

Exercise 3 - Biology 105

Estimating the Size of Cells Using a Compound Light Microscope

Objectives of this lab are to:

1. Learn how to use the compound light microscope.
2. Learn how to make a preparation for viewing on a slide.
4. Make scale drawings.
5. Estimate the lengths of cells seen with the microscope.
6. Draw a bar graph comparing the lengths of the various cells you measure

One principle of biology is that all organisms are cellular. That is, they are made up of one or more cells. Cells live in a wide variety of habitats and perform a wide variety of functions. This is reflected by a multitude of cell shapes and sizes. Today you will observe several different kinds of cells, draw them, and estimate their lengths.

The scopes you will use are compound light microscopes. Since they have two oculars they are called binocular microscopes.

I. Determine the Total Magnification with each objective lens

Each lens (both objectives and oculars) has a particular magnifying power. The ocular lens has a number followed by an X. Each objective lens has a number of a particular color. These numbers are the magnifying power for that lens. Total magnification of the microscope is found by multiplying the power of the ocular lens by the power of the objective lens. Use Table 1 to record your results as you calculate the total magnification obtained with each objective lens.

Table 1: Parameters of a compound light microscope used in Principles of Biology

Power of Ocular Lens	Power of Objective Lens	Total Magnification	Diameter of Field of View (mm)

II. Determine the diameter of the **field of view** for each objective. Use Table 1 to record your results.

The **field of view** is the circular area that one sees as one looks through the microscope. To determine the diameter of the field of view, place a plastic ruler under the low power (4 x) objective. Focus on the ruler and lay it so it crosses the diameter of the field of view. Record the diameter of the field of view in millimeters in Table 1.

Record the diameter of the field of view for the 10x objective lens.

Based on these first two measurements, as the total magnification increases, what happens to the diameter of the field of view? (Does it increase or decrease?)

Try to place the ruler under the 40x objective. It will be difficult to estimate the diameter of the field of view visually at this magnification, because it is hard to see the ruler in focus at this magnification. Make your estimate by doing the following calculations:

1) Divide the total magnification when the 40x objective is in place by the total magnification when the 10x objective is in place: _____.

2) The diameter of the field of view at 10x divided by the quotient obtained in calculation (1) above is _____. This is the diameter of the field under the 40x objective lens.

III. Estimating the Sizes of Cells

A. You will now observe various types of cells. You may use prepared slides, or you may make your own slides. To prepare a wet mount: Obtain a clean glass slide. Place the object you wish to observe on the slide. You may use forceps or a toothpick to manipulate small objects into the proper orientation. Use a pipette to add a drop of water (in some cases, you may add a drop of stain instead of water). Now put one edge of a cover glass into the edge of the drop of water and carefully lower the cover glass into the drop of water as shown in Figure 1. By carefully lowering the cover glass into the drop of water you should be able to prevent any air bubbles from being formed.

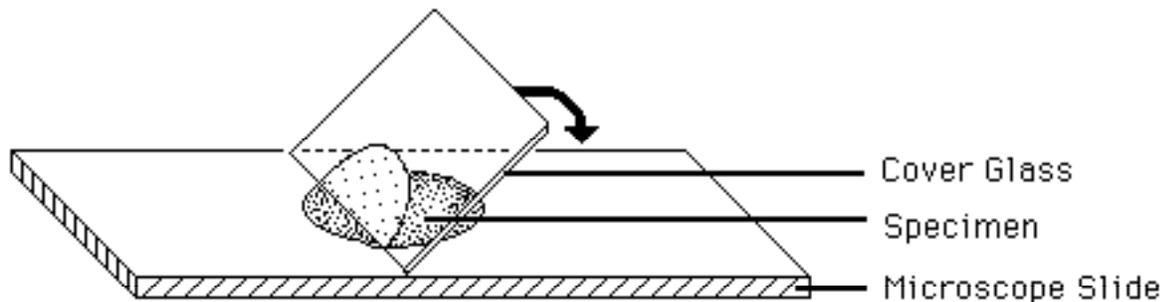


Figure 1: Preparation of a wet mount on a microscope slide

B. Instructions for the particular types of cells you will observe:

1. Human cheek cells -- the inside of your cheek is covered with epithelial cells. These cells are constantly worn away and replaced by new cells. Live cells are easily dislodged from the surface.

Have a slide and cover slip at hand. Gently scrape the inside of your cheek with a toothpick. Rub the scrapings onto the center of a microscope slide. These cells need to be stained with methylene blue to make them easily visible. Try to use about half a drop of methylene blue; just hold the squeeze-bottle over the slide and let a small drop dribble onto the cells. It is not necessary to add water to the slide. Place the cover glass as shown above.

2. *Elodea* cells -- You will find stems of this aquatic plant floating in a glass bowl. Have a slide and cover slip at hand. Tear a portion of a leaf (about half a leaf is fine) from this aquatic plant and place it on the slide. Cover it with a drop of water and a cover slip. The green discs you will see are organelles called chloroplasts; many chloroplasts are inside each individual cell.

3. Bacterial cells -- One of the following bacteria may be available:
 - a. prepared slides of *Sarcinia* or *Bacillus*,
 - b. *Anabaena*, a blue-green algae, a species of photosynthetic bacteria. You will have to make a wet mount of these live bacterial cells, if they are available

C. For each slide that you observe follow steps 1 through 4: Using a pencil,

1. Draw a circle to represent the field of view. (Use a compass or a petri dish.)
2. Carefully and accurately make a **scale drawing** of 2 or 3 representative cells.
3. Beside each drawing neatly record the following information:
 - a. total magnification
 - b. diameter of field of view
 - c. estimated number of cells that will stretch across the diameter of the field of view,
 - d. estimated length of an individual cell*

*To figure the length of one cell, divide the number of cells that cross the diameter of the field of view into the diameter of the field of view.

$$\frac{\text{Diameter of field of view}}{\text{Estimated number of cells that cross the diameter}} = \text{length of one cell}$$

For example, if the diameter of the field is 5 mm and you estimate that 50 cells laid end to end would cross the diameter, then $5 \text{ mm} / 50 \text{ cells} = 0.1 \text{ mm/cell}$. So each cell would be 0.1 mm long.

A note about units of measurement: Items this small are often measured in microns (millionths of a meter) rather than millimeters (thousandths of a meter).

$$1 \text{ micron} = 1/1000 \text{ mm} \quad \text{OR} \quad 1 \text{ micron} = 0.001 \text{ mm}$$

$$1 \text{ mm} = 1000 \text{ microns}$$

To convert from millimeters to microns, just multiply the measurement in millimeters by 1000.

For example, a cell that is 0.1 mm long is also 100 microns long. A cell that is 0.05 mm long is 50 microns long.

By using microns at this scale, one is often able to use whole numbers rather than decimals. When making your graph or solving the problem below, using microns as the unit of measurement will make your task easier.

IV. Construct a bar graph comparing the **lengths** of each cell type you have seen today.

V. **Problems:** Assume an *Elodea* cell is a rectangular prism whose volume is $V = W \times H \times L$. Assume that an *Anabaena* cell is a sphere whose volume is $V = 4/3 r^3$. How many *Anabaena* cells could fit into an *Elodea* cell?

Precautions for Use of the Microscope

- 1. Always carry the microscope in an upright position with both hands, one supporting the base and the other grasping the arm.
- 2. Never clean the ocular and objective lenses with anything but the lens paper supplied.
- 3. Notify your instructor if your microscope is out of order. Do not attempt to repair it yourself.
- 4. Use only the knurled ring to change objective lenses, NOT the lenses themselves.
- 5. Always have the LOW power lens in place before inserting a slide. Always return to LOW before removing a slide.
- 6. Always focus and center the object and adjust the light before turning to the next higher power lens.
- 7. Use the coarse adjustment only when the LOW power objective is in place.
- 8. Clean your microscope slides with lens paper before examining.
- 9. When you are finished using the microscope:
 - a. Make sure that the stage manipulator bar is not extended out to the left.
 - b. Unplug the microscope and coil the cord beside the microscope.
 - c. Put the dust cover back on the microscope.
 - d. Carefully slide the microscope back into place