

Leaf area calculation tutorial

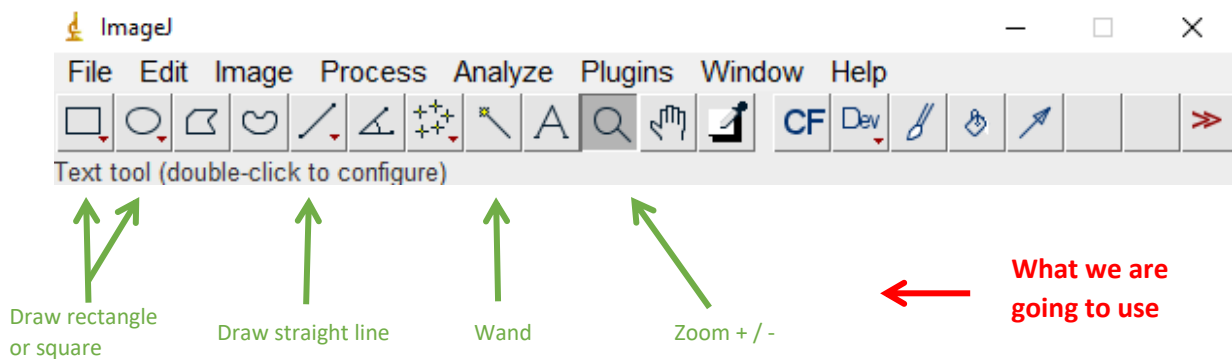
The following tutorial aims to explain how to measure the leaf area with the open-source software *ImageJ*.

ImageJ general download: <https://imagej.net/downloads>

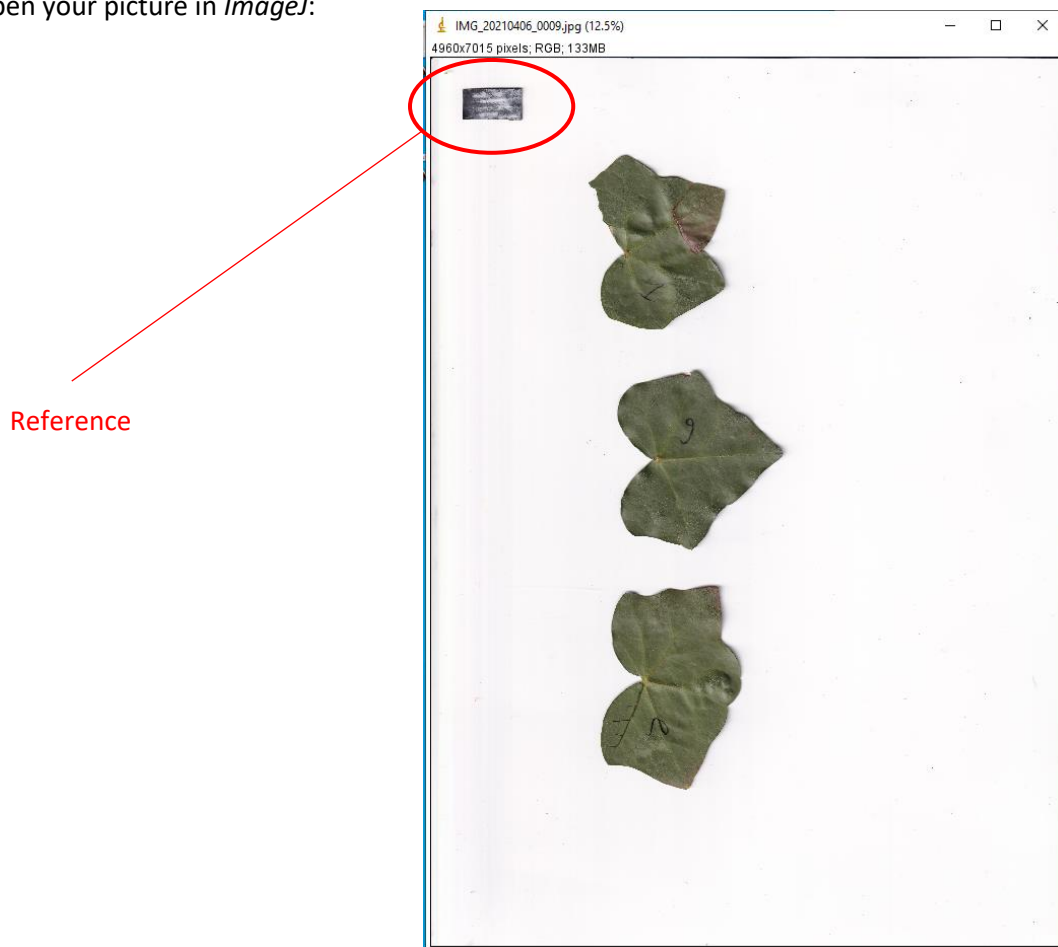
ImageJ general information: <https://imagej.net/ImageJ>

ImageJ documentation: <https://imagej.nih.gov/ij/docs/index.html>

1. Open *ImageJ*. You will get the following interface:

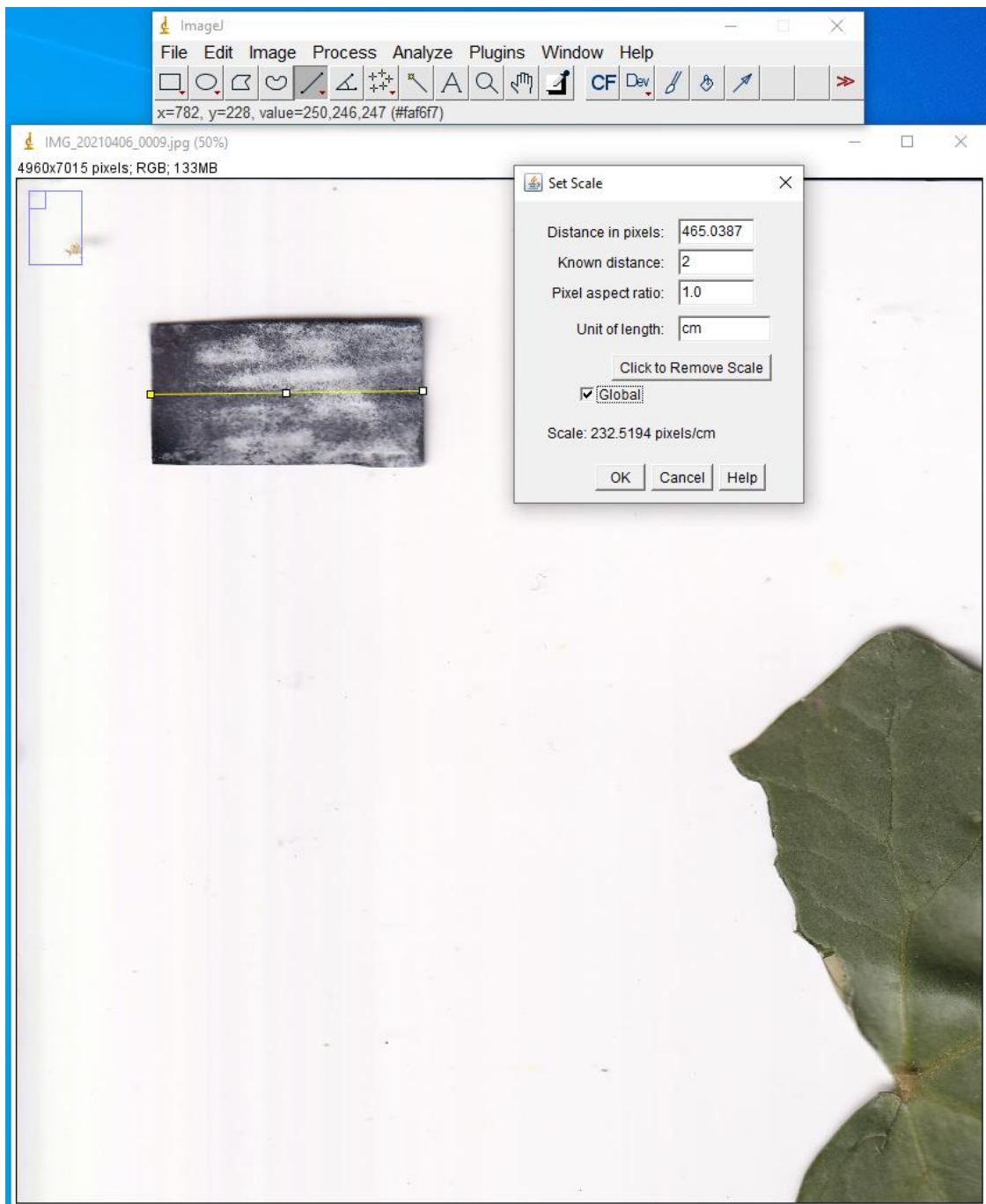


2. Open your picture in *ImageJ*:



You first have to “calibrate” our picture before measuring the leaf area. For this, you will measure the length of the side of the **reference** in the image (on your photos: the black square of 1 cm length; in the present tutorial: the black square with 2 cm length). Then, you will specify to ImageJ what length that square corresponds to in reality:

- Measure the reference (it's better to **zoom** a bit) with the **straight-line** icon
- Open *Analyze -> Set scale*
- “Distance in pixel” is the number of pixels of the straight-line measurement (don't change it!). Change the “Known distance” to 1 cm! Set the unit of length to cm. Tick the box “global”.



3. Go to *Image -> Type -> 8 bit* to change this parameter. 8-bit images allow easier recognition of leaves compared to full RGB images. It is now possible to easily differentiate the leaves from the rest of the image, as all pixels within one leaf are quite similar.
4. To select the pixels that correspond to your leaves, you need to use the function *Threshold (ImageJ -> Adjust -> Threshold)*. It allows setting two categories of pixels depending on the histogram of the image. At the end of this step, the area of your leaves should be covered by a red or a black area. It corresponds to the regions of interest (ROI) of your leaves. ImageJ should automatically provide a correct result (= leaves well delimited, no holes within the area, ...). If needed, you can manually adjust the threshold by moving the sliding buttons in the *Threshold* window.
5. Other objects will also be selected (the reference rectangle, the metal clip, etc.). In step 7, you will “select” the right objects with the **wand**. But if your leaves touch the border of the image or another object, you must separate them either by changing the histogram (see step 4) or by manually erasing them. To erase a zone, draw a **square** or a **circle** and press *Delete*.
6. You will now select and measure the regions of interest (ROI) of our image.
 - a. Open *Analyze -> Tools -> ROI Manager* and toggle “*Show All*” and “*Labels*”
 - b. Click on the **wand** icon in ImageJ main window
7. Select one ROI with the wand, then click on *ROI manager-> Add* (or press “t”). It will store the area of that region of interest. Repeat the operation for all your leaves. Once you selected all the leaves of your picture, you can read the area [cm²] and other results in *ROI Manager -> Measure*. Save the data as a .csv file or directly report the measurements in your datasheet.

