



# SOP: Dissection and measurement of mosquito wings

May 2023



Version	Date	Reviewed by	Institution
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### Version Control<sup>1</sup>

Version	Date	Updated by	Description of update(s)
1	16 <sup>th</sup> June 2023	Jack Gillespie	Added sections on ImageJ

### Related documents

- I2I Best Practice SOP Library, 31/05/2023 (<https://innovationtoimpact.org/>)
- GXCAM ECLIPSE User Manual
- GXCapture-O User Manual

## 1. Purpose

Wing length measurements are used to estimate the size of adult mosquitoes which is a useful proxy for insect quality and can be used to monitor consistency of adult mosquitoes reared for testing. The purpose of this SOP is to detail the wing dissection procedures and detail how to take a wing measurement using the GXCAM software.

## 2. Materials and equipment

### Materials for mosquito wing dissection

- Standard stereo microscope
- Glass microscope slides
- Fine dissection pins
- Fine-tipped dissection forceps
- Permanent marker pen

### Materials for wing photograph and measurement

- Calibration slide containing a micrometer with every division at 0.1mm

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<sup>1</sup> Historical versions of SOPs can be found on the I2I website (<https://innovationtoimpact.org/>)

- Dissection pins
- GXCAM ECLIPSE
- Laptop or PC with GXCapture-O software installed.
- Camera (GXCAM ECLIPSE, GT Vision) and camera mount
- Micro USB cable
- 0.5x minifier
- 30mm adaptor

### 3. Procedure

#### 3.1. Mosquito storage

- 3.1.1. Mosquitoes should be stored in 25ml falcon tubes, with silica beads in the bottom covered by cotton wool, and labelled with the following: colony name, generation number, cage number, sex, date, bioassay information (or relevant corresponding ID sample number).
- 3.1.2. Tubes should then be placed into a zip-lock bag and placed into the freezer for one hour to ensure mosquitoes are killed quickly.
- 3.1.3. After one hour, remove mosquitoes from the freezer and store at room temperature until ready for dissection.
- 3.1.4. *Note: storing mosquitoes at room temperature allows the body to dry out, preventing mosquitoes from becoming sticky. If mosquitoes are stored in the freezer long-term, they will need to be dissected while still frozen, otherwise they will stick together as they defrost.*

#### 3.2. Wing dissection

- 3.2.1. Place mosquito on a glass microscope slide under the dissecting microscope.
- 3.2.2. Using a fine-tipped dissection pin, pierce the mosquito through the thorax to hold it in place.
- 3.2.3. Using either another fine-tipped dissection pin or fine-tipped dissection forceps, tease the right-hand wing away from the thorax at the wing base. Be careful not to tear the wing or the apical node.
- 3.2.4. Follow the same process as in Step 4.2.3, remove the left-hand side wing. Left-hand side wings will not be measured unless the right-hand side wing is damaged. If this is the case, make a note in your record sheet. Both wings will be photographed for future use investigating wing morphometrics.
- 3.2.5. Once both wings have been separated from the thorax, use the fine-tipped forceps to transfer them to the calibration slide, that has the micrometer on, for photographing. Ensure that the orientation of both wings is consistent throughout all wing measurements. Place the left-hand side wing above the right-hand side wing for photographing (Figure 2).

#### 3.3. Microscope set-up

- 3.3.1. Remove the plastic cover at the bottom of the microscope camera.
- 3.3.2. Connect microscope camera with the 0.5x minifier. Attach this minifier into the 30mm adaptor.
- 3.3.3. Put the adaptor, assembled with the microscope camera, on the stereo microscope.
- 3.3.4. Attach the camera to the laptop using the USB cable.
- 3.3.5. Turn the laptop on and open the GX-CAM software.

### 3.4. Taking a picture

- 3.4.1. Place the slide with dissected wings onto the microscope, so it is under the field of view of the camera.
- 3.4.2. Under the 'Preview' tab, you will now be able to see the wings on the screen.
- 3.4.3. Focus the microscope so that both wings are clear and in focus.
- 3.4.4. Click on the camera symbol to take an image of the wings.
- 3.4.5. Check the quality of the image is suitable for taking measurements, then rename the picture by right-clicking on the image thumbnail and selecting 'rename' from the drop-down menu. Images should be labelled with sample ID numbers.

### 3.5. Calibration function

- 3.5.1. Under the same working conditions as for sample pictures (same objective lens, magnification, and camera resolution), take a photograph of the calibration scale. This is called a benchmark picture and it used to set the measurement scale for pictures.
- 3.5.2. Open the benchmark picture and click "Measure" on the left-hand side toolbar of the screen. Then click on the calibration button to display the calibration set up as below.
- 3.5.3. Draw a straight line along the whole 1mm calibration scale on the benchmark picture. Enter the name of the created calibration (date and initials) and selected scale length (mm).
- 3.5.4. Click 'add' to add the criteria defined in the benchmark picture to the calibration sheer of the software and save it for later use.

### 3.6. Wing measurement

- 3.6.1. Open your picture to measure (in the 'Edit' tab) and click the measuring button on the left toolbar. Click on the calibration button to set the criteria saved in the previous steps.
- 3.6.2. Select the straight-line measurement button.
- 3.6.3. Measure the length of the right-hand wing as shown in the image below (green measurement line, Figure 1). Measured data will be shown in the toolbar on the right side of the screen.
- 3.6.4. *Note: each time you finish measuring a wing you will need to go back to the 'Preview' tab to take additional pictures.*
- 3.6.5. *Note: if at any time you make a mistake while measuring a wing, click on either the right or left arrow. This will cause a pop-up window to ask whether you want to save the image. Click 'no' and then locate your image and start the measurement again.*

### 3.7. Exporting the measurement result.

- 3.7.1. Click on either the 'Word Export' or 'Excel Export' button on the right of the toolbar to export measurement data to Word or Excel and save.

### 3.8. Wing Length Measurement using ImageJ

- 3.8.1. If ImageJ is being run for the first time, install the macro "Rename Labels in Results Table.txt". Go to the folder where ImageJ is installed and select the Macros Folder. Copy the .txt file into the Macros Folder. If you do not have the txt file a copy can be found in section five below.
- 3.8.2. Open ImageJ and select Plugins, Macros and Install. Select the "Rename Labels in Results Table.txt".

- 3.8.3. When opening ImageJ, run the macro "Rename Labels in Results Table.txt". This will ensure that measurements are labelled with the file name automatically.
- 3.8.4. Open a folder containing the images of wings you wish to measure
- 3.8.5. Under analyze, select Set Scale and enter the distance in pixels, known distance and unit of measurement, usually 1mm. Ensure that Global Box is ticked.
- 3.8.6. Select the straight line measurement and click and hold the left mouse button whilst measuring the wing as per Figure 1, then press Control + M to measure the distance.
- 3.8.7. You can open the next file in the folder by pressing Control, Shift and O
- 3.8.8. Repeat until all wings are measured.
- 3.8.9. In the results tab, select File Save As, enter a file name describing the data and press save. Note you only require the label and length columns in this spreadsheet.

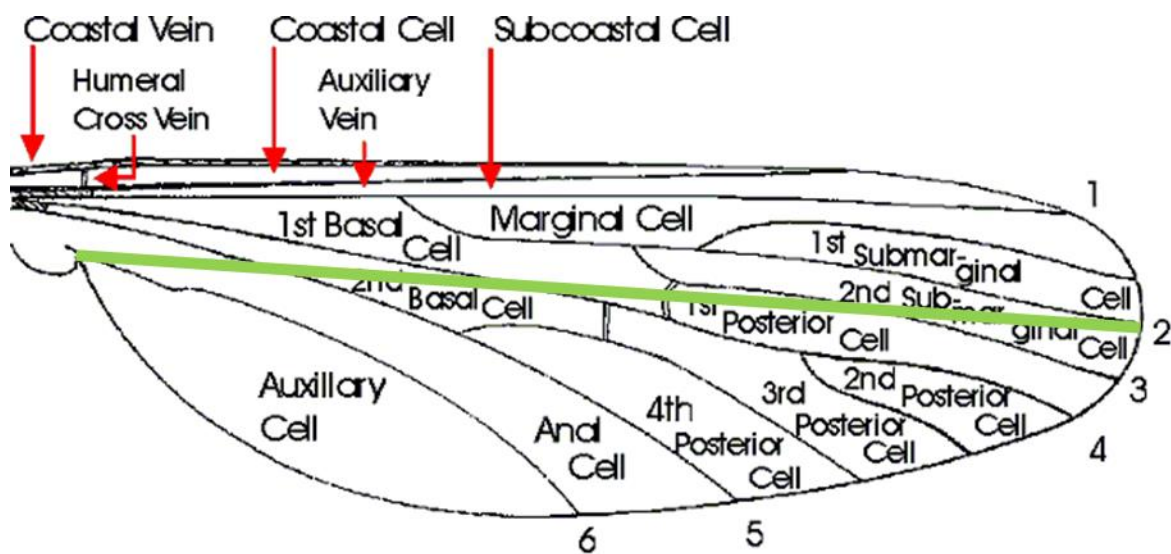


Figure 1. Wing measurement position.

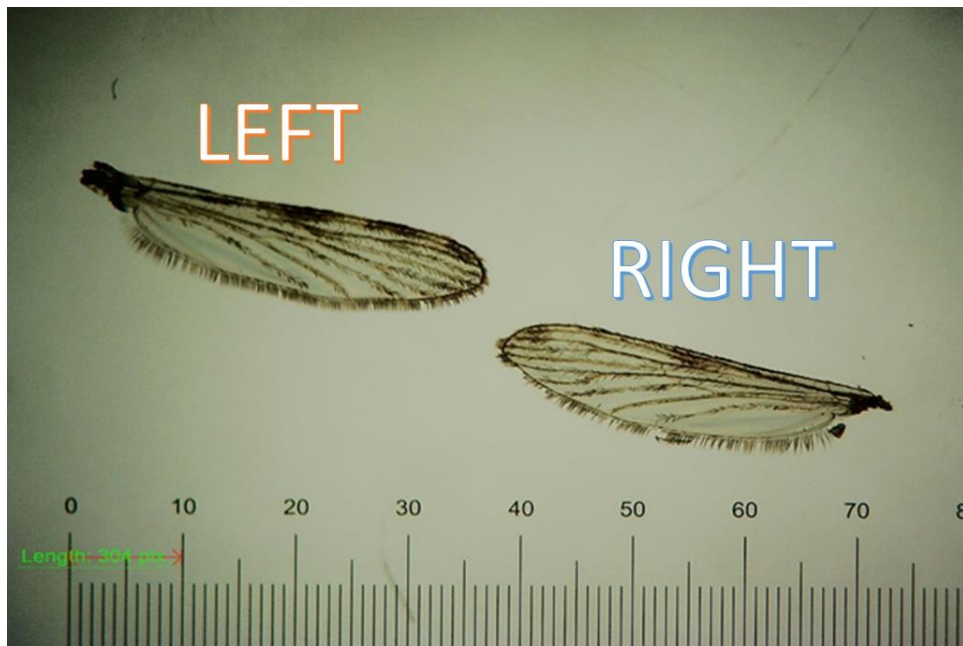


Figure 2. Wing position for photographing with micrometer.

#### 4. Additional data collection

#### 5. Macro to automatically label measurements with the file name

- To install the macro for ImageJ create a .txt file by opening notepad, and copying and pasting following into the .txt file, and saving the .txt as "Rename Labels in Results Table"

```
macro "Rename Labels in Results Table" {
  for (i=0; i<nResults; i++) {
    oldLabel = getResultLabel(i);
    delimiter = indexOf(oldLabel, ":");
    newLabel = substring(oldLabel, delimiter+1);
    setResult("Label", i, newLabel);
  }
}
```

A userguide to ImageJ can be found at this link, as of 16<sup>th</sup> June 2023  
<https://imagej.nih.gov/ij/docs/guide/>

#### 6. Deviations from standard protocol

#### 7. Safety notes

- Do not use alcohol or other organic solvents to clean the camera. If the lens is dirty or damp, use a dry, non-linen fabric or professional lens tissue to wipe clean.

- Excessive temperature and humidity will damage the camera lens. Store and use at conditions of temperature 0°C - 40°C, relative humidity 5% - 85%.

## 8. Glossary of terms

<b>I2I</b>	Innovation to Impact
<b>ID</b>	Identification
<b>PC</b>	Personal computer
<b>SOP</b>	Standard operating procedure

## 9. References