

# Aphid Ruler

## Instructions for the use on an Image-J macroscript for aphid size determination

This script takes standard photographs as a input and measures the pixels attributable to the insect. It was generated using Image J version 1.51k for PCs

### Photo specifications:

- 1) Photographs must be taken against a **BLACK** background.
- 2) The distance of specimen from the lens must be constant throughout the photograph generation process.
- 3) For standardization purposes, a marker photograph is required at every batch processing. We used a one centimetre paper strip of paper fixed on the same platform where Insect pictures are going to be taken (see below).
- 4) The photos must have some sort of sequential numbers, eg. DSC\_0001.JPG, DSC\_0002.JPG ... etc. Note that you will need to adjust the script to match your own sequential file numbers.

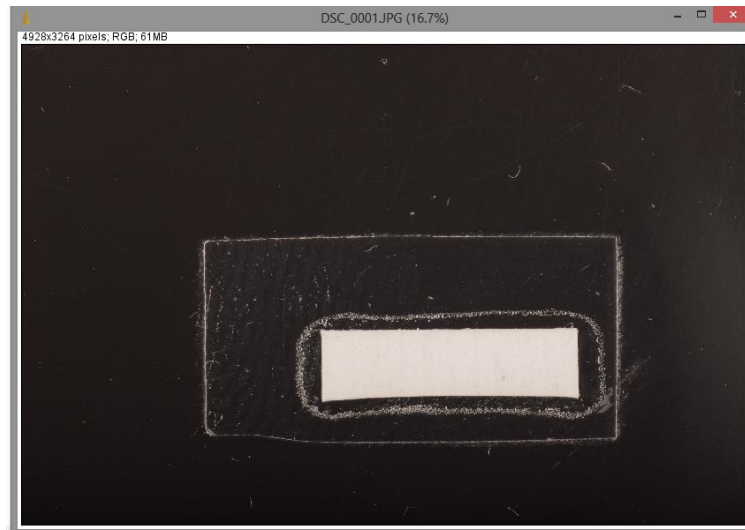
### Before you use the Script in ImageJ

- 1) First change the number of samples according to your requirement on `line 19`
- 2) Change the sample file destination folder on `line 21`.
- 3) Change the output folder on `line 145`.

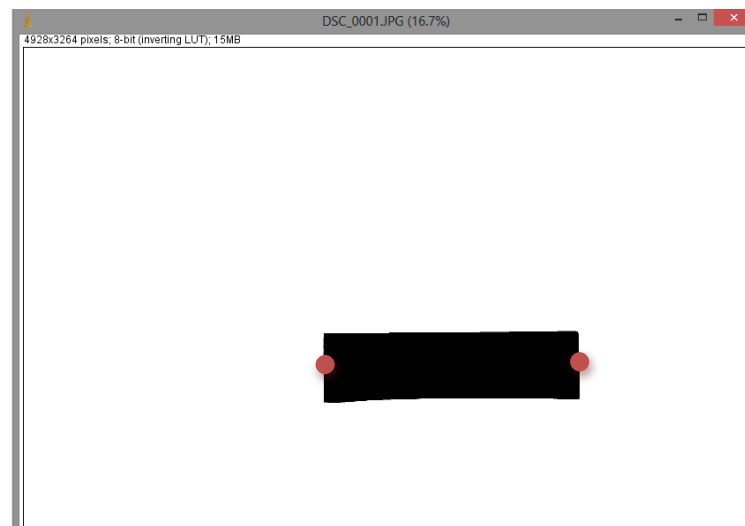
### Calibration Process:

- A) Open ImageJ software
- B) Go to `Plugins` → `Macros` → `Install` → (browse the ImageJ macro script in your system)
- C) You will notice a Blue colour square button (■) appear on the ImageJ panel.
- D) Click on the blue button. Now you can add details of the experiment (day, date etc.)
- E) Then click `OK` by confirming the source folder destination.

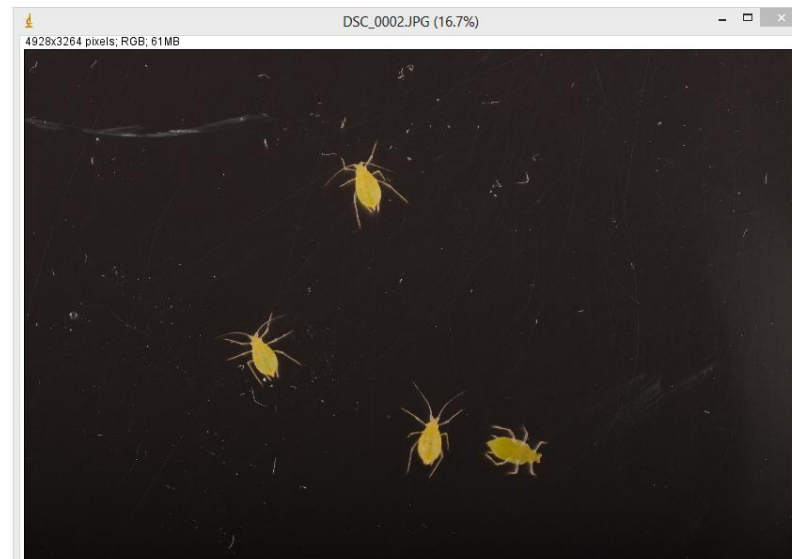
- 1) The software takes the first picture from the folder ( Do not do anything as the software is processing this picture)



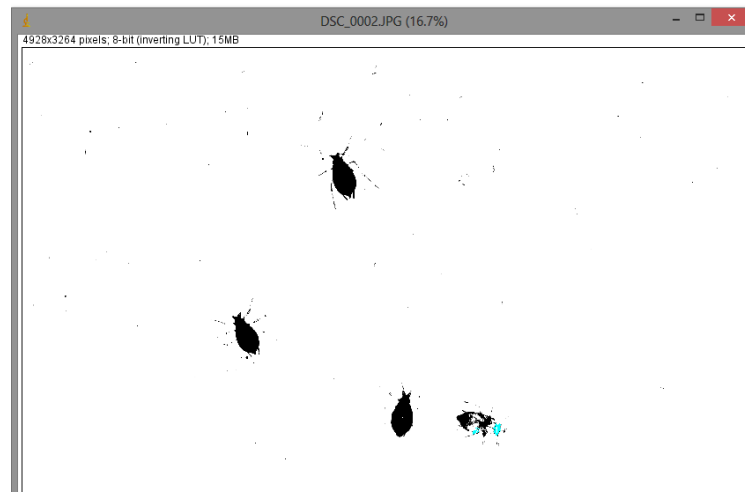
- 2) The software converts the original photo into a binary form. Wait for two points (represented in RED) to be selected on the marker. The software records its co-ordinates and measures distance between two points. This serves as a standard length for the experiment.



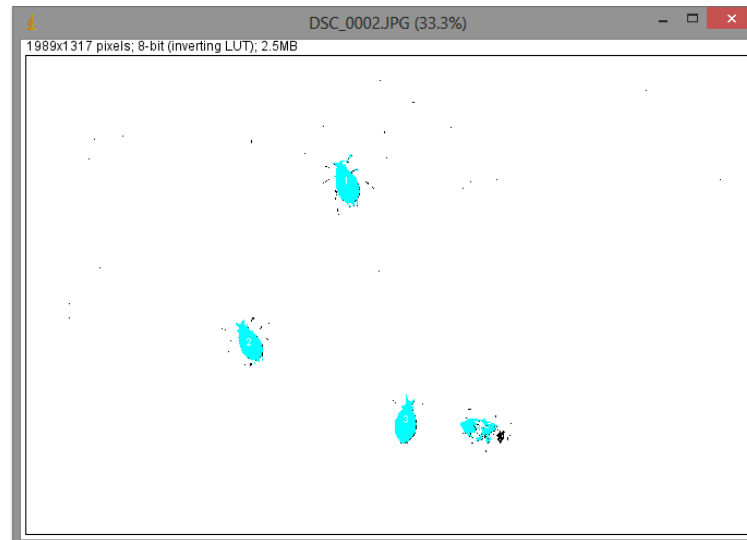
3) Then software will automatically process rest of the photographs. In a similar fashion.



4) It converts images to a binary form according to colour co-ordinates provided in the script.



- 5) Then it measures the pixel in black region and gives a reading in tabular form.



- 6) You can notice that one of the insects is not detected as clearly as other three insects. This is due to slight colour change of that insect. Here you need to do calibration.
- 7) Go to the script `line 115 gives max[2] = XXX (X is number). You can adjust this number from 120 to 200 to optimize the result. Every time you change the coordinates you need to save the file and go through the macro installation process as explained in point B.`
- 8) Once you get the desired result re-run the analysis with calibrated settings.
- 9) You will find a result file in output folder.

## Output file and result:

- 1) The output file will be in .csv format
- 2) The first column gives a number to each insect analysed. All the analysed insect data will be concatenated in a sequence. To find the size of one particular insect you need to count the number of insects on each photograph processed before the particular insect of interest. Careful inspection the analysed picture reveals that there is a number written on each insect in a white font.
- 3) The second column gives total number of pixels occupied by the insect in the picture.
- 4) Copy and paste first two columns from output file in the provided excel template sheet for conversion of number of pixels in to size in  $\text{mm}^2$ .
- 5) Assuming each photograph represents one treatment, You need to manually add the sample names in the third column by referring to the number of insects analysed in each photograph sequentially.

- 6) Once you paste the size in pixels into the second column you will get the size of the insect in cm<sup>2</sup> and mm<sup>2</sup> in columns 5 and 6.

That's it!!! Wish you all the best.

- Amol Ghodke. PhD student The University of Melbourne 2017.

P.S.- \* If someone can create updated this script so that it can do self-calibration or to remove any manual process is highly appreciated. \*

Thank you