Microscopy and Cell Structure

1. Pre-Lab Reading

Be sure to complete the following reading assignment before lab:

- Chapter 4 "Cell Structure" Biology2e, OpenStax College
- Appendix i : Using a Compound Light Microscope
- Guidelines for drawings, lab manual p. 9

2. Purpose and Objectives

The purpose of this lab is to

- learn the proper use of the compound light microscope
- be able to describe differences between prokaryotic and eukaryotic cells and plant and animal cells
- learn how to prepare wet mount slides
- identify components of a eukaryotic cell

Upon completion of this lab, you should be able to:

1. Locate the optic and mechanical parts of a compound light microscope.
2. Identify the function of each part of the compound light microscope.
3. Understand the relationship between magnification and resolution.
4. Calculate the total magnification used to view specimens.
5. Prepare a wet mount slide.
6. Focus specimens on slides using the 4X, 10X and 40X objective lenses.

In all sections of the lab, record observations (things you are asked to note) and answers to questions in your lab book.

3. Getting Oriented

Procedure

1. Using the diagrams of the compound microscope in the Appendix, identify the following parts of the compound light microscope: course adjustment knob, fine adjustment knob, stage, specimen holder, light intensity/voltage control knob or dial, diaphragm knob, objective lenses (4x, 10x, 40x), and stage control/ x and y axis knobs. Briefly review the function of each part.

For this exercise, record all observations in your lab book.

2. Obtain a glass slide and place a newspaper disc containing the letter **e** on the slide. Following the directions provided by your instructor, add a drop of water and a glass cover slip. Record the positioning of the **e** as it appears on the slide.

3. Be sure that the 4X objective lens is engaged properly. ***Always begin slide examination with the 4X objective in place.*** Rotate the course adjustment knob to lower the stage. Place the slide on the stage so that the letter **e** is centered over the hole in the stage.

4. Looking from the side, raise the stage using the course adjustment knob. Look through the eyepieces and focus on the **e** by slowly rotating the course adjustment knob, lowering the stage. You may need to adjust the light by either increasing or decreasing the light intensity or by adjusting the diaphragm lever.

5. Draw the orientation of the **e** as it appears through the microscope. Does its orientation differ from how it appears on the stage? If so, how is it different?

6. Move the slide to the left. How does it appear to move when viewed through the microscope?

7. Center the **e** in your field of view. Increase the magnification by switching to the 10X objective lens. Refocus using the fine adjustment knob **only**. Adjust the light by manipulating the diaphragm lever.

8. What kind of detail is visible now that was not visible using the 4X objective?

9. Before you adjusted the light, did the image appear lighter or darker than under a lower magnification?

4. Background Information

All living organisms are comprised of cells. When examined more closely, cells are one of two types based on several structural differences: **prokaryotic** and **eukaryotic**. One basic difference between the two is the presence or absence of a membrane-bound **nucleus** that contains the cell's chromosomes.

The presence of a nucleus is characteristic of eukaryotic cells. In prokaryotic cells, the DNA is located in a region known as the **nucleoid** that is not enclosed in a membrane. In addition to a nucleus, eukaryotic cells also possess many other membrane-bound **organelles** that are specialized to perform specific functions. Organisms classified in the domains Bacteria and Archaea are prokaryotes. All other organisms are eukaryotes.

5. Prokaryotic Cells: Bacteria

Bacteria are unicellular prokaryotic organisms. Bacterial cell structure is relatively simple and they are typically very small in size. The two most common shapes for bacterial cells are spherical (described as **cocci**) and rod shaped (described as **bacilli**).

Procedure

1. Record the **genus and species name** of the bacterial sample provided.

2. Place a drop of culture on a microscope slide, add a cover slip and examine your preparation under 400x. Your sample will be easier to view if you are careful not to get an excess of liquid on the slide. Also, you may need to adjust the light to create optimal contrast and a clearer image.

3. Make a drawing of your observations at 400x. Record whether or not you see movement by the bacterial cells.

4. Using Table 1 in *Using a Compound Microscope*, estimate the size of a single bacterium. To do this, estimate the number of cells that will fit along the length of the pointer or across the field of view. Then divide the length of the pointer or field of view (given in the table) by the number of bacteria that you estimate will fit. **Record all size estimates and calculations in your lab book. Label using the proper units (usually µm)**

6. Eukaryotic Cells

I. Plant Cells

Plant cells have many features that distinguish them from animal cells. The most obvious feature is the presence of a **cell wall** that provides support and protection to the cell. In addition, plant cells may have a large **central vacuole** that serves a variety of functions, including support and storage. **Plastids** are organelles with a double membrane that are found in various plant cells. Many plastids function in storage of substances like starch or pigments. **Chloroplasts** are prominent plastids in many plant cells and are the site of photosynthesis.

A. Observation of *Elodea*

Elodea is a common plant found in fresh water ponds.

Procedure

1. Remove a leaf from the tip of a sprig of *Elodea* and place it, top surface up, in a drop of water on a microscope slide (cells on the upper surface are larger and easier to observe).

2. Add a cover slip, but do not allow the leaf to dry out. Additional water may be added if necessary by placing a drop near the cover slip.

3. Bring the sample into focus at 40x, 100x and finally 400x. Examine the leaf at 400x magnification.

4. Small green spheres inside each cell are **chloroplasts**. Record your observations concerning the shape of a chloroplast.

5. Are there any cells in which the chloroplasts are moving? This movement is called **cytoplasmic streaming** and is caused by changes in the cell's **cytoskeleton**. Describe how the chloroplasts move.

6. Describe the 3-dimentional distribution of chloroplasts in a cell. What cell structure affects their distribution? (See the plant cell diagram in your text for help).

7. Draw a representative sample of several cells (4-5) as seen under 400X magnification, including all organelles that are visible. Make your drawing large! Label a chloroplast in your drawing. Label the **cell wall**. Leave room for labeling other cell structures.

8. Find the **cell nucleus**. It is usually pushed up against the cell wall and will appear as a faint opaque or grey sphere. Draw and label the nucleus in your diagram. If you are not able to locate a cell nucleus, then state this in your lab notebook.

9. Estimate the length of a single *Elodea* cell. Show your calculations.

10. When finished, remove the leaf from the slide and place the slide in the beaker of water on the lab side bench. Place cover slips in a separate beaker.

II. Animal Cells

Human Epithelial Cells

Epithelial cells form tissues that line organs and body cavities. Those found lining the surface of your body are replaced in 2-3 weeks' time. Cells can be obtained easily by gently scraping the inside of your cheek.

Procedure

1. Using a sterile toothpick, gently scrape the inside of your cheek. One lab partner should both prepare and handle this slide.

2. Wipe the toothpick along the middle of a slide. Add a drop of methylene blue to the slide. Cover with a cover slip. Place toothpicks in an autoclave bag.

3. Observe and record your observations. Draw 3-4 cells and label the cell membrane, nucleus and cytoplasm. **Estimate the size** of the cells and record this calculation in your lab book.

4. **Place this slide in the autoclave bag at your table.**

III. Looking at Pond Water.

Procedure

1. Prepare a wet mount from the pond water sample.

2. Draw and describe the size, shape and movement of **at least three** of the organisms you observe. Record all observations you make regarding the size, appearance and behavior of the organisms.

7. Conclusion and Summary

1. Based on your observations **(things you actually saw, not what your textbook says)**, how do bacterial cells differ from eukaryotic cells?

2. Describe the differences you observed between plant cells and animal cells (at least two **things you actually saw**).

3. In what other ways do plant and animal cells differ (at least two additional differences)? You will want to consult your text to answer this question fully.

4. You wish to view a specimen at 100x magnification. Describe the procedure you should use to place your slide on the microscope and the steps needed to bring it into focus at this magnification.

NOTES: