

# ImageJ Measurement Protocol

## 1. Download ImageJ software for free from NIH.

<http://imagej.nih.gov/ij/>

- Select "Download"
- Determine your platform (Mac/Linux/Windows) then click on the appropriate link to download the program. Note that Chromebooks cannot run ImageJ without installing a new operating system. (You may also download more detailed installation instructions from this page.)
- Follow the prompts to install the program.

This is the basic process you will follow. Detailed instructions are below:

*Open image → Set scale → Outline caterpillar → Measure caterpillar → Record your data*

## 2. Import your Images

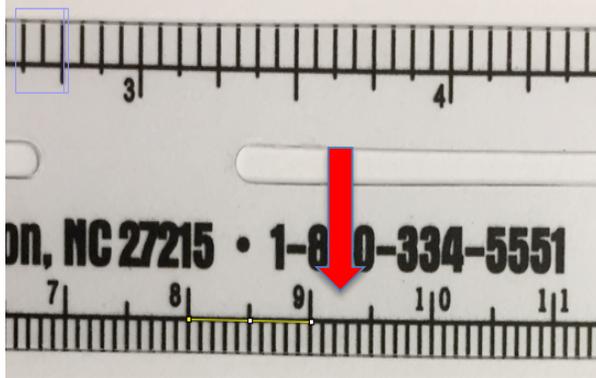
- Import your image into the program:
    - Drag & Drop them into the program <or>
    - File, Open, and then navigate to your images.
- \*Tips:*
- *You may rotate the image for a better view. (Image, Transform, Rotate)*
  - *You may zoom in or out for a better view. (Image, Zoom)*
  - *You may adjust the view by selecting the Scrolling tool, and dragging left or right on the image.*

## 3. Set Scale

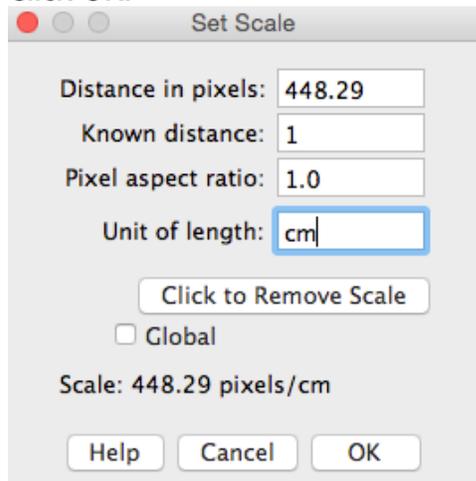
- Select the Straight Line tool (HIGHLIGHTED BELOW)



- Use the crosshairs to trace a line that runs the length of 1cm using your ruler as a guide. It will be displayed in yellow and can be hard to see.



- From the Top menu select Analyze, then Set Scale.
- Enter the Known distance to "1," and the Unit of length to "cm."
- Distance in pixels and Pixel aspect ratio will pre-populate.
- Click OK.



***\*You will need to reset the scale EACH TIME you open a new image.***

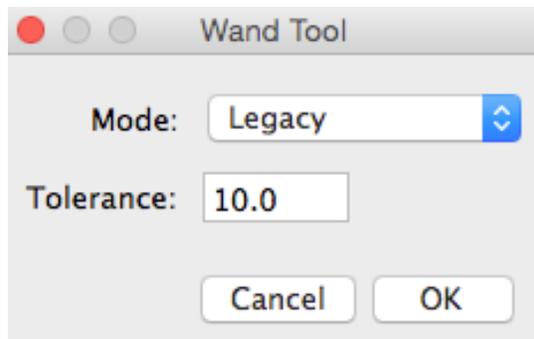
#### 4. Outline the Caterpillar

- To select/**outline the caterpillar** there are two options: Wand and Freehand. You should try both to see which method you prefer. The Wand method is explained first, then the Freehand.

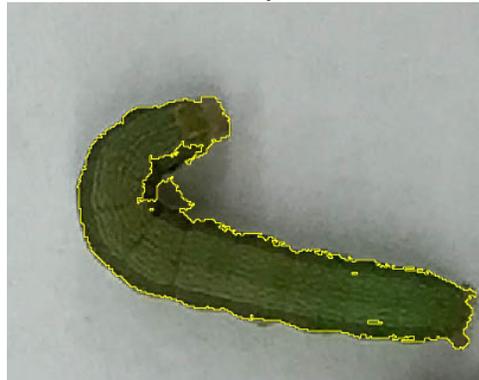
- Select the **Wand tool** (HIGHLIGHTED BELOW)



- Double click the wand tool and set the Tolerance to "10."
- Click OK.



- Click on the inside of the caterpillar to make a rough selection. It will be outlined in yellow.



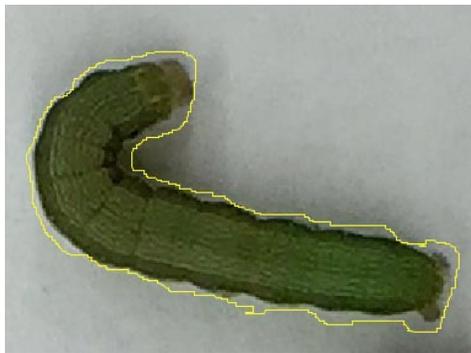
- Refine your selection to be more accurate. Clicking while holding down Shift will allow you to add areas, Clicking while holding down Alt-Option will allow you to remove areas.
- Most of the caterpillar should be outlined in yellow. Be as accurate as you can, but limit yourself to 4 or 5 clicks. It won't be perfect.
- If your tool gets stuck and you cannot remove the yellow outline, click outside of the caterpillar to start over.

<or>

Select the **Freehand tool** (HIGHLIGHTED BELOW)



- Click and drag to outline the caterpillars' body. It will be outlined in yellow.
- Most of the caterpillar should be outlined in yellow. Be as accurate as you can. It won't be perfect, as shown below.
- If your selection tool gets stuck and you cannot remove the yellow outline, try clicking on the outline to remove it.



##### 5. Measure the caterpillar

- Select Analyze from the top menu, then Measure
- The Results dialogue box will show you the area, mean, min and max. We are focusing on the Area, so record this measurement. The units are in  $\text{cm}^2$ .

*\*Note: Measurements should be less 1. If you end up with numbers in the thousands, you need to reset your scale.*

**6. Record** your data in your data table.

**7. Repeat** this process until all the caterpillars in your image have been measured.

**8. Close** your image and Results dialogue box. Do not save changes to either of these.

**9. Repeat** this entire process with each of your images.

## **ImageJ Practice**

Learning new skills takes time. This lesson will give you practice using the ImageJ software. The goal is to ensure that you are collecting quality data that is accurate and usable.

Practice using both the **Wand tool** and **Freehand tool** to measure the caterpillar in the file "Practice Caterpillar for ImageJ". When you begin to feel comfortable with the software, record your measurements below.

Wand tool

Freehand tool

1. \_\_\_\_\_ cm<sup>2</sup>

1. \_\_\_\_\_ cm<sup>2</sup>

2. \_\_\_\_\_ cm<sup>2</sup>

2. \_\_\_\_\_ cm<sup>2</sup>

3. \_\_\_\_\_ cm<sup>2</sup>

3. \_\_\_\_\_ cm<sup>2</sup>

Now average all these together and record here. \_\_\_\_\_ cm<sup>2</sup>

The result should be 0.412 cm<sup>2</sup>. If your results are within the range: 0.330 – 0.494 cm<sup>2</sup> then you have successfully demonstrated proficiency using ImageJ. If your results are outside of that range, keep practicing until you become proficient.

You may notice that you have a preference for either the Wand tool and Freehand tool. You should use which ever is more accurate for you.