Protocol for analyzing leaf images for SLA Nathan Kraft

Overview: This protocol is used for determining the 2- dimensional leaf area of leaves from the Yasuni Forest Dynamics Plot, Ecuador. The area measurements are used to determine leaf area (LA) and specific leaf area (SLA), both important ecophysiological traits. SLA is calculated as LA/ dry leaf mass. Leaves in the field were flattened under a Plexiglas sheet and photographed in the field using a digital camera. The goal of this protocol is to 1. match each photograph to the tree and leaf measurements that we already have, 2. determine the scale each picture, and 3. calculate the area of the leaf in each picture.

Matching image files to existing data

All of the photographs taken on a given day in the field in Ecuador are stored in a unique folder. Days from 2005 are numbered "100YASUN, 101YASUN, 102YASUN, …" and days from 2006 are numbered "100YAS6N, 101YAS6N…" Within each folder (day) pictures are numbered sequentially. Gaps in the sequence are due to photographs that were deleted in the field.

There are usually 3-4 photographs for each tree that we sampled. The first photograph is always of the forestry tag that bears a unique number assigned to that individual tree within the plot. On a few occasions this picture may be of the number written in a data book or a sample envelope if we couldn't get a clear shot at the forestry tag. The next 2 or three photographs are of leaf 1, leaf 2, and (in some cases) leaf 3. We always followed the same sequence in the field with photographs to make matching the images to individuals easy: tag, leaf 1, leaf 2, and (if present) leaf 3. In the case of small leaves, we sometimes tried to fit all of the sampled leaves into the same image. When multiple leaves are in the image, there should be a clear indication in the image (i.e. paper sample envelopes with L1, L2, L3) that indicates which leaf is which. Sometimes other leaves that we did not measure will also be in the image- these are usually partially cut off, covered up by my hand, or otherwise obscured. Just ignore them, and if in doubt, ask me or make a note on the datasheet. The leaf images are distinctive- they should have a leaf that dominates the picture, a white background behind the leaf, a small ruler at some place in the image, and usually a bit of glare (a white circle) from the flash. Occasionally other images are also in the folders, such as pictures of whole trees, one of us, or various creepy crawlies that we found in the field. You don't need to do anything with these images.

Each line of the datasheet corresponds to one leaf that was measured. The order should match the order of the photographs. For each leaf that you measure, first check the tag picture to make sure it matches up against the tag number on the datasheet. Then identify which picture number (or part of a picture) corresponds to leaf 1, leaf 2 and (if present) leaf 3. Record both the folder number and the image number for each leaf that you analyze in the spreadsheet. For this part of the analysis, I really recommend using the application "Preview"- it opens up images much faster than ImageJ or Acrobat. It should be the default way to open .jpg images on the Macintosh.

Analyzing the image

Once you have identified which image corresponds to the next leaf you want to analyze and recorded the folder and file number on the datasheet, open it with ImageJ. Give the image a quick once over to see if it will be easy to analyze- if it looks too hard, just pass on it and make a note for me- I'll handle it. Things that can be tricky are cases where the border of the leaf is broken by the flash glare, my hand, or the edge of the Plexiglas. In these cases it is sometime necessary to draw in or erase lines on the image- something I would prefer to do myself if needed.

Setting the scale

1. Find the ruler in the image. If multiple rulers are in the image, it is best to pick the one closest to the leaf itself. Use the magnifying glass to zoom in until 1 cm of the ruler is quite large on the image.
2. Choose the straight line tool and draw a line 1 cm long on the image.
3. Go to "Analyze > Set Scale" . A dialog box will pop up- you'll need to specify that the line is 1 cm long. This will tell the program how many pixels are in a cm- should be in the vicninity of 60-70 pixels per inch, though it will vary with how zoomed in the lens of the camera was.
4. Zoom out to the original scale with "Image>Zoom>>Original Scale"

Prepping the image

1. If there is a lot of space around the white board, or if the leaf is really small on the image, crop the image down to just a leaf against a white background, as much as you can. Use the rectangular selection tool to draw a box, then choose "Image>Crop".
2. Convert the image to binary. Choose "Process > Binary> Make Binary". This should give you a black leaf outline on a white background. If you somehow got a white image on a black background, invert it by going to "Edit> Invert"
3. Fill any breaks in the edge of the leaf due to the flash using the paintbrush tool. It is best to zoom in tight on the area to be corrected first, then zoom out when you are done.
4. Crop of the petiole if there is one (about half of the images should have one). Do this by selecting an area of the image that includes all of the leaf blade but none of the petiole, using either the rectangular selection tool or the polygon selection tool

Analyze the area.

1. Go to Analyze> Analyze particles. In the box that pops up, set the Size (first box) to be .01 or .05- infinity. Size is left too small you'll measure the size of lots of specks on the image, which is annoying. Make sure that "show" is set to masks, and that "display results" and "clear results" are checked.
2. If there are gaps in the leaf outline due to the flash or errors in how the image was thresholded, make sure that "fill holes" is checked.
3. Click "OK" and two windows should pop. One shows the area (in square cm if you set the scale properly) of all particles in the image that the algorithm found and the other shows a mask, or the shape of what the computer calculated the area of. Make sure that the mask looks right (no holes in the area that shouldn't be there), and then record the area of the leaf shape in the excel spreadsheet.
4. Close all of the windows associated with that leaf before you open up the next file- the cpu is unhappy if it has a lot of these large images open at once.

MOST IMPORTANTLY-

Keep track of anything that looks unusual or confusing or isn't working, make a note of it on the spreadsheet, and let Nathan know!