

Name _____

Date _____

Microscopy - Cells

Prior to lab you should:

- Know the difference between eukaryotic and prokaryotic cells
- Know the major organelles in eukaryotic cells and their basic functions
- Know the basic units of metric linear measurement
- Know the basic parts of a compound microscope
- Know the difference between resolution and magnification

I. Objectives

- Learn to efficiently use the compound light microscope
- Learn to make whole mounts of cells from plant and animal tissues.
- Observe the structures of *Elodea*, *Alium* and human cells.
- Stain cells to improve the visibility of specific organelles
- Calibrate a microscope and determine the size of cells
- Observe cytoplasmic streaming in *Elodea* cells and estimate the rate of chloroplast movement.

II. Background

The light microscope relies on the simple principle of magnification; an object is magnified in size so that it becomes visible to the observer. There is a limit to the useful magnification that can be achieved in a light microscope because of the limitations in the resolving power of the lenses, that is, a limit in the ability to resolve two separate objects as distinct structures. Therefore objects beyond the resolving power of the microscope will not be seen as separate regardless of the degree of magnification.

To allow cells and the structures within cells to be seen in the light microscope, cell biologists developed many dyes or stains that either stain whole cells or preferentially stain various organelles within the cell. For example, methylene blue stains the nucleus of a cell blue. Many stains are applied after the cell is fixed. Various chemicals, including glutaraldehyde or alcohol, are used to cross-link cellular protein and to stabilize cells more or less permanently. However, note that fixation effectively kills cells and can distort cells in some cases.

Several techniques developed over the past three or four decades have enhanced the effectiveness of the light microscope; these include phase contrast microscopy and Nomarski differential interference contrast microscopy. These techniques allow the cell biologist to examine structures in the living cell without fixing the cell or introducing any staining agents. The advantages of contrasting enhancing microscopy will become apparent during examination of cells in this laboratory. More recently, fluorescence microscopy has become an important research tool. Other techniques including, electron microscopy, continue to provide cell biologists with data on cellular structure and function.

III. Materials

Available cells:

Onion skin epidermal cells, cells of the aquatic plant *Elodea*, algal cells, human epithelial cells taken from the lining of the cheek (squamous epithelial cells).

Stains:

Lugols's Iodine, Methylene Blue

Equipment and supplies:

Compound Microscopes, Clear plastic mm rulers, Slides, Cover slips, Lens paper, sterile toothpicks.

IV. Procedures

See appendix on last page for detailed instructions on the use of the microscope

Elodea

1. **Elodea** is the genus name common group of water plants. They have very thin leaves and are used to show the typical structures of a plant cell.
2. Place a portion of a living **Elodea** leaf on a slide in a small drop of water and place a cover slip on top of it. The leaf has several layers and you will have to focus on one layer using your fine adjustment.
3. When you have focused on high power call your instructor.
4. After receiving the OK, diagram your slide on the Lab report sheet.
5. Identify the following cell organelles: cell wall, cell membrane boundary, nucleus, sap vacuoles, chloroplasts, nucleus, and cytoplasm.
6. The heat of the light will cause your slide to dry out quickly. This will cause your cells to die. Work quickly and if necessary add a drop of water to prevent the cell from drying up.

Allium (Onion) Cells

1. The bulb of the onion provides a rich source of plant cells.
2. With a razor blade, carefully peel a thin transparent piece of onion skin from one of the slices provided. Follow your instructor's example. **Caution!** Do not cut yourself.
3. Focus on high power and call your instructor.
4. Next add a drop of iodine stain on one side of the cover slip. Place a piece of lens paper on the opposite side of the cover slip. The paper will "wick" the stain across the specimen causing the nucleus to darken.
5. When the onion cells have been stained, call your instructor again.

- Diagram and label the following cell organelles; cell wall, cell membrane (boundary) cytoplasm, nucleus, nuclear membrane (boundary).

Human Epithelial (Cheek) Cells

The inside of your cheek is covered with epithelium skin cells. In this procedure you will prepare a slide and stain the nucleus of the cell by adding methylene blue.

- Carefully scrape the inside of your cheek with the blunt end of a toothpick.
- Smear the material on the center of a clean slide.
- Stain the cells by adding a drop of methylene blue.
- Cover with a cover slip.
- Focus on high power and call your instructor.
- Diagram and label the cell membrane (boundary), cytoplasm, nucleus and nuclear membrane (boundary).

Calibrating your microscope

Place a clear ruler on the stage and determine the diameter of the field of view while using the scanning objective (the shortest one). Adjust to medium power and again find the diameter of the field of view at that power. Adjust to high power and find the diameter of its field of view. Now determine the total magnification of each objective by multiplying the power of the objective (the even number printed on the objective) by the ocular (the number that has a "x" behind it printed on the eye-piece).

Fill in the table below:

objective	Eyepiece	Magnification	Field dia. (mm)	Field dia. in μm^*
	10x			
	10x			
	10x			
	10x			

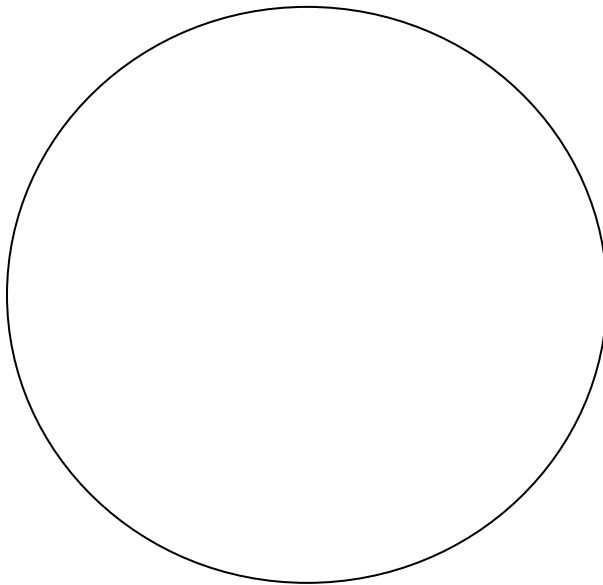
1 mm = 100 μm

Determining the rate of cytoplasmic streaming

1. Make a new mount of *Elodea*. Estimate the length of a cell using the diameter of a field as a guide. Hint: Use the highest power objective that fits the length of the cell. Calculate the length of the cell as fraction of field dia. X dia, in μm .
2. Find an *Elodea* cell that is exhibiting **cyclosis** (cytoplasmic streaming). During this process, chloroplasts (small green disk-shaped organelles) move in a circular fashion around the cell. Determine how long it takes for 1 chloroplast to move from one end of the cell to the other and record your data.
3. Calculate the rate of movement of the chloroplast by using the length of the cell in μm and the time required for the chloroplast to travel the length of the cell.

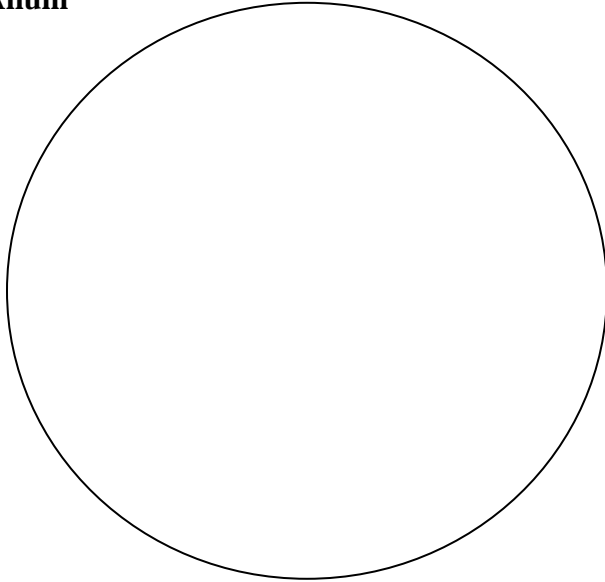
Drawings: Draw cell to scale. Put the labels to the right. Indicate the magnification and field diameter

Elodea



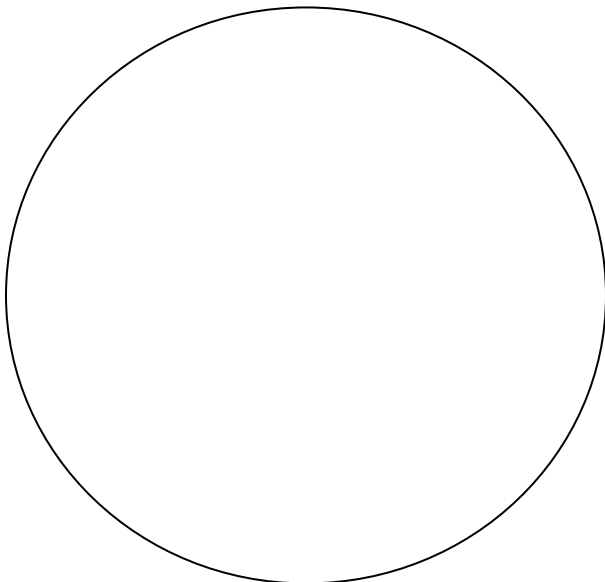
Magnification _____
Field Dia. _____

Alium



Magnification _____
Field Dia. _____

Cheek Epithelium

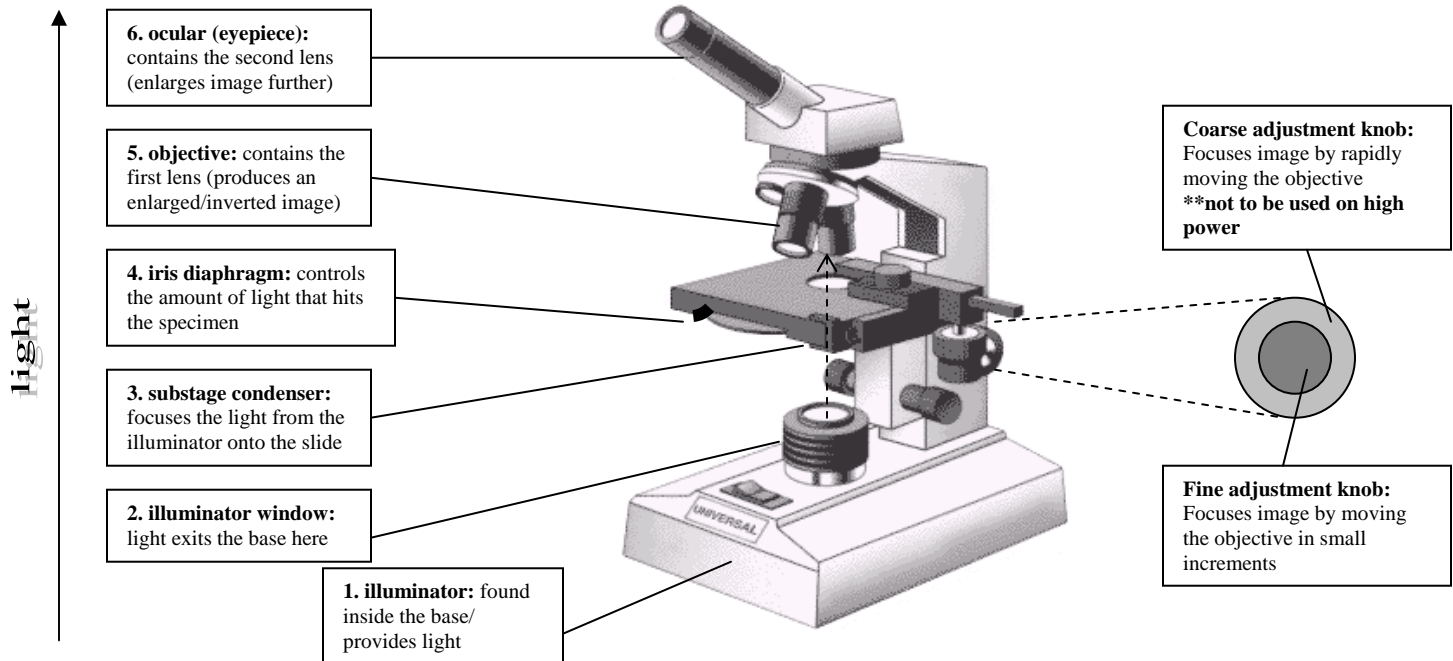


Magnification _____
Field Dia. _____

Appendix- Microscope technique:

The Compound Microscope

Figure 1 shows the parts of a modern bright field microscope:



1. Start with low power

- Place the slide (specimen side up) on the stage so that the specimen lines up with the middle of the opening in the stage

2. Turn the coarse adjustment knob slowly (while looking through the scope) to find the specimen

- You may have to move the slide around if this does not work

3. After finding and focusing on the specimen, clarify the image by using the fine adjustment knob

4. Center the specimen in your field of view while looking through the microscope

5. Once the specimen is focused and centered, move to the next power by turning to the next highest objective lens.

6. After switching power, if you see the specimen then focus the image using the fine adjustment

- If you don't see the image, move back down to low power and start over