Intro to Botany 2017

ImageJ Analysis Protocol by Wes Burtcher

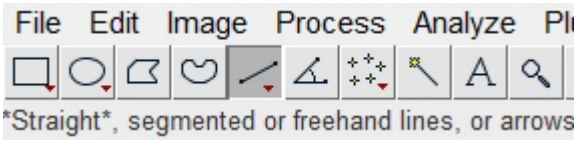
I. Select Desired Measurements

1. Click **Analyze** > **Set Measurements…** and check **Area** and any other desired measurements (e.g., **Perimeter**).

II. Set Scale

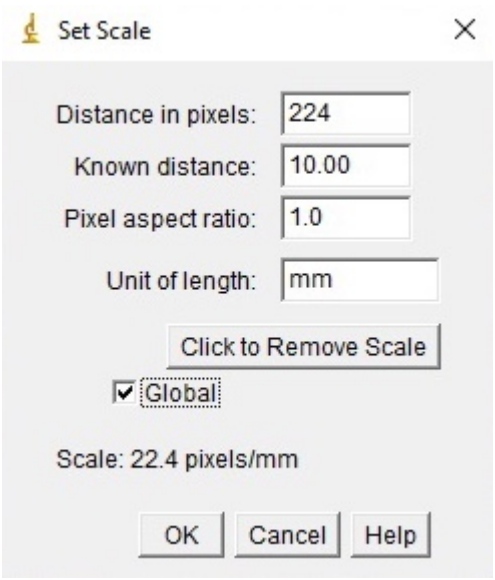
(If measurements are to be left in pixels or converted later, skip to section III.)

1. Open an image taken with the same settings as the images to be analyzed, and that includes a ruler or an object of known length.



2. Use the **Line Drawing Tool** to draw a line along a known distance. (If the **Line Drawing Tool** is not set to ***Straight***, right-click and select **Straight Line**.

3. Click **Analyze** > **Set Scale…**



   a. In the **Known distance** box, enter the length of the line in mm.

   b. In the **Unit of length** box, enter "mm".

   c. If the same scale will be used for multiple images, check **Global**.

   d. Click **OK**.

III. Taking Measurements

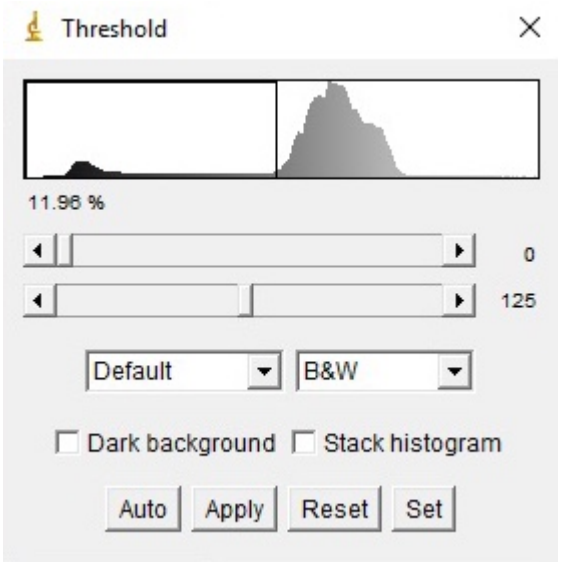
Important: ImageJ uses destructive editing and the Undo command only goes back one step, so make a backup copy of each image before performing the following steps.

1. Open the image to be analyzed (if different from the one used in section II).

2. Click **Image** > **Type** > **8-bit** to convert the image to grayscale.

   a. Optional: Click **Process** > **Enhance Contrast…** and increase or decrease the value in the **Saturated pixels** box as desired. This has no direct on the results, but it may make the edges of the plants and thinner filaments more distinct, aiding in the next step.

3. Click **Image** > **Adjust** > **Threshold…** to convert the image to binary. (Each gray pixel will become black or white.)



a. In the Threshold window, adjust the lower of the two sliders. Everything darker than the selected point will be black; everything lighter will be white. Find the point where the plants are black and the surrounding area is white. This will typically be near the center of the histogram, where it begins to slope upward. Continue adjusting until satisfied that as much of the plant area as possible has been converted to black without including shadows, reflections, etc. around the plants. Use your best judgement as there will not likely be a "perfect" threshold value.

i. The **Reset** button may be used as often as needed to change the image back to grayscale for comparison. The slider will remain in place, so clicking it or the left/right arrows again will return to the binary view at nearly the same value. When satisfied with the results, click **Apply**, then close the Threshold window.

b. If there are any black areas in the image similar in size to the plants (or larger), select the **Color picker** (eyedropper) tool and click on a white are of the image, then select the **Flood Fill** (paint bucket) tool and fill the unwanted black areas with white.

c. Click **Analyze** > **Analyze Particles…**

i. Enter a range of values in the **Size (mm^2)** box. This will vary; try "25-Infinity" as a starting point. (If working in pixels, the minimum value will be much higher.)

ii. Check **Display results**, **Clear results**, **Add to manager**, **Exclude on edges**, and **Include holes**, and click **OK**.

d. In the Results window, there should be a numbered row for each plant and a column for each measurement selected in section I. (e.g., Area).

i. If there are more or fewer rows than plants, close the Results and ROI Manager windows and repeat the **Analyze** step with a different size range and/or use the paint bucket and brush tools to get rid of problem areas.

ii. If the number of entries is correct, note that the numbers correlate with the labels you should now see in the image, but that these numbers may not be in a predictable order. Keep track of this order when copying data from the results window.