BIOLOGY 1101
LAB 2: MICROSCOPES AND CELLS

READING: Please read Chapter 4 in your text book to learn about the history of microscopy and basic cell structure.

INTRODUCTION: The microscope is an important tool for many biologists, without which cell theory would never have been developed. According to cell theory, the **cell** is the fundamental biological unit, that is, it is the smallest and simplest biological structure possessing all the characteristics of life. Cell theory states that all living organisms are composed of one or more cells and all activities taking place in living organisms ultimately depend on processes occurring within cells (e.g., protein synthesis, digestion, energy conversion, reproduction, and growth). Considering the fundamental role played by cells in activities at all higher levels of biological organization, it is easy see why the study of cell structure and function is essential to the study of life. Because most cells are well below the limit of resolution of the human eye, they are studied with the use of microscopes. Biologists use several kinds of microscopes. We will focus on learning about two types of light microscopes used to study cells and cell structures.

LABORATORY OBJECTIVES: The purpose of this set of laboratory exercises is to introduce you to basic microscopy techniques and familiarize you with plant and animal cell organization. In this lab, and from your readings, you should learn:

1. The basic operation of the compound and binocular microscopes.
2. To prepare live specimens for viewing under the microscope.
3. To observe and describe features of unicellular organisms, plant and animal cells.
4. To make scientific observations about organisms and generate scientific hypotheses.

EXERCISES: We will begin by reviewing the parts and proper operation of compound microscopes and dissecting microscopes. Then we will examine several slides of live and prepared specimens to illustrate cell organization. The microscope with a raised mechanical stage and multiple lenses is the **compound microscope** and is used in Biology 1101 strictly for viewing specimens mounted on glass microscope slides. The other is the **dissecting microscope** which is used for viewing larger specimens.

Note: Make sure that you are able to answer all questions on your lab handouts that are **bold** or in *italics*. Make your drawings neat and large enough to see – you probably won't be able to review this material again before the lab exam. Carefully label all structures.

A. Operation of the compound microscope

Materials:

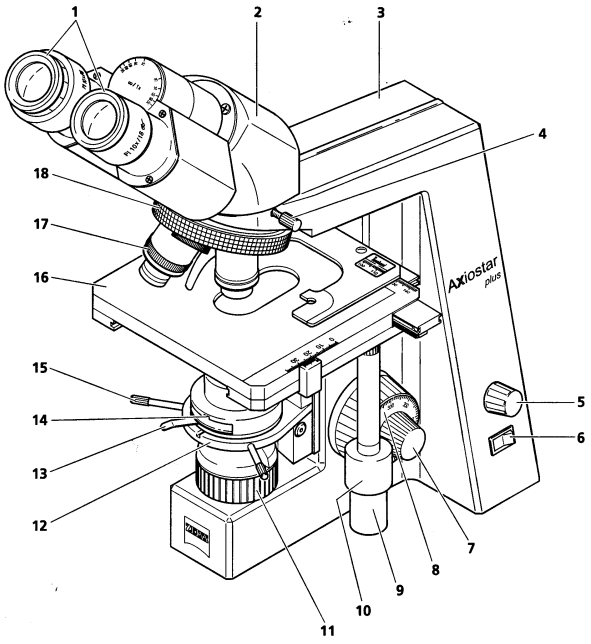
- A Zeiss Axiostar compound microscope
- A packet of lens paper
- Access to glass cleaner (ask your instructor)
- A slide of “newsprint”
- A slide of “colored threads”

Procedure: (Read the entire section before you start.)

1. You may work independently or in small groups, but everyone must be able to demonstrate proper microscope use by the end of class.

2. Precautions!
   a. Always carry the microscope in an upright position with two hands, one hand on the **arm** (3) and the other under the **base**.
   b. *Never slide the microscope across the lab bench*, vibration will damage the lenses and raise your instructor’s blood pressure! If it helps you remember, set the microscopes on the mats provided; these slide easily on the bench.
   c. Clean the eyepieces or **oculars** (1) with lens paper, a few drops of lens cleaner and *nothing else* – do not be afraid to clean them, they often need it. Do not touch the glass with your fingers or you’ll have to clean them again.
   d. Always locate and center the object to be studied using the low power objective before increasing to a higher power objective.
   e. Be careful when adjusting the focus with high power objectives in place, it is very easy to crack a slide and damage the objective.

3. Parts of the compound microscope. Numbers in parentheses refer to numbers on the diagram.
   a. The eyepieces**,** or **oculars (1),** are found at the top of the microscope. They have a magnification of 10X and are removable from the **binocular tube (2)** only with a special tool. The binocular tube can be rotated as needed by adjusting the **locking screw (4)**.
   b. The **objectives (17)** are found on the revolving **nosepiece (18)** and there are four of them. The total magnification for each objective is determined by multiplying the objective magnification by the magnification of the ocular.



1. The **low-power** (or scanning) **objective** has a magnification of 5X.
2. The **medium-power objective** has a magnification of 10X.
3. The **high-power objective** has a magnification of 40X.
4. The **oil-immersion objective** has a magnification of 100X, **and should never be used without a drop of oil on the slide**. It is unlikely that you will need the oil-immersion lens in this course, *so please don't use it*.

c. The slide to be viewed is placed securely in the specimen holder on the **mechanical stage (16)**, and the **knobs (9, 10)** are turned to move the stage front to back and from side to side under the objective.
d. The **aperture diaphragm (13)** and **condenser (12)** are found under the stage. These mechanisms regulate the amount of light that reaches the object being viewed. The condenser can be raised and lowered to focus the light beam from the bulb directly on the specimen.
e. The **light source** is housed in the base of the microscope. It is operated by a **switch (6)** on the side of the microscope and can be adjusted with the **brightness control (5)** or the **luminous field diaphragm (11)**.
f. The **focus** is adjusted by two knobs on the side of the microscope. They work by raising and lowering the stage relative to the objective. The large knob is the **coarse focusing drive (8)** and the smaller knob is the **fine focusing drive (7)**.

4. Using the compound microscope
   a. Obtain a slide of "newsprint" (or similar). Swing the 5X objective into position by turning the nosepiece until it clicks. *Notes: 1) Never turn the objectives using a lens, always use the revolving nosepiece; 2) Slides should be placed on and removed from the stage only when the 5X objective is in place.* Removing a slide when the higher objectives are in position may damage the lenses and/or slide. Lower the stage by turning the course focusing drive.
   b. Open the spring-loaded finger of the specimen holder and insert the slide. Be sure that the cover slip is on top. Release the spring-loaded finger and it should hold the slide in place. **The slide should *NEVER* be jammed under the spring-loaded finger; if you do this, the slide will chip or break.** If you are not clear how this part works, ask your instructor. The sample should be over the hole in the stage, in the path of the light. Raise the stage as far as it will go using the coarse focusing drive while you watch the stage.
   c. Make sure the microscope is plugged in. Switch on the microscope light, (take off your glasses), and look through the oculars. Do not bring your eyes closer than about 2.5cm (1in) from the lens of the oculars. You should see only one round image when you look through both oculars. If this is not the case, you need to adjust the **interpupillary distance** (distance between your pupils). This is different for almost every person, so you will often have to make this adjustment. To adjust this, hold the oculars on either side of the binocular tube and slowly swing them closer or farther apart until you see only one image through the lenses. Note the setting on the scale between the oculars and you can always set it to that number when you are going to use the microscope. *What is your interpupillary setting?* _____

d. Lower the stage slowly by rotating the course focusing drive until the slide is focused and then use the fine focusing drive to sharpen the image. Close your left eye and focus your right eye on the image and not the lens. When the image is in focus for your right eye, close that eye and open your left eye. The image will probably be slightly out of focus. On the left ocular, note that there is a scale – the ocular can be rotated to adjust the image into focus. Carefully rotate the ocular until the image is in focus for your left eye. Now open both eyes and make any minor adjustments to the focus. This is another setting that varies for each person (since it corrects your vision without glasses) and will likely have to be adjusted each time you use the microscope. As you lower the stage you are focusing closer to the coverslip, or moving up through the mounted specimen.
e. Use the aperture diaphragm to adjust the intensity of light until the letter is clearly illuminated. The opening of the aperture diaphragm built into the condenser should be adjusted to obtain optimum depth of focus, image contrast and resolution for each objective. Adjust the diameter of the opening and see how the image changes. The diameter should be such so that there is no diffraction, but the contrast and depth of field is still sufficient for the particular objective and/or specimen. The aperture diaphragm should never be used to control the brightness of the light, only the intensity. The brightness control knob sets the brightness.
f. The function of the condenser is to focus light in the plane of the object being viewed. Rotate the condenser adjustment knob until the condenser is in the lowest position. While looking through the microscope, slowly raise the condenser and see how the quality of the image improves. The condenser height is correctly set when the text on the slide is sharply focused.
g. The microscope should now give a clear and sharply focused image. Small adjustments to the light intensity, condenser and aperture diaphragm must be made for the different specimens and objectives.
h. **STOP! Make sure you can answer comprehension question 1 at the end of the lab before you proceed.**
i. Swing the 10X objective into position. The microscope should still be relatively focused, but a small adjustment with the fine focus knob may be necessary. Note that the distance between the objective and the slide is now less because the 10X lens is much longer than the 5X lens. This distance is known as the **working distance**. You should take note that the thickness of a slide is less than the working distance for the 40X and 100X lenses (0.53 mm and 0.17 mm respectively, compared to the 1 mm thickness of the slide). Consequently, it is not possible to focus a microscope if the slide is upside down. Due to the small working distance of the 40X and 100X lenses, you should only use the fine focusing drive when working with high magnifications. This will prevent damage to both the slide and the objective. Be careful not to damage any slide by squashing it between the stage and the objective. If you break a slide, please tell your instructor immediately. You are not responsible for paying for it (that's what tuition is for); we just need to know so we can replace it for next semester.

j. **STOP! Make sure you can answer comprehension question 2 at the end of the lab before you proceed.**
k. We will probably not use the 100X objective during this course. It should however, be kept in mind that this is an oil immersion lens and it cannot focus properly without a drop of oil between the slide and the objective. If it becomes necessary to use this objective your instructor will help you.
l. You should check the settings of the microscope every time you use it. Not only will this ensure that you see everything you are supposed to, but it will also prevent eyestrain and headaches. Never assume the person(s) who used the microscope before you knew what they were doing.
m. Now for a little competition to see who really knows how to use a microscope. Obtain a slide of "colored threads". Each person in the group should independently figure out which objective is most appropriate to use, and should determine which thread is on top, in the middle, and on the bottom. Keep your answer to yourself. When everyone in the group thinks they have it, call your instructor over to verify the correct answer. Bear in mind that the order may be different on different slides! If you have the correct answer, congratulations, you can move on to part B! If you were wrong, try again and see if you can figure out where you went wrong.

B. Operation of the binocular dissecting microscope

Materials:

- A binocular dissecting microscope
- A slide of "newsprint" and/or "colored strings"

Procedure: (Read the entire section before you start.)

1. You may work independently or in small groups, but everyone must be able to demonstrate proper microscope use by the end of class.

2. Precautions!
   a. As with the compound microscope, always carry the dissecting microscope in an upright position with one hand on the arm and the other under the base.
   b. Never slide the microscope across the lab bench, the vibration will damage the lenses!
   c. Clean the lenses with clean lens paper and nothing else – do not be afraid to clean them, they often need it. Do not touch the lenses with your fingers.

3. Parts of the dissecting microscope.
   a. Two **oculars** are found at the top of the microscope. They may have a magnification of 1X, 5X, or 10X depending on the model you have (it should be printed on the ocular).
   b. Instead of objective lenses, this microscope has a **zoom magnification feature** that usually varies from 7X to 30X (or more). The total magnification

is determined by multiplying the zoom magnification setting by the magnification of the ocular. Because the zoom magnification lacks a "click stop," or fixed setting, any calculation of total magnification is approximate.

c. The object to be viewed is placed on the stage.
d. The light source is in the stage, and there is a switch to direct light up from below (transmitted light), down from above (reflected light), or from both angles depending on the specimen being viewed.
e. There is a single knob on the side of the microscope used to adjust the focus.

4. Using the dissecting microscope – remember that these microscopes vary so you may have to omit steps that refer to features not available on your microscope.
   a. Using the slide of "newsprint," the colored strings, and/or the tip of your pen or pencil, practice using the dissecting microscope. There may even be some odd items around the lab to view. Set the zoom magnification to its lowest setting. Place the object to be viewed on the stage and use both eyes to look through the oculars. Use the single focus knob to adjust the image. Adjust the interpupillary distance by moving the oculars until you see only one image (light spot). If you have problems obtaining binocular vision, it may help to experiment with the distance of your eyes to the oculars. If your microscope has an interpupillary distance scale, note the reading for future use: _____.
   b. While looking through the objectives, slowly increase the magnification. Some adjustments in focus may be necessary as the magnification increases. Note the larger field of view and working distance and the smaller total magnification, compared with the compound microscope.
   c. **STOP! Make sure you can answer comprehension question 3 at the end of the lab before you proceed.**

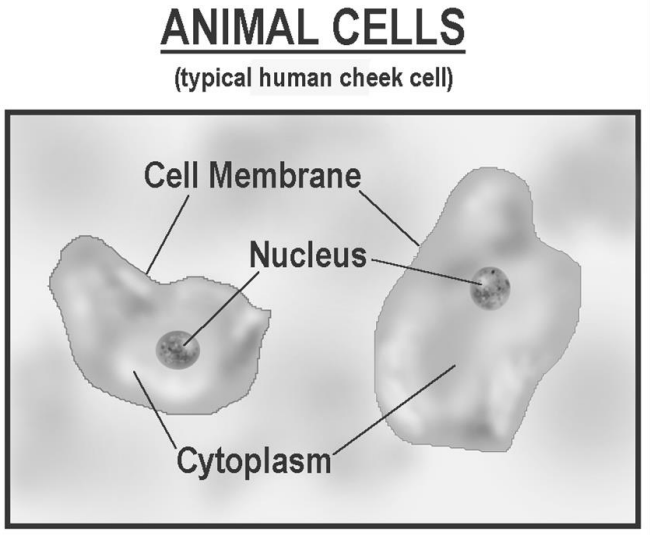
C. Observing cells and cell structures with the compound microscope.

Materials:

- A clean toothpick for one volunteer from your group
- 2-3 clean, regular slides and coverslips and a clean depression slide and coverslip
- A dropper bottle containing methylene blue stain (you may have to share)
- An *Elodea* leaf (obtain it only when you're ready to use it)
- A compound microscope and a binocular microscope
- A sample of pond water [*Note: If weather permits, each group will collect two pond water samples from the campus ponds, so you will need two water sample containers. If not, pond water cultures grown in the lab will be available to observe.*]
- Laptop computer (One group member should log onto the laptop and navigate to a web browser.)

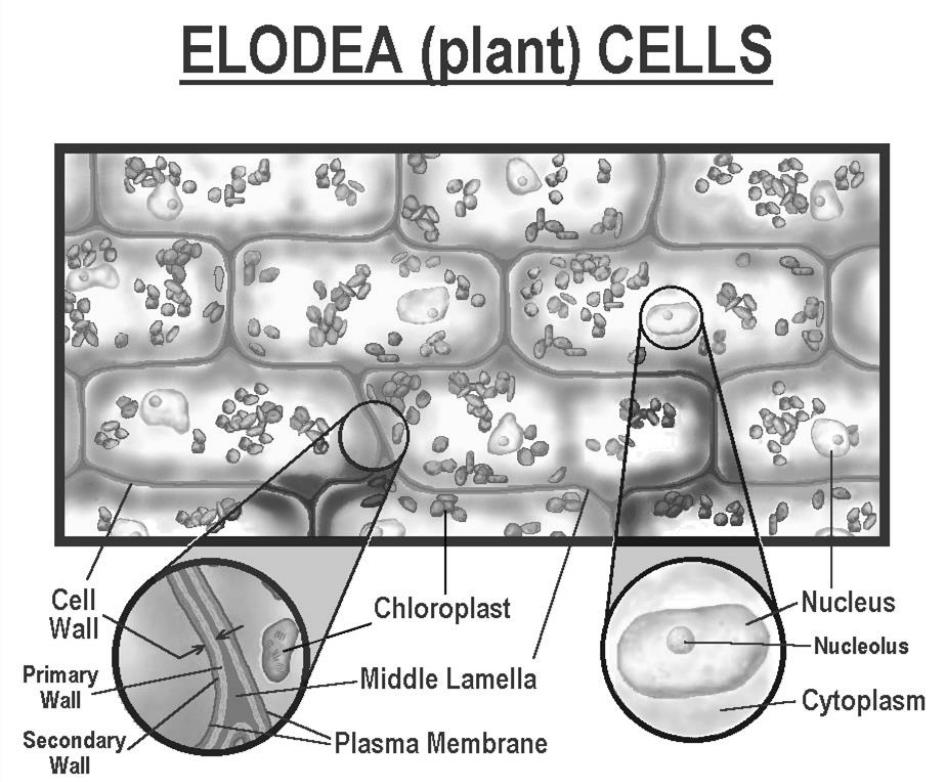
Procedure: (Read the entire section before you start.)

1. Precautions!
   a. Be careful obtaining a cell sample from your cheek – no new body piercing please…
2. Animal cells. Animals are composed of cells that can be categorized into four major tissue groups: **epithelial, connective, muscle, and nervous tissue**. Today we will examine **epithelial cells**. These occur on the outside surface of animals and function to protect the animal from water loss, mechanical injury, and foreign invaders. In addition, epithelial cells line interior cavities and ducts in animals. Examine the epithelial cells that line your inner cheek using the compound microscope.
   a. To obtain a specimen:
      1. With a *clean* toothpick, **gently** scrape the inside of your cheek several times.
      2. Roll the scraping onto a clean microscope slide, apply a drop of methylene blue dye, and cover with a coverslip.
      3. Place a drop of water on one edge of the cover slip and gently touch a piece of paper towel to the other edge, drawing the excess stain away.
   b. Observe the specimen under low, medium and high power with the compound microscope. These cells are extremely flat and may be folded over on themselves. Find several cells that are not folded to study their detail. Check with your instructor if you are having any problems.
   c. *Make sure you can identify the following structures:*
      1. **Cell Membrane** – separates the cell from its surroundings.
      2. **Nucleus** - contains the DNA, the control center of the cell, stains dark blue.
      3. **Cytoplasm** – contains all the material inside the plasma membrane except the contents of the nucleus.



3. Plant cells. *Elodea* is an example of a photosynthetically active aquatic plant.
   a. Obtain a small leaf of *Elodea* from the indicated dishes.

b. Place the leaf on a slide and observe it using the binocular microscope. What can you see?
c. Prepare a wet mount of *Elodea* epidermis. Tear the leaf leaving a thin jagged edge. Place a small piece of the edge part on a slide, add a drop of water and cover with a cover slip. The thicker the leaf piece, the more light you will need to see the cells, therefore, try to get the thinnest piece possible. Your instructor can help you. Examine the tissue under low, medium, and high power using the compound microscope.
d. *Make sure you can identify the following structures:*
    1. **Cell wall** – the rigid outer framework surrounding the cell.
    2. **Cytoplasm** – contains all the material inside the plasma membrane except the contents of the nucleus.
    3. **Nucleus** – contains the DNA, the control center of the cell.
    4. **Vacuoles** – membrane bound sac within the cytoplasm filled with water and stored substances.
    5. **Chloroplasts** – green, spherical organelles often seen moving in the cytoplasm. These organelles carry the pigment chlorophyll that is involved in photosynthesis. As the microscope light heats up the cells, the chloroplasts may begin to move quite rapidly in a process called **cytoplasmic streaming**, or **cyclosis**.



4. Observing live protists. Examine a sample of pond water for protozoans of different sorts (single celled eukaryotic organisms e.g., amoebas, ciliates, flagellates, zooflagellates) that differ in their feeding behavior and mechanism of mobility. While you're at it, look for algae and small plant cells – you may be able to see the cells in action much like the *Elodea* leaves. Try using the dissecting microscope first – how well can you view organisms? Now use your compound scopes for this exercise – is it easier or harder to view the specimens?
   a. If weather permits, go with your lab instructor to the campus ponds and collect two samples of water, in separate containers. For best results, look for algae growing in the water and/or gently scoop up a little mud with your water sample. Be careful not to fall in, and remember, no swimming! If you are unable to collect samples from the ponds, you may observe pond water cultures grown in the lab.
   b. On a depression slide place a drop of pond water. Place a coverslip over the depression slide and examine it under low power. Once you find a few organisms increase the power to see the finer detail. The organisms may swim fast, so you will have to move the stage to follow them.
   c. On your laptop computer, visit the following web site to help identify the organisms you observe: http://www.microscopy-uk.org.uk/pond/index.html
   d. Repeat steps "b" and "c" for your second water sample, observing and identifying a variety of protists and invertebrate organisms.
   e. On the back of the comprehension question page, make drawings of at least two different organisms. Include as much detail as possible.
   f. **After observing your organisms, answer comprehension question 4 for each organism. Write answers neatly next to the drawings.**

Comprehension Questions:

Answer the following questions as instructed in the text above. These questions, or similar questions, will appear later on a lab practical, so make sure you understand them.

1) Is the letter upright or inverted when viewed through the oculars? _____ Move the slide slowly to the right. In what direction does the image in the ocular move? _____ Move the slide slowly away from you. In what direction does the image in the ocular move? _____

2) Compute the **total magnification** of the letters being viewed. To do this, multiply the magnification of the ocular lens by that of the objective lens. What magnification do you get with the 5X objective in position? _____ What if you slide the 10X objective into position? _____ The 40X? _____

3) How are the images viewed with the binocular dissecting microscope different from those obtained with the compound microscope? Is the object upright or inverted when viewed through the oculars? _____ Move the object slowly to the right. In what direction does the image in the oculars move? _____ Move the object slowly away from you. In what direction does the image in the oculars move? _____

4) How does it move? What part of the organism is involved in ingesting food? Does this organism make its own food through photosynthesis or does it ingest food? Why do you think so?

Drawings:

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