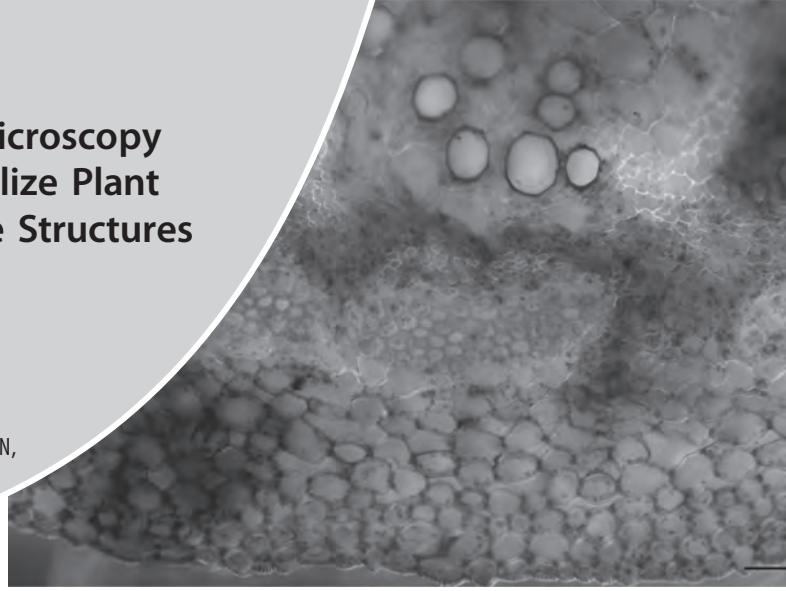
TIPS, TRICKS & TECHNIQUES

Under the Scope: Microscopy Techniques to Visualize Plant Anatomy & Measure Structures



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Abstract

Microscopy and stained specimens engage students visually as they learn about plant anatomy, a topic covered in many biology and introductory science courses. In this activity, students section plant material and prepare specimens to view under a brightfield microscope. Using a camera or cell phone, images of microscope slide contents allow students to label plant parts and engage in discussions with peers. The addition of scale bars to their images will allow a better understanding of the relationships of the various structures observed in the functioning of plants.

Key Words: *microscopy; high school education; undergraduate education; STEM; plant science; training tools.*

○ Introduction

Plant anatomy and cell structure are topics covered in multiple high school and introductory college courses (Yeung, 1998; Peterson et al., 2008). Incorporating microscopy and the use of stains to teach plant anatomy offers immediate hands-on application for high school or introductory undergraduate-level courses and allows students to gain skills in sectioning, sample preparation, and microscopy imaging while creating products that visually reinforce plant structures, rather than simply memorizing lists of parts and their functions. While there are many forms of microscopy, this activity provides guidance and advice on sample preparation for a brightfield microscope, along with safe and easy-to-use stains like toluidine blue to visualize and identify plant parts (Figure 1). Capturing images of prepared samples allows students to discuss what they observe, and scale bars can be added to further quantify visual observations. The cost of microscopy camera equipment may be a limiting factor for many classrooms, but cell phone cameras, a calibration slide, and PowerPoint can be utilized to increase classroom accessibility to microscope images and measurements.

Microscopy is a broad tool, and development of basic techniques can serve to enhance course material but also provide students with foundational skills for future learning experiences. Sample preparation for microscopy includes clearing, fixation, sectioning, and staining. Clearing is the process of treating tissue so that it becomes transparent enough for light to pass through during the imaging process. This allows for enhanced illumination and lets the operator visualize the internal and external structures present. Fixing or fixation halts the growth of the sample in time, allowing for visualizations of growth stages. Sectioning is the process of cutting a portion of tissue to examine. When developing skills in clearing, fixing, and sectioning, soft plant stems make an excellent substrate to safely section under supervision. The stem has enough structure to give resistance for cutting thin sections, but not too much resistance to cause the blade to slip. Additionally, plants are easily grown in the classroom and a single plant can provide enough material for an entire class. Staining samples provides striking visualization of cellular components (Figure 2). In this activity, we focus on utilization of brightfield microscopes that are less expensive, are easier to use, and have fewer safety considerations than fluorescent microscopes. Procedures in this activity demonstrate how older microscopy methods can be adapted to use safe clearing and staining methods paired with phone pictures and PowerPoint to visualize and quantify plant structures in the modern classroom.

○ Methods

This activity is accessible for a wide range of ages, and the level of sample preparation completed by the student can be modified at the discretion of the instructor. Clearing and fixation is an optional step that can be omitted if time or resources are not available. We provide instruction on capturing images with a cell phone camera and on utilizing a calibration scale and PowerPoint to add scale bars to images and set up desired measurements. For further details, see the Supplemental Material available with the online version of this article.

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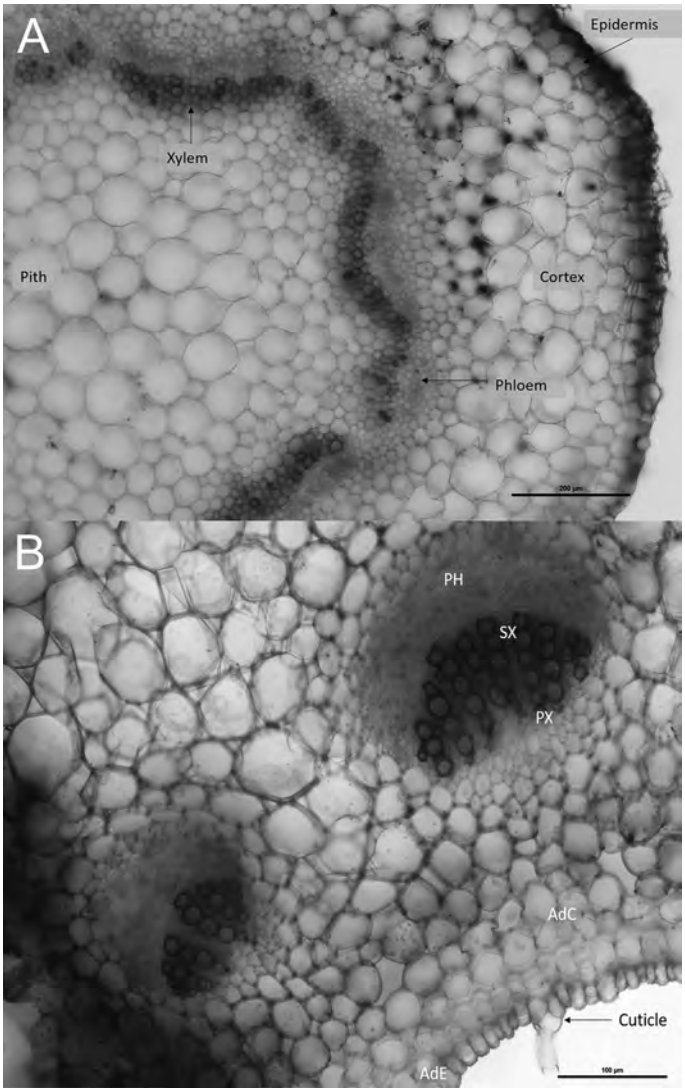

Figure 1. (**A**) Unstained cross section of a stevia stem at 100 × magnification (10 × objective). (**B**) Stevia stem stained with toluidine blue at 200 × magnification (PH = primary phloem, PX = primary xylem, SX = secondary xylem, AdC = adaxial collenchyma, AdE = adaxial epidermis).

○ Materials

- 80% acetic acid (optional)
- 95% ethanol (optional)
- Small beaker (optional)
- Stir bar and stir plate (optional)
- Deionized or distilled water (optional)
- Glycerol (optional)
- Plant material (stevia used here, but any non-woody stem material can be used)
- Razor (single- or double-sided safety razor)
- Forceps
- Stains (toluidine blue to stain cellulose in cell walls, Methyl Blue to stain nuclei, or other prepared following manufacturer recommendations)
- Small Petri dish or well to hold stain and leaf section
- Microscope slides
- Gloves
- Water dropper bottle
- Slide cover glasses
- Brightfield compound microscope
- Microscope camera or cell phone
- Ocular mount smartphone holder (optional)
- Microscope stage calibration slide (AmScope MR095)

○ Clearing, Fixation & Storage

The decision to clear can depend on the sample to be observed, classroom resources available, and the amount of time available for the activity. Clearing and fixation is completed in a 3:1 solution of 95% ethanol to 80% acetic acid. Cut multiple pieces of stem tissue, 2–3 cm long, and place them into a small beaker with a stir bar and fixing solution covering all plant materials. Using a stir plate, mix the beaker contents on low speed for 45 minutes. Then rinse samples in deionized or distilled water for 10 minutes. These steps can be done ahead of time by the instructor if preferred. Samples can be stored for up to one month in a solution of 50% glycerol and 50% water, at 3–5°C.

○ Sectioning Plant Material

If the clearing step is skipped, begin by cutting small pieces of stem (or select previously cleared specimen pieces) and place on a cutting surface in a small amount of water. Using a safety razor or new scalpel blade, begin to cut slowly in a sawing motion to produce thin sections one to two cell layers thick. Always cut multiple sections, trying to make each section as thin as possible until an evenly thin section is produced (video 1: https://youtu.be/UCZdz22CUeI).

○ Staining Plant Tissue & Mounting Samples

After sectioning, put on gloves and place plant samples into a small well or Petri dish with prepared toluidine blue stain for 10 seconds (video 2: https://youtu.be/aR86gE5V4iI). Toluidine blue stains cellulose, is safe and easy to use, and can be used with any plant tissue sample. Once the sample has been stained, wash with deionized or distilled water for 10 seconds. Next, use forceps or a probe to place samples in a drop of water on a cleaned slide and cover with a cover glass.

○ Microscopy & Imaging

Place prepared slides on the microscope stage and explore. Students can observe samples at multiple magnifications (Figure 2) and begin to identify plant structures. If resources are available, a microscope camera and corresponding software can be utilized to capture images. While cell phone use is not typically encouraged in the classroom, mobile-phone images offer a cheaper alternative to microscope

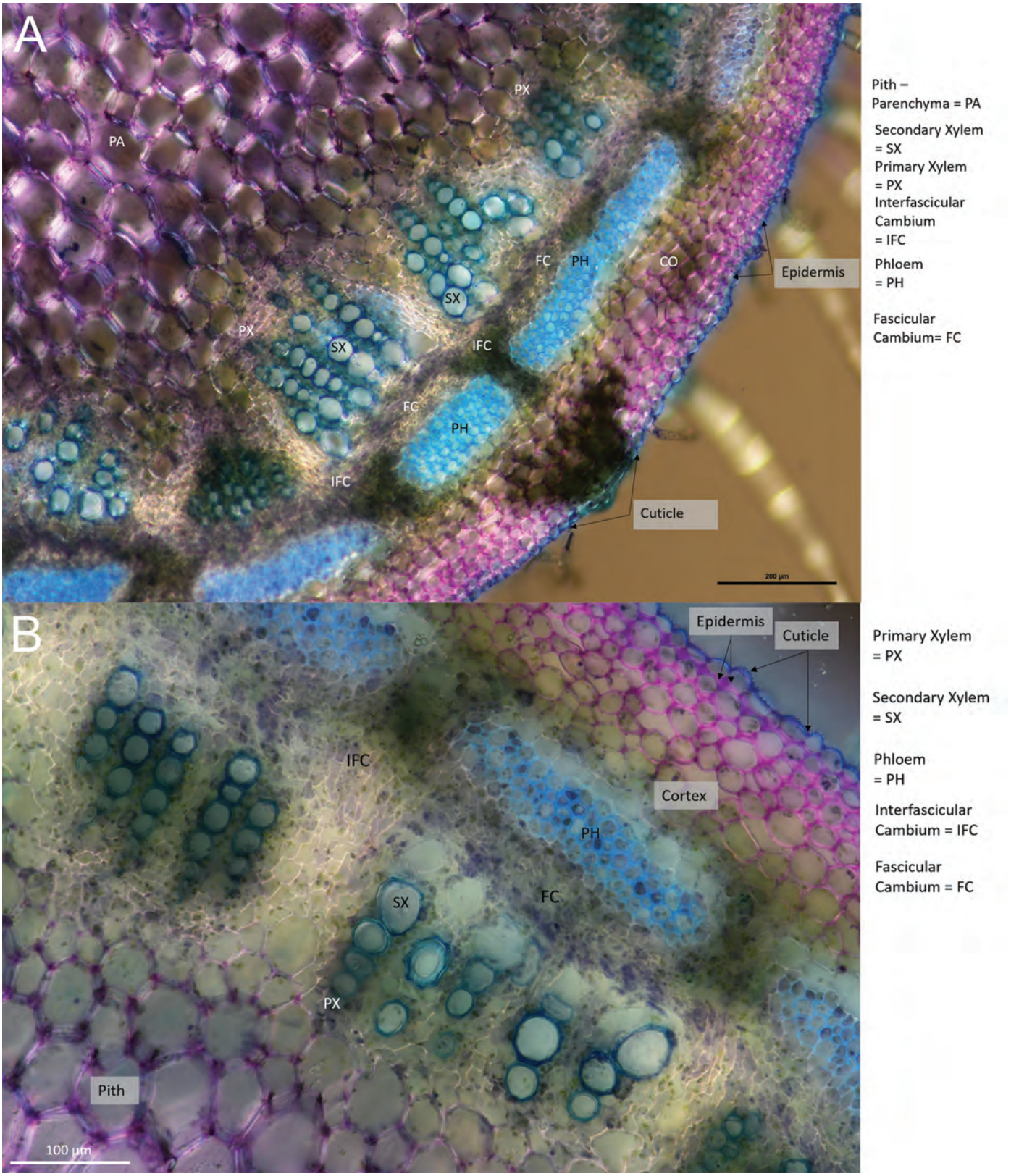

Figure 2. Stevia stem stained with toluidine blue. (**A**) Cross section at 100 × magnification (10 × objective). (**B**) Cross section at 200 × magnification.

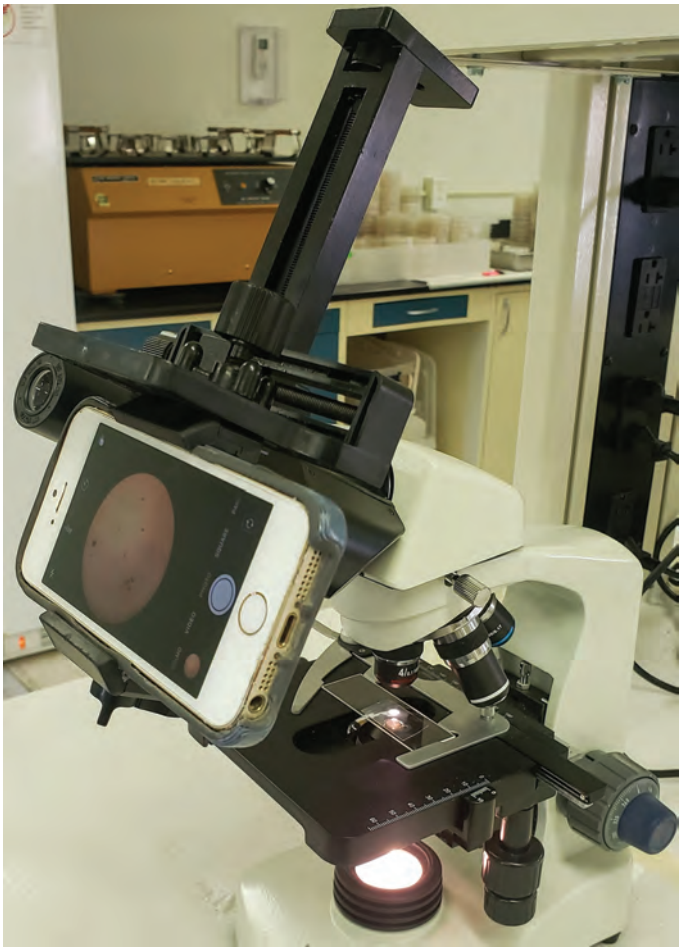

Figure 3. Ocular mount to hold smartphone to stabilize images.

imaging equipment and enhance learning by giving students an opportunity to take their own photos and communicate science with their peers through shared images (Harper et al., 2015). To take mobile images, balance the cell phone camera at the eyepiece and take a picture once you have stabilized. Balancing improves with practice and can be aided by using a smartphone holder that mounts to the camera eyepiece (Figure 3).

○ Adding Scale Bars

To add scale bars, capture an image of the sample and the microscope calibration slide (can be purchased for $10–20) at the same magnification (video 3: https://youtu.be/aR86gE5V4iI). Using a computer, open up PowerPoint and load both photos onto a slide. (If the calibration scale is not straight, hold down the shift key and click "insert-shape-line" to draw a straight line across the image. You can then move your calibration slide image into alignment.) Click "insert-shape-line" to draw a line of desired length within the calibration slide and adjust the color and width of the line to be visible. The length and height of the line in PowerPoint can be found under the drawing tools "format" tab, this measurement provides the equivalency of PowerPoint unit length compared to the calibration slide. To draw a new straight line, press shift, and again click on "insert-shape-line." Under the format tab, enter the correct PowerPoint unit length. Add the new line on top of the desired sample image and insert a text box to label the scaled length of the line. Knowing the equivalence of the scaled length to PowerPoint units, lines can now be drawn to measure any features on the image.

○ Conclusion

Through this activity, students are able to develop skills preparing specimens for visualization and gain experience using a microscope. Microscopy and stains provide students with a hands-on connection to plant anatomy to facilitate discussion about what they observe. Cell phone images and calibration slides offer an inexpensive way to enhance microscope activities to quantify the size of structures and make comparisons among samples. The goal of this activity is to bridge multiple learning styles while enhancing student participation and excitement about fundamental plant structures and organization.

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