

BIOLOGY 3A LABORATORY

Microscopes and Cells

Objectives

- To learn the proper use and care of compound microscopes
- To learn staining techniques used in light microscopy
- To prepare a wet mount
- To determine the magnification and size of the field of view
- To use the microscope reticle to measure the size of objects in the field of view
- To determine the depth of field
- To differentiate between prokaryotic and eukaryotic cells
- To learn the similarities and differences between plant and animal cells
- To identify cellular structures

Introduction

The ability to magnify specimens has been around since 1000 B.C. The first simple compound microscope that utilized two lenses was not invented until the late 16th Century. It was the invention and modification of this microscope that changed the way scientists studied living organisms. It allowed scientists to study the structure of a living organism and to discover numerous species that were not visible to the unaided eye.

Today there are numerous types of microscopes available to scientists that provide greater **magnification** and superior detail (**resolution**). Besides magnifying and resolving an object, the microscope also provides the contrast that is needed to distinguish detail between adjacent objects. Microscopes used in most biology laboratories magnify up to 1000X with a resolving power of 0.2 μm . The microscopes in this laboratory are compound, light microscopes. The light is transmitted through the specimen on the stage and through two lenses before it reaches the user.

These are expensive pieces of equipment. Please handle them carefully.

A. USING THE MICROSCOPE

There are some basic rules that you need to adhere to when using microscopes. These are:

1. Always use two hands when moving your microscope. Use one hand to hold the arm and the other hand should support the base. **NOTE:** The scopes are heavy!
2. Use only **LENS PAPER** to clean the lenses. Do not use tissues, paper towels, Kimwipes, your shirt, etc. to clean the scope. Even though these items may feel soft, they can scratch the lenses.
3. The microscope must be on the lowest power objective lens:
 - a. when starting to use the microscope.
 - b. when you finish using the microscope. Before you return the microscope to its numbered slot: 1) make sure the objective lens is on the lowest power, 2) the power is off, 3) there is no slide on the stage and 4) the dust cover is in place.

4. **NEVER** use the course focus knob on high power; use **ONLY** the fine focus knob. These microscopes are **parfocal**. This means that when the image is in focus on one objective, the image will be in focus with the other objectives. You may need to fine focus the image for sharpness.

Functional Parts of the Microscope:

Materials:

Microscope, lens paper, letter "e" slide, color thread slide, 1 mm gridline slide

Procedure:

1. Remove the microscope from the scopes cabinet and return to your work area.
2. Make sure that you use both hands to support the microscope (arm and base).
3. Place the base securely on the lab bench with the arm towards you.
4. Identify the following parts of the microscope:
 - a. The ocular lens or eyepiece is a 10X lens which is at the upper end of the tube. The scope that you are using is binocular; it has two eyepieces.
 - b. Revolving nosepiece: the objective lenses are attached below the nosepiece. It allows the user to change the magnification.
 - c. Objective lenses: our scopes will either have 3 or 4 objective lenses attached to a revolving nosepiece. The magnification is inscribed on each lens. The powers that you will use are: scanning (4X), low (10X) and high dry (40X). Some scopes will have an oil immersion objective lens (100X). You **will not** use this lens in this lab.
 - d. Stage and stage clamp: the slide will rest on the stage and will be held in place with the stage clamp. The moveable portion of the stage clamp should only be touching one corner of the slide.
 - e. Iris diaphragm lever: on the front edge beneath the stage is a small lever that is used to adjust the contrast by regulating the amount of light.
 - f. Condenser and adjustment knob: the condenser condenses the light rays into a stronger beam. Use the adjustment knob located below and to the side of the stage to increase or decrease the light intensity.
 - g. Coarse Adjustment Knob: On each side of the scope is a large knob use to move the stage up and down to focus the image. This knob is to be **only** used with the scanning and low power objective lenses.
 - h. Fine Adjustment Knob: Located by the coarse adjustment knob, this knob allows for very small changes to the height of the stage. This knob is used to increase the sharp focus of an image and is the **only** knob to be used with **high power**.
 - i. Slide Movement Knobs: To one side of the stage, there are two black or silver knobs that you will use to move the slide. One knob will move the slide to the left and right. The other knob will move slide towards and away from you. The scopes are **parcentral**. This means that when you have the object centered in the field of view on a lower power and then change to a higher power, the image will remain in the center of the field of view.

- j. On/Off Knob/Switch: Located on side of the microscope (Nikon) or the top of the base in the front end of the microscope (Swift).

Determining the Magnification or Power:

The compound microscope utilizes two different sets of lenses to magnify an object. These lenses are the ocular and objective lenses.

Procedure:

1. Determine the magnification for each of the following lenses by looking for the engraved magnification.
2. Calculate the total magnification of object being viewed by multiplying the magnification of the ocular lens and objective lens.

Image Orientation and Brightness:

Procedure:

1. Obtain a letter "e" slide.
2. Place the letter "e" on the stage so that you can read it normally. Use the stage clamp to hold it in place on the stage.
3. Adjust the letter "e" so that the light is transmitted through the letter.
4. Start with the scanning lens; bring the stage as close to the scanning lens as it will go.
5. Look into the eyepieces and use the coarse focus knob to slowly move the stage away (down) until the letter "e" comes into focus.
6. What is the orientation of the letter "e"? Place your answers on the worksheet.
7. Move the slide slightly to the left while observing the letter "e" in the microscope. What direction did the letter "e" appear to move?
8. Re-center the letter "e". Move the slide towards you slightly. What direction did the letter "e" appear to move?
9. Re-center the letter "e". Switch from scanning to low power. Center the letter "e" if needed. Look at the **working distance**, the between the objective lens and the slide.
10. Switch from low to high power. Center the letter "e" if needed. What happened to the image and the brightness?

Size and the Diameter of the Field of View:

If you know the diameter of the field of view for each magnification, you can estimate the size of the object for that field. The high power field may be too small to measure with the ruler, thus you may have to calculate it.

Procedure:

1. Obtain the slide with the millimeter gridlines and place it onto the stage.
2. Adjust the slide so that the light is transmitted through the gridlines.
3. Start with the scanning lens; bring the stage as close to the scanning lens as it will go.

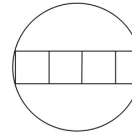


Figure 1. This field of view is 3.5 mm

4. Use the coarse focus knob to slowly move the stage away (down) until the gridlines are in focus.
5. Align the edge of a gridline with the edge of the field of view (Figure 1).
6. Count the number of boxes that can fit across the field of view (or count spaces between the lines). **Do not** count the lines! Each box is equal to 1 mm. Record this value on the worksheet.
7. Change to low power and measure the diameter of the field of view.

8. To determine the size of an object, use the following formula:

$$\text{Size of Object} = \frac{\text{field diameter for that magnification}}{\# \text{ of cells that fit across the diameter}}$$

9. To determine the field of view under high power, use this following formula:

$$(\text{Low power diameter}) \times (\text{Low power magnification}) = (\text{High power diameter}) \times (\text{High power magnification})$$

Using the eyepiece reticle to measure the size of objects:

Your microscope also has a reticle in one of the eyepieces. This looks like a small ruler, scaled from zero to ten. To use this device to make measurements you must first calibrate it. The calibration can be done for 40x and 100x, then you may simply multiply by ten to get the 400x and 1000x.

Procedure:

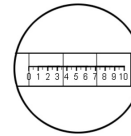


Figure 2. Using the reticle to measure the gridline scale

1. With the millimeter gridline slide in place, start with the scanning (4x, scan power objective).
2. Measure the length of two millimeters using the reticle. The numbers on the reticule scale will be called reticule units (r.u.).
3. Calculate the length of a reticule unit by dividing the number of millimeters by the reticule measurement. In Fig. 2, you see an example. In this example, 2 mm equals 7.0 reticule units, thus one reticule unit equals 0.29 mm. So now you have a ruler, for use at 40x.
4. Complete the same exercise for the 10x objective, 100x total magnification.

Depth of Field:

On a higher magnification, it may be necessary to focus up and down with the fine adjustment knob to view different portions of the object. This is necessary because the entire object may not come into focus. If the object is thick, it will have a greater depth of focus. As you will see later, cells are three dimensional.

Procedure:

1. Obtain the colored thread slide and note the number associated with the color thread.
2. Locate and focus on the color threads on scanning power.
3. Change to low power. Center the point where the three threads cross and focus.
4. Slowly focus up and down to determine which thread is on the top, middle and bottom.

B. PROKARYOTIC AND EUKARYOTIC CELLS

The cell is the smallest unit that possesses all seven attributes of life; therefore it is considered the basic unit of life. All organisms are composed of one or more cells and arise from pre-existing cells. All cells have a cell membrane and contain genetic information (with a few exceptions). These cells can differ in their complexity and structural features. Because of these similarities and differences, living organisms are classified into two major groups: **Prokaryotes** and **Eukaryotes**.

The less complex group is the prokaryotes (1 – 10 μm) which lack membrane-bound organelles and lack a nuclear membrane around their genetic material. Their genetic material is concentrated into an area called the **nucleoid**. Bacteria and cyanobacteria (blue-green algae) are prokaryotes that you may be familiar with. Bacterial cells have different morphologies that include: coccus (round), bacillus (rod-shaped) and spirillum (spiral). Various bacteria may be composed of a single morphological type, a chain of the same morphological type (*Streptococcus*) or a cluster of the same morphological type (*Staphylococcus*). The largest prokaryotes are the cyanobacteria or blue-green algae. These organisms contain photosynthetic pigments, such as chlorophyll *a*, in thylakoid membranes, not chloroplasts.

Eukaryotic cells are larger (10 – 100 μm) in size and have more complex structural features. These structural features include a membrane around the genetic material, a greater quantity of DNA and various membrane bound organelles. Protists, fungi, plants and animals are different types of eukaryotic cells. In this lab, you will have the opportunity to compare and contrast plant and animal cells. You will also have to compare and contrast prokaryotic and eukaryotic cells.

Prokaryotes - Bacteria:

Procedure:

1. Obtain a prepared bacterial slide that contains the three different morphological types.
2. Observe under low and high power.
3. Make a drawing of each morphological type under high power.
4. Label all the components that you can identify.
5. Obtain a slides of the cyanobacteria: *Nostoc* and *Anabaena*. Observe these organisms and draw their cellular shapes on your worksheet.

Eukaryotes - Animal Cells:

Animal (Fig. 3) and plant cells have many of the same membrane bound organelles. There are a few structures that animal cells do not possess such as a cell wall and chloroplasts. The animal cell that you will observe will be a human cheek epithelial cell that lines the inside of the mouth. After staining your cheek cells, you may even notice bacterial cells.

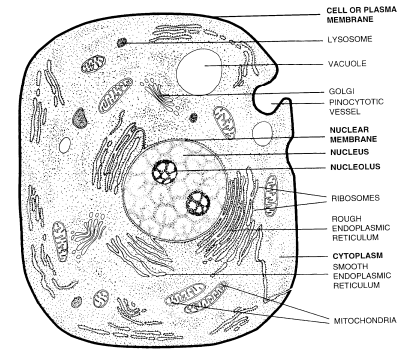


Figure 3. A typical animal cell.

Procedure:

1. To make a wet mount, get a clean slide, cover slip and toothpick.
2. Gently scrape the inside of your cheek and smear your cheek cells on the clean slide.
3. Properly dispose of the used toothpick.
4. Place a few drops of methylene blue stain to your cheek cells on the slide. Allow the stain to remain for a few minutes.
5. Over the sink, tilt the slide with the stain and carefully rinse the excess stain using a dropper bottle filled with DI water. Do not spray directly on your cells.
6. Place a cover slip on the slide at the edge of the "wet" specimen at a 45° angle, and then carefully drop the cover slip onto the specimen minimizing "air bubbles." Compress gently and wipe any excess liquid with a Kimwipe.
7. View the cells first under low power and then switch to high power.
8. Under high power, draw a few cheek cells and **label** all visible components.
9. Discard the cover slip and soak the slide in the bleach solution for 5 minutes.
10. **RINSE YOUR OWN SLIDE BEFORE YOU LEAVE! Any slides left in the bleach solution or anywhere else will result in point deductions from all students!**
11. On a clean slide mix two or three drops of Janis Green B stain with one drop of 7% sucrose solution.
12. Prepare a wet mount of your cheek cells as before.
13. Search each cell to locate stained mitochondria. They are small blue spheres located within each of your cells. The color will fade in 5 – 10 minutes, so make a new preparation if needed.
14. Determine the average size of your cheek cells by measuring at least 10 different (unfolded) cells. Determine the approximate size of the mitochondria.

Eukaryotes - Plant Cells (Onion):

Although plant and animal cells share some common structures, plant cells have chloroplasts, cell walls, larger vacuoles and lack centrioles. However, some parts of plants may not have a particular organelle.

Procedure:

1. Make a wet mount of an onion skin.
2. Obtain a thin piece of onion skin by taking an onion leaf and snapping it in two.
3. Carefully remove the thin skin that will separate from the convex side of the onion leaf.
4. Place the thin onion skin on a clean slide and a few drops of iodine to stain. Allow the stain to remain for a few minutes.
5. Place a cover slip on the slide at the edge of the "wet" specimen at a 45° angle, and then carefully drop the cover slip onto the specimen minimizing "air bubbles." Compress gently and wipe any excess liquid with a Kimwipe.
6. View the onion cells under low and high power.
7. Under high power, draw a few onion cells and label all visible components.
8. Discard the cover slip and onion skin.
9. Rinse the slide.
10. Make another wet mount slide, but use the Janis Green B stain instead. The onion cells must be healthy and metabolically active for the mitochondria to stain well.
11. Determine the approximate size of the onion cells and mitochondria.

Eukaryotes - Plant cells (*Elodea*):

Elodea is an aquatic green plant (Fig. 4). You will notice structures that were not visible in the onion cell. The cell membrane is present; however, it is not visible as it is just inside the cell wall. In another laboratory exercise, you will have the opportunity to view the cell membrane of an *Elodea* cell. The most noticeable organelle will be the small circular chloroplasts. Notice the movement of the chloroplasts around the periphery of each cell. This movement is called **cyclosis** and is the result of the movement of the cytoplasm (cytoplasmic streaming). As cyclosis occurs, this helps the plant facilitate the transport of materials within the plant.

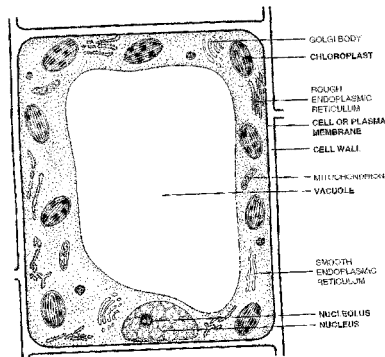


Figure 4. A typical plant cell.

Procedure:

1. Make a wet mount of a leaflet of *Elodea*.
2. Place the small leaflet on a clean slide and cover with a cover slip.
3. View the *Elodea* cells under low and high power.

4. Under high power, draw a small section of *Elodea* cells and label all visible components.
 - a. Determine the size of an *Elodea* cell.
 - b. Look for the area of the central vacuole
 - c. Search for the nucleus, it is usually pressed against the cell wall as a faint gray color (use a drop of iodine to stain to enhance the nucleus).
 - d. Approximate the size of the nucleus and chloroplasts.
5. Notice the chloroplasts moving around the periphery of the cells. This is called **cyclosis**.
6. Using the reticle as a measuring stick determine the speed of cyclosis by timing a single chloroplast for a known distance.
7. Discard the cover slip and leaflet.
8. Rinse the slide and dry the slide.
9. Replace the slide in the slide box.

Eukaryotes - Protists:

Amoeba and *Paramecium* are members of the kingdom Protista. This kingdom is one of the oldest and most diverse of the eukaryotic kingdoms. Protists are microscopic single-celled or multi-cellular (colonial) organisms may have plant-like and animal like characteristics. There are even protists that exhibit fungal characteristics. Members in this group can be separated into one of three general groups: algae, protozoans and slime molds.

Algae may be unicellular or multicellular photosynthetic organisms that use various types of photosynthetic pigments to obtain energy. Alga that are filamentous have their cells attached end to end (you saw this in the blue-green algae, or cyanobacteria, *Anabaena*, *Nostoc*, etc.) ranging from a few cells to many cells in length. Depending on the type of algae, the filament may or may not be branched. Colonial algae are algae that do not attach end to end, but rather attach to each other as a flat sheet, sphere or some other three dimensional shape.

Protozoans have an animal like lifestyle and typically are not photosynthetic. These heterotrophs have numerous types of vacuoles for food storage, expulsion of excess water, digestion, etc. You will observe two of the many protozoans in this laboratory, *Amoeba* and *Paramecium*. *Amoeba* can be found in a variety of environments (terrestrial, marine and freshwater). The most distinguishing feature about *Amoeba*'s are the presence of pseudopods, which are extension of the cytoplasm used for movement and obtain food. *Paramecium*, differ in that they belong to a group that possesses large numbers of cilia for locomotion. They also differ in that they possess two types of nuclei: a micronucleus and a macronucleus.

Procedure:

1. Obtain a prepared slide of a stained *Amoeba* and a *Paramecium* or another protist.
2. You may have to lower the light intensity to see cellular structures.

3. Determine the approximate size of each of these protists.
4. Sketch each of these and label obvious structures (refer to your textbook).
5. Make a wet mount from the pond water sample.
6. Sketch, identify and describe 2 – 3 of the various organisms seen.
7. Rinse and dry the slide.
8. Replace the slide in the slide box.

Saddleback CSI

Unknown Identification:

So now it's your turn again to try your hand at the identification of an unknown. The Saddleback CSI team has returned with several unknowns. Again, these samples were collected in the same food preparation area of the cafeteria where a crime has taken place before. This time, the team wants to know what type of cells these are. The samples collected could range from various prokaryotic to various eukaryotic cells. Some of these samples seem to be from food items, human bodily fluids and strangely, pond water. Using what you've learned in today's lab, you'll need to identify the cells as prokaryotic or eukaryotic; if eukaryotic, identify the cells as plant, animal or protist. The samples may not be exactly like the representative cells that you looked at earlier. You will need to use other resources to aid you in deducing your unknown cell type. Again, you will need to justify how you came to conclude the type of cell that you have and how you excluded the other cell types.

Your lab instructor will assign a particular unknown organism for you to identify.