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Using Foldscopes™ For Offline Exploration in Online Biology Courses

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A Foldscope™ is a portable microscope that is assembled from paper and a small lens; it can be used alone or in combination with a smartphone or tablet camera. Foldscopes™ can magnify up to 140X with a resolution of 2 microns, producing images comparable to those obtained with a basic light microscope. Foldscopes™ can provide students with direct access to the microscopic world via a safe, inexpensive activity that they can participate in without supervision, which makes them especially useful for online courses where students may not have access to standard laboratory equipment. In our online introductory courses for mixed majors, we use Foldscopes™ to observe chloroplasts in plants and algae and pigmented epidermal cells in red onion (*Allium cepa*), and guide students to consider the evolutionary and physiological significance of their observations. In the workshop, participants will assemble their own Foldscopes™, work through our students' activities, and provide suggestions for using Foldscopes™ in other contexts (such as field work, experimental work, face-to-face settings, and with other organisms); these suggestions will be included in the revised workshop manuscript.

Keywords: Foldscope™, microscopy, cell structure, field studies, online, citizen science

Introduction

Light microscopy is an engaging activity that provides novice and expert biologists alike direct access to the microscopic world. In introductory biology, we use light microscopy to explore diversity at the organism and cellular level - which, for many organisms, are one and the same. Our two main learning goals for students are proficiency in (1) using the microscope and (2) interpreting the images. In online courses, these learning goals can be addressed by using virtual tools and digital images (Mione et al. 2011; Mione et al. 2013), but students may not have the opportunity to work with real equipment or specimens.

The Foldscope™ was invented by Stanford University engineer Manu Prakash[1], who, after visiting remote clinics in Thailand where expensive equipment sat unused for fear of damage and the need for repair (Yong 2015), recognized the need for a decent instrument that was also portable, durable, and inexpensive. By 2014 Prakash's group had developed early prototypes, and by 2017 they had refined the manufacturing and shipping process to the point where Foldscopes™ could be produced at scale and distributed worldwide.

Workshop Format

In this workshop, we will present our use of Foldscopes™ in our online courses and, with participant input, explore options for using Foldscopes™ in other contexts. For our online introductory courses, we developed one *de novo* lab using the Foldscope™ (Chloroplast Diversity) and modified two existing labs (Microscopy; Osmosis and Diffusion) to incorporate Foldscope™ work. The student activities in the three labs are similar: for each lab students collect and prepare their own specimens, observe them with the Foldscope™, capture an image, and share their images with their peers via the online platform Padlet (www.padlet.com). Students also work with digital images and other supplemental material to situate their Foldscope work within a broader conceptual context.

During the workshop, participants will assemble a Foldscope™ and work through our students' activities. We will present the Chloroplast Diversity lab in full, and

[1] https://web.stanford.edu/group/prakash-lab/cgi-bin/labsite/

the Foldscope™ activities (estimating specimen size and observing plasmolysis in plant cells) from the other two labs. We will also work with participants to identify ways that Foldscopes™ can be used in different contexts: face-to-face courses, experimental work, fieldwork, upper-level courses, with different specimens, etc. The revised manuscript for the workshop will include participants' ideas for using Foldscopes™.

This is a 'Bring Your Own Device' workshop; participants require a mobile device to take photos of their specimens, upload them to Padlet, and access the online versions of the activities presented. Foldscopes™ will be provided, and participants can keep them.

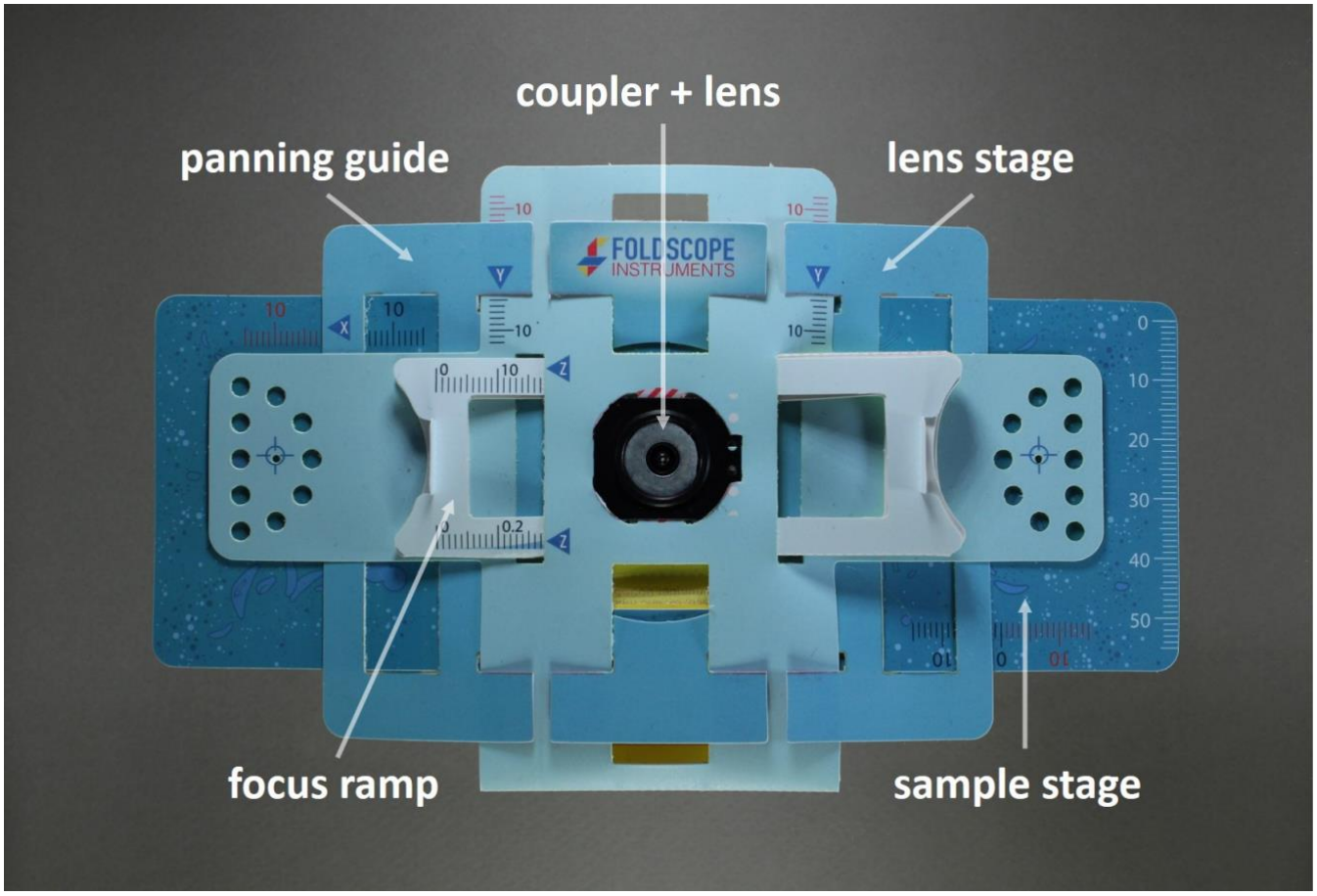


Figure 1. An assembled Foldscope™; photo by Ronnie Van Dommelen.

Student Outline
Activity 1: Chloroplast Diversity Lab

NOTE: This Student Outline is modified from the online student version of the lab. A link to the online workshop version can be provided upon request to the corresponding author (Jennifer Van Dommelen; jennifer.vandommelen@dal.ca).

Students work with a separate questions document concurrently with the online content (Appendix A) and submit that document for grading. For an offline adaptation of the lab, the student questions could be interleaved with the instructional material.

Chloroplast Diversity

In this lab you'll use your own portable microscope to explore the microscopic world and share what you find with the rest of the class. You'll also work with micrographs to explore aspects of chloroplast morphology in different plant and algal tissues.

Learning Objectives

After completing this lab you should be able to:

1. Assemble a portable microscope, collect and prepare specimens, and capture and share images of what you find.

2. From micrographs, recognize and compare chloroplasts from a variety of plant and algal species.

3. Use Fiji/ImageJ software to collect data from images.

4. Relate quantitative and qualitative data about chloroplasts to cell growth, structure, and function, and to phylogenetic relationships among groups of photosynthetic organisms.

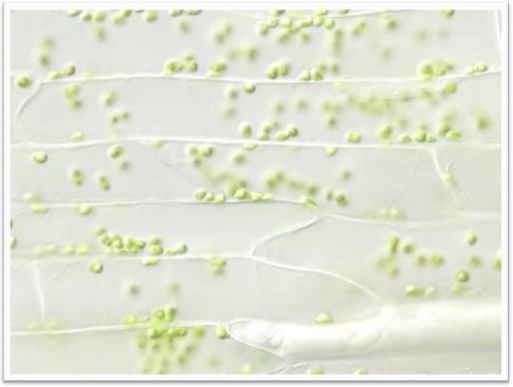


Figure 2. Chloroplasts of lace plant (*Aponogeton madagascariensis*); photo by Jacob Fletcher

Required Tools and Software

- **lab questions document**: download the associated file from the designated assignment dropbox [Appendix A]. This file contains the questions you need to answer and submit.
- a **Foldscope™**: provided free of charge; you should pick one up at your earliest convenience (watch for announcements from the instructor about when and where you can do so).
- **Fiji/ImageJ**: free download via https://imagej.net/Fiji/Downloads; detailed instructions on its use will follow later in this lab. If you already have ImageJ, you can use that.
- **Padlet**: no software or account required; instructions to follow.

Obtaining and Assembling Your Foldscope™

The Foldscope™, invented by Stanford University engineer Manu Prakash, is a contemporary version of the instrument perfected by Antonie van Leeuwenhoek in the late 17th century.

Get ready to channel your inner Leeuwenhoek!

Where to Get Your Foldscope™

Instructions about when and where you can pick up your Foldscope™ will be posted on Brightspace. You will have several opportunities to collect your Foldscope™, but if you anticipate any problems or conflicts, please contact the instructor as soon as possible to make alternate arrangements.

In addition to the Foldscope™, we will provide you with a transfer pipette and non-latex gloves. They are all yours to keep!

Figure 3. "Pencils are everywhere; so should be microscopes." - Manu Prakash (with a Foldscope™ prototype in 2012). Photo by https://www.ted.com [CC BY-SA 4.0], via Wikimedia Commons

Foldscope™ Assembly Tips

Your Foldscope™ kit includes printed instructions for assembly, and there are also video tutorials posted at the Foldscope™ website (www.Foldscope™ .com/tutorials).

Based on our experience with the Foldscopes™, we can also offer these tips:

- The instruction card included in your kit as well as some of the tutorial videos include reference to some items that are *not* included in your kit. This is because there are two different types of Foldscope™ kits available - Basic and Deluxe - and the instruction card is the same for both. You have the basic kit, but it contains mostly everything you need[2] for this lab's activities. We have also provided you with a transfer pipette and latex-free vinyl gloves, which are not included in the basic kit.
- Examine the unassembled Foldscope™ sheet carefully and note where to *detach* components and where to *fold* components. Detachable components are mostly punched out for you already and are attached to the rest of the card at a few points. Folds are marked by fine perforations. Don't detach where you should fold!
- Your Foldscope™ kit includes three *square* couplers and one *circular* lens. All are magnetized and will probably be stuck together. Make sure you pull these pieces apart prior to assembly – when you are finished assembling the Foldscope™, there will be one square coupler left over. (You will use this to attach the Foldscope™ to your phone or tablet.)
- Allow yourself about an hour to assemble and troubleshoot your Foldscope™. It may not take that long at all, but it's not something that you can whip together in a few minutes, either!

Figure 4. Portrait of Antonie Van Leeuwenhoek, [Public Domain], via Wikimedia Commons
Van Leeuwenhoek microscope by Museum Boerhaave, Leiden (Museum Boerhaave, Leiden) [CC BY-SA 3.0], via Wikimedia Commons "Almost everything he saw, he was the first person ever to see." – Douglas Anderson (2014, p.25)

[2] We suggest some other miscellaneous items that you may find useful on the **Finding Specimens for the Foldscope™** page of this lab.

Once you have your Foldscope™ assembled, you're ready to start exploring! See the **Finding Specimens for the Foldscope™**, **Slide Preparation and Photo Tips** and **Sharing Your Discoveries** pages of this lab for additional instructions.

Finding Specimens for the Foldscope™

Notes of Caution

While collecting specimens for this lab is a low-risk activity, you are responsible for using common sense and due caution. You should be able to collect and prepare interesting specimens without entering bodies of water or using potentially dangerous tools such as blades or scissors. Collect only in a location and manner that you judge to be safe, and do not risk injury or misadventure for the sake of this lab.

Please DO:

- wash your hands as soon as possible after your collecting trip
- wash any extra non-disposable equipment that you may have used
- dispose of any disposable equipment you may have used in the appropriate manner (compost, recycling, landfill, etc.)
- pick up some garbage while you're out exploring and dispose of it properly (that's mainly what the gloves are for...)
- tell someone where you're going - or, better yet - bring a friend!

Please DO NOT:

- enter the ocean or lakes, or anywhere where there is moving water (flowing streams, etc.)
- trespass on private property
- collect specimens where you feel unsafe or where it is unsafe to walk
- walk on submerged rocks, or on rocks covered in algae (whether submerged or not)
- use blades or other sharp objects to prepare your specimens
- handle wild mushrooms (see 'Other Specimens to Try (Optional)', below)
- ingest anything you collect
- use any collecting equipment for food preparation or storage

Specimen Collection and Preparation Tips

We've chosen chloroplasts as the organelle of interest for this lab because they are relatively large and easily recognizable. If you can find your own specimens that clearly show chloroplasts, then that's great, but it's not strictly necessary. Flora or fauna, there's plenty to see with the Foldscope™, and the most important aspect about this part of the lab is that you get to experience the thrill of discovery!

Additional Equipment

We provide you with a Foldscope™, transfer pipette, and a pair of gloves. You should also gather together the following items:

- small container with a lid - pop bottle, etc., for wet specimens (spice jars from the dollar store work well)
- small plastic bag or envelope for dry specimens such as leaves, pollen, spores, etc. (but prepare them for viewing as soon as possible after collecting)
- toothpicks, plastic utensils, or other similar items (something you can scrape with) for taking small soil samples or collecting samples from the surface of rocks or trees

- a long, thin stick such as a pencil, chopstick, barbeque skewer, etc. to safely extend your reach if necessary
- paper or a notebook and pencil
- camera
- tweezers (if you have them)
- a grocery bag or other container for collecting garbage

Algae and Plants

Your best chance for getting a good view of chloroplasts is to find some filamentous green algae. At your collecting site (see **Collecting Tips: Locations**, below), look for rocks or muddy patches with a greenish cast or film on the surface. In lakes, you may also see obvious clumps of filamentous algae that are either clinging to rocks or floating freely in the water (but see **Notes of Caution**, above). Onshore, look for a green film on the surface of rocks. If you or someone you know has a fish tank, you might find some algae there.

Plant leaves may also work, but they must be *very* thin - if a specimen is more than a few cell layers thick, then it will be too thick for the cells to be visible. Choose leaves from plants that are naturally very small (rather than using fragments of larger leaves) and look near the exterior margins of the leaf or near the veins, where the leaf tissue is typically thinner. Mosses and other ground-hugging weedy plants are good candidates, as are aquatic plants and fish tank ornamentals such as *Vallisneria* and *Elodea.*

If you collect a small amount of mud from a damp or marshy area, you have a good chance of finding motile organisms, even if you don't find algae. You may even want to try collecting a spoonful of soil in a glass jar, adding some water, and leaving it to sit in a sunny location. After a few days, you may have some algae growing in the jar! (If you try this, choose soil from a damp or shaded area with lots of plants growing).

Other Specimens to Try (Optional)

None of these has chloroplasts, but they could be fun to try...

- mushroom gills: whole mushrooms from a grocery store or the dining hall only - do not collect wild mushrooms!
- flower parts (petals, stamens, anthers): as with leaves, the smaller and thinner the better. There are lots of teeny tiny flowers out there if you look carefully!
- plant roots: try to spot a root cap near the apex. You can also search for root hairs and emerging lateral roots!
- pollen grains: tap a flower head onto your Foldscope™ slide
- fern sporangia (in season): look for the bumps on the underside of larger fern leaves; each bump is a sorus (pl. sori) that consists of many sporangia - gently scrape a sorus into your collecting envelope / bag
- onion epidermis (the moist, membranous inner layers, not the papery outer layer) - the cells of red onions are pigmented, and therefore easier to see
- lichen - macerate a small amount with a spoon or other blunt object in a small amount of water to increase your chances of seeing something recognizable

Collecting Tips: Locations

NOTE: We recommend providing several suggestions for location collections on and near your campus, including directional information, relevant cautions, and photos similar to those in Figures 5-9. The locations should be easily and safely accessible. For our campus we suggested a small marshy area, a wooded area, pathways where we observed algae, and stone walls. We also provided directions to parks and natural areas within walking distance of campus and in other easily accessible parts of the community.

At the Site

Take a few photos of the site in general and the specific spot where you collect your specimen, safely collect the specimen, and make notes about the time, location, and weather conditions.

If collecting wet or muddy specimens, nearly fill your specimen bottle with water from the site; then add your specimen. Or, bring some tap water with you, for algae that you may collect from relatively dry spots as well, such as the surface of rocks or soil.

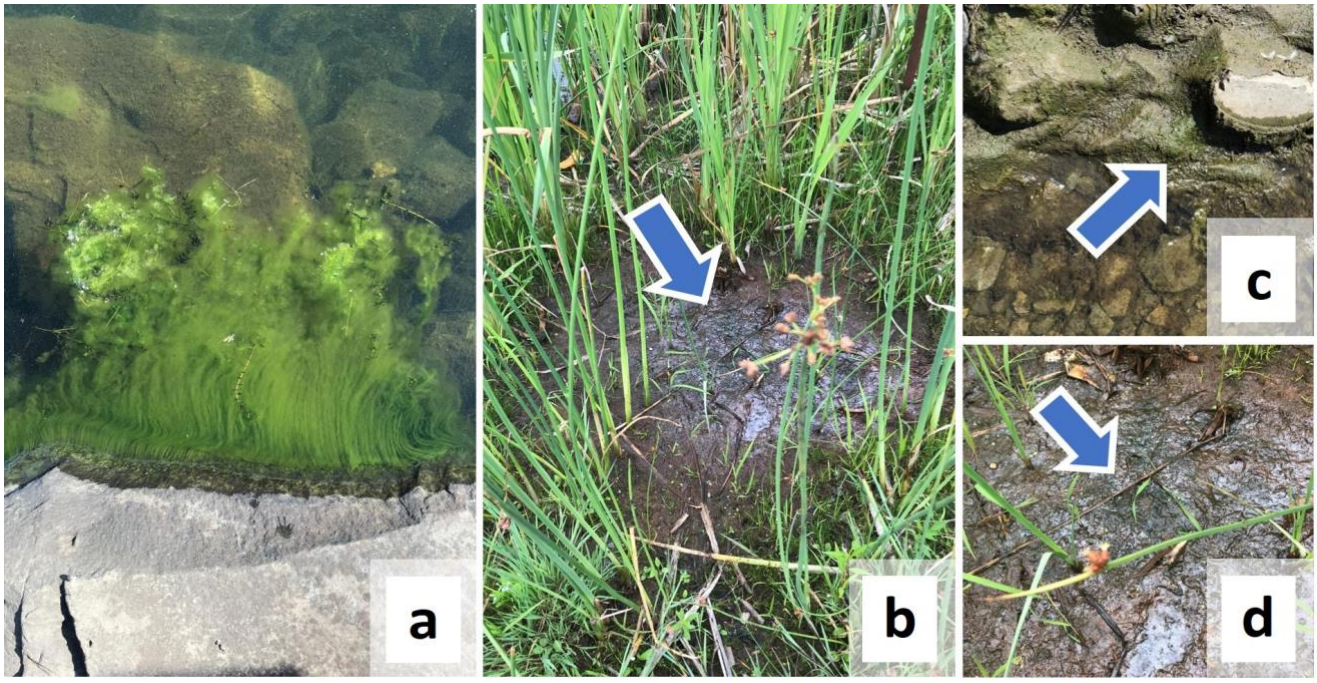


Figure 5. Filamentous algae in various locations. a & c) Attached to rocks in Lake Banook. b & d) Seen on the surface of the mud near the shore of Maynard Lake; look closely for the greenish film on the mud. Photos by Jennifer Van Dommelen.

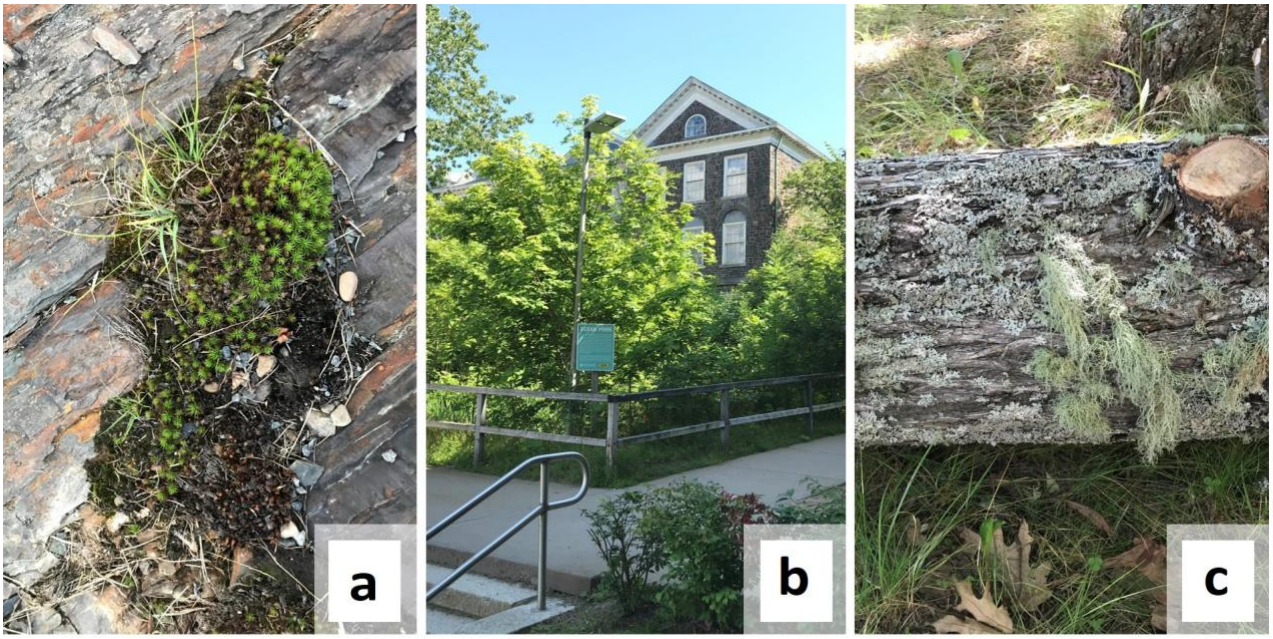


Figure 6. Locations around campus. a) Moss on a rock wall. b) Ocean Pond. c) Lichen on a log behind Sheriff Hall (at the perimeter of the Dalhousie Outdoor Ecolab). Photos by Jennifer Van Dommelen.

Figure 7. Locations around Dalhousie campus: the walkway behind the LSC, between the Psychology wing and Oceanography (Sherriff Hall is in the distance, behind the trees; the Dalhousie Outdoor Ecolab is among the trees); photos by Jennifer Van Dommelen.

Figure 8. Drift algae on the beach at the end of South Street, on the Northwest Arm in Halifax; photos by Jennifer Van Dommelen.

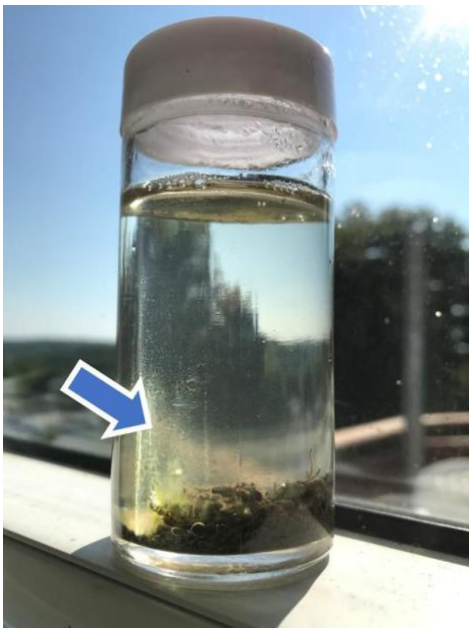


Figure 9. Grow your own algae in a jar (just add water!!); photo by Jennifer Van Dommelen.

Slide Preparation and Photo Tips

View the tutorials at https://www.foldscope.com/tutorials/ for tips on preparing your slides and taking photos. *NOTE:* The videos are also embedded on this page of the online version of the lab.

A Little Luck Goes a Long Way

A good image starts with preparing a good slide, and it's often difficult to know whether you've done that until you actually look through your Foldscope™. If you have trouble locating something interesting, you might do well to start over with a fresh slide.

Then, once you have a good image in view, getting a decent photo may require some improvisation - if your camera lens is raised, you may find that the ring sticker that is meant to attach the coupler to your phone doesn't work very well. You may have to use sticky tack or extra tape to hold the coupler in place or borrow another phone or tablet with a 'flat' lens (i.e., one that is flush with the surface of the device) to take the photo.

Need More Slides?

You'll probably have plenty of slides for this lab, but if you use them up now or in the future, you can make more with some heavier-weight paper and transparent tape. Try greeting cards or other similar material, or you may be able to buy a product called "100-pound silk cover" by the sheet at stationery or art supply stores.

The outside dimensions of the slides are 27 mm x 76 mm; the dimensions of the rectangular "windows" are 8 mm x 16 mm. It's not crucial to stick to these dimensions exactly -- you just don't want the window to be so large that the slide is "floppy" or bends too much.

Sharing Your Discoveries

NOTE: The workshop participants' Padlet gallery can be viewed at https://padlet.com/RustyBlackbird/bimfltt11kum.

Share a photo or video of your Foldscope™ specimen by posting it to the Chloroplast Diversity Foldscope™ Gallery on Padlet. Upon opening the gallery, double-click anywhere on the 'corkboard' or click on the pink circle in the lower right-hand corner of the screen. You will be prompted to add some text and upload your photo/video.

- In the **Title** field, provide as much information as you know about the identit of the organism and the part of the organism pictured. Common names are fine, scientific names are great if you know them. If you don't know what your photo shows, use 'unidentified'
- In the 'Write something...' field, include information about your collection site and who you are. See the example below.

You can also "like" and comment on your peers' posts. And if you post well ahead of the deadline, you might get some help with identification!

Posts to the Padlet gallery are moderated and will not be visible to the rest of the class until the instructor approves them.

Figure 10. Sample Padlet post of filamentous algae (*Spirygyra* sp.) as viewed through a Foldscope™; photo and screen capture by Jennifer Van Dommelen.

Protect Your Privacy

Digital files include metadata with information like when and where a photo was taken, the type of camera used, etc. If you wish to do so, you have some ability to remove this information from your photos by adjusting the location/privacy/photo settings on your device before taking your photos, or after-the-fact by changing the photo file's properties. For more information, see 'Remove metadata from Office files, PDFs, and images' (https://www.cnet.com/how-to/remove-metadata-from-office-files-pdfs-and-images/; provided for information purposes only, and not as an endorsement of links and services mentioned in the article).

Respect Your Classmates' Privacy and Intellectual Property

The Chloroplast Diversity Foldscope™ Gallery is intended to be a private board for members of the class to share their work and to comment on that of their peers. Please do not download, copy, share, or embed individual photos or the Padlet board in whole or in part to any external location without the written permission of the instructor and the student or students whose work you wish to copy/share. The Foldscope™ Gallery Padlet will be archived at the end of the term.

Share with the World (Optional)

If you would like to share your image with a larger audience, you might consider joining the Foldscope™ Microcosmos community (https://microcosmos.foldscope.com/), or creating an account with iNaturalist (https://www.inaturalist.org/), which has a dedicated Foldscope™ community (https://www.inaturalist.org/projects/foldscope). The iNaturalist website/app can also help you with identification - when you upload a photo, it will make suggestions about the identity of your organism.

Creating accounts at these sites is entirely up to you; even if you choose not to, you can still see what others from around the world have discovered with their Foldscopes™!

Downloading, Installing, and Testing Fiji/ImageJ

NOTE: The activities that follow are designed for an online interface; a face-to-face version of the lab could be developed using hard copy images that would not require the use of Fiji/ImageJ, in which case the instructions below could be omitted.

You will need Fiji/ImageJ for the second part of this lab, in which you will collect data from images. If you have taken BIOL 1010, BIOL 1011, or BIOL 1020 you may have used this program and have it installed on your computer already. If not, go to https://imagej.net/Fiji/Downloads to download and install the version of the program that is right for your computer.

These instructions should work for Fiji and older installations of ImageJ:

1. Click on the thumbnail of moss leaf cells to open a larger version of the image. Right-click on the full-size version of the photo and save a copy of it to your computer.

2. Start the Fiji/ImageJ program; a small dialog box will appear on your screen (upper right in the screen capture). Use **File → Open…** to open the photo you saved in the previous step.

3. On the Fiji/ImageJ icon ribbon, click on the **Point Tool** icon. If it is set to 'Point' (a single square with cross-hatches), right-click on the icon to set it to 'Multi-point' (several hatched squares). The Point Tool dialog box may appear; if it doesn't, double-click on the Point Tool icon. Keep the default settings; deselect **Label points** and **Show on all slices**.

4. Move your mouse over to the photo and start clicking on individual chloroplasts. All you have to do is click; a running tally will appear below the **Counter** drop-down menu. Click on as many chloroplasts as you wish to confirm that this function is working properly. If it is, you are ready to proceed with the second part of the lab (beginning on the **Chloroplast Density** page) at any time.

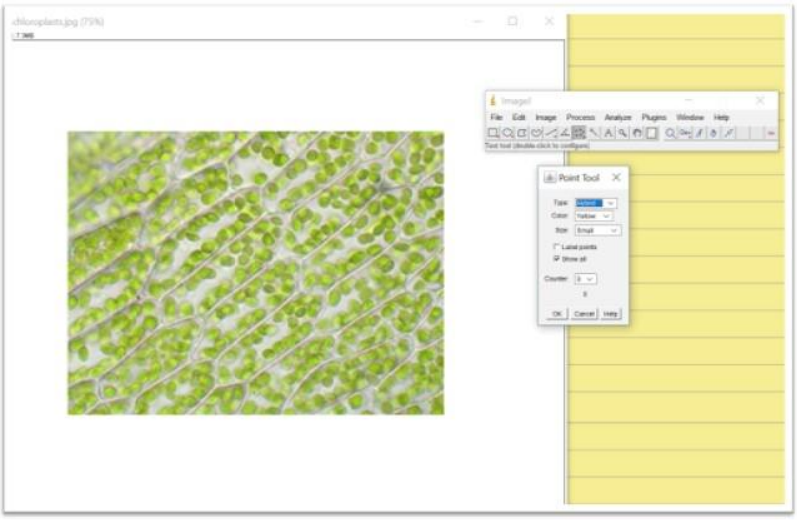


Figure 11. Screen capture showing the Point/Multi-Point Tool in Fiji/ImageJ; image by Jennifer Van Dommelen.

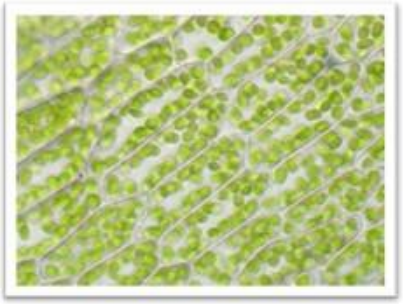


Figure 12. Thumbnail version of moss leaf cells; Funsci.com. (2018). – Lower Plants. [online] Available at: http://www.funsci.com/fun3_en/guide/guide3/micro3_en.htm#3.1 [Accessed 5 Sep. 2018]

Chloroplast Density

NOTE: The activities in this section are designed for an online interface; a face-to-face version of the lab could be developed using hard copy images that would not require the use of Fiji/ImageJ.

For this part of the lab we're going to take a closer look at chloroplasts, and relate that information to cell structure, function, and evolution. Our model organism for this activity is the lace plant (*Aponogeton madagascariensis*), so named for its unique leaf morphology. The characteristic holes in the leaves of the lace plant are due to a process called **programmed cell death**, or apoptosis[3], where cells die "on purpose" during the development of the organism or structure. The lace plant is an emerging model species for cell death research, due to this highly predictable cell death pattern and to its thin transparent leaves which allow for extensive microscopic observation of cellular activity throughout development. There are a few hypotheses about *why* the lace plant forms these holes, such as an anti-predation/camouflage strategy, or to decrease water resistance. The ultimate reason, however, remains a mystery (perhaps one you will solve some day?). Although it is an endangered species in the wild, its natural beauty and uniqueness has made it a popular cultivated plant not only for3

[3]In the online version of this lab the word 'apoptosis' is hyperlinked to the following article:

https://www.ncbi.nlm.nih.gov/books/NBK26873/ .

scientists, but for aquarium hobbyists as well.

Figure 13. Lace plant (*Aponogeton madagascarensis*). Left: botanical illustration by Sir William Jackson Hooker (1856), [Public domain] via Wikimedia Commons; right: ornamental aquarium specimen, [CC0] via Pixabay.

Data Collection

The images below are micrographs of cells from two different parts of the lace plant. Click on the photos to open up larger versions [Appendix A], save a copy of each large version to your computer. Use Fiji/ImageJ (see **Downloading, Installing, and Testing Fiji/ImageJ**) to count the chloroplasts in five different cells from each photo (identified for you on the larger versions). Enter your data into the table in your lab document and answer the associated questions.

Figure 14. Micrographs of unidentified lace plant tissue showing chloroplasts; photos by Jacob Fletcher.

The last question for this section in your lab document refers to the labelled photo of a lace plant below. (The **sheath** is a protective layer of cells found around the base of the stem, and the **corm** is an underground shoot.)

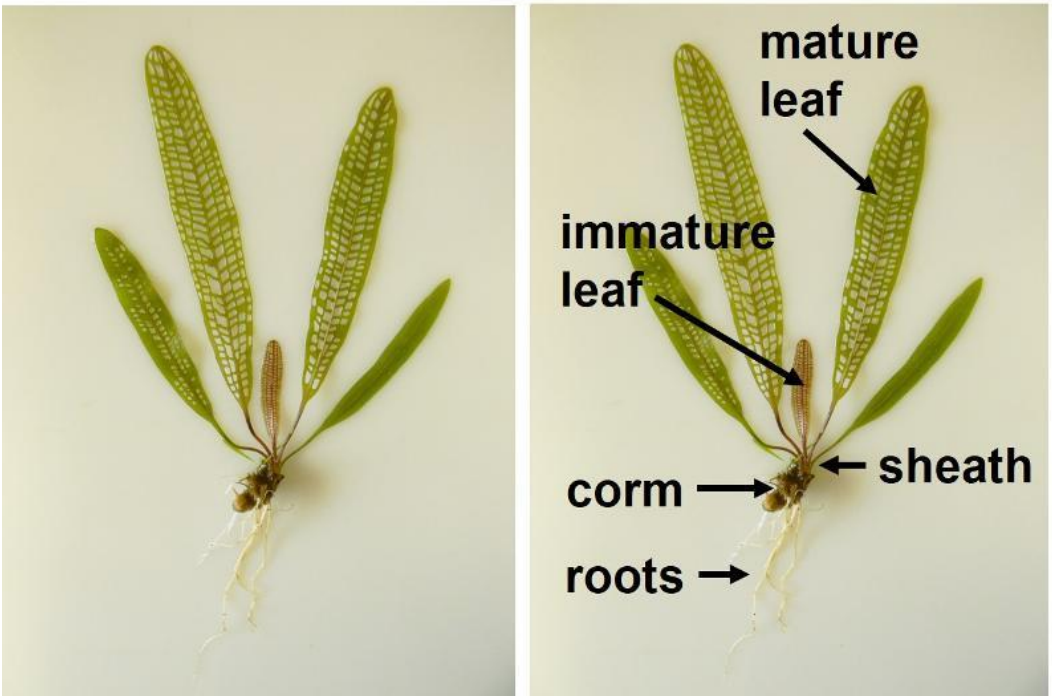


Figure 15. Lace plant whole mount photos by Jacob Fletcher.

Chloroplast Morphology

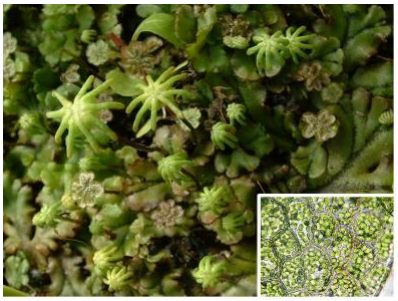
NOTE: The activities that follow are designed for an online interface but could be adapted for hard copy delivery. See Appendix D for image attributions.

Plant Chloroplasts

In the previous activity, you looked at a difference in chloroplast density between different tissues of the same plant. For this activity, we'll consider some differences in chloroplasts among several different species of plants and algae.

First, click through the images below, which feature different species of land plants. Each photo of a plant *in situ* includes an inset of a micrograph of chloroplasts from the same plant species. As you study the pictures, consider whether the variability in shape that you see among the chloroplasts is relatively high (i.e., lots of variation in shape) or relatively low (i.e., not much variation in shape).

a) Lady fern (Pteridophyta)



b) Liverwort (Marchantiophyta)

c) Orchid (Orchidaceae)

d) Sycamore maple (Magnoliophyta)

e) Thyme moss (Bryrophyta)

f) White pine (Coniferophyta)

Figure 16. Various plant species (large photo) and micrographs of their chloroplasts (insets). See Appendix D for image attributions.

Now that you've viewed the photos, what do you think? Mouse over your choice for feedback:

(1) The land plant chloroplasts show relatively high variability in shape among different species (i.e., they all look quite different from each other). [FEEDBACK: High shape variability – Try the next activity about algal chloroplasts, then return to this question.]

(2) The land plant chloroplasts show relatively low variability in shape among different species (i.e., they all look very similar to each other). [FEEDBACK: Low shape variability – Land plant chloroplasts are fundamentally lens-shaped, regardless of which species of plant they are found in.]

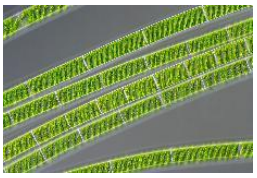
Algal Chloroplasts

Time to count more chloroplasts! You won't need Fiji/ImageJ this time, however. In the interactive below, view each image and make a rough estimate as to how many chloroplasts you see. Click on 'SHOW' to reveal the correct answer.

a) *Cryptomonas ovata*; number of chloroplasts: 1

b) Vaucheria; number of chloroplasts: 1

c) *Glaucocystis* sp.; number of chloroplasts: 0, they contain endosymbionts called cyanelles

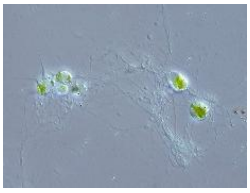


d) *Spirogyra* sp.; number of chloroplasts: 1-4

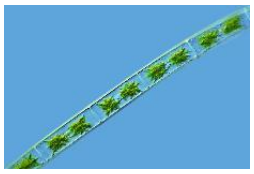
e) *Ulothrix*; number of chloroplasts: 1

f) *Chlamydomonas*; number of chloroplasts: 1

g) *Cladophora* sp.; number of chloroplasts: many, interconnected in a net-like structure



h) *Chlorachnion raptans*; number of chloroplasts: 1



i) *Zynema*; number of chloroplasts: 2

j) *Dinobryon divergens*; number of chloroplasts: 1

Figure 17. Various algal species. See Appendix D for image attributions.

Are you surprised by the results? Click through to the next page of the lab for an explanation...

Chloroplasts and Evolution

Chloroplasts are just one type of **plastid** - a category of closely related organelles found in photosynthetic eukaryotes. Chloroplasts are specialized to perform photosynthesis, while other types of plastids perform other functions, such as starch or oil storage, pigment production and storage, gravity sensing, and production of secondary metabolites (Solymosi 2012).

The activities on the previous page demonstrate that the cells of land plants tend to have relatively large numbers of similar-looking chloroplasts, while algal cells tend to contain relatively few chloroplasts, with much more variety in shape across species. And while we didn't provide any information about chloroplast size, it is also true that algal chloroplasts are relatively large compared with plant chloroplasts. They are also less specialized than plant chloroplasts, in some cases taking on the metabolic functions that are performed by different plastids in plant cells. In other words, algal chloroplasts are multi-taskers (Solymosi 2012)!

A recent paper by Dalhousie researchers Jan de Vries and John Archibald (2018) summarizes the evidence supporting the hypothesis that land plants evolved from the streptophyte lineage of algae. The authors assert that land plants evolved just once, and are most closely related to the Zygnematophyceae, a group of unicellular and filamentous freshwater algae.

Study the summary figure from de Vries and Archibald (2018; Fig. 18) and compare it to the figure from Chapter 29 of your textbook reproduced below (Fig. 19). de Vries and Archibald (2018) provide more detailed information about the evolutionary relationships among the 'Viridiplantae' group.

Answer the last question in the lab assignment.

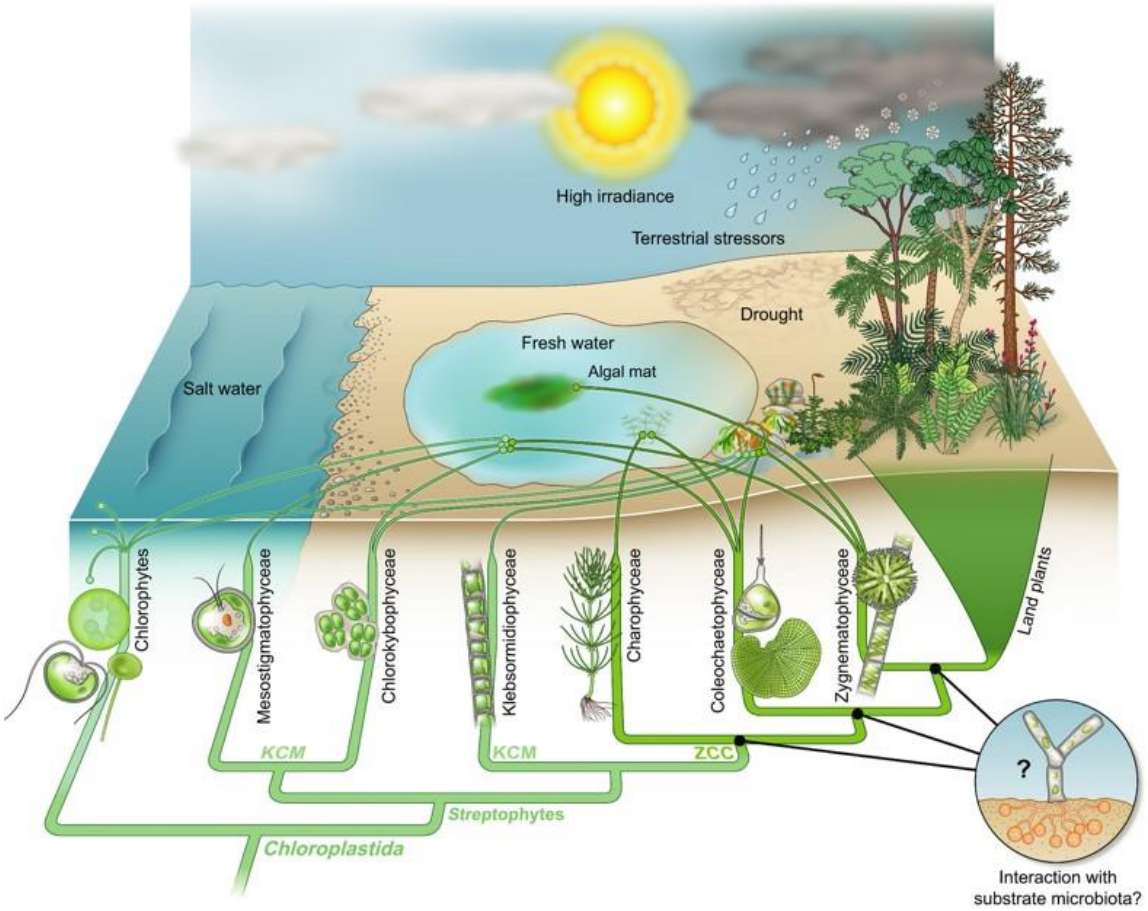


Figure 18. Streptophyte terrestrialization and the colonization of terrestrial habitats by extant Chloroplastida; from deVries and Archibald (2018)[4].

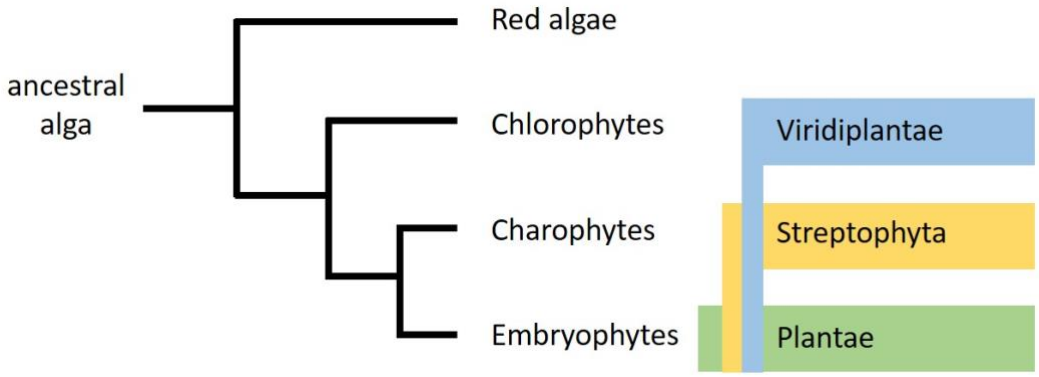


Figure 19. Three possible "plant kingdoms"; after Figure 29.3 in Reece et al. (2018; p. 654). Modified by Jennifer Van Dommelen.

[4] Open access; see https://onlinelibrary.wiley.com/terms-and-conditions and https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/index.html.

Activity 2. Estimating Specimen Size

NOTE: This Student Outline is excerpted from a complete Microscopy lab, as an additional example of how Foldscope™ is used in our courses. This specific use, but not the whole lab, are presented here. The introduction and learning objectives for the full lab are provided for context; the Foldscope™-relevant learning objectives (#5 and #7) appear in bold. Student questions are included in Appendix B.

Microscopy Lab

In this lab, we'll work with two microscopes[5] and some images to explore concepts in microscopy. Working with microscopes requires two sets of skills: (1) manipulating the instrument and specimens and (2) interpreting the resulting images. We'll do a bit of both!

The lab consists of a series of activities in which you will follow some directions and answer some questions. Work through the pages of this module one by one and complete the activities as instructed, then submit the assignment document for grading by your TA.

Learning Objectives

After completing this activity and the relevant readings from the textbook (Chapter 6), you will be able to manipulate a virtual compound light microscope5 and interpret the resulting micrographs, as well as to interpret representative images produced by electron microscopes. Specifically, you will be able to:

1. Identify the structural components of a basic light microscope.

2. Describe the correct procedure for placing a slide on a light microscope stage, illuminating it appropriately, and bringing the specimen into focus.

3. Recognize and distinguish among images produced by a light microscope, a scanning electron microscope, and a transmission electron microscope.

Figure 20. Low-temperature scanning electron micrograph of soybean cyst nematode and egg, USDA Agricultural Research Service [Public domain], via Wikimedia Commons.

4. Distinguish between viewing magnification and image magnification by working with images and answering questions that require you to apply your knowledge of these terms.

5. Use field of view, scale bars, and an ocular micrometer **to estimate the actual size of a specimen or of specific features of a specimen.**

6. From micrographs, identify cell structures and organelles by their appearance, relative size and position within the cell, and/or clues about function.

7. Assemble a portable microscope, collect and prepare specimens, and capture and share images of what you find.

[5] One is the University of Delaware Virtual Compound Microscope (https://www1.udel.e5 One is the University of Delaware du/biology/ketcham/microscope/scope.html); anticipated to be unavailable after December 2020 when Flash is no longer supported. The other is the Foldscope™.

Obtaining and Assembling Your Foldscope™

NOTE: See similar instructions as for Chloroplast Diversity Lab.

Finding Specimens for the Foldscope™

Required: Red Onion

The only specimen you need for this lab is red onion (*Allium cepa*). Red onions contain anthocyanin pigments that make the cells easy to see - no staining required! Simply pull or scrape a tiny piece of the red membrane from any inner layer of the onion (not the dry, papery outer layer), and place it on your slide for viewing (see **Slide Preparation and Photo Tips**).

You may have a red onion at your home, or you can buy one at the grocery store if you want. Even a small piece of red onion that you may find in your salad[6] will have more than enough material for you to make your slide (as long as there is a red layer present).

Figure 21. Red onion (*Allium cepa*). Left: "Red Onion on White" by © User:Colin [CC BY-SA 3.0] via Wikimedia Commons; right: cut red onion by Amada44 [Public domain], via Wikimedia Commons

Notes of Caution (including Collecting Tips and Locations)

NOTE: See similar instructions as for Chloroplast Diversity Lab.

Optional: Other Specimens

NOTE: See similar instructions as for Chloroplast Diversity Lab.

Slide Preparation and Photo Tips

NOTE: See similar instructions as for Chloroplast Diversity Lab.

[6]Or at the dining hall salad bar – just sayin'.

Sharing Your Discoveries

NOTE: See similar instructions as for Chloroplast Diversity Lab. A sample post to the Padlet board relevant to this activity is included at right.

Measuring What You See: Viewing Magnification, Field of View, and Specimen Size

NOTE: Standard instructions about using field of view diameter to estimate specimen size are provided to students. They use the ocular micrometer in the University of Delaware Virtual Compound Microscope, and the field-of-view estimation method on their Foldscope™ specimens.

Figure 22. Sample Padlet post of red onion (*Allium cepa*) epidermal cells as viewed through a Foldscope™; photo and screen capture by Jennifer Van Dommelen.

Activity 3. Observing Plasmolysis in Plant Cells

NOTE: This Student Outline is excerpted from a complete Osmosis and Diffusion lab, as additional example of how Foldscope™ is used in our courses. This specific use, but not the whole lab, are presented here. The full lab includes work with literature, images, and data about osmosis and diffusion in model animal cells (erythrocytes) and plant cells (red onion epidermis). The introduction and learning objectives for the full lab are provided for context; the Foldscope™-relevant learning objective (#5) appears in bold. Student questions are included in Appendix B.

Osmosis and Diffusion Lab

This lab entails studying well known exemplars -- mammalian red blood cells (RBCs), plant cells, and solutions of sodium chloride (NaCl) -- to better understand the processes and physiological significance of diffusion and osmosis. You will work with material that has already been prepared for you, as well as make your own direct microscopic observations with your Foldscope™.

Learning Objectives

After completing this activity and the relevant readings from the textbook (Chapter 7), you will be able to:

1. Define diffusion and osmosis and discuss the physiological significance of these processes at the level of the cell and of the whole organism.

2. Relate molarity to osmolarity and discuss the significance of these terms with respect to biological systems.

3. Define and state the difference between osmolarity and tonicity.

4. Interpret a line graph.

5. Compare and contrast the effects of osmosis on red blood cells and red onion cells and relate your observations to the structural properties of these cells.

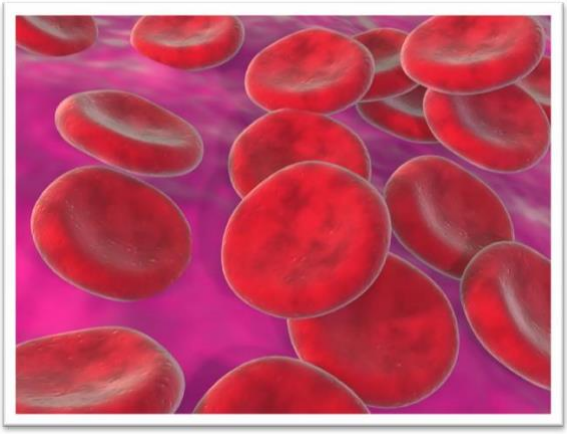


Figure 23. 3ds max Red Blood Cell Render by kingdesmond1337 [CC BY-NC-SA2.0] via Flickr

Required Tools and Software

Before starting this lab, download the associated file from the designated assignment dropbox. This file contains the questions you need to answer and submit. This lab also includes several self-test questions on the supporting background material. You are not required to submit your answers to these questions for grading, but they will help you understand the material and they are relevant to the learning objectives for the lab.

You will also need:

- a Foldscope™; if you don't already have one, please pick one up at your earliest convenience (watch for announcements from the instructor about when and where you can do so)
- a free program called **Fiji/ImageJ**, which can be downloaded from https://imagej.net/Fiji/Downloads; detailed instructions on its use will follow later in this lab
- to post images to a Padlet board; no account is required, instructions to follow
- a red onion, or piece of red onion - enough to collect several small pieces of epidermis
- tweezers (or your fingernails)
- tap water
- table salt

- two small cups or containers
- a teaspoon or similar small tool for measuring, such as a regular small spoon or a pop bottle cap
- a clean spoon, chopstick, or similar tool to use for stirring

Plasmolysis Demonstration

NOTE: Instructions about obtaining and assembling the Foldscope™, slide preparation, notes of caution, etc. have been omitted. A sample post to the Padlet board relevant to this activity is included below.

For this demonstration, you will prepare three slides, each slide representing one treatment: 1. No Treatment, 2. Tap Water, and 3. Salt Water. Follow the steps below.

1. *Prepare the Tap Water and Salt Water treatments.* Add 5 teaspoons of tap water to each of your two cups. (If you are using something other than a teaspoon, add 5 of whatever it is...). To one of the cups, add 1 teaspoon (or 1 measure of whatever you're using) of salt, and stir until the salt is completely dissolved (about 2 minutes).

2. *Obtain your tissue sections.* Remove the dry outer papery layer(s) from your red onion until you get to the purple epidermis underneath (the part that we eat!). Using a pair of tweezers or your fingernails, carve a small square into the surface of the onion and peel off the outermost layer (the purple epidermis). Do this several times so that you have a number of small tissue sections - you only need three, but it's good to prepare a few extras, just in case. The most important notes here are that (a) the thinner you can get your tissue layers, the nicer they will look under the Foldscope™, and (b) they should be small enough that you can comfortably mount them onto the Foldscope™ slide.

3. *Soak the tissues.* Place one or more tissue pieces into each of the Tap Water and Salt Water treatment cups. Allow them to soak for 5 minutes. While these tissue samples are soaking, mount another one (the No Treatment sample) directly onto a Foldscope™ slide.

4. *Record your observations.* Using your phone or other device, capture an image of your No Treatment tissue sample. Mount the Tap Water and Salt Water samples on their own (separate) slides, and capture images of them as well. It is okay to use the optical zoom on your device if doing so improves your image - your photo does not need to include the entire field of view.

5. *Share your observations.* Post your best image from each of the three treatments to the BIOL 1020 Foldscope™ Gallery on Padlet. Upon opening the gallery, double-click anywhere on the 'corkboard' or click on the pink circle in the lower right-hand corner of the screen. You will be prompted to add some text and upload your photo/video. Fill out the text fields as shown in the example on this page, substituting the treatment as appropriate, and your own name. Posts to the Padlet gallery are moderated and will not be visible to the rest of the class until the instructor approves them.

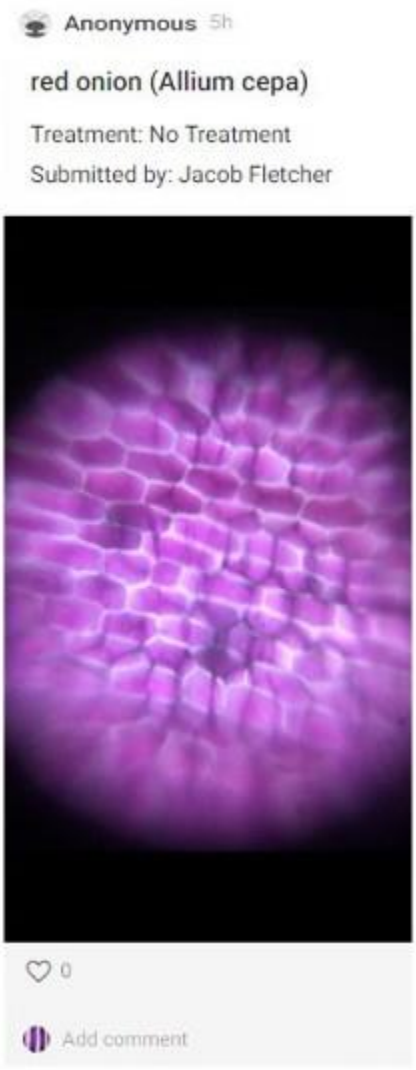


Figure 24. Sample Padlet post of red onion (*Allium cepa*) cells as viewed through a Foldscope™; photo by Jacob Fletcher, screen capture by Jennifer Van Dommelen.

Finally, answer the questions for this part of the lab in your lab assignment document. If your own Foldscope™ images are unsatisfactory, you can use the ones below to help you with the questions:

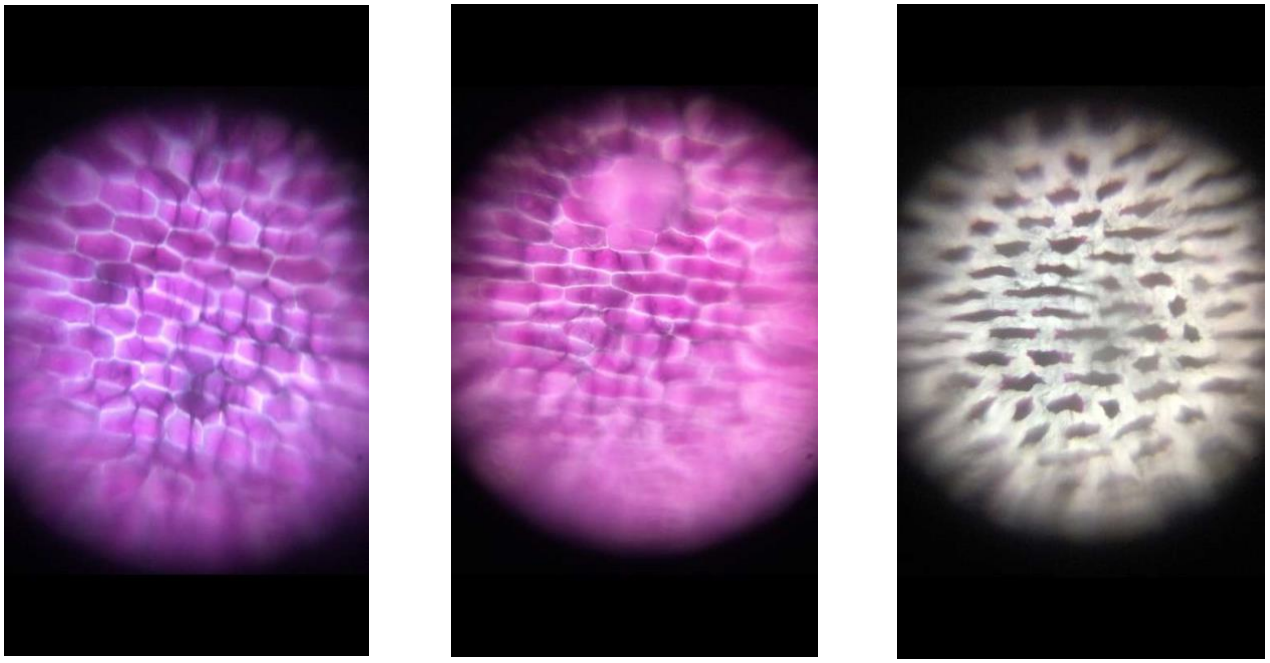


Figure 25. Red onion (*Allium cepa*) epidermal cells viewed through Foldscope™. Left to right: Whole mount - untreated; whole mount – after immersion in tap water for five minutes; and whole mount – after immersion in salted tap water (approximate concentration 16 mM) for five minutes. Photos by Jacob Fletcher.

Cited References

Anderson D. 2014. Still going strong: Leeuwenhoek at eighty. Antonie van Leeuwenhoek 106: 3-26.

de Vries J and Archibald JM. 2018. Plant evolution: landmarks on the path to terrestrial life. New Phytologist 217: 1428-1434. [accessed 2018 October 27]; https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.14975

Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB, Rawle F, Durnford D, Moyes C, Scott K, Walde S. 2018. *Campbell Biology.* Second Canadian Edition. Hoboken, NJ: Pearson Education Inc.

Solymosi K. 2012. Plastid Structure, Diversification and Interconversions I. Algae. Current Chemical Biology 6: 167-186.

Materials

The exact materials required and who supplies them will depend on whether students will be working independently or under supervision. The lists below reflect the former scenario, with the additional expectation that students will be able to use items that they already own and are not required to purchase any of their own equipment. Some of the 'household items' could be replaced or supplemented by standard laboratory supplies in a supervised deployment of the lab.

Chloroplast Diversity Lab

Instructor Provides

- 25 Foldscopes™ (Basic Classroom Kit; $35 USD for 20)
- 25 pairs of non-latex gloves
- 25 disposable transfer pipettes
- access to a Padlet gallery (see **Notes for the Instructor**)
- access to spare devices for taking photos (see **Notes for the Instructor**)

Students Provide / Obtain

- specimens – ideally algae, but other specimens acceptable
- small container with a lid - pop bottle, etc., for wet specimens (spice jars from the dollar store work well)
- small plastic bag or envelope for dry specimens such as leaves, pollen, spores, etc.
- toothpicks, plastic utensils, or other similar items (something one can scrape with) for taking small soil samples or collecting samples from the surface of rocks or trees
- a long, thin stick such as a pencil, chopstick, barbeque skewer, etc. to safely extend one's reach if necessary
- paper or a notebook and pencil
- camera (phone or tablet)
- tweezers (optional)
- a grocery bag or other container for collecting garbage
- Fiji/ImageJ (https://imagej.net/Fiji/Downloads)

Estimating Specimen Size (Microscopy Lab)

Instructor Provides

- 25 Foldscopes™ (Basic Classroom Kit)
- 25 pairs of non-latex gloves
- 25 disposable transfer pipettes
- access to a Padlet gallery

Students Provide / Obtain

- red onion (*Allium cepa*; a small piece is sufficient)
- tweezers or toothpicks
- camera (phone or tablet)

Observing Plasmolysis in Plant Cells (Osmosis and Diffusion Lab)

Instructor Provides

- 25 Foldscopes™ (Basic Classroom Kit)
- 25 pairs of non-latex gloves
- 25 disposable transfer pipettes
- access to a Padlet gallery

Students Provide / Obtain

- red onion (*Allium cepa*), or piece of red onion - enough to collect several small pieces of epidermis
- tweezers or toothpicks (or fingernails)
- tap water
- table salt
- two small cups or containers
- a teaspoon or similar small tool for measuring, such as a regular small spoon or a pop bottle cap
- a clean spoon, chopstick, or similar tool to use for stirring
- camera (phone or tablet)

Notes for the Instructor

Foldscopes™

Foldscopes™ can be ordered online via www.foldscope.org. They offer basic kits and deluxe kits; the basic kits can be ordered in bulk only, in units of 20 (Basic Classroom Kit; $35.00 USD) or 100 (Large Classroom Kit; $350.00 USD). Peculiarly, five Basic Classroom Kits are half the cost of one Large Classroom Kit.

The Deluxe Kits are more expensive ($39.99 USD per individual kit) but also include more equipment and would allow for more complex investigations. They may be an option depending on the number of students you have, your budget, and whether any or all of the cost may be reasonably recovered from the students.

One limitation on students' use of the Foldscope™ is the number of paper slides included with

the kit. If more slides are required, more can be made with heavier-weight paper and transparent tape. Try greeting cards or other similar material, or '100 lb silk cover' available at stationery stores or print shops. The outside dimensions of the slides are 27 mm x 76 mm; to fit the Foldscope™ properly, the width is more important than the length. The dimensions of the rectangular "windows" are 8 mm x 16 mm. It's not crucial to stick to these dimensions exactly - you just don't want the window to be so large that it is "floppy" or bends too much.

An issue that arose among some of our students during the summer and fall of 2019 (after the ABLE workshop was delivered) is that some phone models use magnets in their cameras to stabilize images and prevent blurring. The camera magnet may repel the Foldscope's™ magnetic coupler, making it difficult to take a photo. Additionally, the Apple iPhone 11 camera released in late summer 2019 features three lenses; it is unknown how this design will work with the Foldscope™. Instructors may recommend that students borrow another device, or alternatively provide access to an alternate device for the purpose of taking photos. We recommend that instructors check the Foldscope™ website for the most recent tutorials and updates.

Lab Design and Safety

If the lab is to be completed without supervision, the two main design considerations are (1) safety and (2) provision of "back-up" images in case students have problems with their Foldscopes™ or fail to capture a satisfactory image. Any unsupervised specimen-collection and preparation activities must be low-risk and should not require the use of specialized or potentially hazardous equipment. Additional lab activities should not rely on successful use of the Foldscope™. To mitigate these constraints, we held non-mandatory drop-in help sessions and were prepared to provide specimens upon request.

SoftChalk

The online versions of the labs presented here are built in an authoring platform called SoftChalk, which provides built-in widgets for creating interactive exercises that can be embedded within the online content of the lab. Large-resolution versions of images that students are asked to download are located on HTML pages hosted on a university account associated with the course. SoftChalk is available via institutional or individual licensing; other content authoring platforms may also be suitable. Images used in the interactive elements of the lab are collated in Appendix D.

Padlet

Our main criterion for choosing Padlet as a platform to share Foldscope™ images is that students can access it simply via a link and are not required to create an account. Other platforms may also be suitable, if permitted by your institution. The instructor sets up a Padlet account, creates a gallery, and provides a link to students. The gallery can also be embedded within an LMS (Brightspace, Canvas, etc.), webpage, or blog.

Padlet offers a limited number of archivable, re-usable galleries with a free account, and unlimited galleries with a paid account.

Websites

Foldscope™
https://www.foldscope.com

Foldscope Microcosmos Community
https://microcosmos.foldscope.com/

Foldscope™ tutorials
https://www.foldscope.com/tutorials/

Fiji/ImageJ
https://imagej.net/Fiji/Downloads

iNaturalist
https://www.inaturalist.org/

iNaturalist Foldscope™ Community
https://www.inaturalist.org/projects/foldscope

Padlet
https://www.padlet.com

Prakash Lab
https://web.stanford.edu/group/prakash-lab/cgi-bin/labsite/

Remove metadata from Office files, PDFs, and images
https://www.cnet.com/how-to/remove-metadata-from-office-files-pdfs-and-images/

SoftChalk
https://softchalk.com/

Extension Ideas Contributed by Workshop Participants

The following ideas were contributed via workshop evaluations and an online survey: (1) Foldscopes™ would be an excellent resource for analysis in the field. Also, for living specimens such as *Euglena* or when looking at human cheek cells, hair specimen, and possibly even fingerprints for a forensics lab. – *anonymous; verbatim*

(2) Pollen diversity or density at different times of day/week/year (student picks independent variable,

hypothesizes, and tests). – *Mark, University of Central Oklahoma; verbatim*

(3) observations and data collection on field trips

(4) K-12 workshops and outreach

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Workshop Introduction

Mione S, Bacher K, Thierens H, Cornelissen M. 2011. Virtual electron microscopy in cell biology. Microscopy Research and Technique 74(3):251-256.

Mione S, Valcke M, Cornelissen M. 2013. Evaluation of virtual microscopy in medical histology teaching. Anatomical Sciences Education (6)5: 307-315.

Yong E. 2015 Sep 1. The $1 pocket microscope. The Atlantic (Washington, DC). [accessed 2018 May 12]; https://www.theatlantic.com/science/archive/2015/09/one-dollar-origami-microscope-Foldscope/403156/ .

Chloroplast Diversity Lab Student Outline

Anderson D. 2014. Still going strong: Leeuwenhoek at eighty. Antonie van Leeuwenhoek 106: 3-26.

de Vries J and Archibald JM. 2018. Plant evolution: landmarks on the path to terrestrial life. New Phytologist 217: 1428-1434. [accessed 2018 October 27; https://nph.onlinelibrary.wiley.com/doi/full/10.1111/nph.14975

Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB, Rawle F, Durnford D, Moyes C, Scott K, Walde S. 2018. *Campbell Biology.* Second Canadian Edition. Hoboken, NJ: Pearson Education Inc.

Solymosi K. 2012. Plastid Structure, Diversification and Interconversions I. Algae. Current Chemical Biology 6:167-186.

Acknowledgments

The Chloroplast Diversity Lab and the Foldscope™ portions of the Microscopy and Osmosis and Diffusion labs were developed by Jennifer Van Dommelen and Jacob Fletcher, supported by a Teaching and Learning Enhancement Grant from Dalhousie University's Centre for Learning and Teaching. We thank Beverly Hymes and Herbert Vandermeulen for their feedback on early drafts of the Chloroplast Diversity lab, and the students and teaching assistants of BIOL 1020 and BIOL 1021 for their participation and feedback on all three labs. Special thanks to Ronnie Van Dommelen (Figure 1) and to students Alex Arsenault, Maya Jones, Jenny Kim, Allan Masterson, and Samuel Ogunbayo for their contribution of images to this manuscript (Appendix C).

About the Authors

Jennifer Van Dommelen is a Senior Instructor in the Department of Biology at Dalhousie University. She is the primary instructor in the department's online introductory biology courses and contributes to the development and supervision of the department's face-to-face introductory biology labs. Jacob Fletcher earned his MSc in Biology at Dalhousie University in 2017. He has worked as a teaching assistant in several courses, including introductory biology, plant and microbial diversity, developmental biology, and leadership in science. He has also worked as a teaching associate in Dalhousie's Centre for Learning and Teaching, and has recently obtained a full-time teaching science at the Shambhala School of Halifax.

Appendix A:
Activity 1: Chloroplast Diversity Lab - Student Questions

NOTE: Students work with a separate document concurrently with the online content and submit that document for grading. Other questions are completed online and are self-graded or offer instant feedback. Section titles below correspond with section titles in the instructional material in this article. For an offline adaptation of the lab, the student questions could be interleaved with the instructional material. Links to online versions of the labs presented during the workshop can be provided upon request to the corresponding author (Jennifer Van Dommelen; jennifer.vandommelen@dal.ca).

Chloroplast Diversity Lab

Obtaining and Assembling Your Foldscope

No questions for this section; please see online content.

Finding Specimens for the Foldscope

No questions for this section; please see online content.

Slide Preparation and Photo Tips

No questions for this section; please see online content.

Sharing Your Discoveries

1. In the space below, insert two or three photographs:
   - the general location where you collected your specimen and/or your specimen *in situ* (i.e., a closer view showing your specimen, if you can safely take one – don't risk dropping your camera into the water!)
   - an image of your specimen captured with your Foldscope™

All photos should be taken by you. For photos inserted into this document, reduce the file size to tens or hundreds of kilobytes (there are a range of web-based tools and apps that can resize an image for you).
In addition to inserting the photos into this document, post your Foldscope™ photo to the class Padlet gallery as instructed in the online content for this lab. Feel free to "like" and comment on other posts!
INSERT PHOTOS:

2. Give a brief description of your collecting site, including the location, date, time of day, duration, and weather conditions during your visit. Identify your specimen and the part shown in the Foldscope™ photo to the best of your knowledge. (Pro tip: If you post your photo to the Padlet gallery before the lab is due, you can get some help with identification!).
RESPONSE:

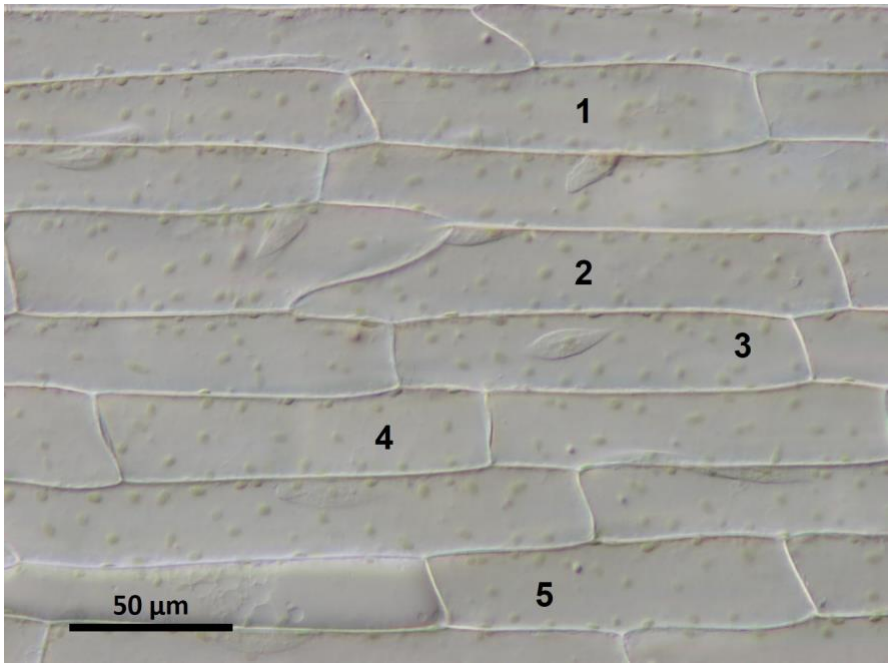
Downloading, Installing, and Testing Figi/Image J

No questions for this section; please see online content.

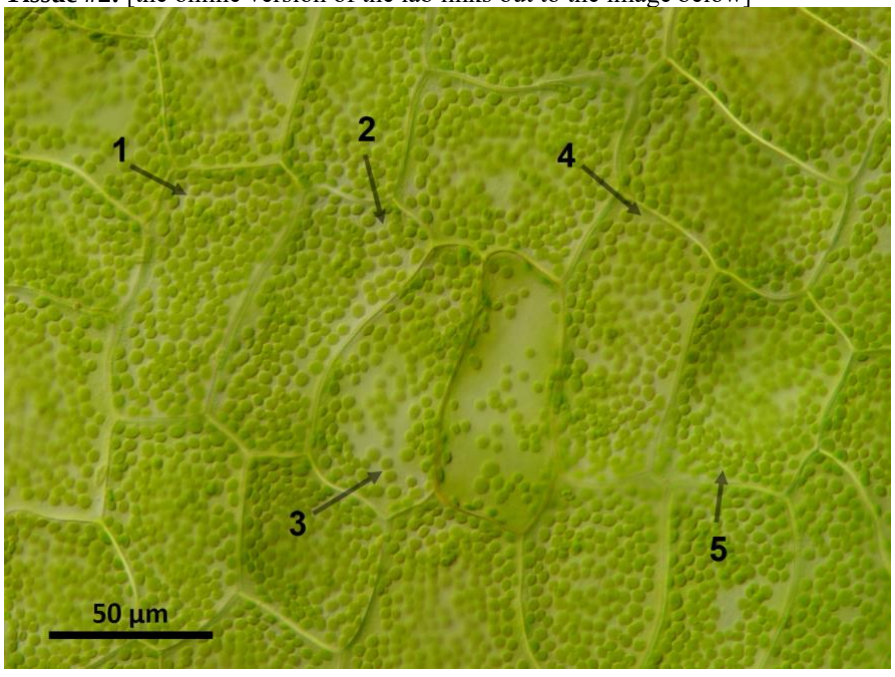
Chloroplast Density

3. Describe three differences that you can identify in the cells between these two tissue types.

RESPONSE:



Tissue #2: [the online version of the lab links out to the image below]



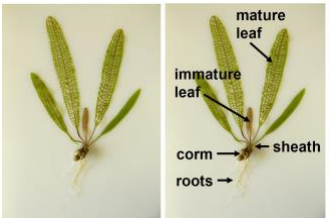
4. Use Fiji/ImageJ to count the chloroplasts in the identified cells in each photo and enter your results into the table below. Calculate the mean chloroplast density per unit area.

Table 1. Chloroplast density in two lace plant (*Aponogeton madascariensis*) tissue types.

	Tissue #1			Tissue #2		
	number of chloroplasts	cell area (μm2)	chloroplast density per μm2	number of chloroplasts	cell area μm2	chloroplast density per μm2
cell 1		3038.4			2767.1	
cell 2		3715.6			2985.2	
cell 3		2937.7			2602.2	
cell 4		3055.5			2995.8	
cell 5		2881.6			2757.4	
mean						

5. Refer to the labelled lace plant photo in the online content [thumbnails provided for reference]. Can you guess which part of the lace plant each tissue (1 and 2) came from? Support your response with reference to Table 1 and what you know about chloroplast function.

RESPONSE:



Chloroplast Morphology

Carefully review the online content for this section of the lab, including the interactive elements. You should also review the following sections of your textbook[7]:

- from Chapter 6: The Evolutionary Origins of Mitochondria and Chloroplasts
- from Chapter 25: The First Eukaryotes
- from Chapter 26: Interpreting Phylogenetic Trees

Chloroplasts and Evolution

6. Compare the information from your textbook to the de Vries and Archibald (2018) figure. In this lab, you learned about some differences between plant and algal chloroplasts – density, characteristic shape, relative size, etc. How is this morphological evidence consistent with endosymbiotic theory and the phylogeny of green algae and land plants as hypothesized by deVries and Archibald (2018)?

RESPONSE:

[7]Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB, Rawle F, Durnford D, Moyes C, Scott K, Walde S. 2018. *Campbell Biology.*Second Canadian Edition. Hoboken, NJ: Pearson Education Inc.

Appendix B:
Activities 2 & 3: Estimating Specimen Size & Plasmolysis Labs - Student Questions

AUTHORS' NOTE: Students work with a separate document concurrently with the online content and submit that document for grading. Other questions are completed online and are self-graded or offer instant feedback. Section titles below correspond with section titles in the instructional material (pp. 20-24 of this manuscript). For an offline adaptation of the lab, the student questions could be interleaved with the instructional material. Links to online versions of the labs presented during the workshop can be provided upon request to the corresponding author (Jennifer Van Dommelen; jennifer.vandommelen@dal.ca).

Microscopy Lab (questions related to Foldscope™ only)

Sharing Your Discoveries

1. In the space below, insert one or two photographs:
   - an image of red onion cells captured with your Foldscope™
   - an image of any other specimen of interest captured with your Foldscope™ (optional)

All photos should be taken by you. For photos inserted into this document, reduce the file size to tens or hundreds of kilobytes (there are a range of web-based tools and apps that can resize an image for you). If you use your device's optical zoom, do not zoom in so far that you lose the 'circle' of the Foldscope™ field of view – you will need this for a question later on in the lab.

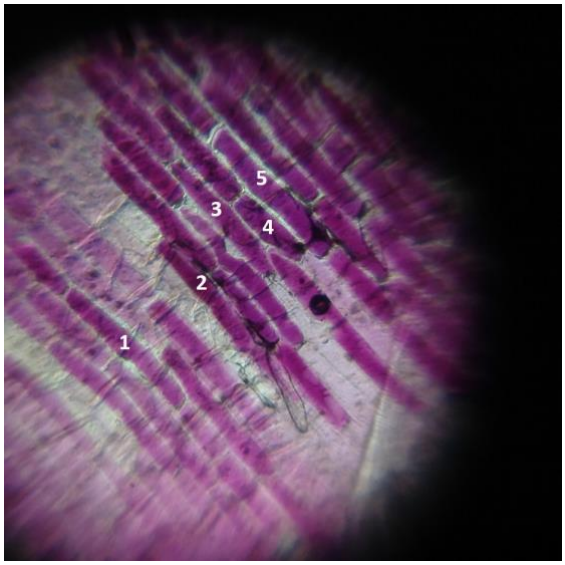
In addition to inserting the photos into this document, post your Foldscope™ photo(s) to the class Padlet gallery as instructed in the online content for this lab. Feel free to "like" and comment on other posts!

INSERT PHOTOS:

Measuring What You See: Viewing Magnification, Field of View, and Specimen Size

6. The approximate field of view of the Foldscope™ is 88 μm. Working from your photo, choose any five cells and determine the average length and width of a red onion cell. Show your work. If you were not able to obtain a satisfactory photo, you may work with the one on the next page.

RESPONSE:



Osmosis and Diffusion Lab (questions related to Foldscope™ only)

Observing Plasmolysis

7. In the space below, insert three photographs: one of each of the three red onion cell 'treatments' described in the online content for the lab.

All photos should be taken by you. For photos inserted into this document, reduce the file size to tens or hundreds of kilobytes (there are a range of web-based tools and apps that can resize an image for you). It is okay to use the optical zoom on your device if doing so improves your image and allows you to see the cell better – for this lab, your photo does not need to include the entire field of view.

In addition to inserting the photos into this document, post your Foldscope™ photos to the class Padlet gallery as instructed in the online content for this lab. Feel free to "like" and comment on other posts!

INSERT PHOTOS:

8. Examine your three best photos, one for each treatment, and describe what you SEE. (This is different from describing *what happened*…that comes next!)

RESPONSE:

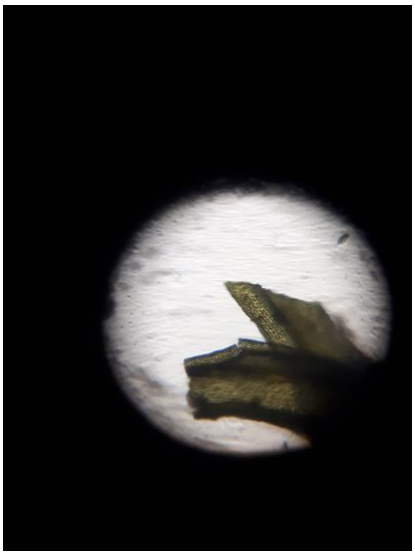
9. Briefly explain your observations in terms of osmosis and what you know about the structure of plant cells. Address the following points explicitly:

- in comparing the Tap Water and Salt Water treatments to each other and to the No Treatment condition, whether there is an apparent change in the size of the cells, and why or why not
- the net direction of water flow for each treatment
- the net direction of solute flow for each treatment
- the tonicity of Tap Water and Salt Water treatments

RESPONSE:

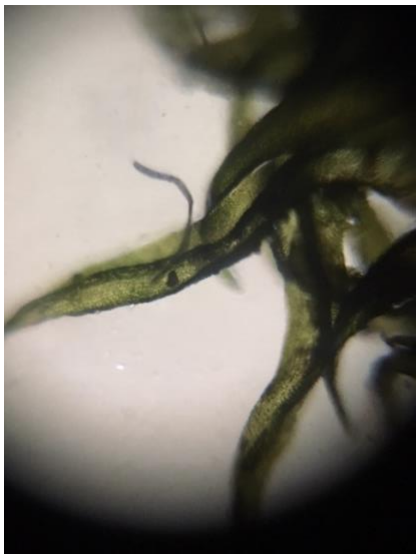
Appendix C: Student Photo Submissions

A selection of photos submitted by students during the Winter 2019 term. (The only criterion for inclusion is that these students replied to a general call for photos for this purpose and gave their explicit consent.)



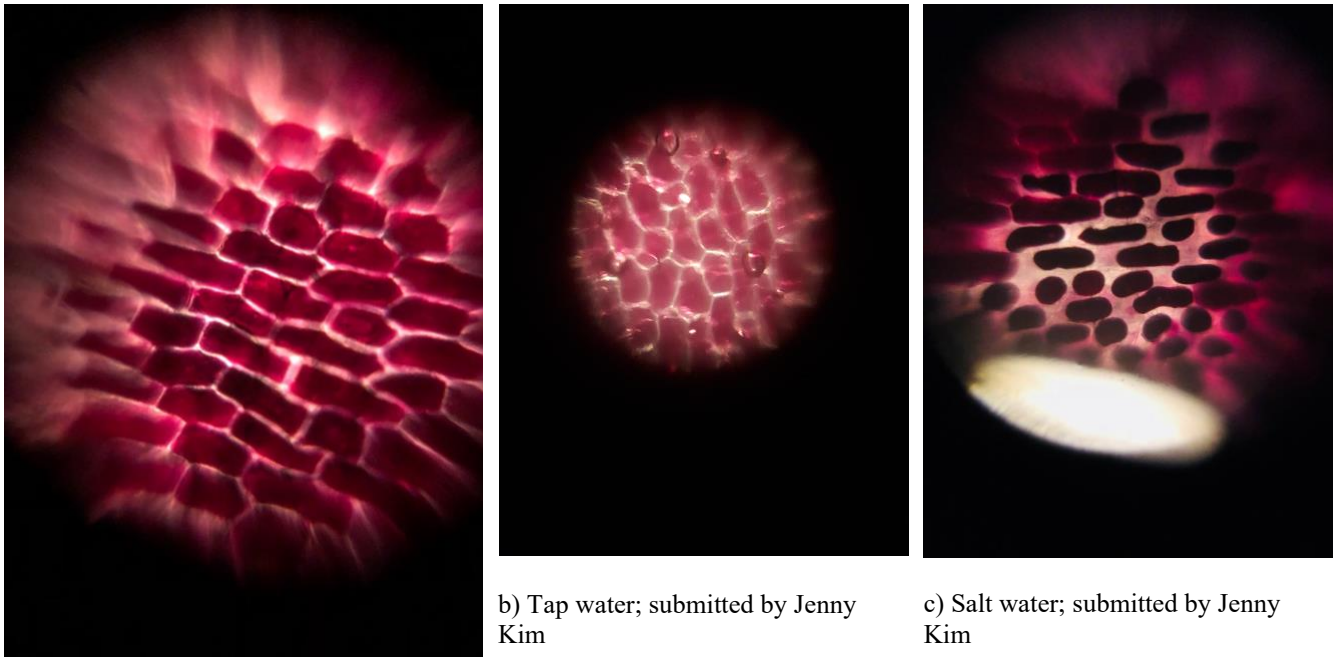
a) Unidentified moss; submitted by Samuel Ogunbayo

b) Curveleaf hypnum moss (*Hypnum curvifolium*) (unverified); submitted by Maya Jones



c) Unidentified moss; submitted by Allan Masterson

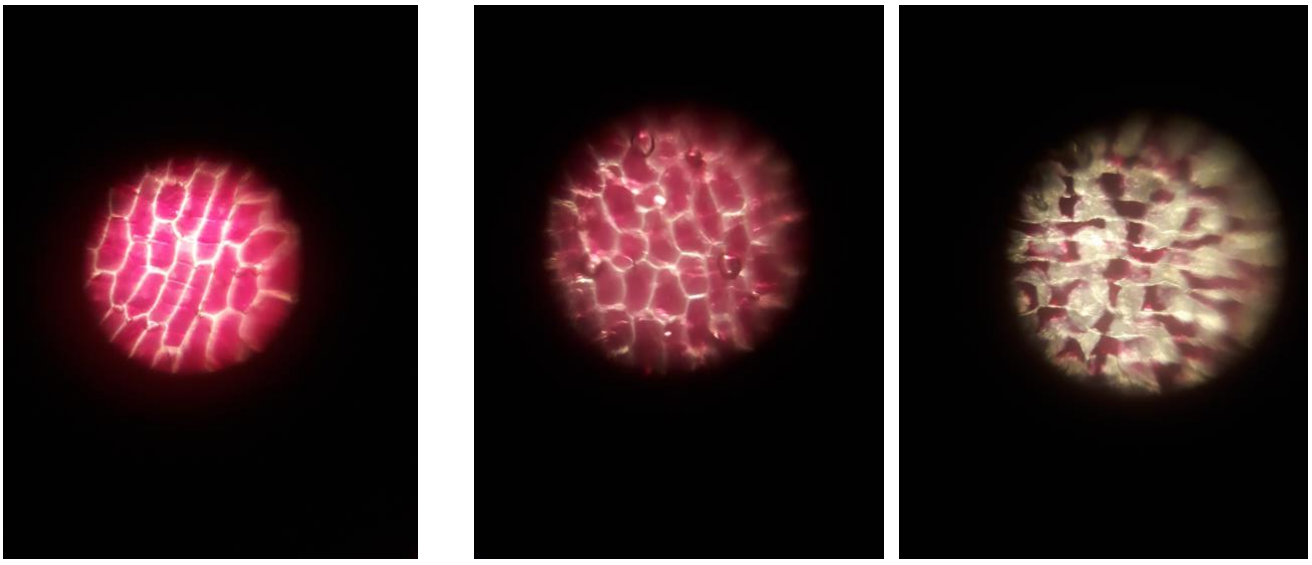
Figure 26. Foldscope™ photos submitted by students to the Chloroplast Diversity Padlet gallery, Winter 2019. Viewing magnification is 140X for all photos.



a) Untreated; submitted by Jenny Kim

b) Tap water; submitted by Jenny Kim

c) Salt water; submitted by Jenny Kim



d) Untreated; submitted by Alex Arsenault

e) Tap water; submitted by Alex Arsenault

f) Salt water; submitted by Alex Arsenault

Figure 27. Foldscope™ photos submitted by students to the Osmosis and Diffusion Padlet gallery, Winter 2019. All specimens are red onion (*Allium cepa*); treatments as indicated. Viewing magnification is 140X for all photos.

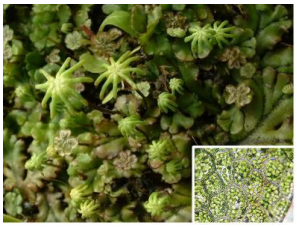
Appendix D: Image Attributions

Image attributions for the **Chloroplast Morphology** exercises of the Chloroplast Diversity lab.

(a) lady fern (Pteridophyta)

in situ: By No machine-readable author provided. MPF assumed (based on copyright claims). [GFDL, CC BY-SA 3.0 or CC-BY-2.5], via Wikimedia Commons

chloroplasts (inset): Courtesy of Carolina Biological Supply Company; www.flickr.com/carolinabio



(b) liverwort (Marchantiophyta)

in situ: By Jeffdelonge [GFDL or CC BY-SA 3.0], via Wikimedia Commons

chloroplasts (inset): Introduction to Liverworts (2018). Biology 321 – UBC. [online] Available at: http://www3.botany.ubc.ca/bryophyte/liverwortintro.html [Accessed 23 Aug. 2018]

(c) orchid (Orchidaceae)

in situ: By Arne and Bent Larsen or A./B. Larsen [CC BY-SA 2.5 dk], via Wikimedia Commons

chloroplasts (inset): Shutterstock. [online] Available at: https://www.shutterstock.com/video/clip-8401891-green-chloroplasts-cells-leaf-orchid-plant-under [Accessed 23 Aug. 2018]

(d) sycamore maple (Magnoliophyta)

in situ: By Willow [CC BY-SA 2.5], via Wikimedia Commons

chloroplasts (inset): MSU.edu (2018). Cell Unit. [online] Available at: https://msu.edu/~lupalisa/webplans/biologyunits/Cells/unitpagecell.htm [Accessed 23 Aug. 2018]

(e) thyme moss (Bryophyta)

in situ: By HermannSchachner [CC0], via Wikimedia Commons

chloroplasts (inset): By Kristian Peters [GFDL or CC BY-SA 3.0], via Wikimedia Commons

(f) white pine (Coniferophyta)

in situ: By US FWS [Public domain], via Wikimedia Commons

chloroplasts (inset): Pine needles under the microscope. Leaf, B. (2018) [online] Available at: https://www.dreamstime.com/stock-photos-pine-needles-under-microscope-image28153803 [Accessed 24 Aug. 2018]

Figure D1. The first part of the Chloroplast Morphology exercise requires images of whole plants from a variety of taxa and their chloroplasts; other images may be substituted.

(a) *Cryptomonas ovata*; number of chloroplasts: 1

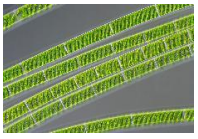
Plingfactory.de. (2018). [online] Available at: http://www.plingfactory.de/Science/Atlas/Kennkarten%20Algen/AndereAlgen/Source/Cryptomonas%20ovata.html [Accessed 23 Aug. 2018]

(b) Vaucheria; number of chloroplasts: 1

By Carolina Biological Supply [CC BY-NC-ND 2.0], via Flickr

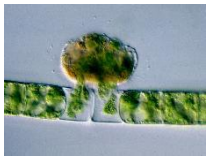
(c) *Glaucocystis* sp.; number of chloroplasts: 0, they contain endosymbionts called cyanelles

By ja:User:NEON / commons:User:NEON_ja [CC BY-SA 2.5 or CC BY-SA 2.5], via Wikimedia Commons



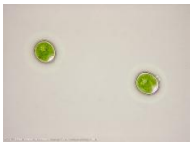
(d) *Spirogyra* sp.; number of chloroplasts: 1-4

By Wiedehopf20 [CC BY-SA 4.0], via Wikimedia Commons



(e) *Ulothrix*; number of chloroplasts: 1

By Norbert Hulsmann [CC BY-NC-SA 2.0], via Flickr

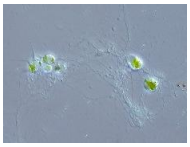


(f) *Chlamydomonas*; number of chloroplasts: 1

By Marc Perkins [CC BY-NY 2.0], via Flickr

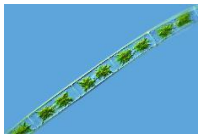
(g) *Cladophora* sp.; number of chloroplasts: many, interconnected in a net-like structure

By Pryoyecto Agua [CC BY-NC-SA 2.0], via Flickr



(h) *Chlorachnion raptans*; number of chloroplasts: 1

By ja:User:NEON / commons:User:NEON_ja [CC BY-SA 2.5 or CC BY-SA 2.5], via Wikimedia Commons



(i) *Zynema*; number of chloroplasts: 2

By Pryoyecto Agua [CC BY-NC-SA 2.0], via Flickr

(j) *Dinobryon divergens*; number of chloroplasts: 1

By Frank Fox (http://www.mikro-foto.de) [CC BY-SA 3.0 de], via Wikimedia Commons

Figure D2. The second part of the Chloroplast Morphology exercise requires images of algae from a variety of taxa that demonstrate diversity in chloroplast morphology; other images may be substituted.

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