

# SCANNING LEAVES AND MEASURING BASIC LEAF MORPHOLOGY

PREPARED BY: TIMOTHY M. PEREZ

LASTED EDITED: NOVEMBER 8TH, 2017

## 1. Scanning leaves:

1.1. Place leaves on a flat-bed scanner to scan the 1-sided projected area of a leaf. Make sure that each leaf is numbered and that each number is visible to the scanner. Make sure that petioles are removed (unless doing  $g_{min}$  measurements)

1.2. When scanning, make sure the resolution is set to 300dpi. This is necessary to ensure the same scale can be used for each scan when measuring leaf morphologies.

1.3. Before or after scanning leaves, scan a rule to use later in the calibration in ImageJ.

1.4. When scanning, multiple leaves can be contained within the same image.

1.5. File naming and format:

1.5.1. Save files as .jpegs

1.5.2. For file name, type the individual's accession number. Treat spaces as underscores. After the accession number, place a period (.) then numbers that correspond to the leaves scanned in that image.

**Example:** A picture with leaves 1-3 from a plant with accession number 83534 C should be saved as 83534\_C.1-3.jpeg. Similarly, leaves 4-5 would be saved as 83534\_C.4-5.jpeg

In the case that a plant has no accession number, record the first three letters of the genus and the first three letters of the species name followed by the leaf assignments.

## 2. Measuring leaf morphology in ImageJ:

2.1. Download the open source image analysis program, "ImageJ" here:

<https://imagej.nih.gov/ij/download.html>, or here: <https://fiji.sc/>

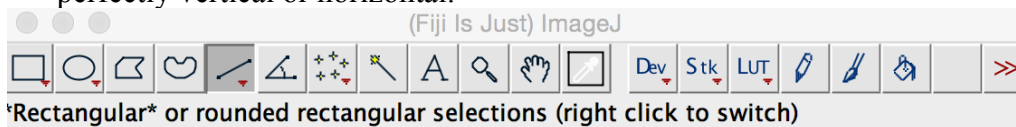
2.1.1. Both programs are free to download for both mac and pc, but the second link to FIJI has more packages built-in and is the one I prefer to use (versions available for both mac and pc)

2.2. Open ImageJ/Fiji application

2.3. **Setting the scale** – Any time you open ImageJ it is important to set the scale that you'll be working. This tells ImageJ to convert a given number of pixels into a distance. We'll be using centimeters.

2.3.1. **Open the Image of the scanned ruler.** File>Open> select image

2.3.2. Then, select the line segment tool (see figure below) and draw a line across the image of the ruler. Draw a line parallel along a known distance of the ruler (e.g. exactly 5 or 10 cm). Holding down the shift key as you draw the line will make it perfectly vertical or horizontal.

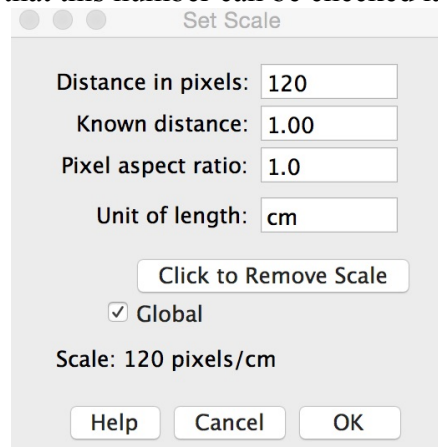


2.3.3. Go to Analyze>Set Scale. A box will pop up and prompt you for information.

2.3.3.1. The Distance in pixels information should already be filled in in ImageJ. This number indicates the span, in pixels, of the line you just drew.

- 45 2.3.3.2. Into the “Known distance:” space, enter the length of your line of  
46 centimeters as indicated by the ruler.  
47 2.3.3.3. The “Pixel aspect ratio:” should be 1.  
48 2.3.3.4. For “Unit of length” enter “cm”  
49 2.3.3.5. Lastly, check the box next to the word “Global.” This tells ImageJ to  
50 apply the same scale to all other images that you open while ImageJ is running.  
51 The scale has to be reset if ImageJ is closed and re-opened. Hit “OK” to close  
52 the box then close the image with the ruler.  
53

54 Note: At 300dpi, the scale should roughly equal 120 pixels/cm –Check to make  
55 sure this is the case in the dialog below “Global”. Also, it is a good idea to  
56 record the “Scale” so that this number can be checked later



57  
58 **2.4. Setting Measurements**

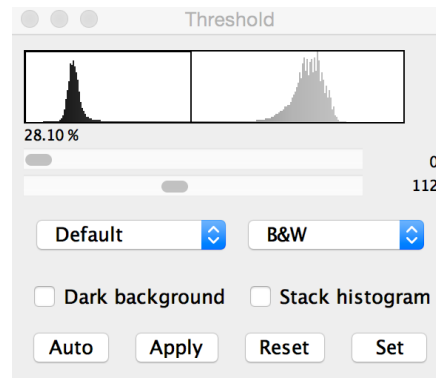
- 59 2.4.1. Go to Analyze>Set Measurements. A box will prompt you to select several  
60 measurements to analyze. For our purposes, we only need to select “Area” and  
61 “Perimeter”. All other boxes can remain unchecked. We’ll use 3 decimal places for  
62 our data – so enter “3” in the space that says “Decimal places (0-9)”. Hit “OK” to  
63 make these adjustments and to close the box.  
64

65 **2.5. Measuring leaf diameter, length, area, and perimeter**

- 66 2.5.1. Open the first image in your file: File>Open  
67 2.5.2. Double check to make sure your scale is set.  
68 2.5.3. **Thresholding an image**  
69 2.5.3.1. Once we are 100% sure the scale is correct, we can prepare the image to  
70 be measured. To do this go to Image>Type> and select 8-bit. This will turn the  
71 image black and white.  
72 Note: Here, be sure to remember how your leaves are numbered if several of them  
73 are in the same image because the numbers will become obscured in the next step  
74  
75 2.5.3.2. Next, hold “shift”, “command” (or equivalent on PC), and “T” to open up  
76 the “Threshold” box. Alternatively, this can be done by going to:  
77 Image>Adjust>Threshold. The silhouette of the leaf should remain. The  
78 threshold box should look like the one below with unchecked boxes.

79  
80  
81  
82

Note: In some cases, it may be necessary to change the threshold by using the sliders to properly showcase the area of the entire leaf, or achieve better definition of leaf edges.



83  
84

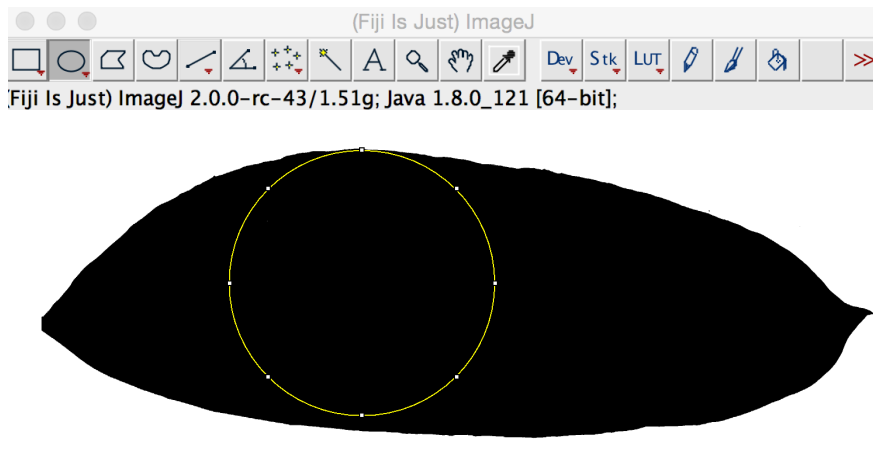
You are now ready to start measuring!

85  
86  
87  
88  
89  
90  
91  
92  
93

2.5.3.3. **Diameter:** We measure diameter of the leaf, also known as the effective leaf width, or leaf width by determining the largest diameter of the largest circle that can be drawn within the borders of the projected leaf area. To do this:

2.5.3.3.1. Select the circle tool and draw a circle in the widest part of the leaf. Hold down the “shift” key to draw a perfect circle, then drag the of your circle to the edge of the widest part of the leaf (see image below)

94



95  
96  
97  
98  
99  
100  
101  
102  
103  
104

2.5.3.3.2. Then, holding “shift” and extend the the edge of the circle to the edge of the leaf. Be sure that the edges of the circle do not extend beyond the edge of the leaf. To be sure that you created the largest circle that can fit within the leaf, drag the circle around to make sure it doesn’t “fit” anywhere else inside the perimeter of the leaf.

2.5.3.3.3. Once you’re sure you have the largest circle that fits within the leaf, hold down “command” & “M” to measure the circumference of the circle. Alternatively, you can go to Analyze>M to do this. A box will pop

105 up and will indicate the circumference of your circle in the “Perimeter”  
106 column. Record this value, which we will divide by pi to get the leaf’s  
107 diameter.

108 2.5.3.4. **Leaf length:** Measure the length of the leaf of interest by selecting the line  
109 tool. Click on the base of the leaf blade where it meets the petiole, and drag the  
110 line to the very tip of the leaf and hold “command” & “M” to measure. Record  
111 the length.

112 2.5.3.5. **Leaf area and perimeter:** Select the wand tool from the toolbar, then  
113 click the leaf of interest. A line will appear around the silhouette of the leaf of  
114 interest. Hold “command” & “M”, and the area and perimeter of the leaf will  
115 appear in the “Results” box. Record these values  
116



117  
118  
119 2.6. When done with measuring all leaves in the image, you can close the image and open a  
120 new one. IMPORTANT: DO NOT save changes to the picture. Doing this will have an  
121 additional copy of the photo, which we do no need.

122 2.7. To quickly open up the next image in the file, hold “shift”, “command”, “O”.

123 2.8. Repeat 2.5 to 2.8 until all leaves have been measured.

124

125 Note: Remember closing out of ImageJ will erase the the scale that you established.