Cells and Enzymes

EACH WEEK YOU WILL CHECK YOUR BEAN BEETLE EXPERIMENT

Take notes in your bean beetle notebook. Count live beetles, eggs, additional observations.

Microscopes and Cells

LEARNING OBJECTIVES

1. Be able to use the light microscope to draw, identify, and describe cell structures.
2. Examine cells from eukaryotic multicellular organisms.
3. Demonstrate osmosis in plant cells.
4. Describe and interpret the animated short film *The Inner Life of the Cell.*

INTRODUCTION

The cell is the basic functional unit of all living organisms—the smallest and simplest biological structure possessing all key characteristics of life. All living organisms are composed of one or more cells, and every activity taking place in living organisms is ultimately related to the metabolic activities occurring in each cell. Thus, understanding the processes of life necessitates an understanding of the structure and function of cells.

The earliest known cells lacked nuclei and membrane-bound organelles. Present in the fossil record 3.5 billion years ago, these earliest cells were **prokaryotes** (Figure 1). They possessed the basics: a plasma membrane, a single

Lab 1

circular DNA, and used RNA and ribosomes to assemble needed proteins. Cells with a nucleus and membrane-bound organelles, called **eukaryotes**, do not appear in the fossil record for another two billion years. Figure 2 shows a typical animal and plant cell—both eukaryotic cells. Eventual evolution of the eukaryote and its internal compartmentalization led to enormous biological diversity in single cells. The evolution of loose aggregates of cells ultimately to colonies of cells paved the way for specialization, whereby certain groups of cells played differing functional roles. Some were involved in locomotion, others in reproduction, yet all worked together as a functional unit.

Multicellularity appears to have evolved more than once among Eukarya, and adaptive radiations have given rise to multicellular protists, fungi, plants, and animals. Today, unicellular organisms exist as prokaryotes in the Domains Bacteria and Archaea, and unicellular, colonial, and multicellular organisms make up Eukarya. Today's lab activities are designed to familiarize you with cells from the Eukarya, teach you techniques that are used to study them in the lab, and explain how osmosis affects cell chemistry.

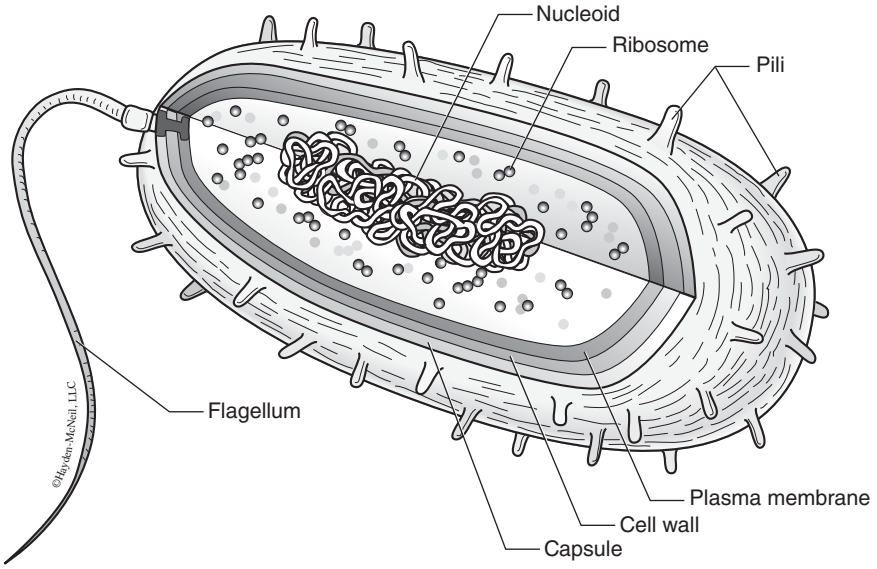


Figure 1. Generalized features of prokaryote cells.

ACTIVITY 1: REVIEW OF LIGHT MICROSCOPY

Microscopes of one kind or another are used to study cells. Cell size and the limitations of the human eye necessitate the assistance of microscopes to allow us to visualize them. Light microscopes, which use visible light for illumination, are useful for viewing most eukaryote cells. They are limited by the smallest wavelength of visible light; thus, when viewing the smaller structural features of cells or even whole prokaryotes, their use is limited. They are essential, however, for examining whole cells and their general features. Given the importance of the light microscope's role in the biological sciences, it is important for you to have a thorough understanding of its proper use. Please review key features of a compound microscope (Figure 5) on page 14 before you get started on today's activities.

PROCEDURE A: PRACTICE USE OF THE COMPOUND MICROSCOPE USING A PREPARED SLIDE

1. Obtain one of the prepared slides at your table. Each student should work with their own slide and microscope.

2. Place the prepared slide on the microscope stage with the lowest objective lens (4×) in place over the slide. Use the mechanical stage adjustment knobs to center the specimen under the 4× lens. Turn on the light switch so that you can see light shining through the specimen on the slide (generally the area under the coverslip).

3. Always start to focus using the 4× objective lens and use the coarse focus knob to bring the specimen into focus. Once your image is focused, you can use the small fine focus knob to fine tune the needed focus.

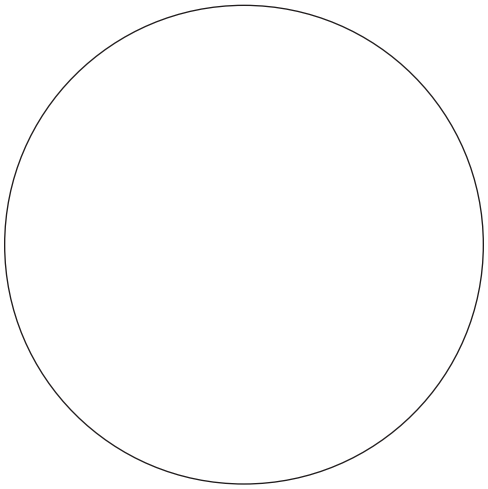
4. Most likely you will want to increase the magnification to better see your specimen or to look for finer details not obvious with the 4× lens. The lenses on your microscope are parfocal, so that you can rotate the objective lens to the next power lens, 10×, and the image should be somewhat close to focus. You may need to move the stage to re-center and find the specimen using the stage adjustment knobs, but do not raise or lower the stage. You should ***only*** use the smaller fine focus knob at any magnification higher than the 4× lens or you risk damaging the expensive lens and/or breaking the slide.

5. Now look at your specimen using the 10×, then the 40× lenses. Again, remember to only use the smaller fine focus knob to better focus on the specimen. Do not use the 100× lens, since special immersion oil is necessary for that level of magnification. Our specimens will all be too large to examine with the 100× lens.

6. In the circle that follows, provide a sketch of the cells, tissue, or organism found on your slide. You will need to determine the best level of magnification to make your drawing. Please include the total magnification used for your sketch. How do you calculate this? Be sure to properly label your drawing indicating the *Genus (*and *species if possible)* or description name as it appears on the slide label and at least two major structures or organelles seen at this appropriate level of

magnification. If you are unsure of what you are looking at and possible structures, you can do a quick Google search. Be sure to sketch from what you see on your slide, and not from the Internet image. Different staining or microscopy may be used to show structures you cannot see in the preparation here.

Total magnification = _____ × _____



Organism: _____

Total Magnification: _____

PROCEDURE B: SIMPLE STAINING OF AN ANIMAL AND PLANT CELL

In order to better visualize key features of some cells, we will employ simple staining. Simple staining is the use of one stain to help visualize a key structure. Simple stains generally adhere to specific types of molecules found in or on the surface of cells.

PREPARING SLIDES OF HUMAN CHEEK CELLS

Cheek cells form the epithelial tissue that lines the mouth of the human and other mammals. They are a good representative of a generalized animal cell and naturally sluff off in your mouth.

1. *Gently* scrape the inside surface of your cheek with the flat side of a toothpick.
2. Place 1–2 drops of toluidine blue stain on the slide.
3. Roll the tip of the toothpick in the stain on the glass slide in order to transfer the cells to the slide and then apply a coverslip. To avoid trapping air bubbles in our slide, place one side of the coverslip to the side of the stain, then gently let the raised end down to cover the stain and your specimen.

4. Examine the prepared cells with the compound microscope. Again, start with the 4× lens and focus with the coarse adjustment knob, then fine focus. Then rotate to 10× and 40× using ***only*** the fine focus knob. Which is the best magnification to view your cheek cells?

5. Locate the prominent nucleus in each cell. The presence of a nucleus is a critical feature that distinguishes the eukaryotic cell from the prokaryotic cell.

6. Use the circles on the following page to illustrate your findings. Please include total magnification and label all structures and organelles you can see in your cells.

7. Do you notice any other structures, organelles, or cells on your slide? What inhabitants of your mouth might you be observing?

PREPARING SLIDES OF ONION EPIDERMAL CELLS

1. Obtain a piece of cut onion bulb. Note the slightly curved shape of the piece. With your fingernails or a dissecting needle, peel off the thin transparent layer of onion epidermis from the inner surface of the onion piece.

2. Place the onion epidermis on a glass slide. Be careful not to wrinkle or fold this too much.

3. Add 2 drops of toluidine blue and apply a coverslip. To avoid trapping air bubbles in our slide, place one side of the coverslip to the side of the stain, then gently let the raised end down to cover the stain and your specimen.

4. Examine the prepared cells/tissue with the compound microscope. Again, start with the 4× lens and focus with the coarse adjustment knob, then fine focus. Then rotate to 10× and 40× using ***only*** the fine focus knob. Which is the best magnification at which to view your onion cells?

5. Locate all recognizable structures and organelles in and around the cells.

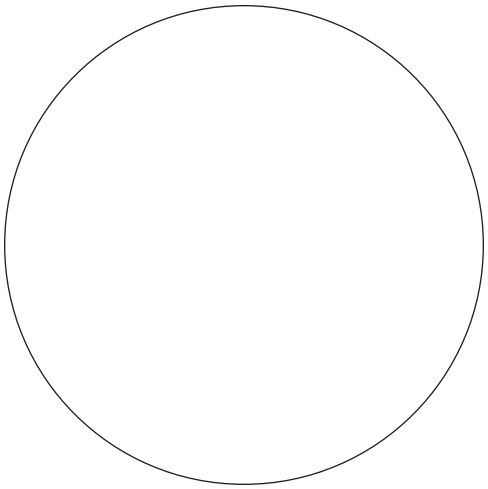
6. Use the circles on the following page to illustrate your findings. Please include total magnification and label all structures and organelles you can see in your cells.

7. Given that this is a plant cell, is there anything missing that you would expect in a typical plant cell? Explain your answer.

8. How do the onion cells differ from the cheek cells? List one similarity and one difference you can see between these two cells.

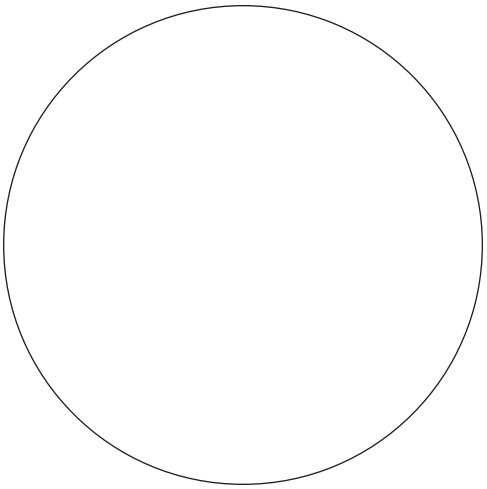
   Similarity:

   Difference:



Human cheek cells

Total Magnification: _____



Onion cells

Total Magnification: _____

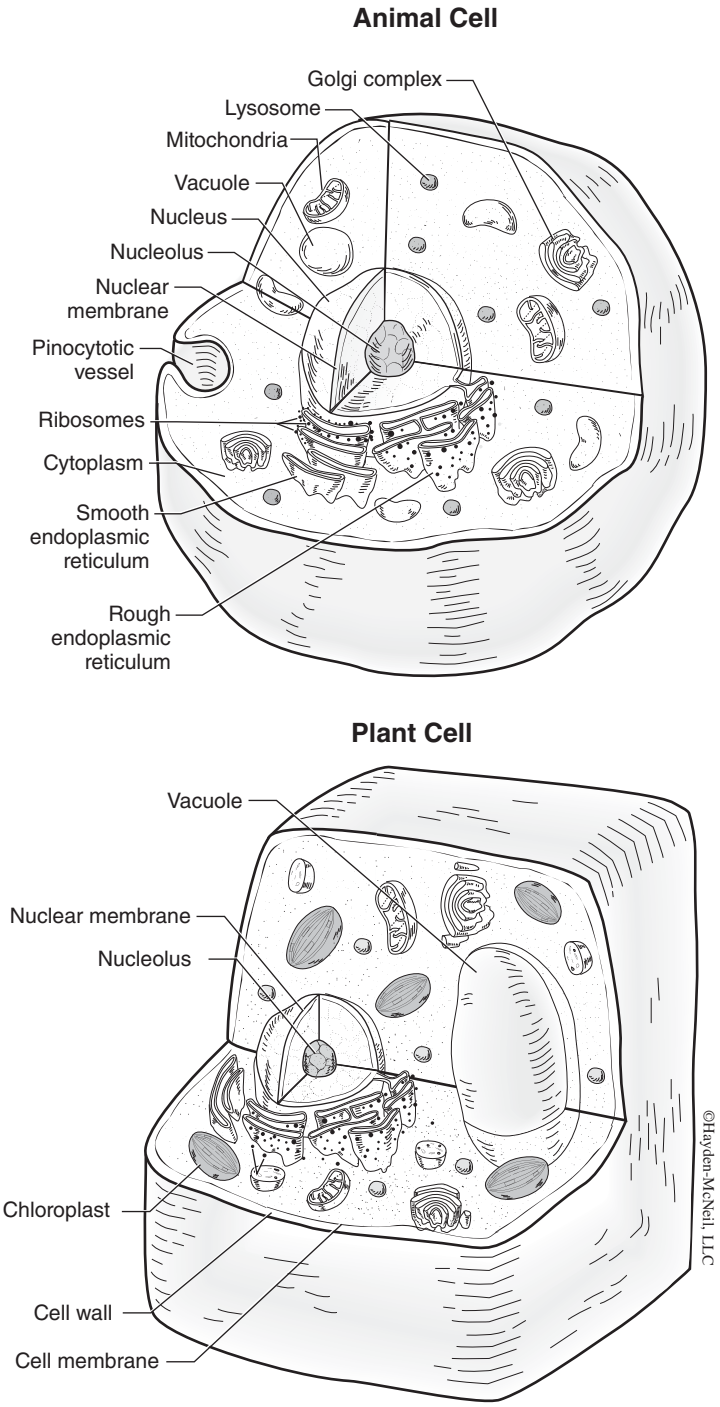


Figure 2. Generalized features of animal and plant cells.

ACTIVITY 2: OSMOSIS IN LIVING CELLS

The internal and external chemical environment of a living cell is aqueous, and there is a concentration gradient across the lipid cell membrane that separates the two. This membrane is semi-permeable, allowing water in particular to move passively in response to the concentration differential. Typically, cells are bathed in an isotonic solution, meaning that the concentration is similar on both sides of the cell membrane. A change in external conditions can have dramatic effects.

PROCEDURE A: OSMOSIS IN PLANT CELLS (*ELODEA*)

1. Prepare a wet mount of an *Elodea* leaf using the deionized water (dH_2O) at your table.

2. Observe the leaf first at low power (4× lens), then at medium and high power (10× and 40× lenses). You will need to decide the most appropriate level of magnification to best see these cells. Record your observations in #7. Draw and label organelles and structures observed (chloroplasts, vacuoles).

3. Place 1–2 drops of either the 5% or 10% saline (NaCl) solution at the edge of the coverslip. One pair of students at each table should use the 5% solution and the other pair should use the 10% solution.

4. Using a small piece of paper towel, carefully touch the *opposite* edge of the coverslip in order to draw the salt solution through your leaf under the coverslip. The paper towel should "wick" up the added solution. Refer to Figure 3.

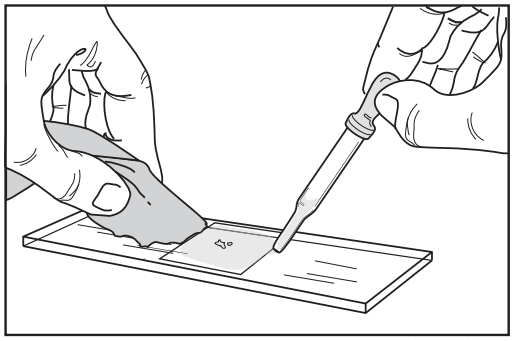
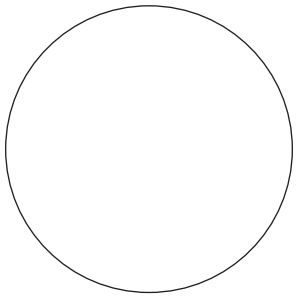


Figure 3. Slide preparation.

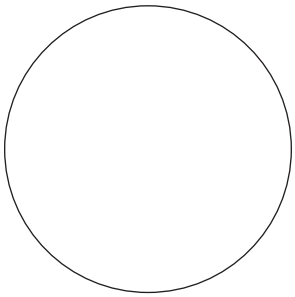
5. Watch carefully. Record and draw your observations in #7. What changes do you observe? Explain why this occurred in the cells.

6. Repeat the process, only this time use dH_2O and wick the fresh water back through the *Elodea* leaf.

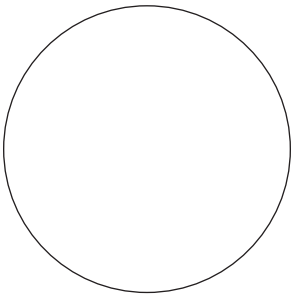
7. Again, draw and record your observations. Now that the cells are bathed in fresh water again, what differences do you see in the cells? Have they returned to their original appearance? Explain what occurred.



***Elodea* cells in water**



***Elodea* cells in _____ NaCl (fill in with the % saline you used)**



***Elodea* cells in water**

8. What differences did you see in the 5% and 10% saline treatments? What would account for this?

9. When the *Elodea* cells were bathed in fresh water, was the recovery the same in both the 5% and 10% treated cells? Explain why or why not.

PROCEDURE B: CELL MEMBRANES: OSMOSIS AND DIFFERENTIAL PERMEABILITY

An essential property of the cell membrane is **differential permeability**. The protein-phospholipid matrix of the membrane of a specific cell type determines which substances are exchanged between the cell's internal environment and the external environment. We demonstrate this property by using two chemical solutions separated by a synthetic barrier, which functions analogously to a cell membrane. In our activity we will use dialysis tubing with a pore size that allows for the movement of molecules from 12,000–14,000 Daltons.

1. Fill a 400 mL beaker with 200 mL of tap water.
2. Using the plastic pipet, add 5 mL of Lugol's solution (IKI) into this water until it develops a distinct deep yellow color.
3. Prepare one 8" length of water-soaked dialysis tubing. Place a clamp on one end of the tube 1" from the end. Using the provided syringe, fill this tube with 10 mL of 5% starch solution.
4. Leave 3 inches of unfilled tube above the starch, then place another clamp about 1 inch from the top/open end of the tube. Check the clamped tube to ensure that it does not leak. Blot the outside of the tube dry of any of the added solution.
5. Weigh the tube and record the mass and time of immersion in the table below.
6. Immerse the filled tube in the beaker of water/Lugol's solution. We will let these tubes sit during the course of the rest of the lab.
7. Write a hypothesis on what you think will occur and explain why:

8. Note and record any color changes that take place in either the starch solution inside the tube or the iodine solution in the beaker. This may take some time, so be patient. You may or may not see a change.

   What is the color solution inside the tube?

   What is the color of the solution in the beaker?

9. At the end of lab OR as specified by your TA, remove the tube from the beaker of iodine solution. Record the time.
10. Weigh the tube and record the final mass in Table 1 on the next page; then calculate any change in mass. Does mass change? If so, what does this change indicate?

Table 1. Group osmosis data.

	Mass	Time
Initial		
Final		
Change (F – I)		

11. Make notes below in Observations of any color change or other observations you may notice during the experiment.

12. Using the plastic pipet labeled "Starch," remove 1 mL of the starch solution from INSIDE the dialysis tube and add it to the yellow Lugol's/water solution in your beaker.

13. Again, note and record any color changes that take place in your beaker.

14. What can you conclude from you own results?

15. Are your results consistent with the results of the other groups in your lab section?

16. What can you conclude from this? How could we improve this experiment?

17. What do these results imply about cell membranes?

MICROSCOPE CAPABILITIES

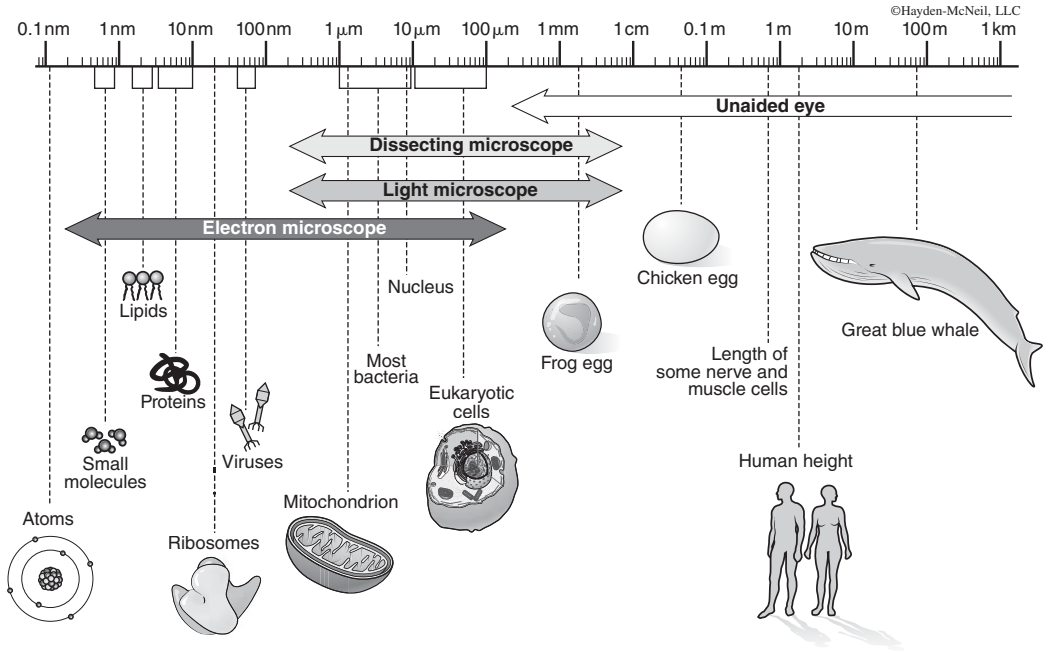


Figure 4. Resolving powers of the human eye and microscopes.

The electron microscope magnifies objects approximately 1000× larger than a light microscope can (up to 1,000,000×). This difference is due to the resolving power of the electron microscope. **Resolving power** refers to the ability of the viewer to see two objects of comparable size that are close together and still be able to recognize that they are two objects rather than one (see Figure 4). The resolving power of light microscopes depends on the wavelength of light passed through the specimen; the shorter the wavelength, the greater the resolution. This is also what limits their use—you cannot use a light microscope to visualize objects smaller than the shortest wavelength of visible light. Because electron microscopes use electrons as a source of illumination, and electrons have a much shorter wavelength than visible light, the resolving power of electron microscopes is much greater than that of light microscopes. Two types of electron microscopes are commonly used, and both use electrons to generate images. The **transmission electron microscope** (**TEM**), which produces two-dimensional images, is useful for viewing details of organelles such as the endoplasmic reticulum and the nucleus that reside within the cell, while the **scanning electron microscope** (**SEM**) provides three-dimensional viewing.

Both technologies are available through the **Center of Electron Microscopy** (www.ncsu.edu/cem/index.html), housed here at NCSU in the basement of Gardner Hall. Courses are available for students interested in learning more about these microscopy techniques, and the center also provides services to NCSU faculty

and graduate students who require electron micrographs for research purposes. Many of the images you will see in your textbook throughout the semester (indeed, many you have already seen) were taken with either a TEM or an SEM. Check out the Gallery link on the EM center website to see both SEM and TEM images/micrographs.

ACTIVITY 3: ANIMATED FILM—*THE INNER LIFE OF THE CELL*

While microscopy has been essential to the visualization of cells for hundreds of years, microscopes have always been limited in their ability to show cell activities occurring at the molecular level. New technologies have opened up other ways to visualize cellular metabolic processes. For instance, video-microscopy techniques and the use of TEM and fluorescence microscopes allow researchers to visualize movement of molecules and structures within a cell. Computer animation can actually provide a medium for showing cell metabolism in a way never before seen, allowing the viewer to visualize important details such as cell-to-cell recognition, protein synthesis, and even transport of proteins from the endoplasmic reticulum to the Golgi apparatus.

A research team at Harvard University designed the short computer animated film you will be watching today in lab. This film was produced with the aid of cell and molecular biologists, and it demonstrates the reaction of a white blood cell within a blood vessel to chemical signals sent from an injury adjacent to the vessel wall. Each process that is shown has been painstakingly determined through countless hours of research efforts from many scientists. You will watch the film twice—the first time, just sit back and enjoy it. Some of the structures should be familiar to you given what you have learned about cells' organelles and cellular processes in lecture and in your textbook. The second showing is more educational and your TA will help you understand better what you are seeing. After the second viewing, answer the discussion questions at the end of the unit.

You may want to watch this film again on your own—here is the web address:

http://multimedia.mcb.harvard.edu

Scroll down to see the clip for *The Inner Life of the Cell.*

In addition, this site has animations showing mitochondrial and ATP synthase function, and protein packaging.

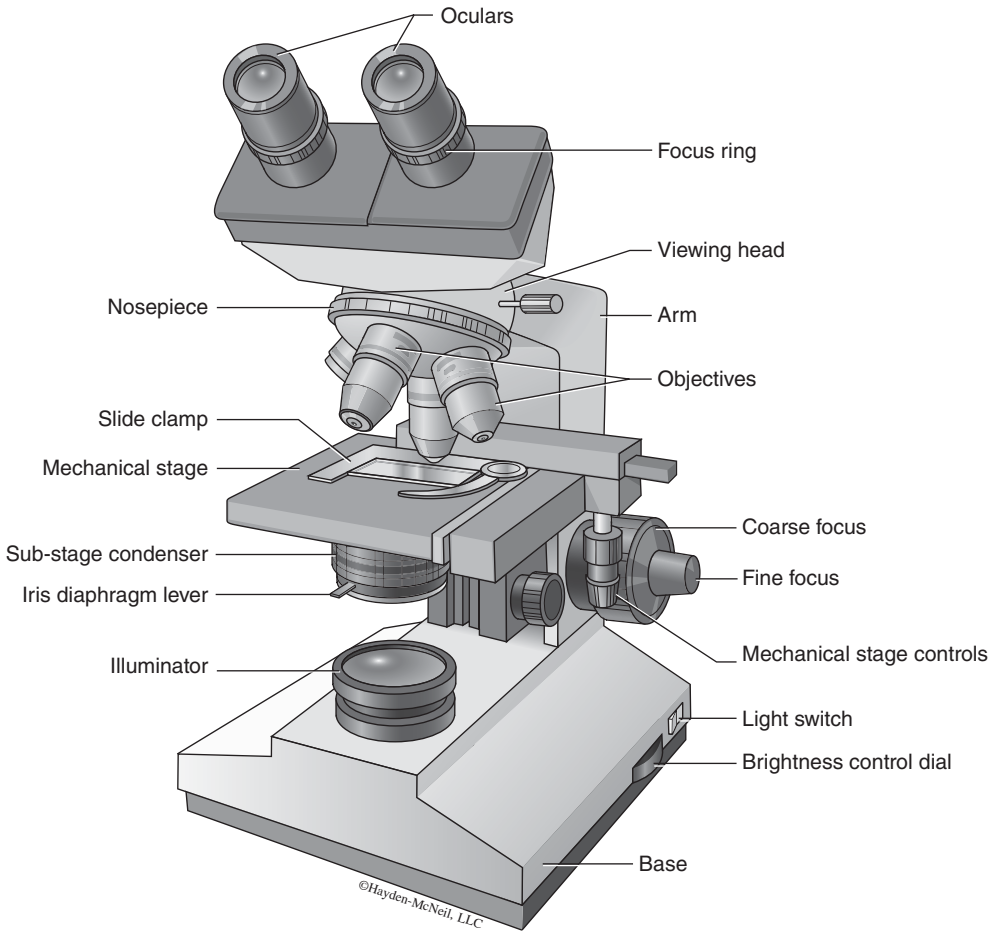


Figure 5. Generalized diagram of a compound microscope with mechanical stage adjustment knobs.

DISCUSSION QUESTIONS

MICROSCOPES AND CELLS

Name

Date *Section*

1. Why did we stain some of the slide preparations in this lab? What would these structures have looked like without the stain?

2. If you could repeat the osmosis experiment with animal cells, what might you observe? Would this be different from the plant cells? Explain why or why not.

3. From what you viewed in this animated film, list at least two roles proteins appear to play in a living cell.

4. Referring to *The Inner Life of the Cell* video, describe one example of how we saw "information" move within the cell.