Nail Polish Peels

Soltis Lab 2023 Update

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Purpose

To visualize and measure stomata cells from herbarium specimen using a minimally destructive approach.

Supplies

Annotation of herbarium specimen

- Pre-cut labels
- Label glue

Stomata peel

- Clear nail polish
- Fine forceps/tweezers
- Microscope slide and coverslips
- Dropper
- Water
- Sharpie

Imaging

- Zeiss Axio Imager M2 microscope (Bartram 421)
- Immersion oil
- Kim wipes
- Extra water + pipette
- A jump drive

Instructions

Annotation of herbarium specimen

1. Make sure to add an annotation label to a specimen before beginning
    a. "Standard practice is to place annotations in a blank space as near to the original label as possible. This is usually to one side or above the label and flush with the right side of the sheet. If other annotations are present, it is best to position the new one above the most recent, unless it will not fit or the most recent is in a very unusual position. When there is not sufficient blank space, an annotation may be glued only at one end and overlap mounted plant material."
    b. A small amount of glue (found in the mounting room) should be applied to the label and spread thinly before placing the label onto the herbarium sheet.

Stomata Peel

2. Apply a thin layer of nail polish to a small area on the bottom of the leaf (when possible) and wait for it to dry.
    a. For *Galax,* the petiole attachment point to the leaf blade can be seen from the bottom of the leaf, but not the top; there can also be a slight color difference.
    b. While the nail polish dries, record information about the specimen including: the FLAS # (found near a barcode) and which side of the leaf the peel is from.
3. Prepare a microscope slide by labeling it and adding a drop of water.

4. Carefully remove the nail polish using fine forceps and place the peel in the water on the microscope slide. Add a microscope slide cover and the peel is ready to be imaged.
    a. You can use a microscope in the herbarium to check on your peel prior to moving to Bartram.
    b. Before leaving the herbarium, make sure to return specimens to their cabinet space, clean the area you used, and return any material to where you found them.

Imaging

- *Note:*
    - Please take images of each specimen and save to jump-drive (upload to the dropbox). Name them by the catalog number and x1, if there are more than one per catalog number. (ex. 263086x1.jpg). Provide the name of the photographer, a description of the picture and magnification or scale.
    - Find a way to save scales onto the photo.

5. To turn on the Zeiss – turn on the X-Cite, then the Power Supply, then the Microscope. After turning on the Zeiss, log into the computer and open the imaging software (AxioVision).
6. Select "Microscope" page on microscope TFT display (control panel), go to "Objectives" screen, and then select "20x".
7. Next, go to "Reflector" screen and select "POS 1" (this setting is for using transmitted light instead of fluorescence). Make sure "Transmitted light" is On, "Reflective light" is Off.
8. Begin looking at your sample through the microscope lens at 20x. Slowly turn fine tuning knobs on left or right of microscope to focus specimen.
    a. You may need to change the "Light" to increase or decrease "Transmitted light"
9. When you are ready to measure your stomata cells, zoom to 40x by selecting "40x" on "Objectives" page. The stage will automatically lower. Add a drop immersion oil on top of the slide cover and tap "DONE" on control panel.
    a. By adding a drop, touch the wand to the slide cover.
10. When ready to capture an image, pull the microscope lever all the way out to direct light to camera, and click "Live View", focus using fine tune knob, click "Snap" to take a picture.
    a. Use the "length" feature to measure cells.
    b. Add a "scale bar" for publication/presentation purposes.
        i. To save the scale bar: "File" -> "Export"
            1. Specify "File name" and "Save as"
            2. Change "File type" to "TIF"
            3. Check "Displayed image"
            4. Check "Burn-in annotations"
            5. Click "Start"
    c. Save image to external jump drive, this computer is not connected to the internet.
11. To turn off the Zeiss:
    a. Return to 20x by selecting 20x from "Objectives" screen on control panel and then "20x". Wait for stage to lower.
    b. Remove the slide when the stage is in the fully lowered position.

c. Carefully use “Lens Paper” in one direction across the lens to remove excess oil.
    i. WARNING: Leaving oil on the lens or wiping to vigorously can cause damage!
d. When done, tap “Done” on the Zeiss control panel screen. Wait for stage to return to default 20x working position.
e. Turn off the Microscope, then the Power supply, then the X-Cite.
f. Replace the dust cover over the microscope.