Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Volume 39, Article 13, 2018

A Rich Introductory Inquiry-Based Cell Motility Lab Utilizing *Dictyostelium discoideum* Chemoattraction

Jonathan E. Moore[1], Melissa L. Petreaca[2], and David A. Knecht[3]

[1]Pomona College, Biology Department, 175 W 6th St, Claremont CA 91711 USA
[2]Depauw University, Department of Biology, 1 E Hanna St, Greencastle IN 46135 USA
[3]University of Connecticut, Department of Molecular and Cell Biology, 91 North Eagleville Rd, Storrs CT 06269 USA
(**jon.moore@pomona.edu; melissapetreaca@depauw.edu; david.knecht@uconn.edu**)

We have developed an introductory to intermediate inquiry-based cell motility lab with extensive microscopy, a relative minimum of financial overhead, and a minimum of specialized software. We use refurbished iPods to capture time-lapse video of *Dictyostelium discoideum* migration toward a chemoattractant. This five to seven session lab develops students' lab techniques, numerical skills, and statistical methods, as well as providing opportunity for literature exploration, scientific inquiry, and presentation of findings. This lab has yielded a wealth of ways that students modify it for their own inquiry. This lab can also be scaled back into several shorter formats including a one to two session non-inquiry based lab.

Keywords: cell motility, cell biology, chemotaxis, inquiry-based learning

Introduction

Using microscopy in an inquiry-based cell biology lab was long an elusive goal in our introductory curriculum. To achieve this goal, we introduced a version of this lab four years ago as the last project in course dominated by second semester students. Yearly, the lab has evolved, but at its core is the under-agarose chemotaxis assay using *Dictyostelium discoideum* (Woznica, 2006).

Dicty and the Under-Agar Assay

D. discoideum, colloquially *dicty*, is a soil dwelling amoeboid model organism that feeds upon bacteria (Chisholm, 2006). Folate is secreted by many of their bacterial prey and chemoattracts *dicty* (King, 2009). This assay takes advantage of this behavior by chemoattracting the *dicty* toward a folate source and forcing them to crawl between the inside surface of Petri dish and an agarose gel, thereby staying in one focal plane. We capture time-lapse videos of the crawling *dicty* and subsequently calculate various cell motility measures, like their speed and the directness of their paths toward the folate (Meijering, 2012). Appendix A shows some representative student data. Set up, videoing, and several other exercises can be accomplished in three hours.

Timeline and Level

As an investigative, inquiry-based project, this module takes us five to seven lab periods:
(1) an introduction of lecture and classroom activities
(2) a practice run in lab
(3) video & data analysis
(4) discussions about the students' proposed projects
(5) inquiry based project, week 1
(6) inquiry based project, week 2
(7) oral presentations of findings

Due to calendar constraints, we routinely shorten some lab sections to five weeks by first compressing the introduction and inserting it into week 2, and second turning week 3 into a homework exercise. Week 2 alone or weeks 2 and 3 make for a fine cookbook style lab.

This lab is at a fairly high level, and most of our students execute this near the end of their first year. These seemed inconsistent to many at the ABLE workshop. The selective nature of our college and the somewhat extended timeline allow this lab to work for us. At other institutions, this might work better as an upper-level lab. More mature students might also manage this with a more streamlined timeline.

Learning Goals

In addition to reinforcing cell biological concepts involving the cytoskeleton, signal transduction, and

chemotaxis, this lab introduces and reinforces many intellectual and lab skills. The intellectual ones include:
- mining the scientific literature
- designing experiments
- interpreting results including handling outliers
- constructing and presenting a scientific talk.

The lab skills include:
- using a compound microscope
- pipetting
- calculating and performing dilutions
- calibrating an instrument
- using a hemocytometer
- extrapolating from a sample
- using cell stains
- accounting for experimental artifacts
- aligning optics
- manipulation of spreadsheet data
- conducting and interpreting t-tests
- keeping a lab notebook

.

Student Outline

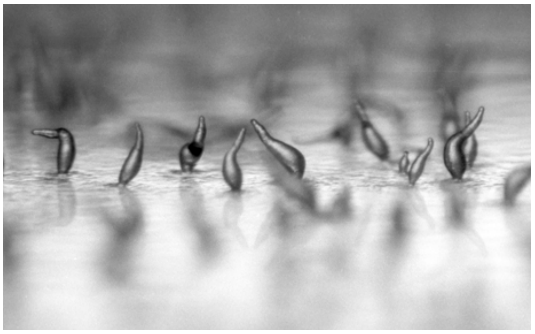

Figure 1. Image of *Dictyostelium* fruiting bodies by Matthew Springer. http://dictybase.org/dictyArt/ and http://news.nationalgeographic.com/news/2008/10/photogalleries/best-microscope-photos/photo4.html

A. Background

Most eukaryotes, including all animals, rely on *cell motility* – the movement of cells on their own – during their life cycle. Processes dependent on cell motility include many stages of embryogenesis, wound healing, synapse formation for learning and memory, the hunting of pathogens by immune cells, and others. Cell motility at inappropriate times is one of the causes of cancer metastasis – the process by which a cancer moves from its original location to others within the patient. Clearly, cell motility is centrally important to modern biology and medicine.

Despite its importance, our understanding of cell motility remains incomplete. Cell motility is a complex process requiring several different cellular processes: the detection of a chemoattractant – the molecule promoting cell migration up its concentration gradient, the detection of this chemoattractant gradient, the transmission of the resulting signal to the inside of the cell, and the mobilization of the cell machinery to promote coordinated movement specifically toward the chemoattractant's source. Studying cell migration is particularly challenging in higher organisms because of the complex nature of whole tissues, difficulties controlling and measuring a cell's microenvironment, and the difficulty of genetic manipulation in higher organisms.

Dictyostelium discoideum, colloquially *dicty,* is a slime mold that lives primarily in a vegetative social amoeboid form (Fig. 2). In its unicellular vegetative cycle, it is a haploid hunter of bacteria within leaf litter and soil. As such, it detects and moves toward its prey, which it subsequently phagocytoses. Obviously, effective prey detection and capture is vital for *dicty's* survival.

Dicty finds bacteria by sensing bacterially released chemicals called *pteridines*, such as *folate*. By detecting the gradient in folate concentration, which increases closer to a bacterium, *dicty* can orient itself and subsequently move toward a bacterium.

During the first week's lab, you will take advantage of folate-induced chemotaxis by:

- placing folate into a set of central wells in a Petri dish and adding vegetative *dicty* into other wells, then
- give time for the folate to diffuse outward forming a gradient of folate concentration for the *dicty* to follow and the
- measure by time-lapse video the resulting migration toward the folate source, and possibly migration away from the folate source. The migration away will serve as a control for non-chemotactic migration. An overview is illustrated in Figure 3.

While you are waiting for the gradient formation and *dicty* migration, you will:

- measure the density of the provided *dicty* culture and the fraction of live cells, and
- familiarize yourself with your compound microscopes, the software for image and video capture on the iPod, and how to work the two together.
- When you are done, you still need a sense of scale. For this, you will take a video of a length reference, a stage micrometer.

Next week you will take measurements of the migration, and calculate various measures of directionality and speed.

After familiarizing yourself with these general techniques, and searching relevant literature, you should be able to propose an experiment or experiments on *dicty* cell motility or chemotaxis. During the third week of lab, you will likely discuss your proposal with your lab professors. In the subsequent two weeks, you will execute the proposed and approved experiments. In the last week, you will present your ideas and findings to your classmates as an 8-minute presentation.

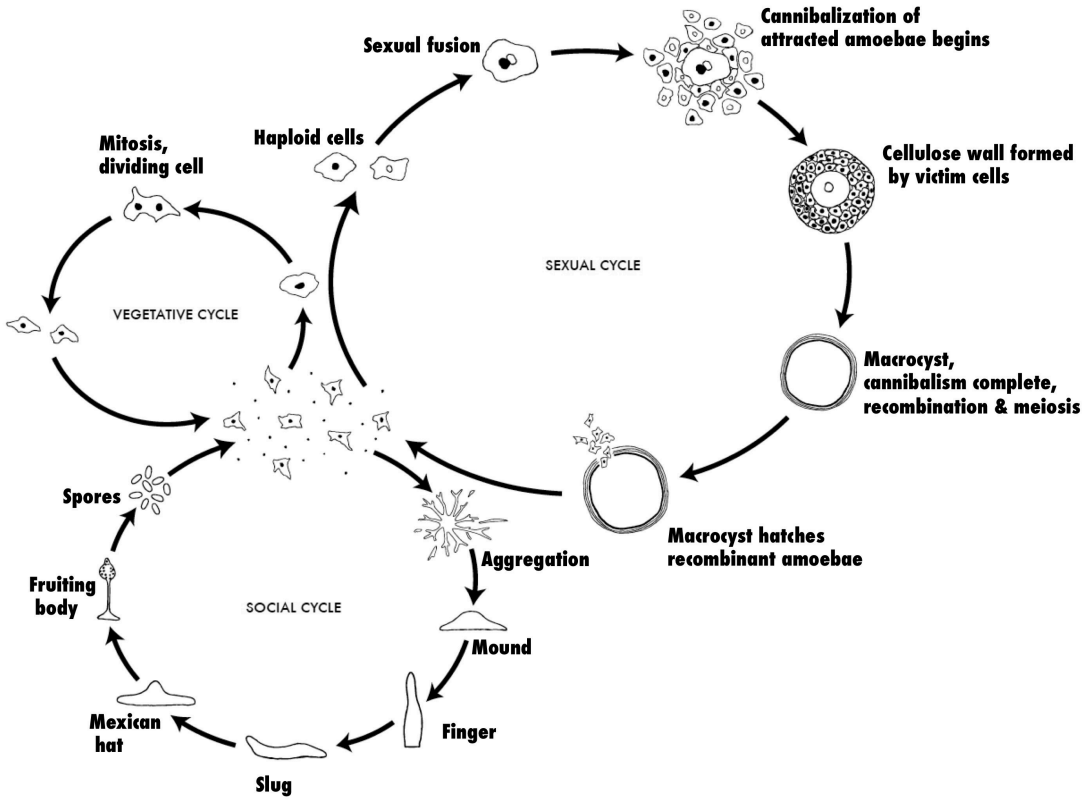

Figure 2. The life cycles of *Dictyostelium discoideum.* Most of its life, this haploid social amoeba undergoes the vegetative cycle, preying upon bacteria in the soil, and periodically dividing mitotically. When food is scarce, either the sexual cycle or the social cycle begins. Under the social cycle, amoebae aggregate to cyclic adenine monophosphate (cAMP) by the thousands, and form a motile slug, which moves towards light. Ultimately the slug forms a fruiting body in which about 20% of the cells die to lift the remaining cells up to a better place for sporulation and dispersal. Under the sexual cycle, amoebae aggregate to cAMP and sex pheromones, and two cells of opposite mating types fuse, and then begin consuming the other attracted cells. Before they are consumed, some of the prey cells form a cellulose wall around the entire group. When cannibalism is complete, the giant diploid cell is a hardy macrocyst that eventually undergoes recombination and meiosis, and hatches hundreds of recombinants. Not drawn to scale. *CC Creative Commons Attribution – Share Alike 3.0, David Brown & Joan E. Strassmann.*

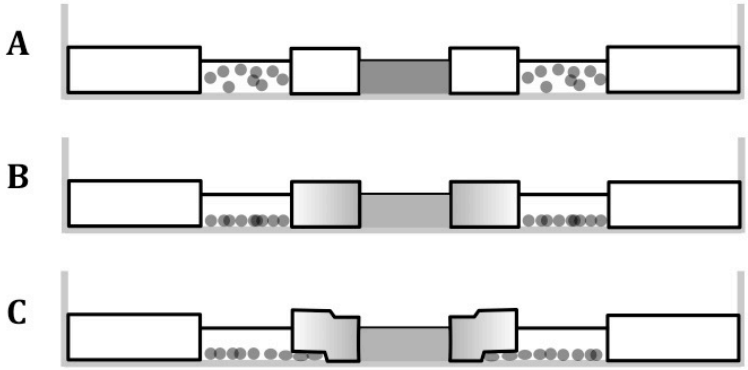

Figure 3. Cross-sectional diagrams of key stages in your experiment. **A.** At the end of procedure step 5, the Petri dish (black) should have folate (dark gray area) in the center wells and the cells (circles) are in the outer wells. **B.** Midway through the assay, the cells have settled to the bottom of the wells, and the folate has diffused to the cells making a gradient. **C.** At the end of the assay (step 28), some cells are crawling toward the folate source between the agarose and the Petri-dish bottom.

B. Experimental Procedure: Measuring Motility & Cell Density

Pouring Agarose Plates

This has already been done before class. Sorensen's buffer (Sor) and agarose are microwaved or heated on a heat plate to melt the agarose. 6 mL of the resulting media is added to each 6-cm Petri dish. The final concentration of agarose is 1.0% w/v.

Cutting the Wells in the Agarose Plates

Now you will cut the wells in which you will place the folate and *dicty*. To be successful, you will want to leave the plastic of the Petri dish relatively unscratched, and move the agarose gel that forms the wall of the wells as little as possible.

1. Lay the plate on top of the template on this page (Fig. 4a) so that you have a cutting guide. When cutting the wells, open this manual so that the plate is resting on a single sheet of paper. Cut aligning the brass punch vertically over one of the rectangles. Smoothly push downward through the gel in a single quick motion while using as little force as possible. Raise the punch. Do not rotate the punch or slide it side-to-side within the gel. Repeat this motion for all seven wells. (The distance between the wells is ~2 mm.)

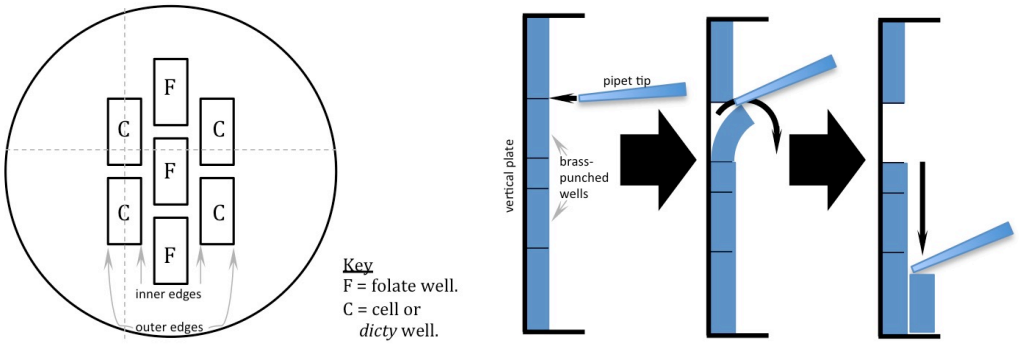

Figure 4a. Template for cutting wells in plate. The horizontal & vertical hatched lines indicate possible cross-sectional views in Figures 3 & 4-right, respectively. **b.** Pick up the plate and hold vertically. Using a pipet tip, pry out each agarose block and move it to the edge of the plate.

2. After the wells are cut, hold the plate vertically. Use a pipet tip to lift up a short edge of one agarose block and remove it. (Fig. 4a). Be careful to not move the agarose gel between the wells. If you do, note the regions of movement. Repeat for all the wells, progressing from the top to the bottom as you do so.

3. Repeat this with a second plate. Label both plates with the date and your initials on both the lid and the bottom.

Adding the Folate and Dicty *Cells*

4. Carefully add 60 µL of the provided folate solution to each of the three wells in the center row, ensuring that it covers the bottom of the well. When pipetting, do not push through to the second stop; doing so causes bubbles which may cause spills. Be careful to not move the agarose gel between the wells or spill folate outside of the wells. Repeat with the second plate.

5. Flick several times the provided tube of *dicty* cells to mix them. Carefully add 60 µL of this cell suspension to each of the four outer wells, using the same methodology as before with the folate.

Place the lid on the plate, and slide to an out of the way place on the bench.

Repeat with the second plate. At this point, your plates should be set up like Figure 3A.

Wait at least 80 minutes and preferably 90. During this time, the folate will diffuse and the *dicty* will migrate (Fig. 3B & C). After this, you will take a time-lapse video and measure the speeds of individual *dicty* cells. In the meantime, perform the next two subsections: "Calibrating your Microscope Setup" and "Measuring Cell Density."

Familiarizing Yourself with Your Microscope

You will first familiarize yourself with your microscope, use it to observe some sample slides, and then learn to how to use it with an iPod to take photographs and time-lapse videos.

As background, the microscopes we use in this lab might appear old. They are. But they have two great attributes for this teaching lab. First, the optics are still great when clean and treated with respect. (Do not touch lens with your fingers, and always turn off and cover your microscope at the end of the day.) Second, they are simple. There are plenty of the more modern microscopes with fancier optical setups, but they are more complicated to use and easier to foul up. In this introductory lab, your instructors find that these work well and are less frustrating.

Most of the microscopes in this lab have only eight parts to change (Fig. 5).

#1. On-off switch: This is found on the power cord.

#2. Lamp position control: The knobs for this are small and under the *stage*, the flat area on which you put your slide. Rarely will lamp position be important in this lab. If the lamp is all the way up, humidity can build up in your plate and fog some of the lenses.

#3. Diaphragm control lever. This slender lever is found just below the stage. This lever adjusts the size of the hole that lets in light. Frequently novices will have this too far open, yielding too much light, particularly at low magnifications, making it difficult to visualize the cells.

#4. Slide clips: These are on the stage and hold the slide so that you can move it around with the micromanipulators. Be sure the slide is flush against the stage when using these, or the spring could catapult your slide off the stage.

#5. Micromanipulators: These knobs to the side of the stage allow you to more easily control the movement of your slide. One controls forward and back, while the other controls side-to-side.

#6. Focus knobs: These raise and lower the stage of the microscope so as to bring the item in focus. The one closer to the stage is the coarse focus and allows for faster control; the one further down and further from the stage is the fine focus and allows for more precise control. When using these, be careful to <u>not</u> ram the lens into your sample; this is especially easy to do at higher magnifications.

#7. Objective lenses: These lenses on the nosepiece allow you to control magnification. Most of the microscopes in this lab have three objective lenses of different powers: 3.5x, 10x, and 40x. <u>Check these</u> by looking on the side of the lenses. To get the total magnification to your eye, multiply the power of the objective lens by the power of the eyepiece, which in all cases in this lab is 10x. To change the magnification, turn the nosepiece so it clicks in place and the desired lens is down.

#8. Eyepiece: You have two of these. You use the one to which you mount the iPod to video your cells and stage micrometer. The one without the iPod is useful for seeing greater detail and finding the migrating cells. Last, when moving a microscope, use one hand to grab it by the arm – the solid part between the tube and the stage.

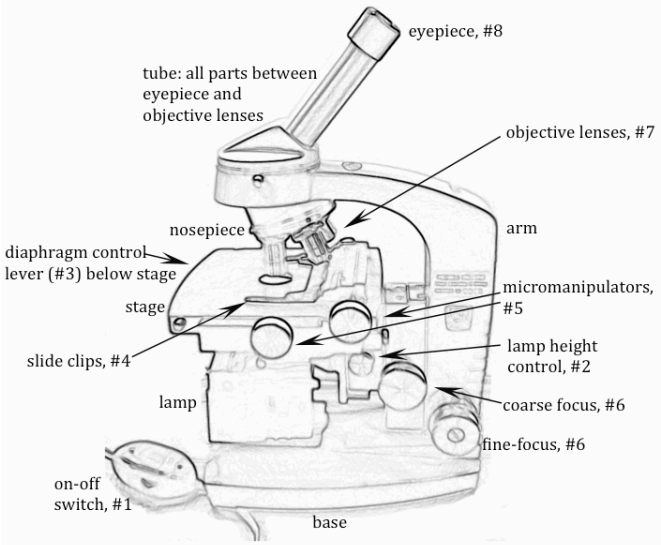

Figure 5. See the text for descriptions of the numbered parts of a microscope, the parts you can adjust.

the stage – and the other hand to support it beneath the base. <u>Do not</u> drag it across the bench top, as vibrations misalign the optics.

6. Pick a specimen slide from the red slide box. Handling the slide near its edges, clip to the stage.

7. Turn on the microscope. Pull the diaphragm lever all the way clockwise, closing it as much as it allows. Turn the nosepiece so the lowest magnification lens is down, but do not grab the microscope by the objective lens. (It is often best to start with the lowest magnification, since that is typically easier to focus and provides more context.)

8. Use the micromanipulators to bring the interesting part of the slide underneath the objective lens.

9. Using the coarse focus, bring the objective lens and stage as close together as possible. Do not ever allow the objective lens to touch the slide.

10. Now while looking through the eyepiece, use the coarse adjustment to bring the stage and objective further apart until the specimen is roughly in focus. Now fine-tune the focus with the fine focus knob. What do you see?

11. Choose another slide and let the other lab partner perform steps 6 through 10.

Taking Pictures with your Microscope

12. Now mount the iPod adaptors (Figure 6) following the steps below.

a. Pull the eyepiece out of your microscope and stand it on your bench, lens up.

b. Slide two tube clamps (Figure 6, insert) onto the tube of your microscope (Figure 5).

c. Replace the eyepiece.

d. Place the cylindrical part of the adaptor over the eyepiece. Gently push on it until it is snug.

e. Raise one tube clamp. Tighten its thumbscrew to secure the adaptor to the eyepiece.

f. Raise the other tube clamp to just below the eyepiece. Tighten its thumb screw (i) tightly enough that the eyepiece does not wiggle and (ii) loosely enough that you can still easily slide out the eyepiece.

g. Slide the iPod onto the platform from the left with the touchscreen toward you and the back camera roughly over the camera hole.

h. If you unplugged the iPod, re-plug it back in so that it will not lose charge while taking video.

13. On the iPod, slide to unlock, and select the camera. Slide the iPod left and right until you can see something through the eyepiece. Adjust until you get a desirable image.

Sometimes the iPod is too far up or down the platform. If that is the case use a small piece of paper as shim: folded it 4- or 8-thick and place it between a bracket and the iPod to tweak it into position.

14. Take a picture. Note what this picture is in your notebook.

15. Change the objective lens to 10x, and possibly adjust the focus and the diaphragm, if needed. Take another picture. Note again.

16. Change to the highest magnification and adjust as before. Take another picture and notate.

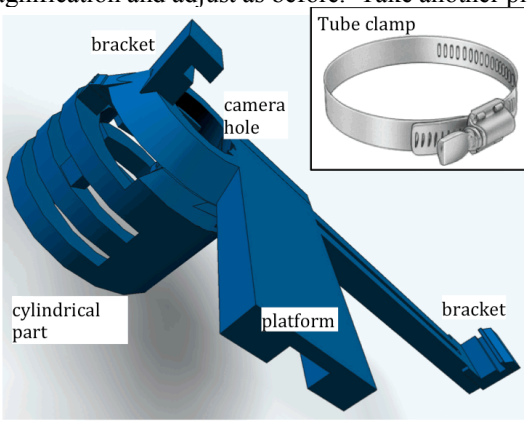

Figure 6. A 3D-printed adaptor and tube clamp.

Calibrating your Microscope Setup

By themselves, any photographs or videos will not provide a sense of scale. Given the importance of distance in this lab, you will need to determine how many *pixels* there are per some length, and do this for each magnification. This is the calibration process.

17. Locate the stage micrometer – slide containing a tiny 2-mm long ruler (Fig. 7). It looks like a microscope slide except its label, and is in your red slide box.

18. Set aside your specimen slide for a moment. Mount the stage micrometer on the microscope stage. Adjust to the lowest magnification. Adjust the focus as needed to find the 2 mm ruler.

It might help to set the iPod aside and use the other eyepiece, since your eye by itself has much better resolution and sensitively than the iPod screen. If you do this, when focused, exchange the eyepieces again.

19. Press the home button on the iPod, and tap "Lapse It." You will now create a calibration video using Lapse It.

20. Choose "New Capture." The frame interval and resolution should be set to 6 seconds and 720p, respectively. If not, tap them and adjust.

21. Tap "capture." While it is clicking and taking pictures, adjust the microscope so that you get at least one good picture at each magnification. Press "stop."

22. At this point, you have a set of photos, and no video. To render (make) a video:

a. Tap "rotate project" so it will later fit your computer screen better.

b. Tap "render."

c. Tap "copy to camera roll."

d. Tap "adjust settings." Scroll down to "Render Settings" and adjust frames per second to 1. Click back.

f. Name your video.

g. Tap "render." Set the frame per second to one. When completed, choose "Cancel."

h. If you get an error, likely you named your file with a forbidden symbol.

23. To view your photos and videos, hit the home button, and choose "Photos." Your photos will typically be in "Photo stream," and your rendered videos in an album named, "Camera roll."

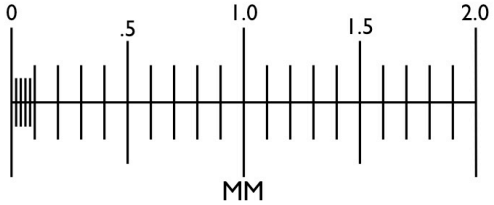

Figure 7. The 2-mm ruler within the stage micrometer.

Measuring Cell Density

Measuring cell density and rate of death serves a multitude of purposes: (1) If your cells are dead, they certainly will not move on their own. Though hopefully not a problem this week, your treatments during your independent projects could kill them, and that will make interpreting the rest of your data easy. Thus, you should do this with each different treatment you have then. (2) Also, if your cells are considerably less dense one week compared to another, you might expect to see fewer cells migrating in the former case. Again, the additional measurement eases the interpretation of other data.

You will use a compound microscope and a hemocytometer — a microscope slide modified to allow for the counting of cells in a known volume. For this, we will follow a procedure very similar to the one here: http://www.youtube.com/watch?v=pP0xERLUhyc

The trypan blue used in this assay will penetrate the cell membranes of dead cells, thereby staining them blue. This allows us to additionally determine what fraction of the cells is dead. Safety Note: There is some evidence that trypan blue causes cancer. When using the trypan blue solution, wear gloves.

24. Mix the provided dead stained cells and add 6 µL of them to the hemocytometer (Fig. 8).

25. Exchange the eyepieces to remove the iPod from your microscope. Use your microscope to observe the dead cells in the counting grid (Fig 8). Note to yourselves how blue they are. Unless you need a reminder of how blue dead cells are, you will only need to do this your first week of lab.

26. The density of the provided *dicty* cells is too high for easy counting, and we will need to add a trypan blue to allow us to determine which cells are alive and which are dead. Combine in a microfuge tube 80 µL Sor, 10 µL trypan blue solution, and 10 µL provided *dicty* cells, freshly-mixed. Mix this. Add 6 µL of these stained cells to another counting grid of the hemocytometer. Again, use your compound microscope to look at them. Count cells within 1-mm^2 fields until you have either at least 50 cells or four 1-mm^2 fields counted (Fig. 9). Hand counters make this process easier and more reliable. If there are any, count any dead cells separately.

When engaged in research, it often occurs that you have *dead time,* time in which you're waiting for something else to happen. When this happens, researchers often do other experiments, work on their lab notebooks,
research new projects, make new reagents, or clean up While you are waiting for your migration to finish, recall that you could be working on your lab notebook or discussing ideas for your project.

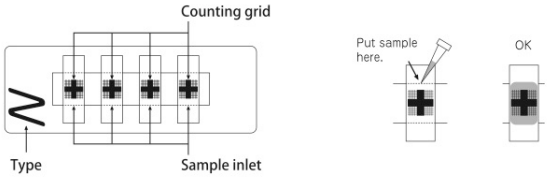

Figure 8. A diagram of the hemocytometer showing where to load your cells — the sample inlet — and where to look for them under the microscope — the counting grid. The right half depicts the sample loading.

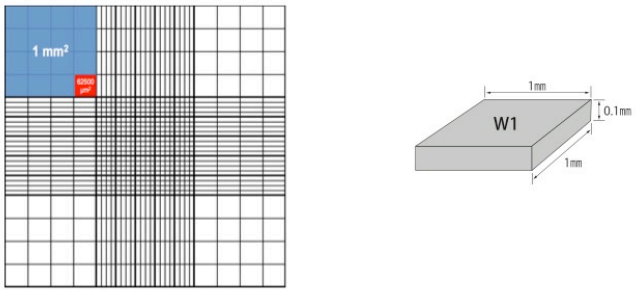

Figure 9. The grid pattern of an Improved Neubauer hemocytometer. The largest square is 3 mm on a side. This largest square is subdivided into nine 1-mm^2 fields. A 1-mm^2 field is also 0.1 mm high, yielding a volume of 0.1 mm^3 = 0.1 µL.

Time-lapse Videoing Cell Movement

27. After at least 80 minutes of migration, and preferably 90 or more, remove the lid from the plate. Slightly over-fill the all the wells of one plate with additional Sor. The fluid in the wells should dome up; a bit of overflowing is Ok. (Fig. 10)

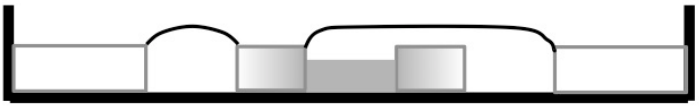

Figure 10. The left thin black line shows fluid in a well slightly domed. The right thin black line shows the liquid from two wells flowing into one another, which is Ok if not too extensive.

28. Place a new, clean coverslip across the four cell wells. To do so, place one short edge of the coverslip on the plate. Carefully lower the coverslip as close as you can above the plate, and drop it to minimize bubbles.

29. Put on the lid. Invert the plate to let excess fluid drain.

30. With a Sharpie, make 4 small marks on the plate in the cell wells near but not on the inner edges, like the C's in Figure 4.

31. Pick up the plate and place it still upside-down on the bench. Slide the blue-gray plate adaptor over it (Fig. 11). Clip these to the stage.

32. Following the same general procedures as before, focus on a mark at the lowest magnification.

33. Increase the magnification to 10x. With the course adjustment, slowly raise the stage to focus in on the cells still on the bottom (now top) of their well.

34. Move the plate to view an inner edge. Do you see cells migrating outside the well? Migrating cells should be flatter, bigger, and less refractive (lens-like) relative to their counterparts still in the well (Fig. 12). Cells in the well will be smaller, rounder, and more refractive. If you see cells like those inside the well outside the well, likely these cells did not migrate but flowed under the agarose due to issues when cutting the wells.

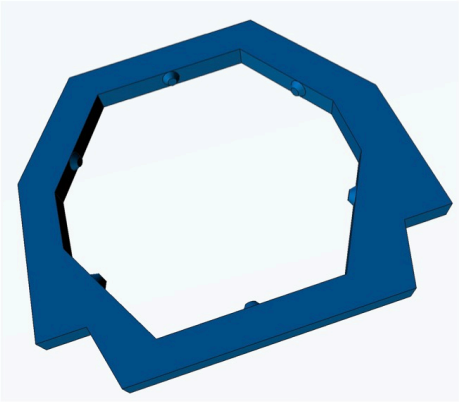

Figure 11. The plate adaptor slides over the plate and clips to the slide clips of your microscope, allowing you to move the plate with the micromanipulators.

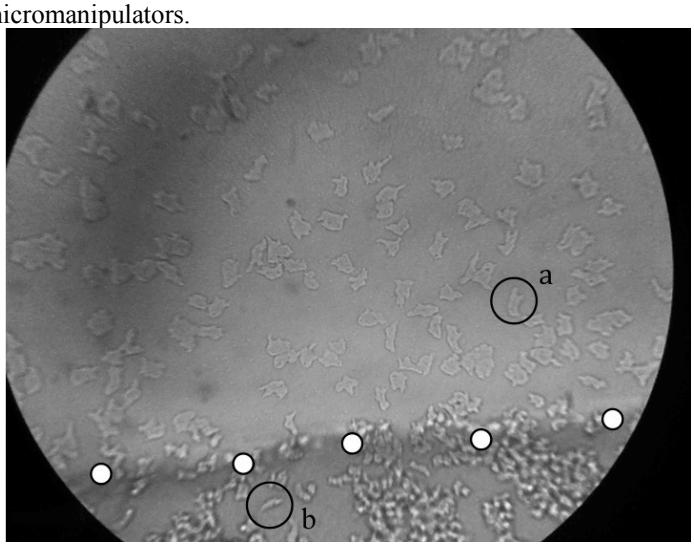

Figure 12. Live *dicty* are photographed at 400x. The folate source is up. White circles show the edge of the well. a: migrating cell. b: non-migrating cell.

If you don't see cells outside the well right away, this is normal. Sometimes a small adjustment to the focus is needed. Play with the fine focus for half a minute. If you see migration, that is great. If not, return the focus to the original position.

If you are in doubt if what you see are cells, they are often more distinctive with the highest objective. However, using the higher objective reduces the field of view, thereby making it more difficult to search.

35. Exchange eyepieces so as to mount the iPod. With the highest objective, take a time lapse of an inner- edge region with good migration. Rotate the plate so that the folate source is to the left, right, up or down, and not off at an angle. Note the direction of both the folate source and the well in your lab notebook. This information will be critical when assessing distance and directionality of migration later.

Set the frame interval and resolution to 6 seconds and 720p, respectively. Take this for 10 minutes. Render it as before but with frames per second set to 10.

If you see an outer-edge region with migration, take a similar video. While the iPod is collecting data, start step 37.

36. Time permitting, repeat the process with your second plate. Other than the 10 minutes of time-lapse, the process is often considerably quicker the second time. While the iPod is collecting data, start the clean up process.

Cleaning Up

37. Put away the stage micrometer, other sample slides, the spare eyepiece, and the plate adaptor.
38. Leave the tubes of dead cells, Sörensen's buffer, and trypan blue in your rack for tomorrow's class.
39. Throw away any remaining provided dicty cells, folate solution, and your plates.
40. Place your used hemocytometers in the "Used hemocytometers" Tupperware.
41. Remove the iPod and the adapter from the microscope.
42. Turn off and cover your microscope.

43. Transfer your videos off the iPod. (If one of you and your partner has a laptop, that could be handy to have.) You should have one video for microscope calibration and likely more than one of migrating cells. You will analyze these videos next week in lab following the procedure in Section C.

C. Experimental Procedure: Analyzing Your Videos

Last week you created videos of migrating cells and another for calibrating those videos. This week you will (1) track some of the videoed cells, recording their positions at different times, (2) convert pixels to µm for various distances, and (3) calculate various quantitative motility measures.

Spreadsheet software for these calculations will remove much tediousness. The instruction here is for Microsoft Excel. If you are would like help with Excel skills, like general functions, the auto-fill feature, and intermediate-level graphs, please look at the HHMI BioInteractive tutorials available at
http://www.hhmi.org/biointeractive/spreadsheet-data-analysis-tutorials

Tracking Cells

1. Open a video of migrating cells and bring it to actual size. First, we need to find the initial and final positions of a cell.

a. Choose a cell away from the folate.

b. Determine the initial screen position of the cell, $p_0 = (x_0, y_0)$ (Fig. 13). On a Mac, position the mouse over the center of the cell. Press Shift-Command-4, which will bring up crosshairs with the screen coordinates. The top number is the pixels from the left edge of the screen. The bottom number is from the top of the screen. Press Escape to make this go away.

For Windows, there is a similar free tool called Mofiki's Coordinate Finder which can be found at:
http://www.softpedia.com/get/Desktop-Enhancements/Other-Desktop-Enhancements/Mofiki-s-Coordinate-Finder.shtml
Site registration is required. Once downloaded and open, simply press the space bar to report the mouse coordinates.

c. Let the video run and follow the cell until the end of the video.

Was this straightforward? If not, did it wander out of the field of view before the video was done? Or did it run into, over, or under another cell so that it was difficult to follow? If it was not straightforward, decide if this is a problem. If so, choose another cell and start over.

d. Now determine the final screen position of the cell, $p_f = (x_f, y_f)$ (Fig. 13).

2. Repeat step 1 for nine more cells. A table in your lab notebook could be very useful.

3. Choose at random one of the cells you tracked. Now pause your video each second, and record the position of the cell each of those times (Fig 13). It might help to slow down your video as you do this.

4. Repeat step 3 with one additional cell.

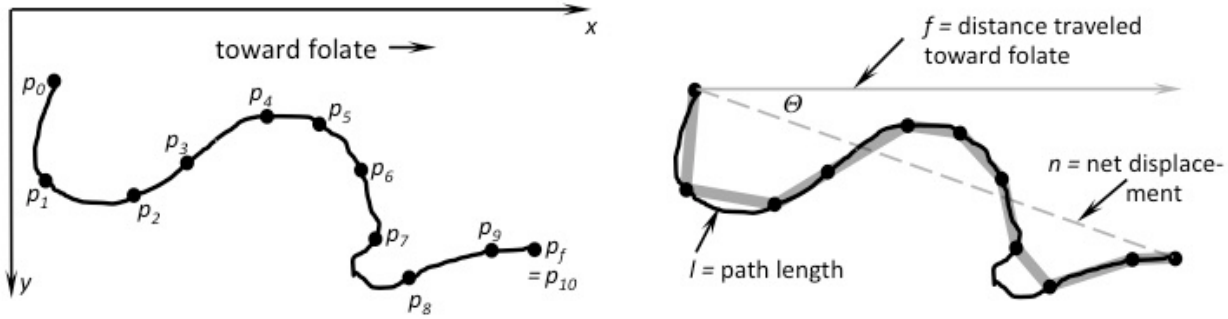

Figure 13, Left. The path of a cell and its position each second. p_0 indicates the initial position of the cell, and p_f its final position. **Right.** Different tracking measures: the net displacement (n), the distance traveled toward the folate (f), and the path length (l), which is by necessity an approximation. Also, the chemotactic index (c) is the cosine of Q.

Calculating Useful Intermediates

5. Determine the *net displacement, n*, of each of the ten cells (Fig. 13, dotted line). To do so, subtract the initial screen position from the final screen position and apply the Pythagorean theorem to get the net distance traveled. For any distance calculation in this section, that yields the formula

$$\text{distance} = \sqrt{(x_a - x_b)^2 + (y_a - y_b)^2}$$

What are the units? Is there a shortcoming of this as a unit?

6. The *distance traveled toward folate, f*, is the difference in either your initial and final *x* values or your initial and final *y* values, depending which way it is toward the folate (Fig. 13, gray arrow). For example, if toward the folate is to the right, $f = x_f - x_0$. Note that unlike a true distance which can only be positive or zero, if your cell moves away from the folate, this value can be negative. Calculate this distance for each of the ten cells.

7. For the two cells which chosen in steps 3 & 4, determine the distance traveled each second, and sum these values for each cell. These are our estimates of the *path length, l,* for each cell (Fig. 13, thick gray line).

$$l = \sum_{i=0}^{f-1} \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2}$$

Converting to Distances to μM

Thus far, all your distances have been in pixels. These distances will vary depending on magnification you used and your microscope setup. In order to compare results from one group to another and from one day to the next, we need to convert pixels to μm.

8. Open the video of the stage micrometer and let it play until you reach the magnification that you used for your videos of migrating cells. Press pause.

9. Determine the screen positions for two points far apart on the ruler. Determine the distance in pixels.

10. Now determine the distance you measured in mm. Figure 7 might help to orient you. Convert that mm measurement to μm.

11. Calculate the number of μm per pixel.

12. Now multiply this by your net displacements (*n*'s), distances traveled toward folate (*f*'s), and path lengths (*l*'s).

Calculating the Motility Measures

Last you will calculate five motility measures for some or all your cells. For all these, use the distances in μm.

13. *Average speed. l' = l / t*

For the two cells tracked in steps 3 & 4, divide the path length by the time the cell was tracked, *t*. Usually this is ten minutes, but it might be longer if your video is extra long or shorter if your cell left the field of view, for example.

14. *Average straight-line speed. n' = n / t*

For all ten cells, divide the net displacement by the time the cell was tracked.

15. *Average speed toward folate. f' = f / t*

For all ten cells, divide the distance traveled toward folate by the time the cell was tracked.

16. *Chemotactic index. c = f / n*

This is the ratio of the distance traveled toward folate to the net displacement. Note this is the cosine of θ in Figure 14. Calculate this for all ten cells. This measure can range from -1 to 1. Higher positive values show chemoattraction, more negative values show chemorepulsion, and values near zero show neither.

17. *Straightness index. s = l / n*

This is the ratio of the net displacement to the path length. Calculate it for the two cells. It ranges from 0 to 1. When near 0, the cell is meandering quite a bit, and when near one, the cell is traveling in a rather straight path. This measure also goes by many names including the chemotactic ratio, the meandering index, the confinement ratio, and the McCutcheon index.

18. Your instructor may ask you to make similar calculations using your other video of migrating cells. Please pay attention to her instructions.

T-Test

The central limit theorem of statistics states that when many small independent sources of variability are added together, the resulting distribution tends toward a normal distribution or bell shaped curve (Figure 14). The commonness of normal distributions gives considerable power to statistical tests like the t test, which assumes that two data sets are samples drawn randomly from a single population of normally distributed data. If the two sets are more different than one would expect in 95% of such drawings (assuming a P-value cutoff of 0.05), then we conclude - with caution - that the two samples instead come from different populations with different means. The measure used by the t test for this difference of two populations is the t statistic, which is given by this formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

The *X*'s are the means of the samples, the *s*'s the standard deviations, and *N*'s the sizes of the samples.

When *t* is large, we reject the hypothesis that the two samples come from populations with the same mean, and likewise when *t* is small, we accept the hypothesis that the two samples could come from one population. What happens to *t* when the

means are equal? What if the means are very different? Do these make intuitive sense with the first sentence of this paragraph? When the *s*'s are smaller, what happens to *t*?

When the *s*'s are smaller, *t* gets bigger, which makes it less likely that we will accept our hypothesis. Why should the t test behave this way?

When the *N*'s are larger, what happens to *t*?

19. Partner with another lab group. Together decide which one of average straight-line speed, average speed toward folate, and chemotactic index to compare. Share your ten data points with your partner group.

20. Perform a t-test to see if your data sets are consistent with being taken from the same distribution. In Excel, use the function T.TEST(array1,array2,tails,type).

"Array1" & "array2" are your two data sets. "Tails" in this case should be 2, since your hypothesis is that the data sets would be similar; if you chose 1 tail, then you would be hypothesizing that one particular set would be faster or more attracted than the other. "Type" should be set to 3, which means "two-sample (unpaired) with unequal variance." Your data is not paired, like weight of one person at age 30 and weight of the same individual at age 70. Variance is the spread of the data; one could test to see if the variances of the data sets are the same, but for both brevity here and greater generality, we will choose unequal variance.

21. The output of this function is a P value. Until recently, we generally used a P-value cutoff of 0.05. See Baker (2016) and Cumming (2013) for more on this debate. If using this cutoff and Excel's P value is greater than 0.05, then we conclude that we cannot say that the data sets are from different populations. If less than 0.05, then we conclude (with some caution) that the data sets are not effectively from the same population and there is a difference in their means.

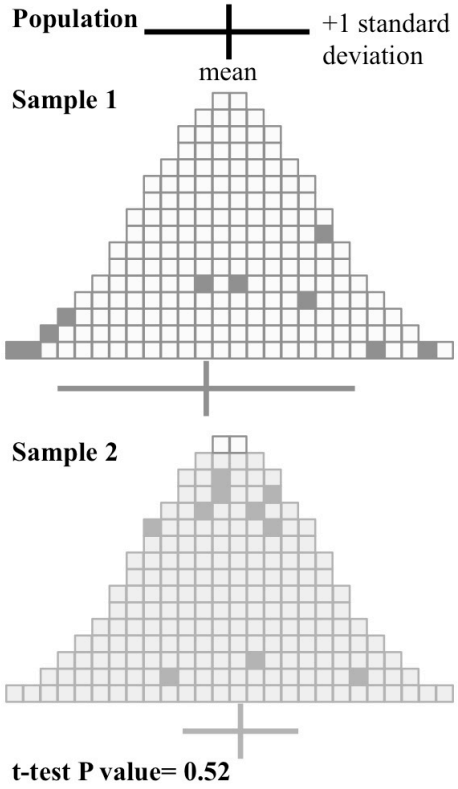

Figure 15. Depicted twice is a normally distributed population with its mean and standard deviation shown at the top. Two samples were chosen at random (darker boxes), and their means and standard deviations are shown below the normal distributions.

More on Motility Measures

22. Likely you should not use all five of the motility measures in your independent projects. Think about what you want to know for your project. Which measure would be most affected if the speed of motility were impacted? Which would be affected if the cells had the signal to move but could not determine which direction they should go? Consider this before making measurements & calculations. To do otherwise could lead to poor experiments and wasting time.

D. Independent Project Discussions and Execution

Introduction

In sections B & C, you measured various motility measures of *dicty* toward and away from a folate source as well as cell densities and percentage of live cells. Now that you are familiar with the equipment and methods of carrying out these assays, you should able to formulate a hypothesis or hypotheses about some aspect of chemotaxis or mobility that interests you, and then conduct experiments of your own design. Your hypothesis must be testable using the available equipment and supplies.

In most sections, you will discuss your experiment with an instructor the week after you perform Section C. Then you will have two more weeks to complete your experiments and interpret the results. The week following those you and your partner will present your findings to the class in an eight-minute oral report. Please see sections B & C of the Introduction on pages 4 & 7, respectively, for guidance.

Experimental Design

As before with the previous lab module, first decide what aspect of *dicty* chemotaxis of you wish to study. Then reread the section in the SDH lab on independent projects. All general guidelines stated there in about experimental design, feasibility, and approval are relevant for this lab as well. Here are the notable exceptions:

- If choosing to treat your cells, consider how long that treatment will take. An hour is the maximum time you have to treat them; otherwise you will run over the designated lab time. Thirty minutes is often sufficient and less frustrating.
- Cell viability assays with the hemocytometer are very valuable, since the cells could be immobile because the treatment killed them.
- Due to time constraints, it is important that you have any dilutions already calculated before lab.
- Each week you will have cells and plates enough for up to 4 plates. Ask yourself if two experimental conditions can be on the same plate, or might the conditions of one experiment taint the results of the other.
- Which motility measures will you choose to examine?
- Standard *dicty* solutions are mildly acidic, pH 6.0 to 6.8. *Dicty* are generally not happy at temperatures much above room temperature, and do not like high solute concentrations or high pH.
- As stated before, you will present your report instead of writing a paper on your *dicty* experiments.

E. Reagents

Dicty *Cells*

Strain: NC4, one of the many wild-type strains.
An actively growing and feeding culture is washed twice with Sor (see below) before it is used in this lab.

Sörensen's Buffer (Sor)

8.00 g KH_2PO_4
1.16 g Na_2HPO_4
Water to 4 L.
pH should be 6.0 ± 0.1

Trypan Blue Solution

0.4% trypan blue, w/v , dissolved in Sor.

Folate Solution, Provided

1 part concentrated folic acid
44 parts Sor
The final concentration of folate is 0.2 mM.

Concentrated Folic Acid

folic acid, 4 mg / mL
pH'd to 6.5-6.8 with NaOH or KOH.
Frozen in small aliquots.

Cited References

Baker M. 2016. Statisticians issue warning on P value. Nature. 531:151.

Cumming G. 2013. Intro Statistics 9 Dance of the p Values. https://www.youtube.com/watch?v=5OL1RqHrZQ8 Accessed Jan 9, 2016.

Materials

The materials below are for 12 student groups working in pairs, 24 students.

(1) 6-cm disposable Petri dishes with 6 mL Sorensen's buffer and 1.0% agarose, w/v. Count: ~30. These should be prepared the day of lab, since drying of the plates tends to sometimes cause the solutions added to the wells to be absorbed by the plates.

Sorensen's buffer recipe: 8.00 g KH_2PO_4, 1.16 g Na_2HPO_4, distilled water to 4 L. pH should be 6.0 ± 0.1. This can also be prepared as a 50x concentrate.

Petri dishes: Genesee Scientific, catalog #: 32-105, $92.50 for 500. The important facet of the plate is the thickness of its bottom. It needs to be consistently smaller than the focal length of the highest magnification lens you want to use.

(2) Rectangular brass punches, 3/16" (4.78 mm) x 3/8" (9.53 mm) x 12". Count: 12. These are made by a company called K & S Precision Metals, Chicago, IL, 773-586-8503, Stock # 8268.

These can also be found in Ace Hardware Stores, frequently in racks of other materials from K & S Precision Metals.

(3) Lab markers. Count: 12.

(4) Pipettors: P20, P200, & P1000. Count: 12 of each.

(5) Tips for pipettors.

(6) Folate solution, 0.2 mM. 3 mL. Typically we make this by mixing 1 part concentrated folate and 44 parts Sorensen's buffer (See item 1.)

Concentrated folate recipe: folic acid, 4 mg / mL, pH'd to 6.5-6.8 with NaOH or KOH. (Note this is well beyond the pKa of folate, so achieving this pH is challenging.) Frozen at -20 C, it is good for at least three years.

(7) *Dictyostelium discoideum*, strain NC4, actively growing and washed, 2-10 million cells per mL, ~4 mL. (See Appendix B on preparation and culturing.)

(8) Compound microscopes, student quality. Count: 12. At least 100x total magnification is good, and more than 500x is likely not very useful.

(9) Extra microscope eyepieces (optional). Count: 12.

(10) Sample specimen slides for microscope familiarity. Count: 12 or more. Alternatively, have students make their own.

(11) microscope-to-phone adaptors. Count: 12. We have extensive experience with two varieties and some experience with a third.

(11a) SkyLight adaptor, which is currently not commercially available. We used these for years before using the homemade 3D-printed adaptors (11b). http://www.skylightscope.com/

(11b) Homemade 3D-printed adaptors. (Figure 7 in the student outline. These are nearly free except for the nominal cost of printing. They are also easier to use, though not as versatile. They do need an appropriately sized tube clamp.

(11c) SnapZoom 2016 adaptor. JM has personally used this though not in a student lab. It works fine. Available for $65 from Amazon. There are at least three other commercially available adaptors at this time, but we have not tried them and they are more expensive than the SnapZoom 2016. http://snapzooms.com

(12) plastic washers (optional). These are just shim material to match the focal lengths between the eyepieces and the iPods. They can make mounting the commercially available adaptors easier. Many similar items are available from mcmastercarr.com

(13) iPods, 4th generation or any such device and associated cables and chargers, 12. We purchased ours refurbished from Apple for $80 each. You can purchase 5th generation refurbished ones from Apple for $129 in September 2017. Most phones will work in the commercially available adaptors.

(14) stage micrometers, 12 or fewer since they can be shared. Ward's Science: item number 949910, $22.50 each. The disposable hemocytometers, item 16, could also be used for the same purpose to save money.

(15) dead *Dictyostelium* cells in 0.04% trypan blue and Sorensen's buffer, <0.5 mL. A live culture of *Dictyostelium* is left at 60 C for 30 minutes and then mixed with Sorensen's buffer and trypan blue. They will persist as cellular "ghosts" for several days but not a week.

(16) "disposable" hemocytometers, 12. Bulldog Bio: Item number DHC-N420, $115 for 50 slides with 4 assays per slide. Note that we reuse these by shaking them slowly in distilled water for at least thirty minutes, replacing the water, repeating three more times, and then drying them with Kim-wipes and compressed air. Fisher Scientific also sells a 10-chambered variety for $144.45 per 100 count.

(17) Sorensen's buffer, <10 mL. See item 1 for the recipe.

(18) Trypan Blue solution, 0.4% w/v in Sorensen's buffer, filtered, <0.5 mL.

(19) coverslips, 22 mm by 40 mm. VWR: catalog number 48393-172 , $113 per case which is 10 oz of glass.

(20) 3D-printed plate adaptors (optional). These were made in house and allow standard microscope slide micromanipulators to move a 6-cm Petri dish around. See figure 12 in the student laboratory handout.

(21) Laptops for data analysis, or a computer lab. Usually our students provide these, but we have some spares for others.

Notes for the Instructor

Order and Length

This lab and variants of it have been run for four years in our Introductory Cell Biology and Cell Chemistry class at Pomona College. Each year, we have had 100 to 150 students in four or five lab sessions. When the time is available, we run this lab over seven weekly sessions:
(1) An interactive lecture on chemoattraction and cell motility.
(2) The dry run lab outlined in in Section B of the Student Laboratory Handout.
(3) The data analysis outlined in in Section C of the Student Laboratory Handout. Some instructors include a discussion of a t-test, and have pairs compare their data using it.
(4) Small group discussions about the students' independent project proposals. For us, this is their second inquiry-based project, so they are familiar with this process.
(5 & 6) Execution of their experiments, and a bit of advice about their upcoming presentations.
(7) Presentation of their background, experiments, data, and interpretations in 8-minutes per group.

Behind the Scenes

Once all the equipment is obtained and working together, there are few issues behind the scenes. If one ever changes the growth conditions of the cells, one should expect to spend a few days learning their new relative growth rates. Also, bacterial concentrates left at room temperature overnight tend to yield lower *Dictyostelium* growth rates. Due to this, we tend to keep the bacterial concentrates in smaller aliquots in case they are accidentally left out.

Again, the Sorensen's buffer plates need to be poured the lab of lab, or they tend to absorb the fluid added to the wells.

The Dry Run Lab

When pulling out the agar blocks to make the wells, it is important to not move the agarose between the wells. Doing so often forms air pockets beneath the agar which can fill with *Dictyostelium* and be mistaken for cells that have migrated.

Mounting the SkyLight has been frustrating to the students in the past. Alignment of the iPod's camera to the eyepiece at the right focal depth does take patience, but it also helps to emphasize the importance of instrument calibration and alignment. The use of the washers for shim (see "Equipment") and the use of a spare eyepiece so they do not need to mount the SkyLight more than once helps with this. Once they mount it once or twice, they do like the reward of seeing some of the prepared slides on the iPod, which tends to mitigate their frustration.

Note that though trypan blue is a widely used vital cell dye, it is widely believed to have some adverse health effects. Gloves and lab coats are required.

When adding the coverslip to the plate, students almost universally have bubbles in their wells. Bubbles only matter when they interfere with seeing migration, which happens at the inner edges of the cell wells. (See figure 4 of the "Student Laboratory Handout.") Also, they only need to video one or two regions. Therefore, rarely are there so many bubbles that hope is lost for their experiment that day.

Some students struggle to find regions of migration. Not all locations near the cell wells on the folate side will show migration; the *Dictyostelium* stack up near the edge of the well but cannot seem to find their way under the agar. Nearly always there is some migration to film; some persistence and confidence is needed.

Sometimes the difficulties finding migration stem from confusion in using the microscopes. Sometimes students focus on cells still in the wells and not migrating under the agar. Other times they focus on cells that have landed on the coverslip, and are therefore not migrating and not in right focal plane. If they follow the instructions carefully within the "Student Laboratory Handout," they will avoid these issues. Often the instructor still needs to provide additional assistance.

The iPods are great for filming and for two or more people to discuss what they can see. iPods are not good for searching for migration. Their screens' low resolution, low contrast, and so-so response time make them vastly inferior to using a microscope without them.

If filming has not started within 30 or more minutes of adding the coverslip, migration may be rather feeble. Once the coverslip is added, folate is spread across the surface of the plate. This subsequently disrupts the folate gradient that the migrating cells are moving toward.

Data Analysis

This is relatively straightforward. However, some students have a relative unfamiliarity with spreadsheets. This year, we plan to utilize HHMI BioInteractive Spreadsheet Data Analysis Tutorials with students who seem to need extra familiarity. http://www.hhmi.org/biointeractive/spreadsheet-data-analysis-tutorials

Independent Project Discussions

We reject a small fraction of proposals due to safety concerns, impracticalities in terms of timing, and expense. The students usually have had another idea that they had not explored as extensively, and we then pursue that one. Despite some rejected proposals, this lab still lends itself to an array of projects as shown by the list of extra reagents used in spring 2016 (Appendix C).

Depending on the chemicals used, additional safety protocols are needed.

In discussing their proposals, we discuss incubation times and dosages; some groups have those covered already, and others do not. One very common discussion is about solvent controls.

The initial year when we ran this lab, we spent about $3,000 for new chemicals for the lab. Though a significant outlay, most of these chemicals were far from used up, and provide a basis for subsequent years' projects. Such expenses could be kept more modest by simply denying more student proposals due to expense.

Cited References

Chisholm RL, Gaudet P, Just EM, Pilcher KE, Fey P, Merchant SN, Kibbe WA. 2006. dictyBase, the model organism database for Dictyostelium discoideum. Nucleic Acids Res. 34(Database issue): D423-7.

King JS, Insall RH. 2009. Chemotaxis: finding the way forward with *Dictyostelium*. Trends Cell Biol. 19:523-30.

Meijering E, Dzyubachyk O, Smal I. 2012. Methods for cell and particle tracking. Methods Enzymol. 504:183-200.

Woznica D, Knecht DA. 2006. Under-agarose chemotaxis of *Dictyostelium discoideum*. Methods Mol. Biol. 346:311-25.

Acknowledgments

Thank you to Fabien Jammes, Karl Johnson, Sara Olson, Ruben Petreaca, Rou-Jia Sung, Bruce Telzer, approximately 400 Pomona College students, and the ABLE 2017 workshop participants, particularly Michael Edgar, who all provided valuable feedback on various versions of this lab. Thanks, too, to Pomona College for providing support and funding for this lab, and to David Tanenbaum and David Haley for assistance with 3D printing.

About the Authors

Since 2012, Jonathan Moore has been an assistant professor of biology at Pomona College, where he teaches genetics and cell biology and coordinates introductory lab courses. Melissa Petreaca was at Pomona College for five years and since 2015, has been an assistant professor at Depauw University where she teaches immunology and cell biology. David Knecht is a Professor in the department of Molecular and Cell Biology at the University of Connecticut where he does research with *Dictyostelium* and teaches courses including upper-level cell biology.

Appendix A
Representative Student Data

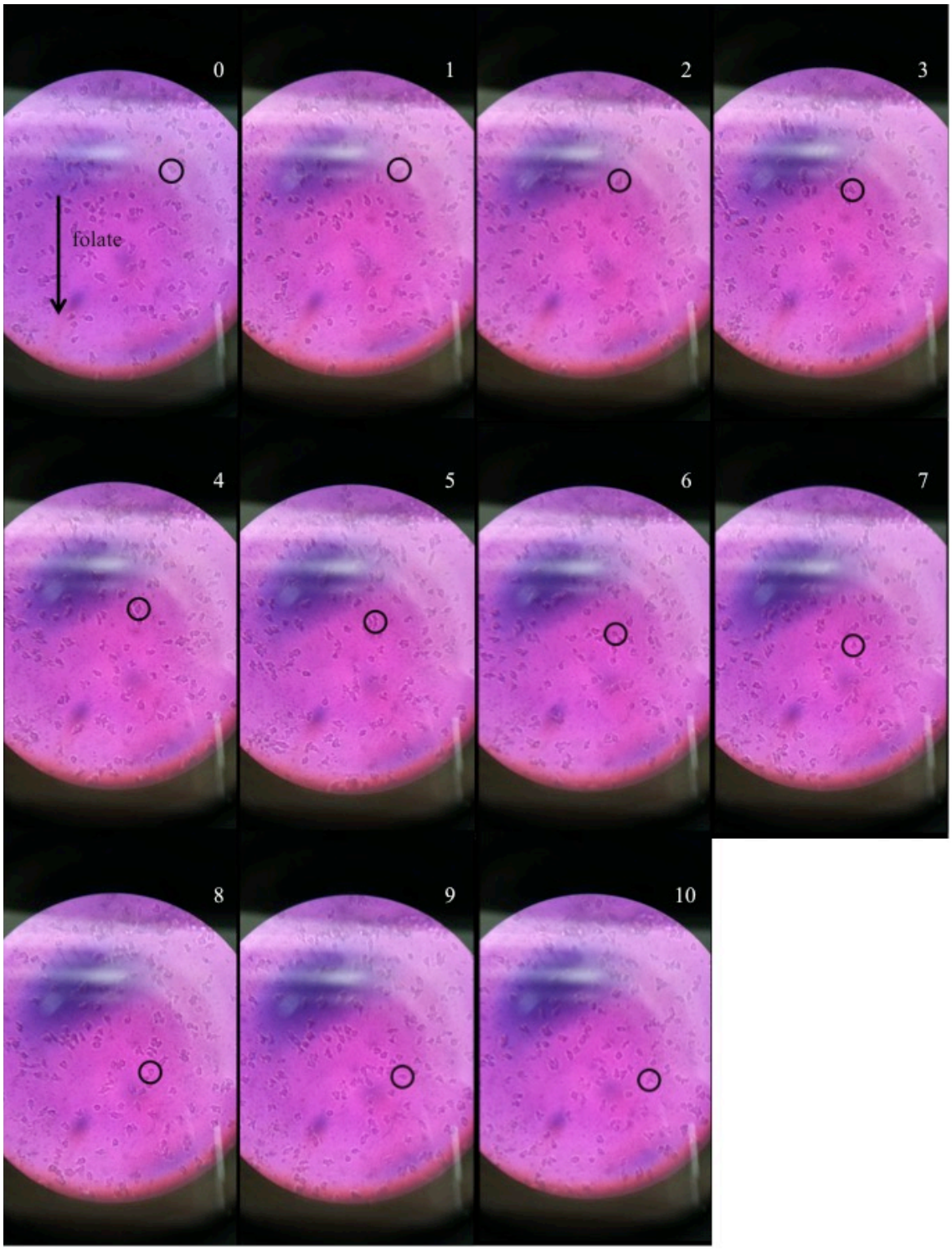

Figure 16. Typical time-lapse images from a video taken by a student group on the first day of lab. The black circles track a single cell over 10 minutes. The pink color is due to a red Sharpie mark in the way of their imaging. The gray blob near the top of each image is an optical artifact due to the alignment of the camera and the iPod.

	initial x	initial y	final x	final y	distance (pixels)	net displacement (uM)	average straight line speed (uM/min)	distance towards folate	uM	average speed towards folate	chemtactic index
cell 1	844	412	980	543	188.8306119	123.4187006	12.34187006	131	85.620915	8.562091503	0.693743449
cell 2	**942**	**390**	**944**	**629**	**239.0083681**	**156.2146196**	15.62146196	239	156.20915	15.62091503	0.999964988
cell 3	715	395	587	516	176.1391495	115.1236271	11.51236271	121	79.0849673	7.908496732	0.686956877
cell 4	842	267	829	556	289.2922398	189.0798953	18.90798953	289	188.888889	18.88888889	0.998989811
cell 5	1030	280	1004	514	235.4400136	153.8823618	15.38823618	234	152.941176	15.29411765	0.993883735
cell 6	994	421	1044	659	243.1953947	158.9512384	15.89512384	238	155.555556	15.55555556	0.978636953
cell 7	915	431	992	674	254.9078265	166.6064225	16.66064225	243	158.823529	15.88235294	0.953285756
cell 8	**717**	**488**	**834**	**745**	**282.3791777**	**184.5615541**	18.45615541	257	167.973856	16.79738562	0.910123764
cell 9	749	366	698	503	146.1848145	95.54563041	9.554563041	137	89.5424837	8.954248366	0.937169845
cell 10	780	365	798	589	224.7220505	146.8771572	14.68771572	224	146.405229	14.64052288	0.996786917

Magnificatio	43x				
		distance (100 um)			
pt 1 x	777	725	uM	100	
pt 2 x	930	725	153 pixel	153	
				0.653594771	

cell 8	**second**	**x**	**y**	**distance travelled**		**cell 2**	**second**	**x**	**y**	**distance travelled**	
				uM						uM	
	0	717	488				0	942	390		
	1	716	500	12.0415946	7.870323254		1	939	401	11.40175425	7.452126961
	2	707	529	30.3644529	19.84604765		2	966	421	33.60059523	21.96117335
	3	716	556	28.4604989	18.6016333		3	975	459	39.05124838	25.52369175
	4	707	594	39.0512484	25.52369175		4	961	488	32.20248438	21.04737541
	5	712	636	42.296572	27.64481828		5	948	503	19.84943324	12.97348578
	6	725	650	19.1049732	12.48691057		6	946	516	13.15294644	8.596697018
	7	749	675	34.6554469	22.65061889		7	942	530	14.56021978	9.516483515
	8	767	698	29.2061637	19.0889959		8	932	554	26	16.99346405
	9	796	706	30.0832179	19.66223393		9	934	585	31.06444913	20.30356153
Path length	10	834	742	52.3450093	34.21242439		10	944	629	45.12205669	29.49154032
				317.609178	207.5876979	Path length				266.0051875	173.8595997

cell 8		**cell 2**	
Average Spe	20.7587698 uM per minute	**average speed**	17.38595997 uM per minute
straightness	0.88907751	**straightness index**	0.898510177

Figure 17. The spreadsheet created by the students from the video shown in Figure 16.

Appendix B
Care of *Dictyostelium discoideum* strain NC4

NC4 is one of the first isolated strains of *Dictyostelium discoideum* and is considered wild type. These protocols take advantage of the phagocytic nature of *Dictyostelium.* One very nice feature of this approach is normal issues of contamination are relatively few, since the *Dictyostelium* are grown in a media containing no simple chemical nutrients.

General Propagation of NC4

NC4 cells are propagated 1x Development Buffer (DB) plus a 20-fold dilution of *E. coli* concentrate. 24 hours before the next split or harvest, cells should be diluted to 25,000 cells per mL. Cells are then incubated with shaking or tumbling at 18 C. Under good conditions, NC4 can undergo six doublings in 24 h.

The initial seeding of a culture is with several fruiting bodies of NC4 harvested with a sterilized, wet inoculating loop. It usually takes two to three days to get a healthy 10 mL culture of NC4.

Preparation of Cells for the Cell Motility Assay

In order to clear most of the bacteria and metal ions from the sample before lab, cells are centrifuged in 50-mL conical tubes at 500 g for 5 minutes, the supernatant removed, and the cells resuspended in Sorensen's buffer. This process is repeated once or twice depending on the time before lab and the desired level of bacteria, which tend to diminish with each wash. On the last resuspension, the cells are usually brought to 5 million cells per mL. Shake or tumble the cells until close to lab time so as to keep them from settling, clumping, and becoming less likely to migrate. The assay works reasonably well with 1.5 million cells per mL and just as well with 10 million cells per mL.

E. coli Concentrate Preparation

Grow a near-saturated 1 L culture of DH5alpha strain *E. coli* overnight in SM media with shaking at 37 C. Distribute this amongst 250 mL centrifuge tubes and centrifuge for 10 minutes at 6000 g. Carefully pour off the supernatant and resuspend each pellet in approximately 50 mL of Sorensen's buffer. Pool the samples into only two tubes repeat the centrifugation. Resuspend these two pellets in a total of 40 mL Sorensen's buffer. Measure the OD_{600} of a 1:20 dilution of this, and dilute the bacteria to achieve an OD_{600} of the 1:20 dilution of approximately 0.6. Transfer to one or more sterile tubes. Normal yields are about 50 mL.

The goal here is to make concentrate that when diluted 1:20 is a less salty, nutrient-free suspension of bacteria at a density like a nearly saturated culture.

Preparation of NC4 Fruiting Bodies

Grow *Enterobacter aerogenes* in SM media overnight. Spread culture over SM plates and allow to grow overnight at room temperature or 37 C. Seed plates with NC4 spores near one edge. Incubate plates at 18C. Within several days, some fruiting bodies will appear near the seeding, and the NC4 will spread across the plate. These can be kept at 4 C for several months.

Development Buffer

Prepare a solution of 25 mM each Na_2HPO_4 and KH_2PO_4.
Prepare a 10 mM $CaCl_2$ solution.
Prepare a 20 mM Mg Cl_2 solution.
Autoclave all three solutions.
To make 1 L Development Buffer, mix 600 mL sterile water, 200 mL of the phosphate solution, and 100 mL of each of the other two. The pH should be 6.5.
Note that a 10x stock of all three together cannot be prepared because of precipitation.

Sorensen's Buffer

Mix 8.00 g KH_2PO_4, 1.16 g Na_2HPO_4 and distilled water to 4 L.
The pH should be 6.0 ± 0.1.
Autoclave.
This can also be prepared as a 50x concentrate.

SM Media

For 1 L, use

1.9 g KH_2PO_4
0.6 g K_2HPO_4
0.5 g $MgSO_4$
10 g glucose
10 g bacto-peptone
1 g yeast extract
distilled water to 1 L.
The pH should be 6.0 to 6.4 Autoclave.

SM plates

Prepare 1L SM media. Before autoclaving, add 15 g agar. Pour into Petri dishes.

Bacterial and *Dictyostelium* Strains

NC4 and *Enterobacter aerogenes* are common in many *Dictyostelium* labs and available from the DictyBase stock center. http://dictybase.org/StockCenter/StockCenter.html.
DH5alpha *E. coli* are likely the most common *E. coli* used in molecular cloning and available as competent cells from many biological suppliers including Thermo Fisher.

Appendix C
Special Reagents Requested by 51 Lab Groups in Spring 2016

With a couple of exceptions, the ideas represented by this list were arrived at by students looking at the primary literature. Many are rather specific drugs that modulate cell signaling, cytoskeletal elements, and focal adhesions. Many

Many of these reagents are quite benign, and others are rather toxic. However, we consider most reasonably safe when provided to the students (a) pre-dissolved, (b) in the quantities they need which are often tens of microliters, and (c) with appropriate precautions and personal protective equipment.

caffeine
8-CPT-Cyclic AMP
Polysphondylium
decaf green tea
green tea
$MgCl_2$
colcemid
cAMP
naringenin
epigallocatechin gallate
zoledronic acid
ibuprofen
capsacin
starving *Dictyostelium*
2,3-Butanedione monoxime
catechin
cycloheximde
$CaCl_2$
LY-294,002
plastics of differing hydrophobicity
temperature
formaldehyde, highly dilute
ammonium chloride
curcumin
nocodazole
arachidonic acid
buffers at pH's 5.4, 6.1, & 7.1
cytochalasin D
caffeine
phenylthiocarbamide
naringenin
latrunculin A
wortmanin
potassium proprionate
pterine
concentrated folate

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/.**

Papers published in *Tested Studies for Laboratory Teaching: Peer-Reviewed Proceedings of the Conference of the Association for Biology Laboratory Education* are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

Citing This Article

Moore J E, Petreaca M L, Knecht D A. 2018. A Rich Introductory Inquiry-Based Cell Motility Lab Utilizing *Dictyostelium discoideum* Chemoattraction. Article 13 In: McMahon K, editor. Tested studies for laboratory teaching. Volume 39. Proceedings of the 39th Conference of the Association for Biology Laboratory Education (ABLE). **http://www.ableweb.org/volumes/vol-39/?art=13**