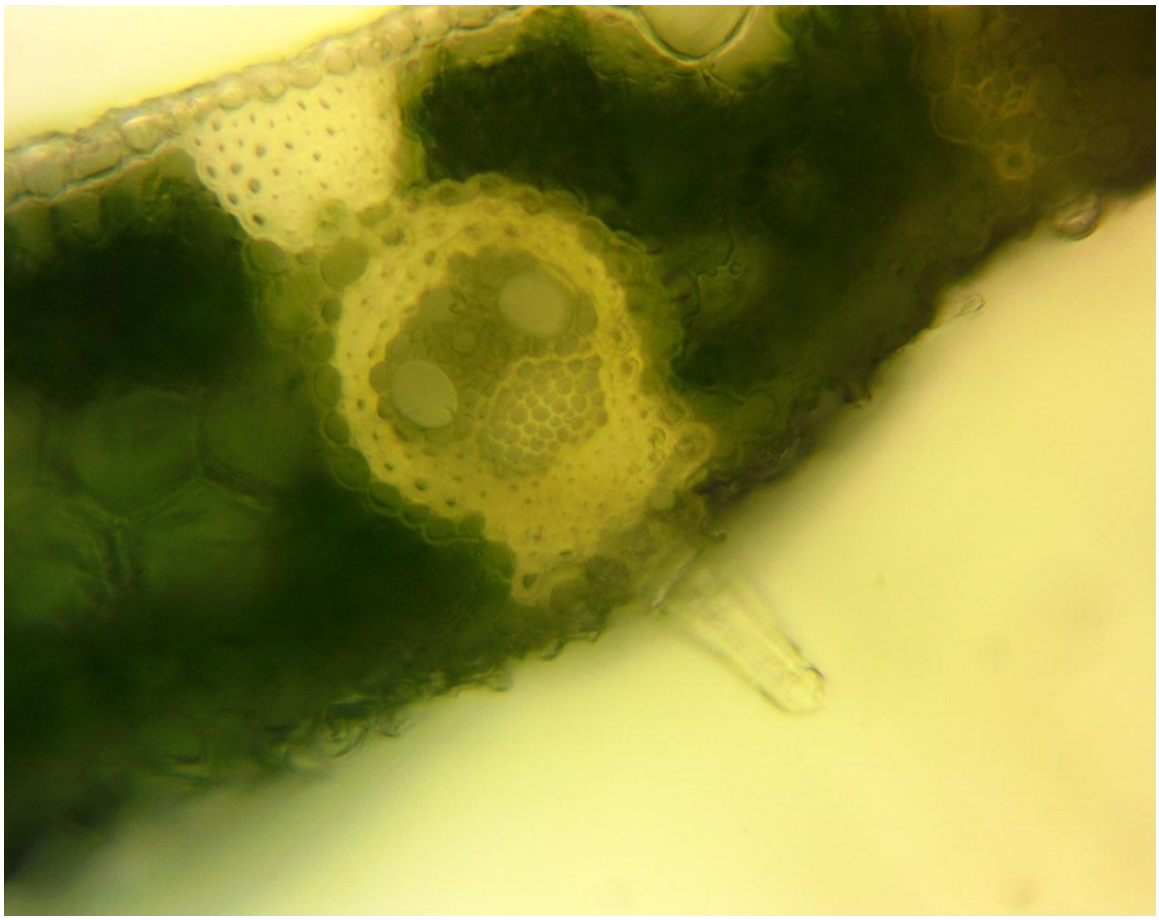

Figure 29. An unstained cross section of a bamboo leaf at 400x total magnification, which shows a vascular bundle. The image was generated using an OMAX 16-MegaPixel digital microscope camera (and its related TOUP software) when viewing the slide using an Olympus CH-style microscope.

Hazards

Obviously, razor blades are extremely sharp and dangerous. As a result, anyone working with them should exercise a great deal of caution and care, especially if using a blade to slice plant tissue. Also, while standard nitrile or other similar safety gloves will not protect completely against a razor blade cut, readers should consider wearing these types of examination gloves while making their hand sections (and when making the OPN Microtome and its accompanying knife) in order to provide at least one additional layer of protection over their bare skin.

For similar reasons, readers should consider wearing these types of safety or examination gloves when working with any glass slides, especially when breaking the glass slides in half to make the face plate for the OPN Microtome. Likewise, readers should wear the appropriate eye and face protection (e.g., safety glasses or goggles, masks or a face shield, etc.) to protect against any potential harm or damage that any airborne pieces or particles of glass may cause when the slide is scribed or broken in half.

Also, quick-drying epoxy consists of two types of chemical compounds in liquid form (a resin and a hardener), and both of these substances are potential skin and eye irritants. These compounds may further cause certain allergic reactions on contact (e.g., red, itchy, and/or blistered skin). Plus, the related vapors can cause headaches, nausea, dizziness, respiratory irritation, and other potential harms if inhaled. These chemicals further should not be swallowed or ingested (whether alone or in combination with each other) since they could cause internal irritation or damage as well. Epoxy resin also contains chemicals that are known to cause cancer, birth defects, and other reproductive harms. As a result, readers should wear the proper protective equipment when working with epoxy (e.g., gloves, safety glasses or goggles, a lab coat or other appropriate clothing, a mask or respirator as necessary). Readers should also work in a well-ventilated area (or under a fume hood) when using epoxy in order to reduce the hazards posed by any related vapors.

In addition, as explained in a recent manual that describes how to prepare leek root tips for epifluorescence viewing, Toluidine Blue can irritate the skin and eyes as well as the digestive and respiratory tracts. As a result, the stain may be harmful if absorbed through the skin, swallowed, or inhaled. Toluidine Blue has also exhibited mutagenic effects in bacteria and yeast, and it might negatively affect genetic material in humans as well (**8**). Also, if introduced intravenously, Toluidine Blue can cause various blood diseases (e.g., hemolytic anemia, leukopenia, methemoglobinemia) and adverse effects on the cardiovascular system (e.g., hypertension and dysrhythmia), the central nervous system (e.g., headaches and convulsions), and the renal system (acute renal failure and hematuria) (**8**). Thus, readers should wear the proper protective equipment (e.g., gloves and goggles) when working with this stain as well.

Moreover, readers should make sure to review the Material Safety Data Sheets for any epoxy, Toluidine Blue solution, or other stain that they might use before beginning any related lab activities or exercises. Teachers should also have their students read these Material Safety Data Sheets in advance of lab and wear the proper protective equipment throughout the lab session. Readers should also make sure to dispose of any excess epoxy (especially if in liquid form) or biological stains as chemical waste (and not pour these materials down any drains).

Finally, as explained in several papers on our OPN Lab "Equipment" website, there are a number of safety hazards associated with 3D printing. In particular, the 3D printing bed can become very hot when it is in use, so readers should exercise great care when removing parts from a 3D printer. Also, the 3D printing process can release harmful nanoparticles into the air, which can cause several serious health issues, including asthma attacks, respiratory arrest, strokes, and even cardiac arrest. As a result, readers should keep their 3D printer in a well-ventilated area and further wear the proper protective equipment when operating the printer (e.g., masks or respirators and goggles or safety glasses to protect their respiratory systems and eyes from these nanoparticles). We further encourage readers to review the following websites and PDFs (or search for their own information online) to learn more about 3D printing safety: Carnegie Mellon University PDF on 3D printing safety; University of Florida Website on 3D printing policies and safety; and University of Vermont Website on 3D printing safety.

Acknowledgements and Disclosures

I would like to thank Chris Stewart for helping to design the 3D-printed versions of the OPN Microtome and for his comments on an earlier draft of this manual.

In addition, I declare that I have no conflicts of interest related to any product, brand, company, website, or other item discussed in this manual. In fact, as with other open source instruments and equipment described on the OPN Lab "Equipment" web page, I encourage readers to improve upon the designs and methods set forth in this manual by using other materials or equipment and by bringing their own insights and inspirations to the project.

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