# Independent Project II: *Dictyostelium* Chemotaxis & Motility

## Chapter 5: Entirety of Project

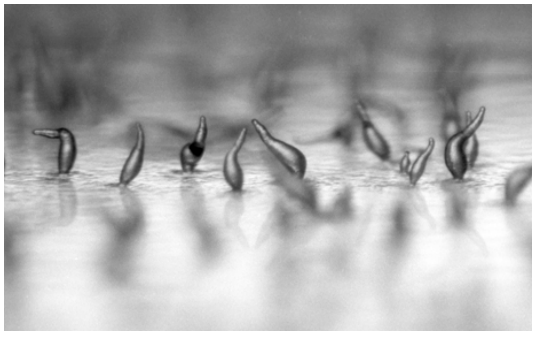

**Figure 1.** Image of *Dictyostelium* fruiting bodies by Matthew Springer
http://dictybase.org/dictyArt/ &
http://news.nationalgeographic.com/news/2008/10/photogalleries/best-microscope-photos/photo4.html

## 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. (See Figure 2 for a brief overview of *dicty's* life cycle.) 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 then
- 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

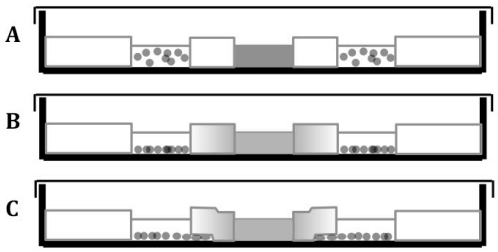


**Figure 3.** Cross-sectional diagrams of key stages in your experiment. **A.** At the end of procedure step 5, the Petri dish (black) should have folate (dark gray area) in the central wells and the cells (circles) in the outer wells. **B.** Midway through the assay, the cells have settled to the bottom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

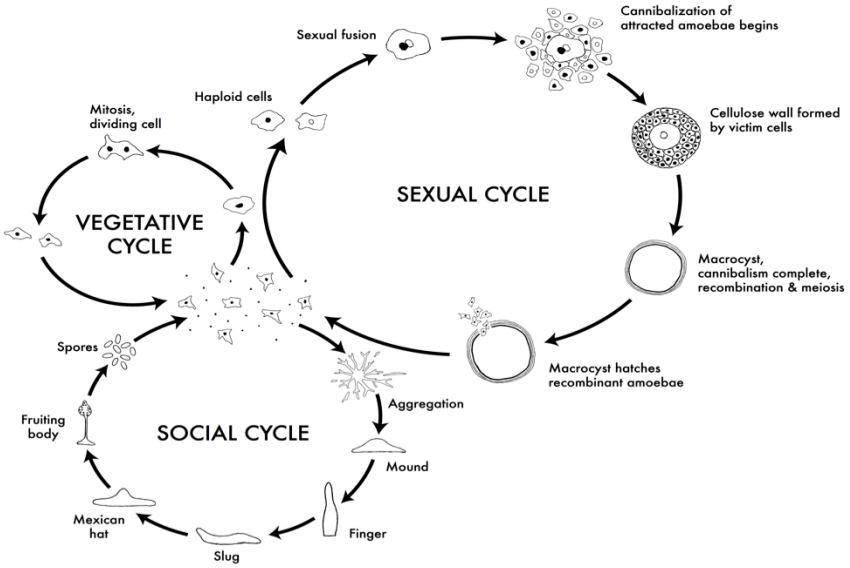


**Figure 2.** The life cycles of *Dictyostelium discoideum.* Most of its life, this haploid social amoeba undergoes the vegetative cycle, preying upon bacteria in the soil, and periodically dividing mitotically. When food is scarce, either the sexual cycle or the social cycle begins. Under the social 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. 

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.

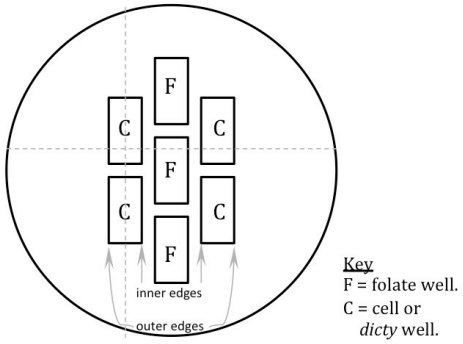


**Figure 4.** Template for cutting wells in plate. The horizontal & vertical hatched lines indicate possible cross-sectional views in Figures 3 & 5, respectively.

## B. Experimental Procedure: Measuring Motility & Cell Density

**Pouring agarose plates: This has already been done before class.**

Sorenson's buffer (Sor) and agarose at 1.0% w/v are mixed in a flask. The flask is placed in a water bath as it warms to 70 C. The agarose is melted by bringing the mixture to a boil on a heat plate, and the solution returned to the hot water bath. 6 mL of the resulting media is added to each 6-cm Petri dish.

**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. 4) 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.)

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. 5 and *dicty* lab YouTube tutorial 1 of 6 on the Pomona College Bio41C YouTube channel: Either search in YouTube for "Pomona College 41C"

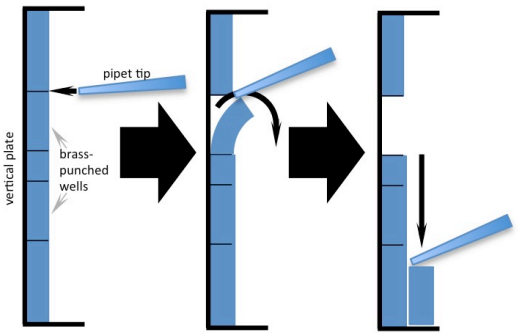


**Figure 5.** Pick up the plate and hold vertically. Using a pipet tip, pry out each agarose block and move it to the edge of the plate.

or go to https://www.youtube.com/watch?v=Bz1LqlcIco4&list=PLXgqUHWYdxrcflDaAKL-ItHL7rkfrBBMk

*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 all the way to the second stop. 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."

**Calibrating your microscope setup**

You will familiarize yourself with your microscope and learn to how to use it with an iPod to take photographs and time-lapse videos.

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.

Note that the sidebar on the next page might serve as a useful reference throughout the rest of this lab.

6a. Uncover the microscope. (*dicty* lab YouTube tutorial 2 of 6) If you need to move the microscope, use one hand to grab it by the arm – the solid part between the tube and the stage – and the other hand to support it beneath the base. Do not drag it across the bench top, as vibrations misalign the optics.

b. Pick a specimen slide from the red slide box squeezing the box on the sides and lifting the lid. (*dicty* lab YouTube tutorial 3 of 6) 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 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 allow*

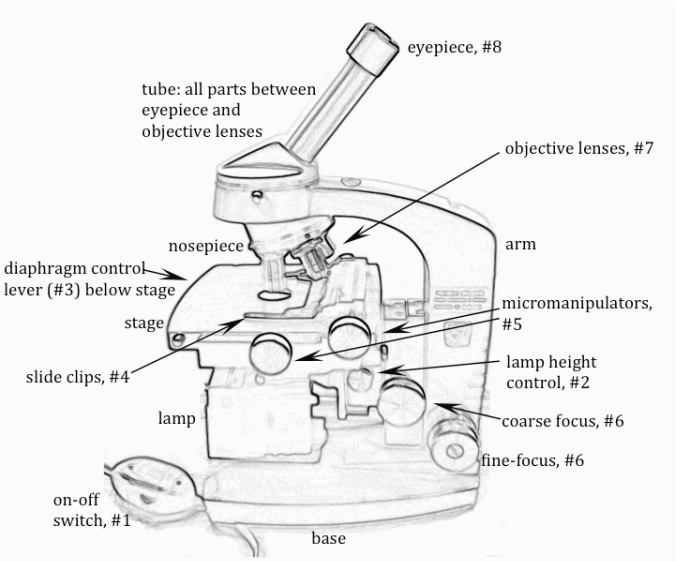


**Figure 6.** See the text for descriptions of the numbered parts of a microscope, the parts which you can adjust.

**Using your microscope**

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. 6).

1. On-off switch: This is on the black power cord. There is also a dimmer switch on the white 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.

*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. Change the objective lens to 10x, and possibly adjust the fine focus and the diaphragm, if needed.

    Change to the 43x objective lens and adjust as before.

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

13. Mount the iPod adaptors (Figure 7) following the steps below.

    a. Become familiar with how the eyepiece fits into the iPod adaptor by (i) carefully pulling the eyepiece out of the microscope and (ii) gently and with some side-to-side jiggling, inserting the thicker end of the eyepiece into the cylindrical part of the adaptor.

    The adaptor should finally sit within a few mm of where the eyepiece becomes thinner. Now, undo what you just did and put the eyepiece back into the microscope.

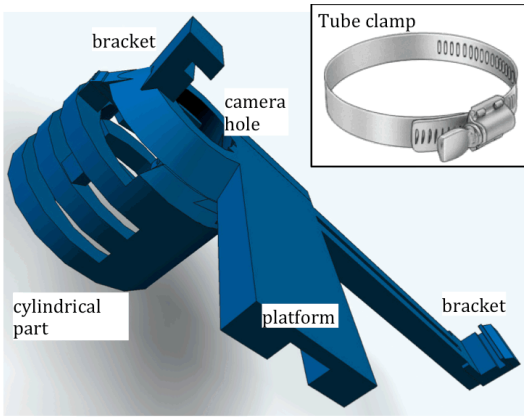


**Figure 7.** A 3D-printed iPod adaptor and a tube clamp.

    b. Insert the plug into the bottom of the iPod. Turn it on.

    c. There should already be a small tube clamp (Figure 7, insert) on the tube (Figure 6) of your microscope. If not, slip one on and attach it to on the top of the tube. (*dicty* lab YouTube tutorial 4 of 6)

    d. Slip a large tube clamp over the cylindrical part of the 3D-printed iPod adaptor (Figure 7).

    e. Jiggling the adaptor slightly, gently place the cylindrical part of the adaptor over the eyepiece, and push on it until it sits as it did in step (a).

    f. Loosely tighten the thumbscrew to secure the adaptor to the eyepiece.

    g. Plug in your iPod.

14a. On the iPod, slide to unlock, and select the camera.

    b. Gently slide the top of the iPod left and right until you can see <u>something</u> through the eyepiece. Adjust until you get a desired image.

    Once you're done adjusting left and right, you might still have dark regions within the field of view, particularly at the bottom. Loosen the tube clamp that you put on the adaptor and press gently on the bottom of the iPod. If this yields the desired image, then you might try re-clamping it so it stays in place. If this makes the image bad again, undo this.

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

16. Now change to the 10x & 43x lenses and adjust, taking a picture at each magnification.

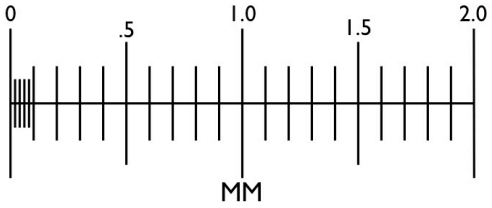


**Figure 8.** The 2 mm ruler within the stage micrometer.

17. Locate the stage micrometer – slide containing a tiny 2-mm long ruler (Fig. 8). 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. 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."

**Safety Note:** There is some evidence that prolonged exposure to trypan blue causes cancer. When using the trypan blue solution, wear gloves.

**Measuring cell density**

Measuring cell density and rate of death serves a multitude of purposes:

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.

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.

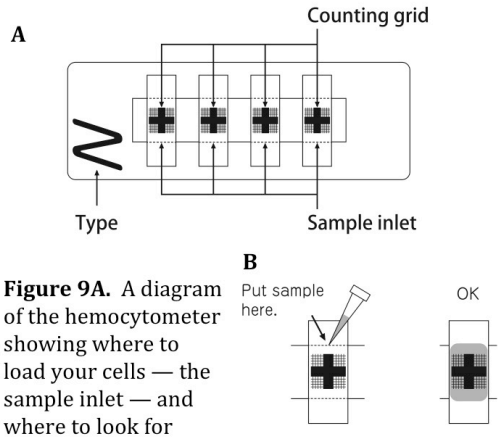


**Figure 9A.** A diagram of the hemocytometer showing where to load your cells — the sample inlet — and where to look for them under the microscope — the counting grid. Part **B** depicts the sample loading.

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.

24. Mix the provided dead stained cells and add 6 μL of them to the hemocytometer (Fig. 9).

25. Exchange the eyepieces to remove the iPod from your microscope. Use your microscope to observe the dead cells in the counting grid (Fig 9A). 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.

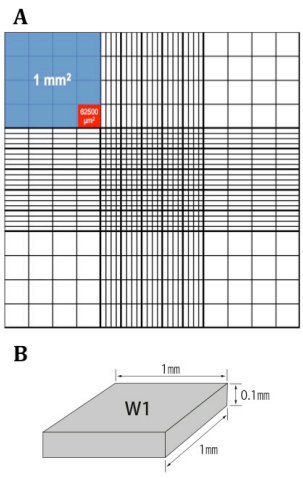


**Figure 10A.** The grid pattern of an Improved Neubauer hemocytometer. The largest square is 3 mm on a side. This largest square is subdivided into nine 1-mm² fields.

**B.** A 1-mm² field is also 0.1 mm high, yielding a volume of 0.1 mm³ = 0.1 μL.

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-mm² fields until you have either at least 50 cells or four 1-mm² fields counted (Fig. 10). Hand counters make this process easier and more reliable. If there are any, count any dead cells separately.

27. Each 1-mm² field is 0.1 mm high, so each square contains 0.1 mm³, or 0.1 μL. Remembering the 10-fold dilution that you did in the last step, calculate both the concentration of *dicty* cells / mL in the tube provided and the percentage of cells alive.

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.

**Time-lapse videoing cell movement**

28. 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. 11)

29. 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.

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

31. 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.

32. Pick up the plate and place it still upside-down on the bench leaving the lid behind. Slide the blue-gray plate adaptor over it (Fig. 12). Clip these to the stage.

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

34. 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. The stage should rise only about 0.5 mm.

35. Move the plate to view an inner edge.

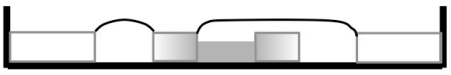

**Figure 11.** The left thin black lines shows fluid in a well slightly domed. The right thin black line shows the liquid from two wells flowing into one another, which is Ok if not too extensive.

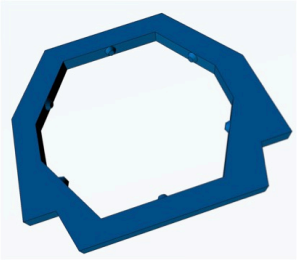

**Figure 12.** The plate adaptor slides over the plate and clips to the slide clips of your microscope, allowing you to move the plate with the micromanipulators.

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. 13). 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.

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.

Next, look in other locations along the inner edges. As you look, again tweak the fine focus. Usually, between 20% and 60% of the inner edge has migration.

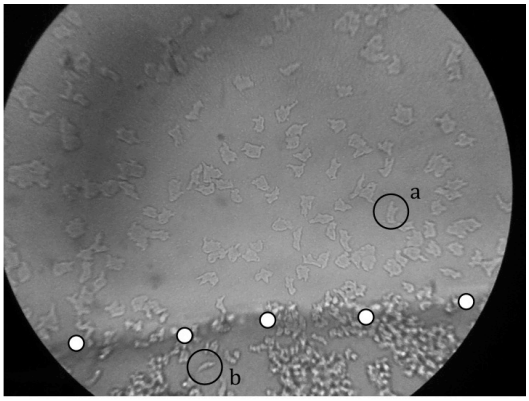


**Figure 13.** Live *dicty* are photographed at 400x. The folate source is up. White circles show the edge of the well. a: migrating cell. b: non-migrating cell.

If you 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.

36. 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 38.

37. 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.**

38. Put away the stage micrometer, other sample slides, the spare eyepiece, and the plate adaptor.

39. Leave the tubes of dead cells, Sörensen's buffer, and trypan blue in your rack for tomorrow's class.

40. Throw away any remaining provided *dicty* cells, folate solution, and your plates.

41. Place your used hemocytometers in the "Used hemocytometers" container.

42. Remove the iPod and the adapter from the microscope.

43. Turn off and cover your microscope.

44. Transfer your videos off the iPod using one of these options:

(a) Attach the iPod to a laptop with the cable and use your favorite picture organization app.

(b) Mail it to yourself by choosing the "Publish" option within LapseIt or clicking the icon to the bottom left of your picture in Photos on you iPod. *

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.

* What if mail is not working?
(a) Check the internet connection by going to your favorite website. If that works, go on to (c).

(b) If (a) failed, go to "Settings," then "Wi-Fi," and connect to "Pomona-Guest."

(c) Go to the "Mail" app and send an email to yourself. If that worked, you should be Ok.

(d) If (c) failed, check your internet connection again by going to google.com.

(e) If (e) worked, but you are getting the message "Your gmail password is wrong," go to "Settings," then "Mail, Contacts, Calendars." Click on the gmail account in "Accounts." Click on "Account pombio41cipod@gmail.com," and re-enter email: pombio41cipod@gmail.com & password: XXXXXXXX

(f) If you tried all this and it still doesn't work, talk to the TA or professor.

## 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 autofill feature, and intermediate-level graphs, please look at the HHMI BioInteractive tutorials available on Sakai in the "Lab" folder under "Resources."

### 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.

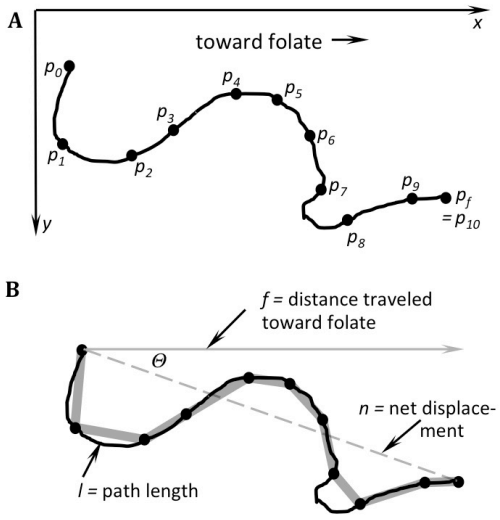


**Figure 14A.** The path of a cell and its position each second. $p_0$ indicates the initial position of the cell, and $p_f$ its final position.
**B.** Different tracking measures: the net displacement ($n$), the distance traveled toward the folate ($f$), and the path length ($l$), which is by necessity an approximation. Also, the chemotactic index ($c$) is the cosine of Θ.

a. Choose a cell away from the folate.

b. Determine the initial screen position of the cell, $p_0 = (x_0, y_0)$ (Fig 14A).

On a Mac, position the mouse over the center of the cell. Press Shift-Command-4, which will bring up cross-hairs 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.

b. 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.

c. Now determine the final screen position of the cell, $p_f = (x_f, y_f)$ (Fig. 14A).

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 14A). It might help to slow down your video as you do this.

4. Repeat step 3 with one additional cell.

**Calculating useful intermediates**

5. Determine the *net displacement, n*, of each of the ten cells (Fig. 14B, 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. 14B, 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. 14B, thick gray line).

$$l = \Sigma \sqrt{(x_{a+1} - x_a)^2 + (y_{a+1} - y_a)^2}$$

**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 8 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 (*f*ns), 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. When planning your independent projects, you should consider which of the five are the most useful given your hypothesis.

13. *Average speed ( l' )*

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.

$$l' = l / t$$

14. *Average straight-line speed ( n' )*

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

$n' = n / t$

15. *Average speed toward folate ( f' )*

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

$f' = f / t$

16. *Chemotactic index ( c )*

This is the ratio of the distance traveled toward folate to the net displacement. Note this is the cosine of Θ in figure 14(b). Calculate this for all ten cells.

$c = f / n$

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 )*

This is the ratio of the net displacement to the path length. Calculate it for the two cells.

$s = n / l$

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**

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 (sidebar on next page and Fig. 15) 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 the news article "Statisticians issue warning on P value" and the video "Intro Statistics 9 Dance of the p Values," both of which are on Sakai.) 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.

## 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.

### About the 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 15). 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 $t$?

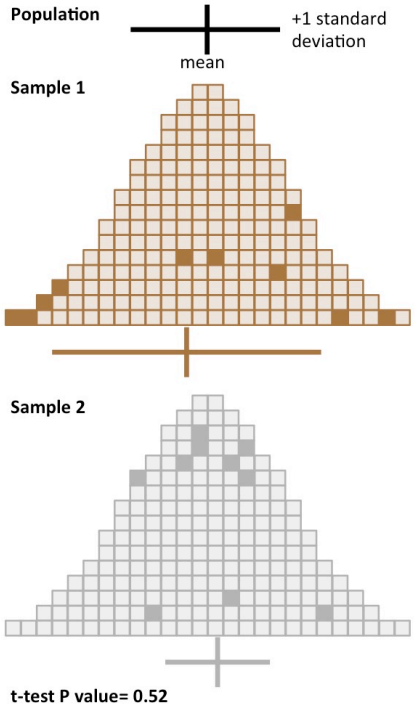


**Figure 15.** Depicted twice is a normally distributed population with its mean and standard deviation shown at the top. Two samples were chosen at random (darker boxes), and their means and standard deviations are depicted below the normal distributions.

# 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 F & G for more guidance.

### Experimental Design

As before with the SDH lab, 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 <u>important</u> 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.

- As stated before, you will present your report instead of writing a paper on your *dicty* experiments.

**<u>Safety Note:</u>** Many additional compounds requested for this lab are dissolved in dimethyl sulfoxide (DMSO). DMSO can cause compounds to be absorbed through the skin. Therefore it is very important to wear gloves when dealing with DMSO and compounds dissolved in it.

## 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.

## F. Preparing an Effective Oral Presentation

An important method of communication to other scientists of research results is the presentation of short talks at scientific conferences. Most scientific societies hold regular meetings and symposia which involve oral presentations, commonly lasting 10-20 minutes, including the time allowed for questions. Quite often, there are multiple concurrent sessions, and attendees move between sessions to hear particular talks of interest. For that reason, it is necessary for speakers to strictly adhere to the time limitations. In many disciplines, oral presentations are the fastest way to learn about new findings and ideas. Real skills are needed to make these oral presentations effective, and these skills are best learned through practice.

### Content of the talk

An oral presentation has the same general format as that of a research paper (Introduction, Materials and Methods, Results, Discussion), but the relative emphasis is quite different. In an oral presentation, the Introduction is quite brief, and one spends the majority of the time describing and analyzing experimental results.

#### *Introduction*

In an oral presentation, you must briefly introduce your subject so that everyone can quickly understand the nature of your topic and the significance of your main question. It is particularly important that you capture the full attention of your audience at the very beginning. Do not assume that your audience is fully familiar with the general and specific contexts of your topic; provide sufficient background such that your questions and approach are clearly understood. Try to anticipate beforehand who your audience

will be, and tailor your talk accordingly. For example, you would prepare a very different talk for an audience of first year college students than you would for an audience of professional biologists. Be sure to clearly state your hypothesis and predictions, and do so with even greater clarity and emphasis than in a written paper. Concentrate on concepts, and eliminate confusing details.

***Materials & methods***

Present the materials and methods very briefly, just enough so that people will know how you did the work. If you are using methods with which most people in the audience are familiar, then focus on the unique aspects of your experimental protocol.

***Results & discussion***

Spend most your time presenting your results and your interpretation of those results. If you are presenting the results of multiple experiments, it often works best to present the purpose and results of Experiment #1 followed immediately by your discussion of it and its connection to your main hypothesis, and then go on to the purpose, results, interpretations and connections of Experiment #2, etc. This is a pattern that you would rarely use in a written paper. Keep in mind, however, that you do NOT have to present the experiments in the chronological order in which you performed them. Put them forth in the most logical order for making your point(s). Present important ideas in different ways, even at the risk of repetition. To finish, briefly summarize what you have done and what it all means, making your most important points right at the end of the talk. Be sure not to overstep your conclusions – they should be appropriate to the data you have collected.

Though less formal than paper writing, biology is still a quantitative science. It is very important that you are as quantitative as possible.

**Presentation**

Write out the talk in advance so that your ideas are logically organized and your points are clear. At the very least, write out a detailed outline. Your speaking notes, on the other hand, should consist of the major points that you want to make. Regardless of whether you decide to use note cards or notes written on 8 1/2 x 11" paper, <u>do not read your talk</u>; the expectation is that you are familiar enough with the material and the points you want to make that you can, indeed, "talk" about them. Rehearse the talk. If possible, give the talk to one or more colleagues and ask them for suggestions for improvement. If the talk runs longer than the allotted time, eliminate the least essential material and rehearse again. The ticket to success is to prepare a good presentation and to practice, practice, practice– but not to the point of sounding like an automaton. Practice is also the most effective way of minimizing stage fright. Speak slowly and clearly— more slowly than you would in normal conversation. Word choice should be simple.

Use active words and short sentences. Words should *reinforce* visual material. Do not include material on a visual that you never explain, and do not simply read from the visual material. Speak toward your audience at all times—not toward the screen, the floor, or your notes. Avoid distracting mannerisms, such as weaving back and forth, clicking the coins in your pocket, or using repetitive distracters like "basically," "um," "you know," "like," etc.

Put yourself in your audience's shoes. As you prepare and practice consider: What information is critical for understanding the main points of your talk? Which parts

of your talk are a bit confusing, or move too quickly or slowly? Remember, if you are confused about a concept you are discussing, your audience will be even more confused. Be responsive to your audience, and adapt accordingly. Good presenters see nuances of audience members' expressions, and hear and see audience members with questioning looks in their eyes. React and respond to them and the audience will know that you are alive.

Out of consideration for the other speakers and the audience, stay within your allotted time. This is essential to ensure adequate time for questions and to keep on schedule.

In responding to questions, listen carefully to the question and then repeat it, being as accurate as possible and rephrasing it only enough to help clarify the question. Repeating the question serves several purposes: it allows everyone in the audience to hear the question, and gives you a bit more time to think about the question and frame an appropriate answer. It also enhances the likelihood that you will answer the question that was asked.

**Visual aids: PowerPoint & like presentations**

***General points***

1. Before starting, ask yourself what do you want your audience to know about your experiment and your data when you are done. While following the format outlined above, outline your presentation with that goal in mind. Tell a relatively comprehensive story about your data.

2. When done with your visuals and perhaps writing out key parts of your talk, practice. Practice with your partners and with your slides. Make sure you finish in the allotted time, preferably at a calm pace. Practice your parts with friends as an audience, and ask them for feedback. Practice some more. Practice.

***Visuals***

3. Use a font large enough to see from the back of the lecture room. Do not use ornate or fancy serifs, or all capitals.

4. Use as few slides as needed and can be discussed in the time allotted. As a general rule, one slide for each minute of presentation is generally effective, though you might spend more or less time, depending.

5. Devote each slide to a single fact, idea, or finding. Each slide should remain on the screen at least 20 seconds.

6. Use the absolute minimum number of words in title, subtitles, and captions. Remember that standard abbreviations are acceptable. Be sure to define your more unique abbreviations.

7. Avoid using a lot of text on a slide. You don't want the audience to be "following the bouncing ball" as you read your main points from your slide.

8. Color adds attractiveness, interest, and clarity to slide and illustrations and should be used whenever possible (but don't overdo it!). When using color, remember that contrasting colors are easier to see, yellow is almost impossible to see, and contrast is usually lower on computer projectors than on screens.

9. If you are going to be using the blackboard, then (1) think about how you will orchestrate the changes in room lighting/blackboard lighting, and practice them if possible; and (2) plan how you will arrange any drawings etc. on the board, and practice this as well.

10. An introductory slide and a concluding slide greatly improve the

focus of your talk.

***Tables***

11. Think about whether a table is the best way to present the data of interest. Whenever possible, present data by bar graphs or line graphs instead of tables.

12. If using a table, do not use more than three or four vertical columns, or more than six to eight rows. The information will be too difficult for your audience to digest in the short time they will see it.

13. As in papers, do not use ruled vertical lines. They distract the eye and clutter the table.

***Graphs***

14. Consider whether or not to show more than one curve on one diagram. Will it be cluttered or hard to follow? Would having curves on the same graph be helpful for comparison?

15. Label each curve; do not rely solely on symbols and a legend.

16. Use conventional units on your axes - e.g., millimolar units to express concentration of a substance, rather than "we added 10 mg of substance X to our reaction mixture." Only by using conventional units will you be able to compare your data to that of your colleagues (and vice versa).

**Acknowledgment**

*These comments are modified from "The Paper; How to Make Better Scientific Oral Presentation" by Larry Oglesby, former Professor Emeritus, Pomona College Dept. of Biology*

# G. Sample Oral Presentation Evaluation

**Name of evaluator:**

**Names of presenters:**

**Evaluation of Presentation Content - Bio41C Independent Project on Dictyostelium Cell Migration**

**Introduction and background information effectively "set the stage" for the project**

| Inadequate | Fair | Good | Excellent |
|---|---|---|---|
| Very little background. | Some background provided. | Background provided and explained well, but lacks sufficient elaboration and depth. | Complete, relevant and accurate background provided. |
| Difficulty with explanations. | Some explanations are inadequate. | | |
| No inclusion of primary literature. | Little inclusion of primary literature. | Decent amount of primary lit included. | Inclusion of sufficient primary literature to support the hypothesis. |
| (I am very confused) | (I am confused about a few things) | (I have a good idea of what's going on) | (I could give this talk now!) |

**Comments?**

**Hypothesis is clearly stated, and sufficient rationale is provided to support the hypothesis**

| Inadequate | Fair | Good | Excellent |
|---|---|---|---|
| Hypothesis not stated or alluded to. | Hypothesis alluded to but not clearly stated. | Hypothesis clearly stated. | Hypothesis clearly stated. |
| No rationale for hypothesis. | Little to no rationale for hypothesis. | Some rationale for hypothesis. | Clear connection provided between hypothesis and primary literature. |

**Comments?**

**Experimental procedure and data are clearly presented**

| Inadequate | Fair | Good | Excellent |
|---|---|---|---|
| Experimental procedure not explained. | Experimental procedure unclear. | Experimental procedure mostly clear. | Experimental procedure very clear. |
| Data not presented logically or clearly. | Data presented in logical manner but could be improved. | Data presented in logical manner but could be improved. | Data presented logically and in a visually appealing manner. |
| Interpretation of data questionable. | Interpretation of data reveals lack of full understanding. | Interpretation of data was for the most part correct. | Interpretation of data was spot-on. |
| No conclusions. | No conclusions. | Conclusions provided. | Conclusions provided. |

**Comments?**

**Presentation skills**

| Inadequate | Fair | Good | Excellent |
|---|---|---|---|
| Slides unorganized, difficult to understand, and/or text-heavy. | Slides are fairly well organized but not visually appealing and/or are text-heavy. | Slides are organized but not as visually appealing as they could be. | Slides are organized, visually appealing, and easy to follow. |
| Presentation style needs improvement (i.e. limited eye contact, speaking inaudibly, reading directly from slides or notes, nervous gestures, lack of practice). | More than 2 aspects of presentation style need improvement. | Presentation is good overall, but with some hesitance. | Presentation style is clear, natural, and engaging. |
| Difficult to follow overall message | Difficult to follow overall message. | Presenters stay on message. | Overall message is clear throughout. |
| Struggles to answer questions. | Answers questions, but incompletely. | Answers questions well. | Answers questions with authority. |

**Comments:?**

**What did these presenters do particularly well?**

**What could these presenters improve upon?**

## H. Acknowledgements

Julia Pitkin (PO '13) help to develop this lab with financial assistance from the Pomona College Jack Steller fund.

The Pomona College Hahn Grant provided the iPods.

The plate adaptors and iPod adapters were made with the 3D printers in the Pomona College physics department. David Tanenbaum and David Lahey graciously gave their advice and instruction.

David Knecht at the University of Connecticut provided invaluable advice and *dicty* strains; the heart of the lab is a modification of the under-agarose assay he co-developed.

Cells, much general *dicty* knowledge, and some initial reagents were generously provided by Susan Lee and Rick Firtel at the University of California at San Diego.

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