Atoms to Ecosystems

Lab 2: Using Microscopes to Study Cell Structure & Diversity

OBJECTIVES:
- To learn how to use and care for the compound microscope.
- To learn the names and functions of the parts and of the microscopes.
- To observe some of the structural and functional differences of various cell types.

We humans are highly visual creatures, obtaining much of our information about the world around them by using our eyes. To understand many things we need to visualize them: we must see them. Not surprisingly, then, some of the most useful tools in biology, and indeed in science in general, are those that allow us to visualize objects, processes, and phenomena. This is one of the main advantages of the powerful computers of today, because their power allows us to visualize things, and thus explore them, as never before possible. But even that technology cannot replace one of the most fundamental tools of biology, the microscope. With the microscope we can see things that are otherwise too small for us to see, and therefore study them.

There are many different kinds of microscopes. The microscope we will be using is the compound light microscope. In this lab we explore how to use and care for this most basic of biological tools.

Understanding the nature of cell structure and function is important to an understanding of organisms. All organisms are composed of cells, whether they exist as single cells, colonies of cells, or in multicellular form. Cells are usually very small, and for this reason, a thorough understanding of subcellular structure and function has been possible only through advances in electron microscopy and molecular biology.

There are two general types of cells: prokaryotic and eukaryotic. These two words have their root in the Greek word karyon (nut), which refers to a cell's nucleus. The prefix pro- means "before" or "prior to." Thus, prokaryotic means "before having a nucleus." Prokaryotic cells do not have a membrane-bound nucleus and their genetic material (DNA) is only loosely confined to a nuclear area within the cell. Bacteria, including the cyanobacteria (formerly known as blue-green algae), are prokaryotes. All other organisms are eukaryotes. The prefix eu- means "true." The cells of eukaryotes have true, membrane-bound nuclei containing their genetic material.

Prokaryotic and eukaryotic cells also differ in several other ways. Eukaryotic cells are generally larger and contain additional specialized compartments (membrane-bounded organelles) in which cell functions such as energy production may occur. Prokaryotic cells lack membrane-bound organelles; their cell functions are carried out in the cytoplasm.

GENERAL
1. Work in pairs.
   - If you do not have experience with a compound microscope, pair up with someone who does.
2. Obtain microscopes from the microscope cabinets.
3. Calculating magnification (for the Compound Microscope):
   - Multiply the power (magnification) of the eyepiece by the power of the objective lens.
   - The power of the eye piece is 10x.
   - The power of the objective lenses is written on them.
   - Always include in any sketches the power or magnification under which you observed the specimen. Always include the "x" as part of the magnification (e.g., 10x, 400x).
4. When finished, be sure that:
   - the scope is turned off.
   - all slides are removed and returned to their proper container in the correct orientation.
   - the stage is wiped clean.
   - the scope is set with the lowest power objective in place.
   - the stage is lowered (objectives raised) as much as possible.
   - the cord is properly wrapped.
   - the scope is returned to the proper microscope cabinet and space.
PRELAB
Before coming to lab, review chapter 6 in your biology text. Using this information, answer the following questions on a separate piece of paper. (Remember that pre-labs are always due at the start of the lab period!)

1. Describe two differences between prokaryotic and eukaryotic cells. Do you expect to be able to see these differences? Why or why not?
2. Describe two differences between plant and animal cells. Do you expect to be able to see these differences? Why or why not?

PROCEDURES- PART 1
1. Obtain slide with a letter “e” mounted on it.
2. How to use the microscope:
   • Leave the microscope on low power – that means having the smallest lens in place. It should already be set this way. What magnification is this? (see instructions above for calculating magnification) _______
   • Work with your partner to be sure you can both answer the following questions. Note that you do not need to write out your answers!
     1) How do you turn the microscope on?
     2) What part of the microscope does the light come from?
     3) Where do you look into the microscope?
     4) Where do you put the slide?
     5) How do you focus? (Where are the focus knobs?)
     6) What happens on the microscope when you focus (what moves)?
     7) What do the two focus knobs do differently?
     8) Is the image of the "e" right-side up?
     9) How do you increase the amount of light? How do you decrease it?
    10) How do you move the slide around without touching it?
    11) If you move the slide to the right, which way does the image in the microscope move?
    12) If you move the slide to the left, which way does the image move?
    13) If you move the slide up (away from you), which way does the image move?
    14) If you move the slide down (towards you), which way does the image move?

3. The instructor will show you how to change the power of magnification on the microscope, and how to focus.
   • Increase magnification (power) to the next strongest lens, what magnification is this? ______
   • As before, be sure you and your partner can both answer the following questions, again while still looking at the “e”:
     1) What are the names of the parts of the microscope that change the magnification?
     2) After you increase magnification, which focus knob should you use? Why?
3) After you increase magnification, is the image still in focus?

4) Is it as bright as it was under lower power?

4. Return the “e” slide to its box, and obtain a “threads” slide.
   - Observe under medium magnification. What magnification is this? ________ Are all three threads simultaneously in focus?
   - Change the focus (remember, use only the fine focus knob!): focus in and out. What do you observe?
   - How would focusing in and out help you to determine the three-dimensional structure of a specimen?

5. Return the slide to its box, taking care that the slide is properly aligned in the box.

**Part 2: Examining Plant Cells**

The cells of plants are eukaryotic, containing both a membrane-bounded nucleus and membrane-bounded organelles. A cell wall composed of cellulose surrounds the plant cell. A large central vacuole surrounded by a membrane (the tonoplast) is used for storing water, pigments, and wastes. Within the cytoplasm are membrane-bound organelles unique to plants called plastids. In this lab, you will look at various types of plastids responsible for photosynthesis (chloroplasts), for storing starch (amyloplasts and leucoplasts) or for storing accessory pigments (chromoplasts). Chromoplasts contain several types of pigment including carotenoids, which give plants an orange or yellow color.

1. **Prepare a wet-mount slide of an Elodea leaf.** Observe the thick cell wall, thinner cell membrane, cytoplasm, nucleus, and chloroplasts. A large central vacuole may be apparent. These structures characterize a generalized plant cell.

2. In your lab book, draw a representative Elodea cell as observed under high power, and label its parts. As with every drawing you do, you should be sure to identify the specimen (what you looked at), the magnification you used, and any distinguishing features (especially color if you’re sketching in black and white!). You don’t need to draw everything in your field of view, but can choose to draw just one or two cells.

3. **Prepare a wet-mount slide of onion epidermal tissue.** Onions (Allium) have layers of modified leaves (scales) that can easily be separated from one another. Peel off a portion of one layer and examine the concave side of the piece you have obtained. The surface is covered by a thin layer of cells, the epidermis.

4. Remove a small piece of the epidermis (approximately 3 x 8 mm) by breaking the scale gently, leaving the epidermis intact. Peel the epidermis from one of the halves of the scale. Prepare a wet-mount slide of the isolated epidermis.

5. Observe the onion cells using low power (10X objective) and then high power (40X objective).

6. Add a drop of Lugol’s solution (I₂KI) at the edge of the coverslip. The iodine in the Lugol’s solution will stain starch blue/purple.

7. Repeat steps 3-7 with a potato, taking care to cut a very thin slice using a razor blade. You should also stain your potato with Lugol’s. Be sure to take notes on the results of these experiments in your lab notebook!

8. Again in your lab book, draw a sample stained onion cell, and a stained potato cell. As always, be sure to note your total magnification, and label any parts of the cells you can identify.
Part 3: Examining Animal Cells

Animal cells can be studied using the light microscope, but most of the cellular organelles within the cytoplasm are not visible without the use of special staining techniques. The nucleus and nucleolus, where ribosomes are manufactured, are usually apparent in most cells.

1. **Prepare a wet mount slide of your cheek cells.** You will examine cheek cells obtained from your mouth. Instructions for obtaining and staining the cheek cells will be provided by your instructor. Sketch a few cells in your lab book, and label the plasma membrane, nucleus, and cytoplasm.

**POSTLAB**

Answer the following questions on a separate page.

1. Explain why you should always begin your work on a compound microscope using the 4X objective. What advantages does this provide?

2. When you compared the onion and Elodea, which organelle was conspicuously absent in the onion cell? Why is it not present?

3. How does the reaction of iodine with the potato cells compare with what you observed in your onion epidermis preparation?

4. What does this difference (question 2) tell you about the differences between the storage products in onions and potatoes?

5. Do you see any chloroplasts in the onion or potato? Why or why not?

6. When observing your cheek cells, did they appear to have a cell wall? How can you tell?

7. List the similarities and differences between the plant cells and the animal cells you have observed. (You might find this easiest in table format.)

8. List the similarities and differences between the plant cells and the animal cells you expect to see, but weren’t able to observe. (You might find this easiest in table format.)
The Olympus CX30/CX31 Biological Microscope

- Eyepieces (Fixed at 10X)
- Interpupillary distance scale (Page 11)
- Observation Tube
- Tube clamping screw (Use the provided Allen wrench)
- Revolving Nosepiece
- Objective
- Specimen holder (Page 10)
- Transport lock pin*
- Aperture iris diaphragm knob (Page 13)
- Condenser centering screws (Page 13)
- Condenser
- Filter holder
  Place a 45 mm filter on this.
- Field iris diaphragm ring
- X-axis knob (Page 11)
- Y-axis knob (Page 11)