Oak leaf morphometric project - 2013

Ackerly Lab

Instructions for morphometric analysis with ImageJ

A) Record keeping: In excel file analyses/photolist.xlsx - keep track of status of each step for each photo. Add your initials and the date for each step (e.g. 'DDA 6/21/13')

B) Analysis steps in ImageJ

1) Open jpg image in photos/analysis\_XXXXXX (XXs are date photo was taken)

2) Number individual leaves

Select text tool in menu

Draw rectangle by each leaf and type 'leaf X' (where X is 1, 2, 3)

hit control-D to save text to image

3) Save file to same folder - ImageJ will make it a tif file, so it won't replace original jpg

4) Open tif file in one window and jpg file in second window

5) SET SCALE: In jpg file, move image down until you can see the ruler

Using the line select tool, draw a line 10 cm long (e.g., from 2 to 12)

Select Analyze > Set Scale....

Set 'known length' to 10 and units to cm

Click OK

6) Clear shadows: Zoom in one one leaf (command-5 brings image to full size); carefully select areas of shadow around edge of leaf and Edit > Clear to erase shadow

Erase petiole (short stem at base of leaf)

If you make a mistake, you can undo JUST THE LAST STEP with command-Z

If you can't undo a mistake, you have to close the file (without saving), reopen, and start again from where you were at the last save

Save file!

7) Threshold: In tif file, select Image > Adjust > Threshold

• Move lower slider below 'Brightness' towards '128' until leaves are captures as fully as possible, without picking up background

• Close thresholding window, and SAVE FILE (say yes to 'Replace existing file')

8) Fill leaf edges:

Zoom in on one leaf, in both the tif and the jpg file so you can see them side by side.

Carefully exam the margin of the leaf to be sure it is fully filled

Compare thresholded tif file to original full color jpg to make judgments

Use freehand select tool to select areas of margin and use Edit > Fill to fill them black

Save file after each step!

9) In Analyze > Set Measurements check that the following are selected:

Area, Shape Descriptors, Area fraction, Perimeter, Fit ellipse, Display label

10) Select Analyze > Analyze Particles

In dialog window, check: Display results, Clear results, Include holes, in situ Show

Select: show outlines

Click OK

11) Results table pops up with hundreds of objects, many tiny!

Zoom into tif image to see object number for each leaf

Copy results for Area, Perimeter, Major and Minor axis for each leaf to spreadsheet