



# Investigating MicroStructures

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[MicroPlants.fieldmuseum.org](http://MicroPlants.fieldmuseum.org)

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A Citizen Science Project

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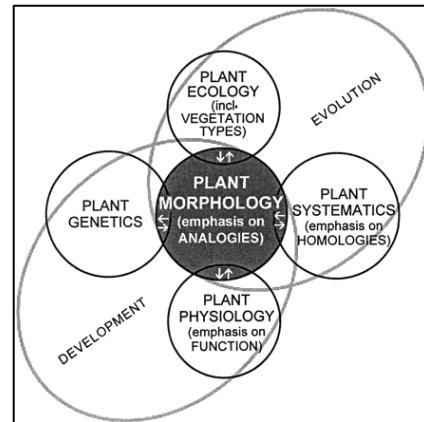


# Early Land Plants Today

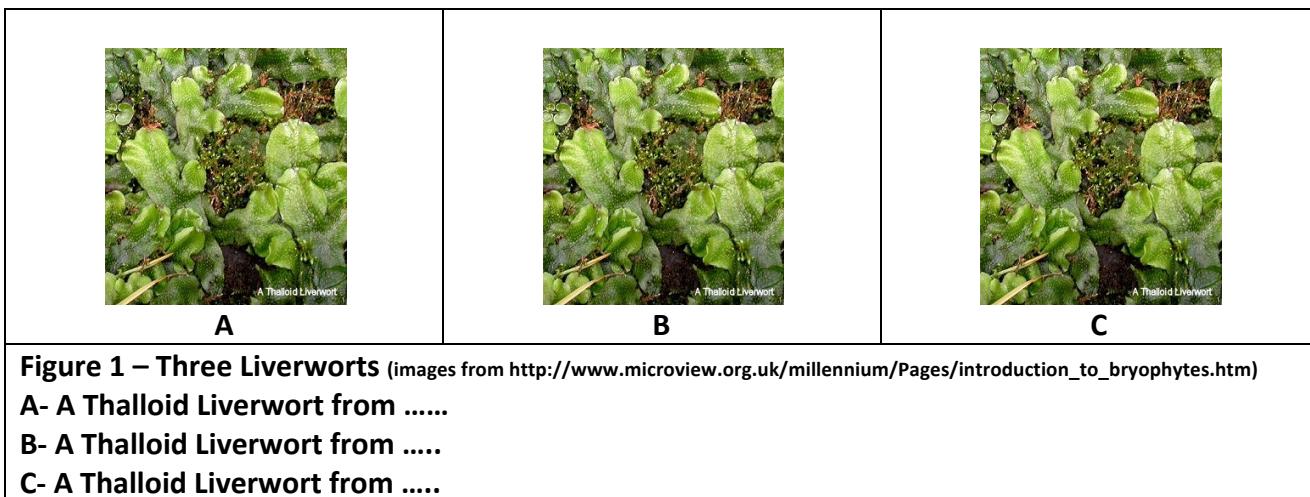
## Contributing Data to an International Project Documenting Biodiversity

### Introduction

The classification of organisms based on natural relationships utilizes multiple lines of evidence such as morphology and anatomy, chemistry, physiology, DNA, ecology, distribution patterns etc. This laboratory exercise focuses on morphology - a branch of biology dealing with the study of the form and structure of organisms and their specific structural features. Morphology is fundamental in disciplines such as systematics (the study of the diversification of living forms, both past and present, and the relationships among living things through time). Here we investigate some morphological characteristics associated with selected early land plants (liverworts), focusing on a genus called *Frullania*.



**What's a Liverwort?** All land plants are classified into one of two groups. Vascular land plants, characterized by the compound lignin, contain special water transport cells that give them the ability to grow to great heights and in arid environments. Non-vascular land plants, on the other hand, are typically found in wet areas and are much smaller. Non-vascular land plants are called *Bryophytes* and can be further classified as Mosses, Hornworts, or Liverworts. Did you know that every Bryophyte spends most of its life as a haploid organism? These special organisms can be found all over the world from Fiji to Alaska. There might even be some in your backyard! With so many different organisms, scientists need your help to learn about each one. Your participation in the project will help groups of scientists learn more about each of these species and the differences between them. Who knows what we will discover?!



**Figure 1 – Three Liverworts** (images from [http://www.microview.org.uk/millennium/Pages/introduction\\_to\\_bryophytes.htm](http://www.microview.org.uk/millennium/Pages/introduction_to_bryophytes.htm))

**A- A Thalloid Liverwort from .....**

**B- A Thalloid Liverwort from .....**

**C- A Thalloid Liverwort from .....**

**The Ecological and Biological Significance of Early Land Plants (Bryophytes):** Bryophytes play a major ecological role. The small size of these organisms enables them to respond rapidly to environmental and ecological change offering them great utility in conservation science. They have been used as indicators of past climate change and as early indicators of global warming. A growing body of evidence also identifies liverworts as the earliest diverging lineage of bryophytes, so they are considered evolutionary very significant. Chemical compounds extracted from liverworts also exhibit important biological activity. For example, chemical extracts derived from *Frullania* species have been found to have cytotoxicity against certain cancer cell lines, and significant antimicrobial and antifungal activity

## Objectives

- **Develop** an understanding of using software to capture and measure data electronically.
- **Evaluate** the accuracy of the software Image J.
- **Perform** measurements on a series of morphological characters from digital images.
- **Record** observations on a series of morphological characters from digital images.
- **Discuss and analyze** the variability of morphological characters.

## Materials

- Computer with Image J installed.
- Folder with digital images of selected species of early land plants.
- Instructions for Image J and laboratory.

## Instructions and exercises

### Exercise 1

- 1) Read the instructions on how to set the scale of the software.
- 2) Open Image J.
- 3) Using the image file labeled “scale bar”, set the scale and test it as instructed in the manual. Measure the full length of the scale bar in the image.

#### Record the following:

Distance in pixels: \_\_\_\_\_

Known distance: \_\_\_\_\_

Unit of length: \_\_\_\_\_

Scale: \_\_\_\_\_ (pixels/unit of length)

#### How many microns in a millimeter?

#### Why is it important to calibrate the software with the images?

### Exercise 2

- 1) Open the folder named “Plant habit”.

- 2)** Using Image J, for each of the images, measure the length and width of FIVE structures called lobules, as indicated. Remember you have to set the scale. Use the scale bar embedded in the image to set the scale. Record the units.

**Results:**

Lobule No.	Species a		Species b	
	Length	Width	Length	Width
1				
2				
3				
4				
5				
Mean				

Provide the mean of both the length and the width for both species. Which species has the larger lobule? \_\_\_\_\_

**Observations:**

Looking at each image, would you describe the margin of the underleaves as toothed or entire?

Underleaf margin	Species a	Species b

**Exercise 3**

- 1)** Open the folder named “Cell anatomy”.
- 2)** Using Image J, for each of the images, measure the cell length and width as indicated. Measure the five cells indicated in the image. Remember to set the scale. In the image, one unit = 10 µm

Cell no.	Species a		Species b		Species c	
	Length	Width	Length	Width	Length	Width
1						
2						
3						
4						
5						

Mean

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- 3) How does it compare to cell size in other organisms?
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### Observations

Count number of oilbodies for five cells, as indicated. These are specialized structures containing various chemical compounds within each cell.

Cell No.	Number of oilbodies per cell		
	Species a	Species b	Species c
1			
2			
3			
4			
5			
Mean			
Range			

Do you observe any other note worthy differences between these structures from the different species?

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The chemicals extracted from the oilbodies have active biological properties. List three types of biological activity these chemicals exhibit.

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### Exercise 4

- 1) Open the folder named "spores".
- 2) Using Image J, for each of the images, measure the length and width of the spores, as indicated. Remember you have to set the scale.

Species a		Species b		Species c		Species d	
Length	Width	Length	Width	Length	Width	Length	Width

**3) Count number of rosettes**

No. of rosettes	Species a	Species b	Species c	Species d

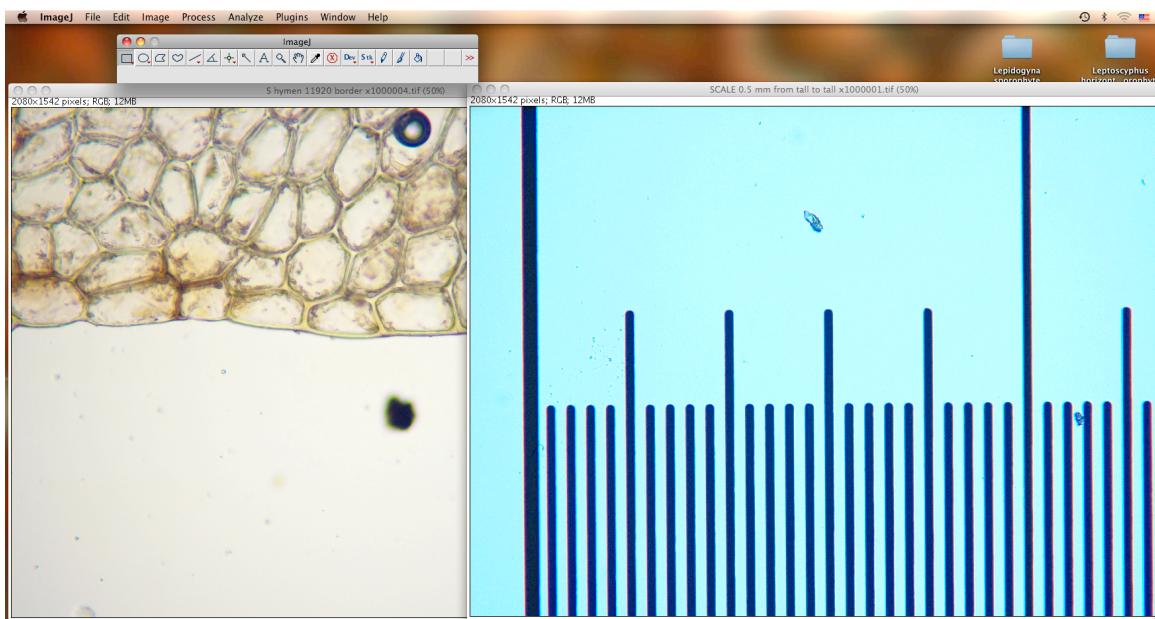
# **Image J for Students: How to set a scale and do basic measurements**

ImageJ is a freely available program developed by NIH, for the purpose of making data from images easily measurable. This has given the scientific community a way of creating measurements easily replicable and comparable across diverse fields. From measuring astronomical data to analyzing gel electrophoresis to measuring cell sizes to creating 3D models, ImageJ streamlines the process of turning an image into a set of numerical data. This data, in turn, can easily be analyzed and compared to other data sets.

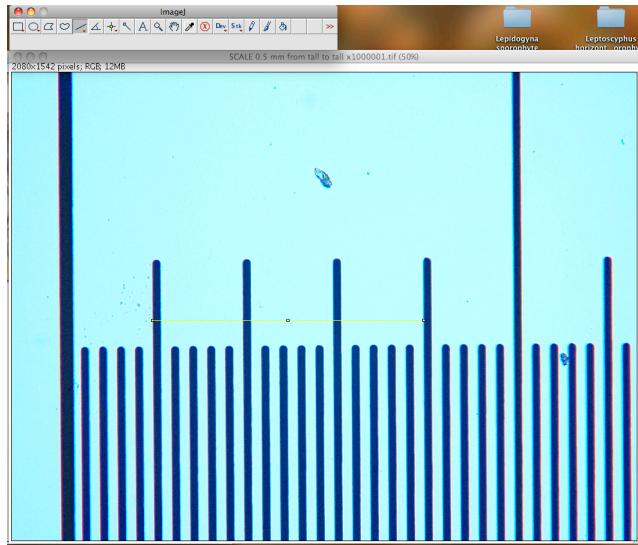
Image J is therefore used in a vast array of the sciences, including medical and biological sciences.

Using this program, you can easily gather measurements from an image using real values ( $\mu\text{m}$ , cm, etc.). The process involves calibrating a single image against known values, then applying that calibrated image to your unknown image. In order to accomplish this, both images must be the same magnification. Here's an example.

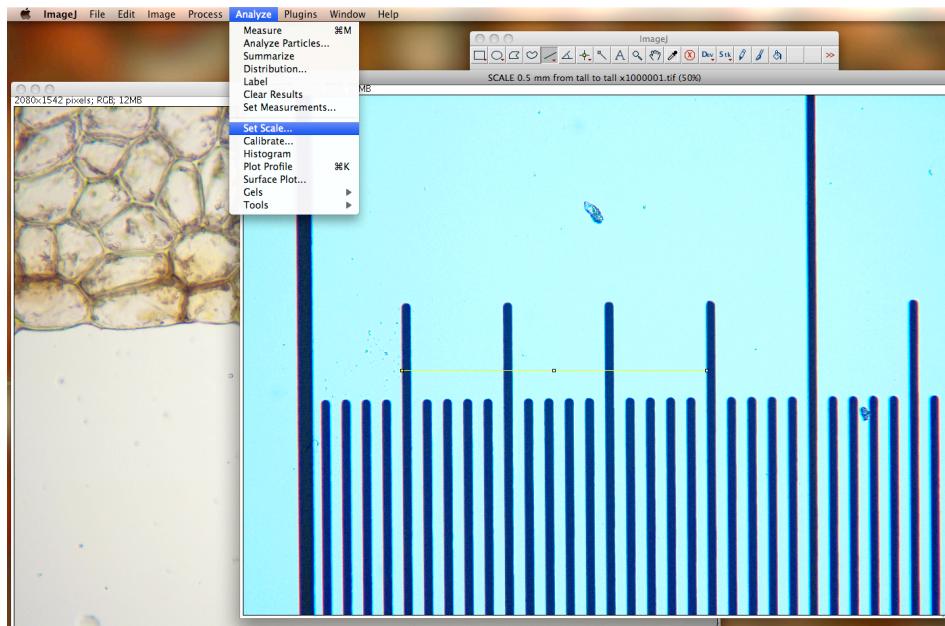
- 1) Open your image as well as a calibrated image in ImageJ. This can be done by dragging your images over the ImageJ icon and dropping them.



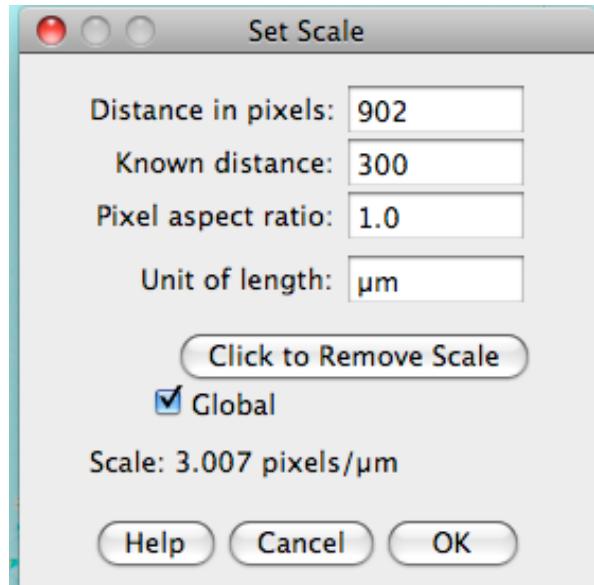
2) Use the line selection tool to draw a selection a known length. Here we have drawn a 300 $\mu$ m line



3) With the selection line, select “Set Scale from the Analyze menu...



4) ...which will bring up the dialog box. Fill in the known distance you measured and the unit of length. Checking “global” will apply this scale to all open windows.



4) Now you are ready to measure! Use the line tool to draw the desired length/width to be measured, hit command+m or go to “Analyze” menu and select “Measure”. Your measurement will appear in units you chose previously in a new box, under “Length”.

