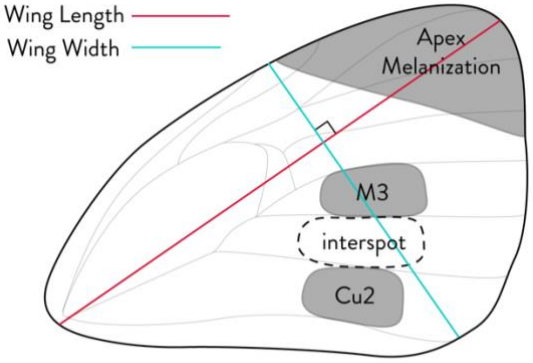
BCEENET
BIOLOGICAL COLLECTIONS IN ECOLOGY AND EVOLUTION NETWORK

# Measuring Wing Size using ImageJ

Created by: Elissa Sorojsrisom, Columbia University; Janice L. Krumm, Widener University; Paula A. Trillo, Gettysburg College

## INTRODUCTION

These directions will walk you through how to use ImageJ to measure **wing length** and **wing width** of *Pieris rapae* butterfly wings.




This may be used as a standalone activity to gain practice using ImageJ, or as part of the BCEENET Sexual Dimorphism CURE: Exploring Melanized Wing Patterns of Pieridae Butterflies (Trillo, Sorojsrisom, Jordan, & Krumm, 2022). After completing this activity, you will be able to:

- Measure a ruler or reference length in an image to set the scale
- Use the line tool to measure wing length
- Install and use the fixed angle line tool to measure wing width at a specific angle

### *Related documents*

| |
|---|
| Part 1- Installing ImageJ and Tools |
| **Part 2- Measuring Wing Size using ImageJ** |
| Part 3- Measuring Wing Spot Area using ImageJ |
| Part 4- Measuring Apex Melanization using ImageJ |

This is the second part in this series. **Be sure to complete Part 1, Installing ImageJ and Tools, before you begin.** Your instructor may ask you to skip parts 3 or 4 or to complete them in a different order.

## INSTRUCTIONS

### 1. Open ImageJ and install the fixed angle macro tool

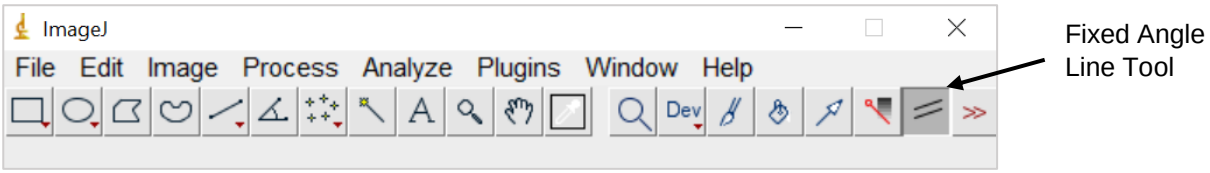
*Reminder: You must reinstall this tool each time you open ImageJ.

a. Hopefully, you have saved the Macro file (*Fixed Angle Line Tool.ijm*) somewhere convenient on your computer. If not, revisit Part 1- Installing ImageJ and Tools.

b. In ImageJ, navigate to **Plugins >> Macros >> Install** and locate the file.

c. Click Open.

d. The macro tool should install automatically, and a new button should appear in your ImageJ Toolbar. The button has two parallel black lines.




### 2. Open your first *Pieris rapae* image

a. You should have previously downloaded your image of *Pieris rapae* from the iDigBio website or your course website and saved it on your computer.

b. Navigate to **File >> Open…** and select your image file. Click Open.

c. The image will now be visible on your screen.

### 3. Open your Spreadsheet for data collection

a. Your instructor will have provided you with a spreadsheet where you will record your measurement data. Find the spreadsheet and open it.

b. Next, find the associated specimen record (represented by the row on your spreadsheet with the same **coreID** as your image. This is the row where you will record your data.

c. Find the group of columns where you will record your measurement data.

d. In the **Units** column, record the units we will be using. We will be measuring in millimeters (mm).

## 4. Determine if your image can be used in the analysis

a. Make sure your image has the characteristics listed below. If your image is missing any of these characteristics, describe the problem in a **Morphology Notes** column and ask your instructor for a replacement specimen.

Image from British Museum of Natural History through iDigBio

i. **Image shows a butterfly**. *Images of caterpillars or labels only will be unusable.*

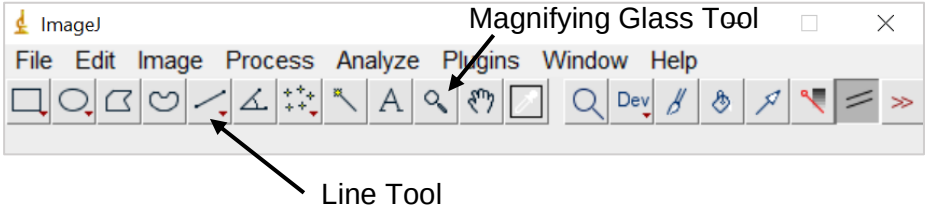
ii. **Dorsal surface of butterfly is shown**. *A good way to determine this is to look for the butterfly's legs. If it is a dorsal view, the legs should be hidden under the wings and not visible. Also look at the overlap of the wings. If it is a dorsal view, the forewing should cover the anterior part of the hindwing.*

iii. **A ruler or other scale is included in the image.** *Most images will have some sort of ruler. Some color bars or labels can also be used to set the image scale. More information is included in the **Set the Scale** section below. If you have any concerns about your image having a scale, ask your instructor.*

## 5. Set the scale

a. Every image you use to measure *Pieris rapae* wings will be different in scale. Therefore, we need to determine how many pixels are in a known distance (example: 12.6064 pixels/mm) for every image.

b. In your butterfly image, you should see a scale bar or ruler. First, we will use the **Line Tool** to draw a line of known distance.



i. You should use the **Magnifying Glass Tool** to zoom in and help you draw your line more precisely. Click twice on the tool button to zoom out.

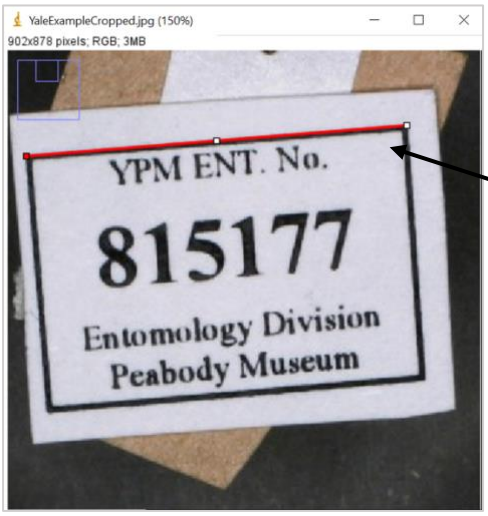
ii. Draw your line using the **Line Tool**. Usually, it's best to use an even measure, such as 10 mm.

iii. Make sure you are measuring from the end of a tick mark to the end of the other tick mark (in other words, your drawn line should be perfectly perpendicular to both tick marks). Do not measure from the middle of tick marks.

Line shown may be a different color and thicker than the line you create.

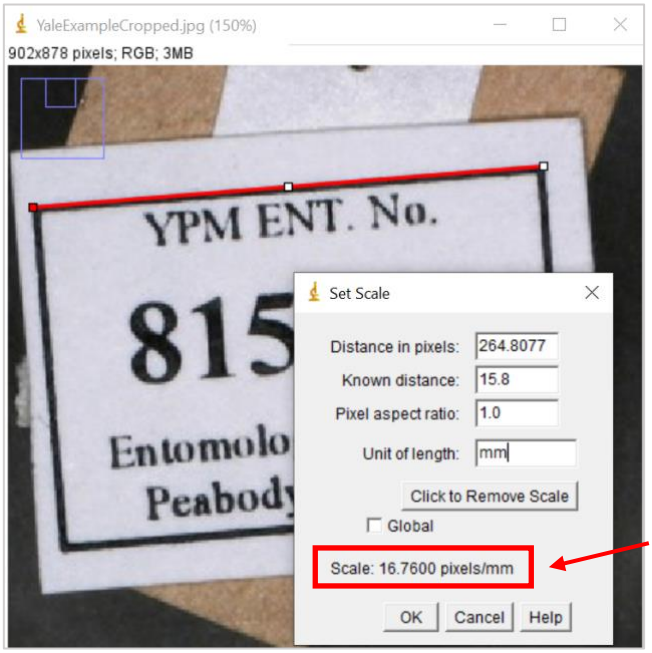
iv. You can adjust the location of the ends of your line by hovering until you see a pointing finger symbol. Click and drag to move the end points around.

v. Some of the images from Yale Peabody Museum are missing their scale bar. However, we do know the width of the black box framing the label information. If you have one of these specimens, draw your line to measure the total width of the top of the black box. This distance = 15.8mm.




Red line measuring the top of the black label box = 15.8mm

c. Once you've drawn your line of known length, navigate to **Analyze >> Set Scale…**




i. The distance in pixels is already filled in for you based on the length of your line.

ii. Fill out the **Known Distance** (this is the measurement of the line you drew based on the scale bar).

iii. Fill in **Unit of Length**: this is the unit of the scale bar (e.g. mm).

iv. Once you fill in your *Known Distance* and *Unit of Length*, Image J will calculate the pixel/mm at the bottom of the dialog box for you. Record this value in the **pixel/unit** column.

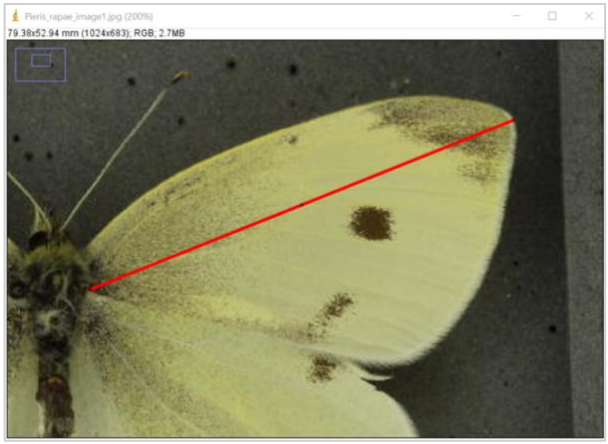
v. Click **OK**.

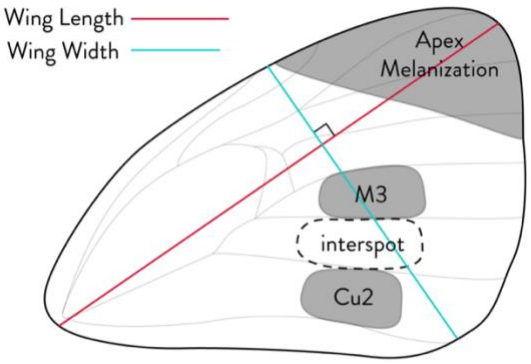
*Note: if you ever need to repeat measurements on this image later, you can set the scale without measuring the scale bar over again using this value. Simply put the **pixel/mm** value into the **Distance in pixels** field and the value **1.0** into the **Known Distance** field. Add the unit and set the scale.

## 6. Measure wing length

a. Use the **Line Tool** to draw a line from the point where the forewing and hindwing touch to the apex of the wing.

i. You are trying to *maximize* this measurement. Place the point at the point where the wings touch first and then click and draw to the wingtip.





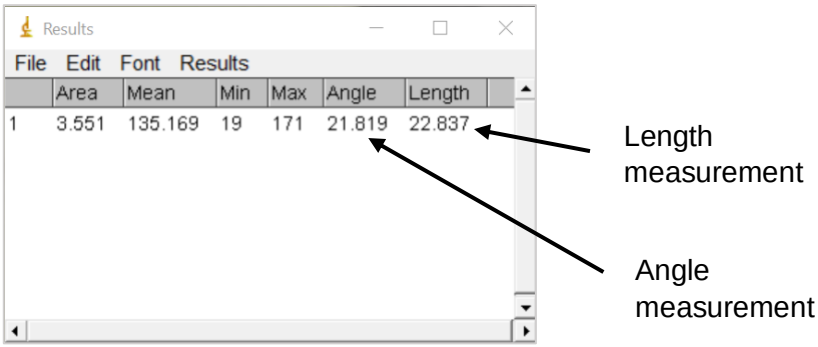

ii. The line should extend to the last pixel that shows well defined butterfly wing color. This may include what appears to be a fringe at the outer edge of the wing.

iii. If you need to adjust your points you can click and drag to edit them.

iv. As you move your cursor around, the length measurement will be displayed in the ImageJ toolbar. Keep an eye on this measurement and place the end of the line where the measurement is maximized. Of course, make sure your point is still on the edge of the wingtip.

b. **Analyze >> Measure** and record the length in your spreadsheet.

i. Take note of the *angle* measurement, you will need it in **step 7**.

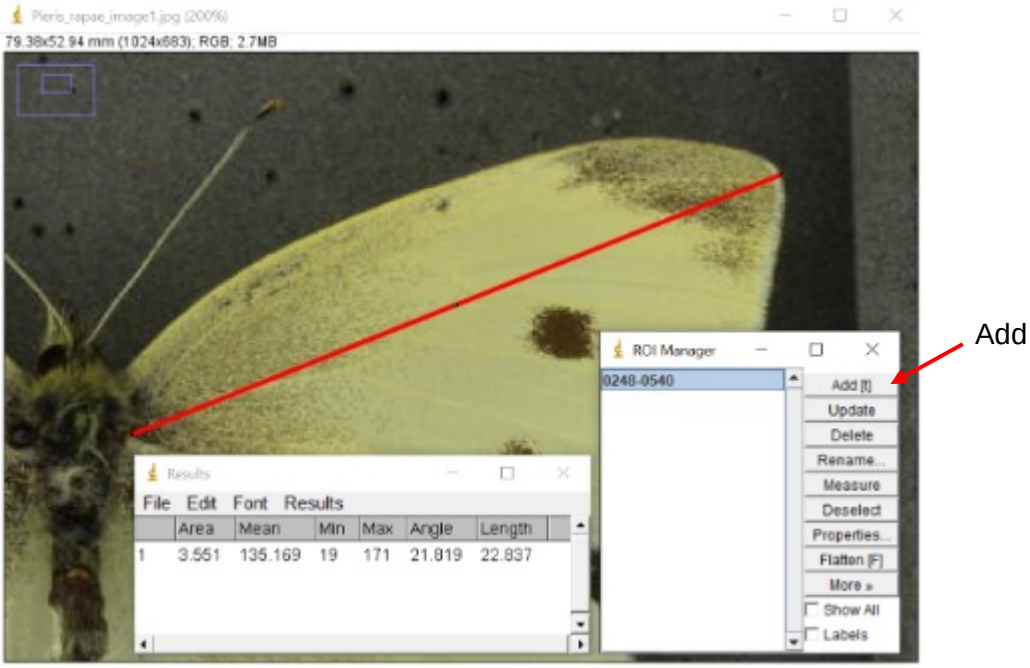


c. Once you're all set, navigate to **Analyze >> Tools >> ROI Manager**.

   i. On the **ROI Manager**, click **Add**.

   ii. This will save this measurement for the session.

   iii. Leave the **ROI Manager** window open.




d. Navigate to **Image >> Overlay >> From ROI Manager**.

   i. This will keep your old lines on the image as you take new measurements.

   ii. Check the **Show All** box in the **ROI Manager** to overlay all your measurement lines.

   iii. You can change the colors and names of measurements saved in the **ROI Manager** to help you keep track of them, if you want, using **Properties**.

## 7. Measure wing width

a. This measurement will be the maximized width of the wing taken ***perpendicular to the wing length measurement***. Find your angle measurement from step 6b to complete the following calculation.

   1. Right wing: $|angle - 90|$
      Example: $|21.819 - 90| = |-68.181| = 68.181$

   2. Left wing: $-(angle + 90)$
      Example: $-(141.246 + 90) = -231.264$

b. Double-click the **Fixed Angle Macro Tool** (looks like two parallel lines).

i. Set the angle by entering the **absolute value** of the angle you calculated above (Example: 68.181).

ii. When you click and drag, a line of fixed angle will be drawn. This line should be perpendicular to your wing length measurement.

c. Draw your width measurement. Use **Analyze >> Measure** to measure your line. You will have to repeat this several times to ***maximize*** the length of this measurement. Record the width in your spreadsheet.

d. When you have drawn and measured the maximized wing width, click **Add** on the **ROI Manager**.

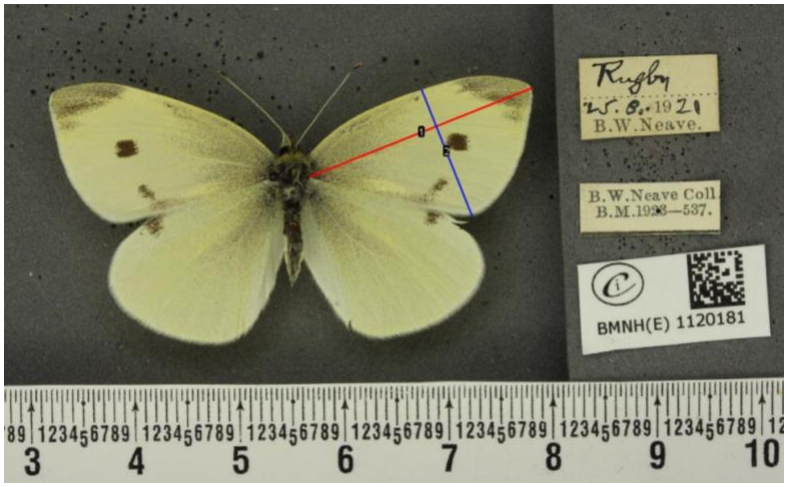
e. Save a jpeg of the image including the length and width lines.

i. Method 1: **File >> Save As >> Jpeg.** Include 'measured' in the file name to distinguish it from the original downloaded image.

ii. Method 2: Save a screenshot showing your image.

1. Name the file using your course's naming convention and submit the image if required by your instructor.

2. Double check to see if the lines are on your image. If not, you probably skipped step 6d (**Image >> Overlay >> From ROI Manager)**.




## ADDITIONAL RESOURCES

ImageJ User Guide

## REFERENCES

ImageJ. (1997). National Institutes of Health. https://doi.org/10.1038/nmeth.2089

Trillo, P.A., Sorojsrisom, E., Jordan, C.N., & Krumm, J.L. (2022). Sexual dimorphism CURE: Exploring melanized wing patterns of Pieridae butterflies. BCEENET- Biological Collections in Ecology & Evolution Network, QUBES Educational Resources. https://qubeshub.org/publications/3250/1