PLANT SECTIONING PROTOCOL

COMPRESSTOME™ FROM PRECISIONARY INSTRUMENTS

Protocol Packet Includes:

1. Complete procedure for using the Compresstome™ slicer for sectioning plant materials
2. Examples of plant sections cut at varying thicknesses (5 μm – 100 μm)
3. Examples of plant leaf sections
4. Examples of plant seed sections
5. Additional protocols for treatment of sections
6. Additional protocols for visualizing plant vasculature in sections

Sectioning Slices of Plant Tissue

This document summarizes the basic laboratory techniques needed for sectioning plant materials (both fixed and fresh specimens) with the Compresstome™ slicer. The Compresstome™ VF-200, VF-200-0Z, VF-300, and VF-300-0Z models are all compatible with sectioning plant tissues. Solid plant materials can be sectioned in several planes for researchers to explore the various tissues within plant leaves, stems, roots, and seeds. Creating slices with the Compresstome™ is an excellent alternative to paraffin methods.

Please note that the VF-200 and VF-200-0Z have a precision setting of 10 μm, whereas the VF-300 and VF-300-0Z have precision of 1 μm. Therefore, it's recommended to use the VF-300 if sectioning slices < 50 μm.

Protocol for Plant Sectioning

Embedding and Sectioning of Plant Materials:

1. Select the plant material (leaf, flower, bud, stem, root, or seed) that you would like to section. As an example, below is an image of a newly sprouted sweet pea seed that will be sectioned (**Figure 1A**).
2. Glue the plant specimen to the top of the Compresstome™ specimen tube, and let the plant briefly dry to adhere to the tube top (**Figure 1B**).

Figure 1. Preparation of plant specimen sectioning using the Compresstome™ slicer. (A) Newly germinated sweet pea specimen consisting of sprout stem, root, and seed pod. (B) Pea specimen is glued to the Compresstome™ specimen tube in an upright position ready for sectioning.

3. Place the agarose loading cap onto the specimen tube, making sure that it is level across the top of the tube.
4. Pipette agarose into the loading cap so that the entire plant specimen is covered in agarose. Gently tap the sides of the tub and loading cap to dispel any air bubbles from inside the agarose.
5. Gently draw down the specimen tube so that the plant specimen enters the metal tube casing along with the agarose.
6. Please the tube containing the specimen in ice, or chill it in the refrigerator for 5 min.
7. Remove the tube from the chiller and remove the loading cap. The embedded specimen in agarose is now ready to be sectioned.
8. Please follow the instructions manual for your Compresstome™ for sectioning your desired thickness of slices.

Treatment of Sections:

Sections of fresh plant material after cutting should be placed in water as floating sections before mounting onto glass slides or for other experimental use. Preserved and treated plant materials after cutting should be immersed into alcohol. Some experimental techniques will require long rinses or dehydration methods for plant materials. In these cases, sections may be moved to different dishes or incubators with a small watercolor brush.

Mounting of Slices Containing Plant Materials:

After sections are completed, you can stain or treat the plant slices as desired for your experimental needs. To mount the slices, place sections into water or alcohol so that they are free-floating. Using a small watercolor brush, gently sweep the section onto a glass slide (preferred slides are Fisherbrand Superfrost Plus Glass Slides). Wipe any excess liquid off with a tissue and let the slide air dry. Once dried, place 2-3 drops of Vectashield Mounting Media (from Vectashield) onto a glass coverslip, and then coverslip the top of the mounted specimen. Use nail polish to paint the edges of the coverslip to help hold the glass in place and seal in the mounting media so that your specimen can be preserved for imaging (**Figure 2**).

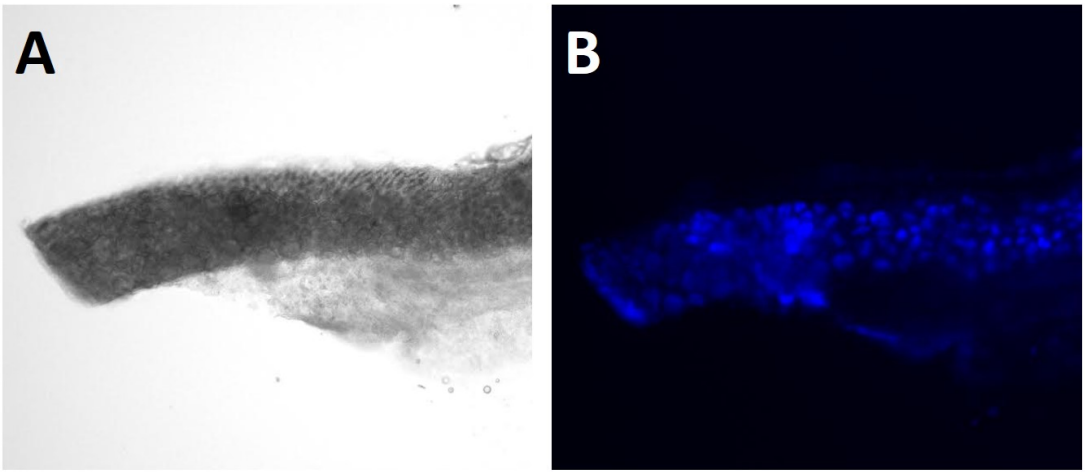

Figure 2. Examples of sectioned plant leaves. Sections that were 50 µm thick were taken from a sprouted sweet pea plant. (A) Transection slice of the leaf shown in bright field microscopy. Note that individual plant cells are distinguishable. (B) Same section shown through a fluorescent blue/cyan filter for DAPI staining of plant cell nuclei.

Best Compresstome™ Settings for High Quality Plant Slices

The Compresstome™ VF-300 is capable of sectioning plant materials from 5 µm to 100 µm thick. Examples of plant leaf slices are shown in **Figure 2** above, and slices of varying thicknesses are shown in **Figure 3** below. To achieve the best plant slice, try using these troubleshooting techniques:

1. Set the oscillation to a range of 1-2. A lower oscillation setting for slicing helps prevent tearing of plant tissue during the cutting process. Whereas a higher oscillation is generally preferred for mammalian tissues, plant tissues are much more prone to tearing at higher oscillation settings.

2. Set the speed to a range of 7-8. A high speed setting for slicing plants helps make a clean cut through the plant material. This is very different compared to mammalian tissue sectioning, because lower speeds are generally preferred for mammalian specimens. However, plants require a more rapid cutting motion to prevent tearing. This is especially true for sectioning germinating seeds, because a slow cutting speed causes the seed material to disintegrate during slicing. A faster speed helps preserve the seed tissue and allows it to remain intact and embedded in the agarose as an entire section (See **Figure 4** for examples of seed sections that have preserved ultrastructure made using the Compresstome™).

3. When attempting to slice very thin sections (<30 µm thickness), it is best to begin sectioning at 50 µm to 100 µm- thick slices, then gradually decrease the thickness down to the desired thickness. The gradual decrease in sectioning thickness helps you make consistent slices as you begin to create very thin slices that are much more delicate to maneuver (See examples in **Figure 3**).

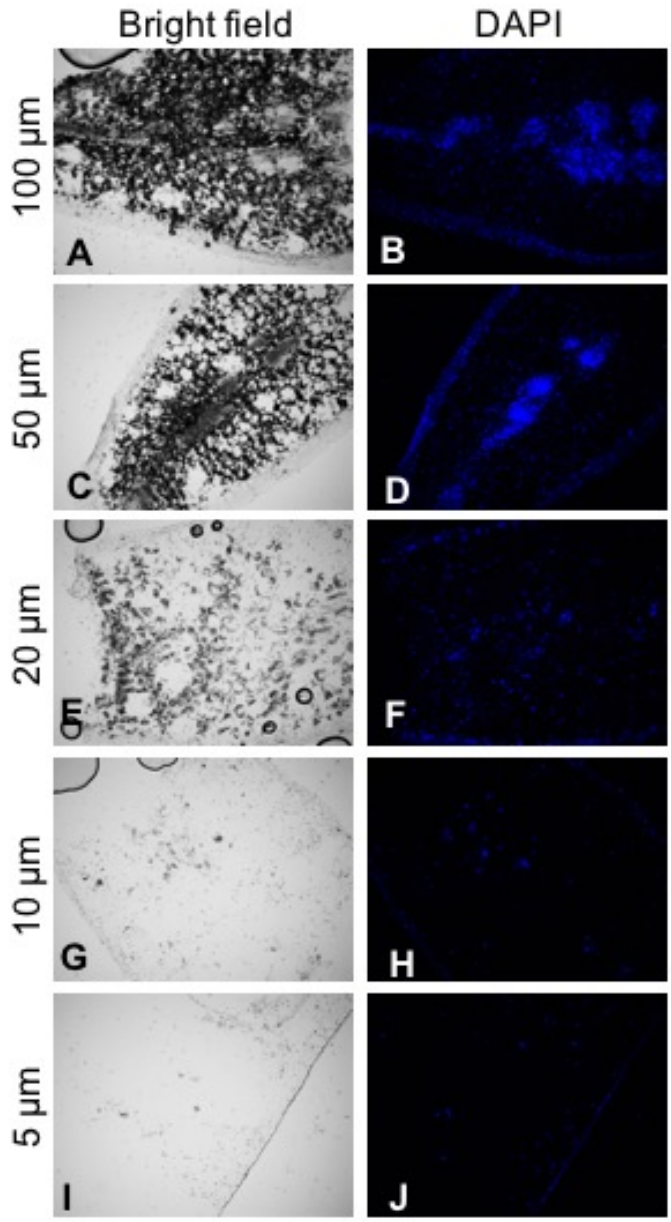

Figure 3. Sections of plant leaf at various thicknesses cut using a Compresstome VF-300. A sweet pea plant leaf was embedded in agarose and sectioned to yield slices that are 100 µm (A-B), 50 µm (C-D), 20 µm (E-F), 10 µm (G-H), and 5 µm (I-J) thick. Images in the left column were taken with a bright field microscope; images in the right column are corresponding sections taken with blue flouroscence to depict DAPI staining.

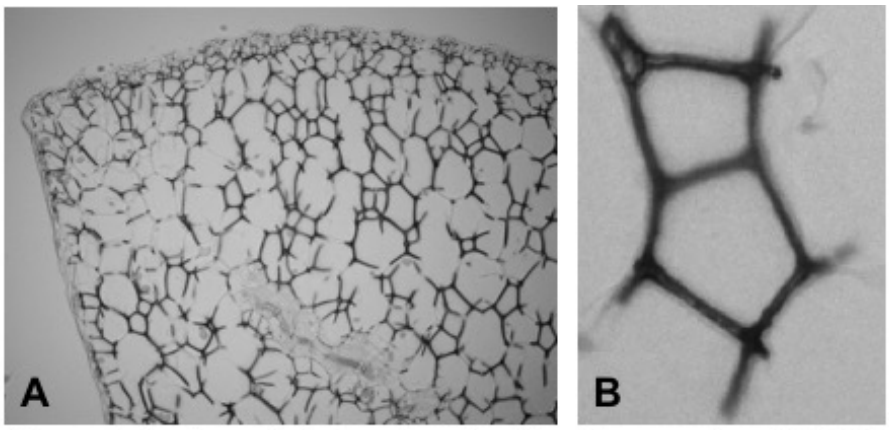

Figure 4. Sections of plant seed made with a Compresstome VF-300 slicer. (A) Depicts a 50 µm-thick section cut from a sweet pea plant seed. Image taken at 40X with a bright field microscope, showing preservation of the cell wall ultrastructure. (B) Magnification of the plant seed section, showing the preservation of the honeycomb-pattern of plant cell walls that comprise the seed.

Visualization of Intact Vascular Systems of Leaves and Flowers

Some experiments require the ability to clearly visualize the vascular systems of leafy plants and floral parts. The simplest method for achieving clarity of plant specimens while preserving the intact vascular systems is with the following technique, which aims to remove hydrophobic pigments such as chlorophyll:

1. Clear plant specimens in 5% NaOH in a Petri dish in an oven set to 37 °C. The time needed for clearing will vary from one to several days depending on the texture and composition of the plant material.
2. Remove plant specimens from the oven, and rinse 3-5 times in distilled water carefully with a pipette.
3. If additional clearing is needed, place the plant specimens in a saturate aqueous solution of chloral hydrate for 24 hours. Then wash 3-5 times in distilled water.