Root scanning using a flatbed scanner with transparency unit

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This guide is meant to allow reproducible scanning of clean roots, with soil or media already washed away. The produced images are intended to be directly ready for batch analysis in RhizoVision Explorer.

Equipment

The equipment that we use includes:

- Scanners: Epson Expression 12000XL (large format) photo scanner with transparency unit or Epson Perfection V850 (normal letter format) photo scanner with transparency unit
- Manufacturer's scanning software, Epson Scan 2 for 12000XL or Epson Scan for V850
- Plastic forceps / tweezers (not metal so won't scratch plastic tray)
- Wooden skewers, chopsticks, pipette tips, or similar (for 'spreading' roots)
- Scissors
- Water in large pitcher, empty pitcher, pasta strainer
- Clear acrylic tray, plans and possible fabricator available here
- Water (between 200 to 450 mL)

Scanning software

Once you have the following equipment gathered, make sure the "Epson Scan 2" or "Epson Scan" software is operational. Both software can be downloaded from Epson.com after looking up the scanner you are using and have online manuals available for detailed usage – here we focus on root scanning. Only one Windows user can operate the software at a time, so if you lock or switch accounts while it is open, the software will not be accessible to anyone else until it has been closed.

Another detail to keep in mind is that the Epson scanner you are operating needs to be turned on, otherwise the software will not open and an error message will be given.

Note on image format and resolution

Higher resolutions are ideal but take longer to scan, take up more disk space, and at some point, offer no better data quality. Ideally, the researcher should ensure the finest roots have at least 5 pixels in width for accurate measures – samples with finer roots need greater resolution. A less described issue with high resolution is the possibility that root hairs become too resolved and therefore are reflected in the measurements. Some researchers believe no compression should be used, thus prefer TIFF. TIFF can be used with lossless LZW compression without losing information, however, or PNG. However, low levels of lossy compression (as in JPG) will not adversely affect the data quality and greatly reduce storage use.

Note on choosing file names

When naming, consider how data will be analyzed. Typically, I encode experiment info needed for analysis in the file name if possible and separate by an underscore '_' including at the end since it is appended by a number. Assuming the file name follows the data extracted from the image, this allows the strings to be split in R and used for factor columns directly, making analysis much more efficient.

Epson Scan 2 for 12000XL

- Scan Settings: Not selected
- Mode: Photo Mode
- Document Source: Transparency Unit
- Document Type: Color Positive Film
- Image Type: 16-bit Grayscale
- Resolution: **minimum** 800 dpi (ensure fine roots are 5 pixels wide)
- Scanning Quality: Standard
- Image format: recommend high quality JPEG, quality set to 95% or above (note quality for JPEG is backwards in Epson Scan 1, be careful)
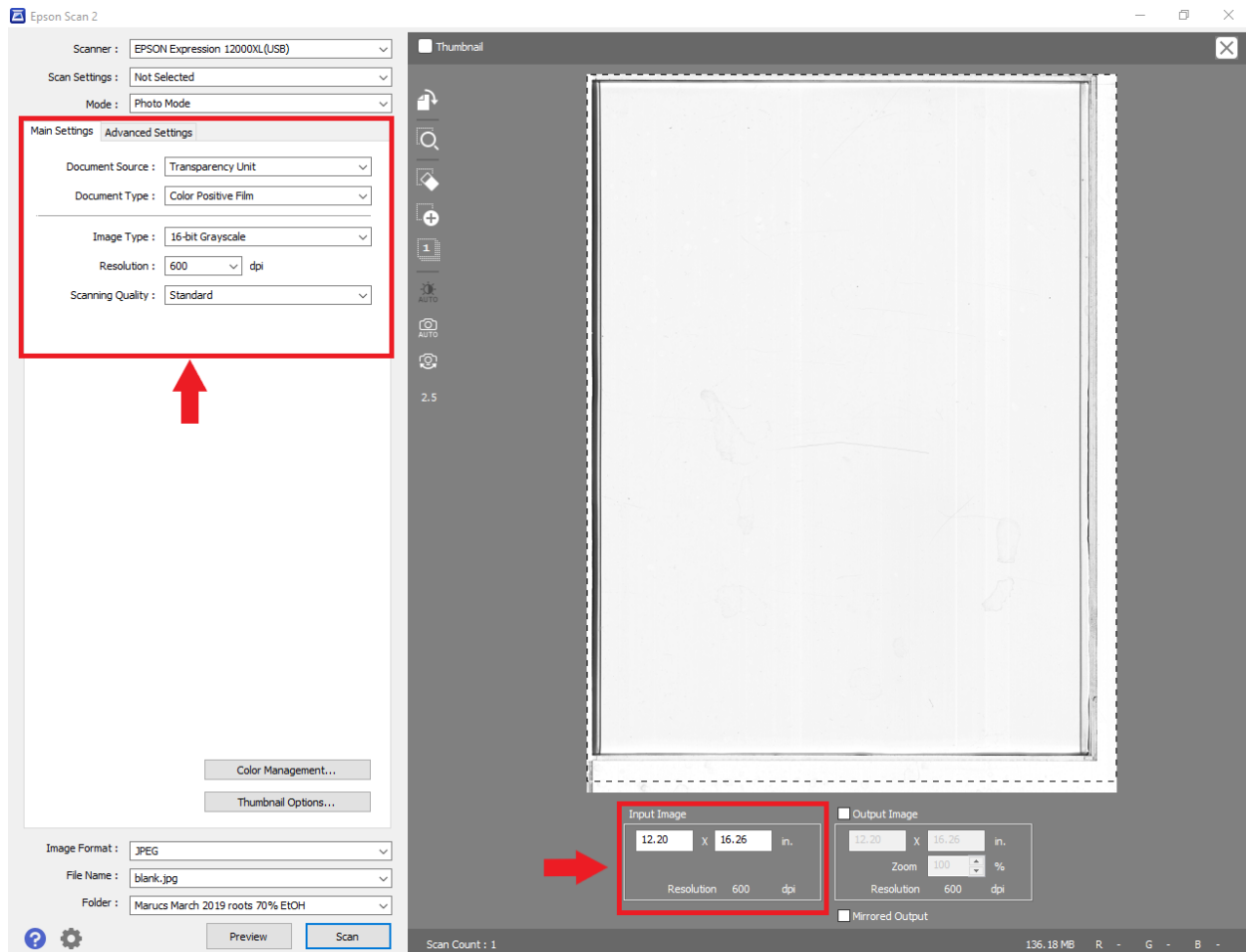

Use the preview button to select only the region of interest and excluding borders. You can type in the image area, or simply use the mouse to click and drag.

Epson Scan for V850

Mode: Professional Mode Document Type: Film (with Film Area Guide) Film Type: Positive Film Image type: Grayscale is typical for root scans Resolution: 600 dpi minimum Adjustments: NONE CHECKED	
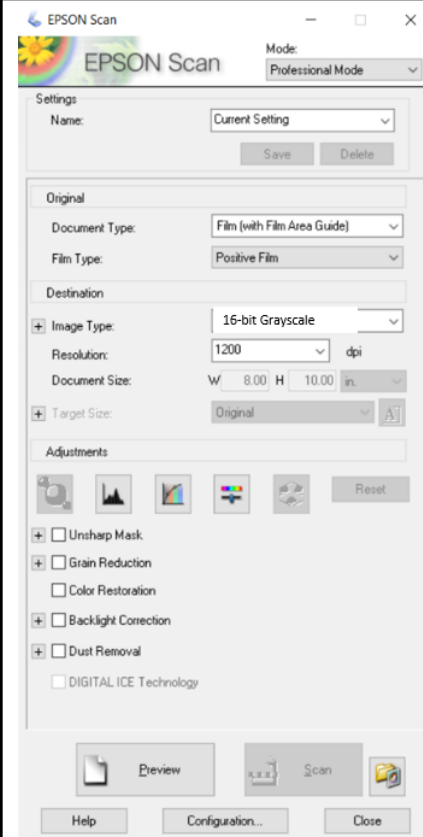
Press Preview on first scan to check image and to draw a bounding box around your scan, so that images are cropped to that area. Leaving approximately 1 cm boundary of 'dead space' is acceptable.
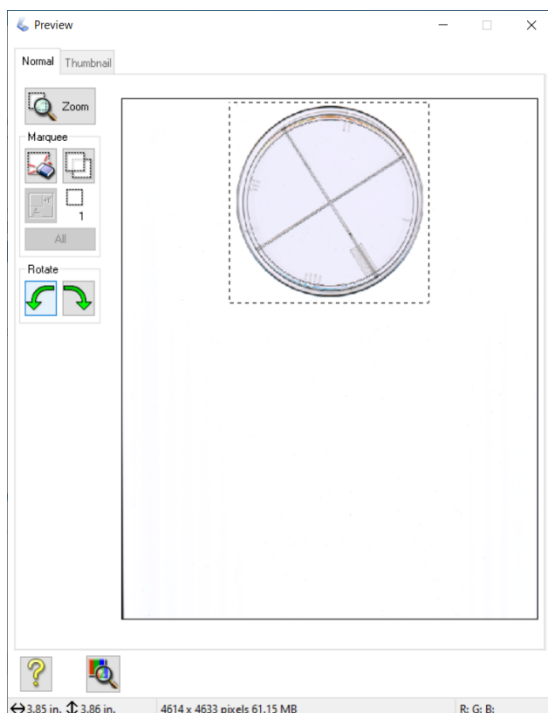

First time scanning (if batch scanning), press Scan

Scan options can be set. JPEG with high quality has minimal compression artifacts but TIF none. Note that the setting for high quality is opposite in Epson Scan 2. Note the 'prefix' field allows to give a file name. Unfortunately, the file is ALWAYS appended by the number which is why I leave a trailing underscore in the file name to clip off the number in R.

<u>Notes on excluding tray borders</u>

Using preview to exclude the tray borders by selecting about 1 cm inside of all borders is necessary, because otherwise borders are included in the image (below, left). RhizoVision Explorer analyzes the sillouhettes of the roots and will interpret the dark lines as roots. So clean images are recommended (below, right).

With border	Without border
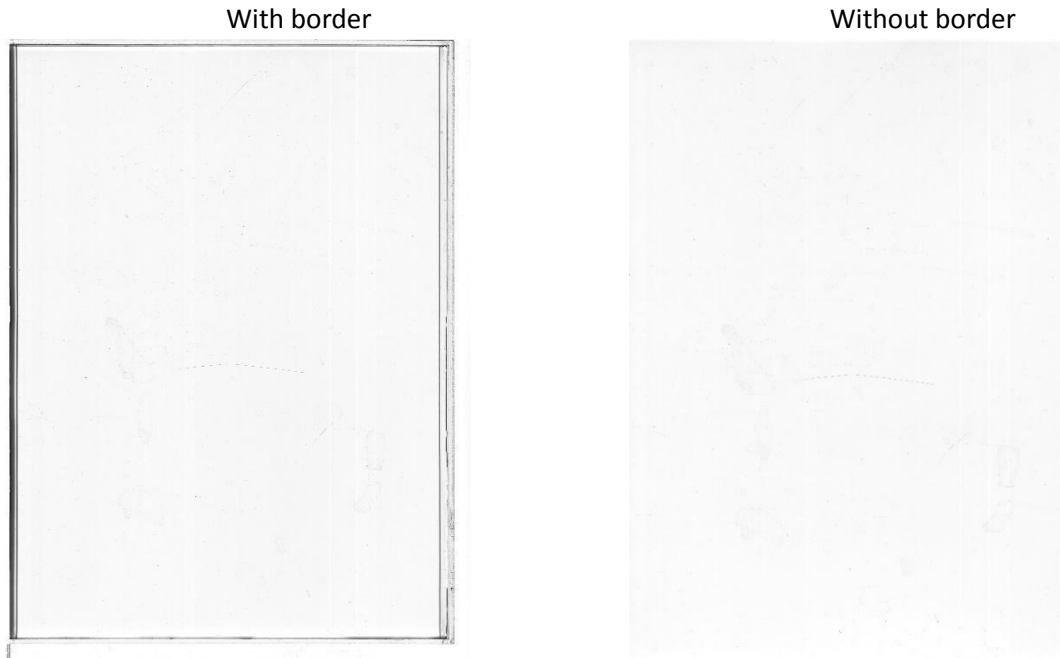
No border included in the scan. The white streaks in the image indicate that the scanner glass is dirty near the scan initiation and should be cleaned. [need image of scanner and this area to clean]

Procedure for scanning on 12000XL or V850

1. Place the acrylic tray onto the scanner at a consistent position, such as a corner, and make sure the tray is not located in the scanner calibration area. The manufacturer will denote this with an arrow. Placing objects here will result in dark or streaked images.

2. Fill the tray with about 400 ml of water for the 12000XL or 200 ml for the V850 – generally enough to allow roots to be submerged in water with no root sticking out of water.

3. Place your clean root sample into the water.

4. Begin by using your forceps / wooden skewers / fingers to gently pry and separate each root from one another. Make sure to minimize root overlap, clumps of roots will be analyzed as a single large object. If that turns out to be increasingly difficult (too many roots, or if they are too large) then you may have too much sample for the tray [Delory *et al.* determined that 1 cm of root per cm^2 of tray is the maximum]. If that is the case, you may need to divide your sample into two or more multiple samples and take multiple images. The data must be aggregated during statistical analysis [R code available from yorklm@ornl.gov (watch for public release) for this or the images can be concatenated before analyzing in RhizoVision Explorer].

5. Once you have successfully separated the roots and evenly distributed across the scan area, bring the cover down onto the acrylic tray and proceed to press, "Preview" and review the sample. Examine, and make sure there is sufficient space between your sample and the boundaries you set up on the Input Image. The scan does not occur along the dotted line, and will crop part of your sample if the root is too close to the edge. Make adjustments to your sample as necessary. "Preview" settings are honored so can be reused to reduce the scan area for subsequent scans if the trays are placed consistently in the same corner.

6. Once you've made your necessary adjustments, press the "Scan" button. The scan should take up to 1 minute to complete under these settings, to which you should be prompted with the option of viewing the scan soon after. Double-click on the file to make sure the scan captured the image properly while checking for bubbles, tray edges, and roots out of water. If the scanned sample is to your satisfaction, proceed to the next sample. For Epson Scan 2, the file name and file locations are given in the main screen before pressing "Scan", while for Epson Scan 1 these options are given after pressing Scan.

7. Proceed to clean the tray by bundling up your sample in order to scoop it out and back into its container. Be sure to get as much of the roost into the container as possible, otherwise they will show in the next scan. Some sample loss is inevitable so be prepared to change the water in your tray after a certain number of samples. Once the previous sample has been removed, proceed to the next sample, repeating steps 1-7, 6 being optional.

8. Once all of your scanning has been completed, proceed with cleaning your acrylic tray: empty the water through a filter into the sink, and throw the debris into the trash. Proceed to rinse the tray with water until clean, then dry with paper towels. Set the tray with the others when finished.

Pro tips: bent roots that reach out of the water can be cut at the bend

The desired output with roots is: