



Biology 151

What is Life? – Instructors' Notes

MICROSCOPY & CELLS

Before lab:

Check to make sure all supplies and materials are available in lab, both on the front bench and at the student desks. Refer to the note in lab for the materials that should be available.

At the beginning of lab:

1. Make any announcements needed, e.g.:
 - a) **Quiz scheduled for following week.** (See attached copy of sample quiz questions which will be available for students in lab.)
 - b) **Have students begin in small groups by working out prelab questions 4, 5 and 6.** Give them about 10 min. Then choose "volunteers" from several groups to do the calculations on the board and discuss how they arrived at their answers (check with rest of class to see if they got the same answers).
 - c) **How this lab will be set up, e.g.**
 - i. Overhead/board collection of data for Exercise 4?
 - ii. Questions that will be addressed in end-of-lab discussion?
2. Let student know where microscopes are located and demonstrate proper handling techniques.
 - a) How to transport microscope(s)
 - b) Always focus away from slide
 - c) Always use a coverslip on wet preps (compound scope only)
 - d) Note: There may be two types of dissecting scopes in some labs.
3. Indicate where material is located for work with:
 - a) compound microscope
 - b) dissecting microscope
 - c) prokaryotes and eukaryotes and behavior exercise
4. Indicate how points will be granted for the behavior exercise? E.g. As part of the quiz 20 quiz points for next week or as separate points.

Overview:

That all living things are composed of cells is one of the most fundamental of all biological generalizations or principles. The two major cell types that exist, prokaryotic and eukaryotic, differ greatly. However, both carry out all of the functions of life. In this lab, students will review/learn some of the basics of microscopy in order to study these cell types in more detail.

- Students will determine object orientation, field diameter and resolution for both compound and dissecting microscopes.
- Using these tools and others, students will investigate the structure and behavior of prokaryotes and eukaryotes.

Microscopy

Most students will have used some type of microscope before, but using an unfamiliar scope always takes some getting used to. In addition, the scopes they used may not have had:

- substage condensers
- phase objective and phase ring
- mechanical stages
- diopters, etc.

They also may not have looked at:

- the difference between resolution and magnification.
- how resolution is determined and calculated for a given scope
- numerical aperture and how it affects resolution
- refractive index and its effects on resolution

Resolution vs Magnification

Since students are working with microscopes where numerical aperture (and therefore resolution) changes with each change in magnification, they have a difficult time imagining how magnification could increase without increasing resolution.

Demo – Use an overhead projector to demo how a letter 'e' on an overhead can be magnified (simply move the overhead further from the screen for greater and greater magnification). Calculate total magnification by measuring size of original 'e' vs size of 'e' on screen. Then take the same 'e' and put it on the demo microscope and magnify to the same extent. Can more information be seen, e.g. do the edges appear more ragged under the microscope than they did on the overhead?

⇨ **Other ways of looking at resolution: GOLF VS BASKETBALL? --**

Resolution distance = wavelength/2NA

The smaller the distance apart two objects are and can still be discerned as separate -- the better the resolution.

Therefore, low R_D s are better, i.e. resolution is more like golf (low score = good) than basketball (high score = good).

⇨ **This is the first lab where you will encounter student with "math difficulties"**. The lab requires that they calculate resolution and NA (given the formulas for these), that they calculate field diameter for the 10 and 40X objectives. As noted above – at beginning of lab have students compare answers to question 4 to 6 on the prelab. Then have volunteers go to the board and explain **how** they got the answers.

⇨ **CAUTION: As students work on the microscopes, be sure you go around the lab and actually look at what they're seeing.** It is not uncommon for students to observe air bubbles, dust and eyelashes and think these are the specimens they are to observe.

BEGIN BY USING PREPARED SLIDES FOR WORK WITH COMPOUND AND DISSECTING SCOPES --

Have the students begin by using prepared slides this week because they're easier to observe than moving specimens. This will allow them to concentrate on how to use the scopes and their properties. We will have some small organisms, e.g. Planaria and algae, that can be observed using the dissecting scopes.

After they've mastered the basics of the microscopes they will move on to examination of the live prokaryotes and eukaryotes.

IN FUTURE LABS THEY WILL NEED TO REMEMBER FIELD DIAMETERS, etc THEREFORE GOOD NOTE TAKING IS ESSENTIAL

⇒ Remind students that they will need the information from this lab in subsequent labs so they should keep good notes in places that will be easily found for reference.

COMPARISON OF PRESERVED AND LIVING PROKARYOTES AND EUKARYOTES

Students compare the amount of information that can be gained from live wet mounts vs prepared slides of various single celled prokaryotes and eukaryotes.

Prokaryotes = *E. coli*, *Anabaena* and *Oscillatoria*

(The latter two are cyanobacteria and much larger than the *E.coli* which look like dancing specks (-) under 40X phase.

To help you and students find *E.coli* and other small specimens.

Use an indelible marker (Sharpie) to make a circle on the top of a slide. Place a sample of bacteria inside of circle and apply coverslip.

Now focus on the ink line first.

Then focus on the culture and look for movement.

Some of this movement is Brownian movement. Some is generated by the bacteria themselves. If you have trouble finding these, see your coordinator.

NOTE: YOU NEED TO USE A FRESH CULTURE OF *E.COLI* EACH DAY. CULTURES THAT ARE MORE THAN 24 hours old can become dormant/immobile. Unless they are motile, they are very difficult to see with a 40X objective.

You can also help by setting up a sample on the demonstration microscope and allowing the students to view it on the monitor.

Eukaryotes = *Euglena*, *Volvox*, *Paramecium*, *Amoeba*

Euglena can be found throughout the culture and make good subjects for experiments on effects of different colors of light.

Volvox are among the most magnificent looking of the algal protists. Students will often see new colonies developing inside an older one. It is better to use a depression slide for these so they are not crushed under a coverslip.

Paramecium - Be careful because the food source (***Chilomonas***) in this culture is often mistaken for *Paramecium*. *Paramecium* is 5 to 10 X larger than the *Chilomonas* (length), however, the *Chilomonas* are more numerous. Taking samples carefully from top 1/3 of culture vial gives the best results.

Amoeba - *Amoeba* are found on the bottom and sides of the culture dish. Usually if you pick up a piece of detritus from the bottom in a drop of culture medium, you've also picked up at least one *Amoeba*. Place a coverslip on the drop and focus on the detritus, then wait a few minutes. The *Amoeba* do not like the light and become more active and extended after being in light for a time, so become more easily seen.

Protoslo - is a mixture of methyl cellulose and water. It is viscous and is used to slow down fast moving protists, e.g. *Euglena* and *Paramecium*. To use - apply a drop of protoslo to a slide. Apply a drop of the culture next to the protoslo. Mix with a clean toothpick. Apply a coverslip and observe.)

WHAT KINDS OF BASIC MORPHOLOGICAL OBSERVATIONS NEED TO BE MADE?

Students observe 3 prokaryotes and 3 eukaryotes and draw them. Drawings should include a key indicating the diameter of each. Students then compare the amount of detail visible in the live specimens to the detail visible from prepared slide of the same types of specimens.

One additional cell type they could look at would be cheek cells from humans.

To do this, scrape (gently) inside of cheek with the blunt end of a toothpick. Add this to a slide. Add a drop of 0.9% saline and a drop of methylene blue. Mix slightly with toothpick. Apply coverslip and observe.

Periodically students will also observe bacterial cultures attached to cheek cells.

DISPOSE OF TOOTHPICKS, CHEEK CELL SLIDES AND BACTERIAL SLIDES IN 10% BLEACH SOLUTION !!!!

Thicker tissues need to be sectioned to view under microscopes --

You can demonstrate a simple hand held **microtome** and explain that thicker tissues need to be sectioned to be observed under the microscope. Put sample in hollow, add melted wax. Let this harden. Then turn the screw to expose part of the wax. Slice this off with a razor blade/knife supplied. To section turn the screw enough to expose the next thin slice of material and slice this off with a razor blade.

Possible Sample Tissues = Coleus or geranium stem, carrot, mushroom

BEHAVIORAL OBSERVATIONS – Single celled Prokaryotes and Eukaryotes

If students are comparing the reactions of organisms to specific stimuli, be sure they compare similar types of organisms. E.g. compare a photosynthetic prokaryote and a photosynthetic eukaryote. Or compare two nonphotosynthetic organisms (prokaryote vs eukaryote or two eukaryotes, etc.). In a sense this is a type of control, i.e. limiting the differences in your test subjects. It would be less useful to compare a photosynthetic eukaryote with a nonphotosynthetic prokaryote because you would not be able to determine if any difference was due to a difference in cell type or nutrition type, etc.

Available equipment/supplies:

To test for effects of colors or wavelengths of light:

Theater gels - These transparent colored gels have specific wavelength characteristics (see handout below). A slide or Petri plate can be set up with 2 or more different color "sections" (see samples available in lab). Organisms can be placed in the middle of the slide, etc. and observed over time for(# of organisms/location). They can also determine rate of travel (from knowledge of field diameter and time to move Xmm, etc), form of travel, e.g. straight line vs zig zag etc pattern and how this differs from color/wavelength zone to color zone.

To test for effects of differences in intensity of light:

Students can use different wattages (comparable to intensities) of light (e.g. using different settings on dissecting scope transformer). They could check specific intensity by using a light meter. Alternatively students could use the same light source but filter out some of the light using one of the more neutral theater gels, e.g. the grey or brown. Again they would need to determine numbers/location over time. They could also determine whether rates and patterns of travel differ depending on intensity of light, etc.

To test for effects of specific chemicals on organisms' behavior:

Students could introduce the chemical along one edge of a coverslip (use a capillary tube to do this so the amount introduced remains small). Then observe (as above) numbers/location over time, rates and patterns of movement.

Chemicals can also be introduced by soaking a thread in the chemical. A drop of culture containing the organism(s) is placed on a slide. The thread is removed from the chemical and run through the fingers to remove excess fluid. The thread is then placed across the middle of the drop of culture. A coverslip is applied and students observe as noted above.

