

Leaf Imaging Protocol

Picture-taking

Prior to beginning any of this protocol, make sure to get in touch with Tom Harper as he will supply the camera and the lighting set-up for taking pictures.

1. Obtain leaves to be imaged from Ziploc bags in the refrigerator (if leaves have been stored since removed) or directly from growth chamber if imaging is to be done immediately following leaf removal.
 - a. If leaves are in the refrigerator, you will also need some dry paper towels.
 - b. Also make sure to get a 24-well plate if the leaves are to be dried in the dry-oven after being photographed.
 - i. Label the bottoms of the wells with the plant numbers that will go in them.
 - c. You will also need to bring a small ruler with centimeters on it.
2. Go to Tom Harper's room (145 Crawford). Tom will set the camera up on the stand in his room that is equipped with a lighting set-up.
 - a. The camera must be on the following settings for quality images to be taken:
 - i. No flash (on the wheel next to the on/off switch, it shows a picture of a flash with a line through it)
 - ii. Remote Control Shutter Release (Tom will set this for you and also give you the remote control)
 - iii. Make sure Tom sets the camera to the "jpeg" setting. This will make retrieval of the images much easier later.
 - iv. Make sure the camera is on and has the lens cap removed. Make sure the remote control is on and has the cover removed.
 - b. In order to achieve the best contrast in the images, the lighting set-up should be set as such before taking any pictures:
 - i. Make sure the lights are plugged into the green voltmeter which should then be plugged into a wall outlet.
 - ii. Flip the switch **down** to turn on the voltmeter.
 - iii. Turn the knob on the voltmeter to 70. This is where the lights should be set when pictures are actually being taken. If the area around the camera gets too hot to work in, the lights can be turned down while setting up leaves, but **remember to turn the voltmeter back to 70 before taking a picture.**
 - c. In Tom's room, there should be a piece of museum glass and some black construction paper. Make sure you have these before you start. Pictures can't be taken without them.
3. You are now ready to begin taking pictures. If the leaves have been stored in Petri dishes in a Ziploc bag, proceed to the next step. If the leaves are coming directly from the growth chamber, proceed to step 3c.
 - a. Place the black construction paper under the camera with the ruler.
 - b. Take the first leaf from its wet paper towel wrapping in the Petri dish and use the dry paper towel to gently dry the excess water from the leaf.
 - c. Put the leaf on the black construction paper under the camera.

- i. Look through the viewfinder of the camera (do not use the screen) and adjust the construction paper until the leaf is in the middle of the frame.
 - ii. You can now adjust the height of the camera. Move the camera as low as you can while still leaving enough distance on each side of the first leaf to include the ruler and tag in the photo. Also, you want to leave about the leaf width's distance on all sides of the frame so as to accommodate bigger leaves without having to move the camera later.
 - iii. Focus the camera using the rubber circle on the lens. Focus until the edges of the leaf and lines on the ruler are sharp.
- d. Put the tag (either came from Petri dish with leaf or was created before photographing) showing the plant number on the black construction paper above the leaf, but still in the frame of the shot.
 - i. Make sure the tag is straight. During image analysis you will be cropping out everything but the leaf and this is much easier to do when the tag and ruler are straight.
- e. Move the ruler so that it is below the leaf but still in the frame of the shot. Make sure the ruler is straight and that you can see centimeter and millimeter markings in the image when you look through the camera's viewfinder.
- f. Put the museum glass on top of the leaf/ruler/tag area.
 - i. Make sure that you are careful when you do this – it is easy to move the things in the image and then you need to remove the glass and set up the tag, leaf and ruler again.
- g. Make sure the leaf is as flat as possible under the glass and that none of it is folded under itself.
- h. Look through the viewfinder of the camera. The square in the middle of the frame should be centered on the leaf somewhere. **This is very important.**
 - i. Press the shutter button on the camera **halfway down**. When you do this, the dot in the middle of the square that is centered on the leaf will light up red and the camera will beep. The camera has now auto-focused so that the leaf is the part of the image in the best focus.
 1. **If you do not hear a beep, simply move the construction paper slightly so that a different part of the leaf is centered under the square and try pushing the button halfway down again.**
- i. Stand back from the set-up without bumping it and grab the remote control.
 - i. Put the remote control next to the camera (don't lean your whole body over the set-up or you will block out too much light) and hit the button on the remote control.
 - ii. You will hear a shutter noise and within a second or two, the image you just took will appear on the screen of the camera. **It is now safe to begin setting up the next leaf.**
- j. Remove the museum glass and fold up the tag and put it in the appropriate well in the 24 well plate.
- k. You can now fold up the leaf until it will fit into the well and put it with the tag.
- l. Repeat steps 3b to k on all leaves. You should not have to change the camera height for the duration of the images on a given day of pictures.

4. When all leaves have been photographed, turn off the camera and remote control. Turn the voltmeter down to 0 and flip the switch to the middle position.
5. Alert Tom that you have finished so that he can download the pictures from the camera to his computer.
6. Take the well-plates to the drying oven and place all Ziploc bags with empty Petri dishes in them back in the Tonsor lab.
7. Put the museum glass and black construction paper back on top of the file cabinet next to the camera set-up (or wherever you got them from in Tom's office).
8. Supply a flash drive so that Tom can transfer the pictures to it.
9. Set up with Tom when you plan to come back to take more pictures.

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