BCEENET
BIOLOGICAL COLLECTIONS IN ECOLOGY AND EVOLUTION NETWORK

Instructor Guide- Measuring Size and Area of Digitized Specimens using ImageJ

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INTRODUCTION

In this activity, students will learn to measure morphological characters using the open access program ImageJ. Students will practice using essential ImageJ tools as they analyze wing size, wing spot areas, and wing apex melanization in images of *Pieris rapae*, the cabbage white butterfly. While a single species is used in this example, the image analysis skills learned are transferable to other organisms and other morphological measurements. This resource can be used as part of the implementation of a BCEENET CURE, or as a standalone guide for introducing students to specimen analysis using ImageJ.

***Intended Audience*:** Undergraduate, all levels. Suitable for in-person, hybrid, or online courses in biology or environmental science.

***Learning Time*:** Flexible, 2-3 hours

***Required Resources*:** Access to computers and the open access program ImageJ

STUDENT LEARNING OBJECTIVES

- Interpret images of *Pieris rapae* to determine suitability for image analysis
- Practice using several measurement tools (line, fixed angle line, wand, versatile wand) in ImageJ
- Gather data on wing size, spot area, and apex melanization of *P. rapae* specimens

CURRICULUM MATERIALS PROVIDED

- Installing ImageJ and Tools
- Fixed Angle Line Tool macro file
- Measuring Wing Size using ImageJ
- Measuring Wing Spot Area using ImageJ
- Measuring Apex Melanization using ImageJ
- Image Analysis Assignment Spreadsheet
- Image Analysis Assignment Key

IMPLEMENTATION NOTES

Installing ImageJ

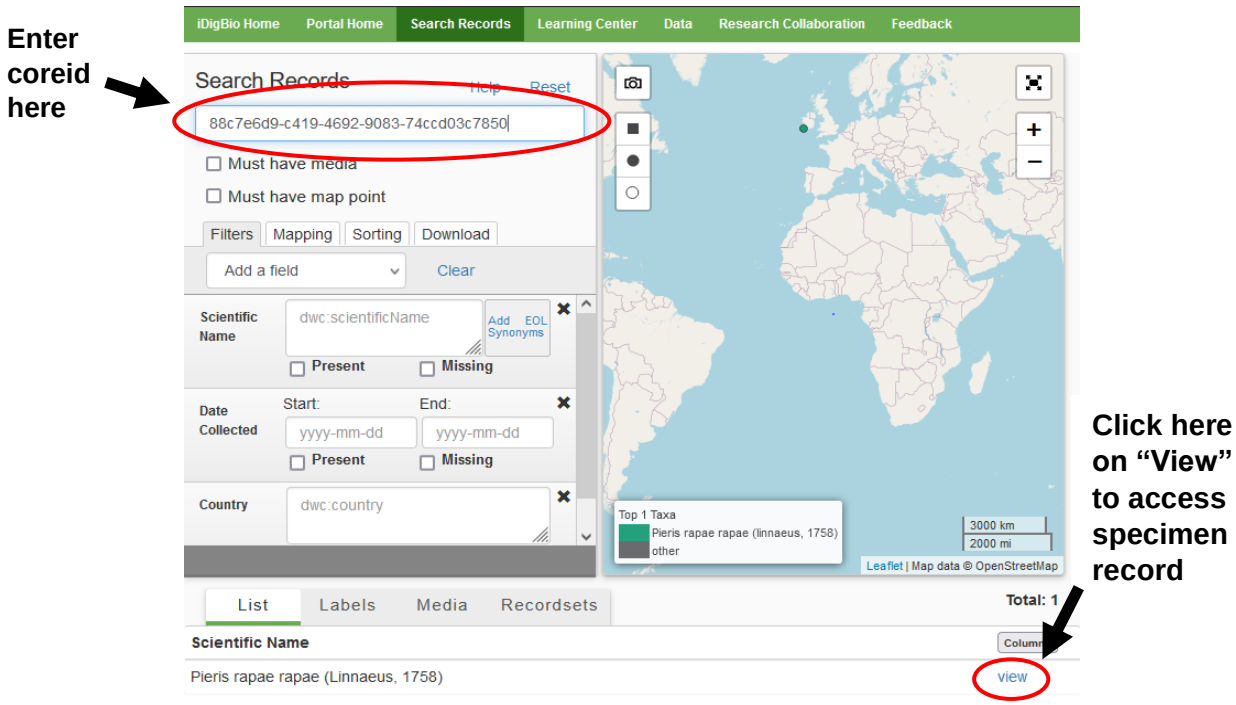
Installing ImageJ should take place **before** the class period in which you intend for your students to complete the assignment. If you are planning on your students installing and collecting data on their own computers, be aware that ImageJ cannot be installed and used on Chromebooks or mobile phones. Students without access to appropriate electronic resources should be identified confidentially in advance and alternative computer access arranged.

Installation of ImageJ on student computers usually requires some basic troubleshooting. Solutions to common issues are addressed in the **Installing ImageJ and Tools** tutorial. Additionally, more extensive instructions and a troubleshooting guide are available on the ImageJ website for both Windows Installation and Mac OS Installation.

To complete the **Installing ImageJ and Tools** tutorial, students will need access to the ***Fixed Angle Line Tool.ijm*** file. A copy of the file is provided as one of the curriculum tools and it is suggested that you provide access by uploading it to your course management system or course webpage. If instead you choose to provide students with a link to the activity QUBES page, be aware that students will also find the **Image Analysis Assignment Key** in the same location.

ImageJ Assignment

Students can access digital images for morphological analysis through the iDigBio search portal. If you are using the Image Analysis Activity as a stand-alone assignment, the **Image Analysis Assignment Spreadsheet** provides students with a selected set of specimen images through iDigBio. For each specimen, students will copy the “coreid” from their spreadsheet and paste it into the “search all fields” search field at the top of the webpage. This will produce a single specimen record result, which can be viewed by clicking the “view” button on the bottom right of the page. The specimen image can then be downloaded from the specimen record page.



Alternatively, the specimen images can be downloaded in advance and provided to the students through the course management system.

Students can enter their specimen measurements on the **Image Analysis Assignment Spreadsheet** and a key to the measurements is provided in the **Image Analysis Assignment Key**. There should be a small amount of variation between student results and the answers provided in the key.

If your students are using the ImageJ Analysis Activity as part of the BCEENET Sexual Dimorphism CURE or another research project, you can substitute images from your project and your own data collection spreadsheet in the assignment.

Classroom Management

This is a flexible assignment- you may include as many or as few specimen records as you wish. Additionally, the sections on Measuring Wing Spot Areas and Measuring Apex Melanization can be excluded from the activity if they are not needed to meet your student learning goals or to reduce the amount of time needed for this activity.

Students can complete the activity individually or as a 2-4 person team, depending on the number of specimen records included in the activity.

The sections of this activity should be completed in the following order:

1. ***Installing ImageJ & Tools***
    - This step should be completed prior to the class session where data is collected on the specimen images.
    - If students are installing the ImageJ on their own computers, we recommend providing time in class or office hours to help students troubleshoot the installation.
2. ***Measuring Wing Size***
    - This section includes directions on setting the scale and determining the suitability of an image for data collection.
    - Directions for measuring wing length and width are usually the most straightforward for students to understand, making this a good starting point for analyzing images.
3. ***Measuring Wing Spot Area*** and/or ***Measuring Apex Melanization***
    - These two sections can be included in either order.
    - One or both sections may be excluded from the assignment to reduce work time or if not needed for your course learning objectives.

Potential Student Challenges

Low-resolution images

When students are downloading their images from iDigBio, they need to be sure to download the high-resolution images, not copies of the thumbnail images. If your students complain of low-resolution images, make sure they click on the images to access the high-resolution versions before downloading and saving.

The upside-down butterfly

The last specimen record on the assignment spreadsheet (row 5) only shows the ventral surface of the butterfly. Therefore, the measurements cannot be completed on this specimen. This specimen record was included to raise student awareness of the challenges associated with using specimen data from online natural history collections data portals and similar resources. The data must always be assessed with a critical eye to determine its usability in your study.

Carefully follow the directions

If students are rushing through the assignment, they often skip steps, leading to ImageJ problems. This can lead to high levels of frustration. It is important for students to walk through every step in the procedures carefully. We recommend walking through data collection on the first specimen with your students, step by step, to increase their chances of success the first time through.

Potential Discussion Topics

Discussions on the accuracy, precision, and repeatability of student measurements can be helpful for student self-assessment of new research skills. You may consider looking at the variability between measurements of a single specimen across the students in your classroom.

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