Lab 2. Microscopic Observation of Cells

Prelab Assignment

1. *Before* coming to lab, read carefully the introduction and procedures of each part of the experiment. If you and your group members are not familiar with the procedure before coming to lab, you may have difficulty completing this exercise during the lab period.
2. Answer the Prelab Questions on the first three pages of the report sheet and be prepared to hand them in at the start of your lab class. **Please be aware that you need to go online to answer prelab question #3**.

Goals of this Lab Exercise

After completing this lab exercise you should be able to.....

1. Identify the parts of a compound light microscope and use a microscope to competently examine biological samples
2. Determine the diameter of the field of view for the various objectives of a microscope
3. Accurately sketch, describe and cite the major functions of the structures and organelles of the cells examined in this lab exercise
4. Estimate the size of specimens viewed with a microscope.

The Microscope

The microscope is one of the principal tools of the biologist. Without the microscope, many of the great discoveries of biology would never have been made. The light compound microscope, illustrated in Figure 1, is the type of microscope most commonly used. Proper, comfortable use of the instrument demands practice. The practice afforded you in this exercise depends upon familiarity with the parts of the microscope and with their interactions.

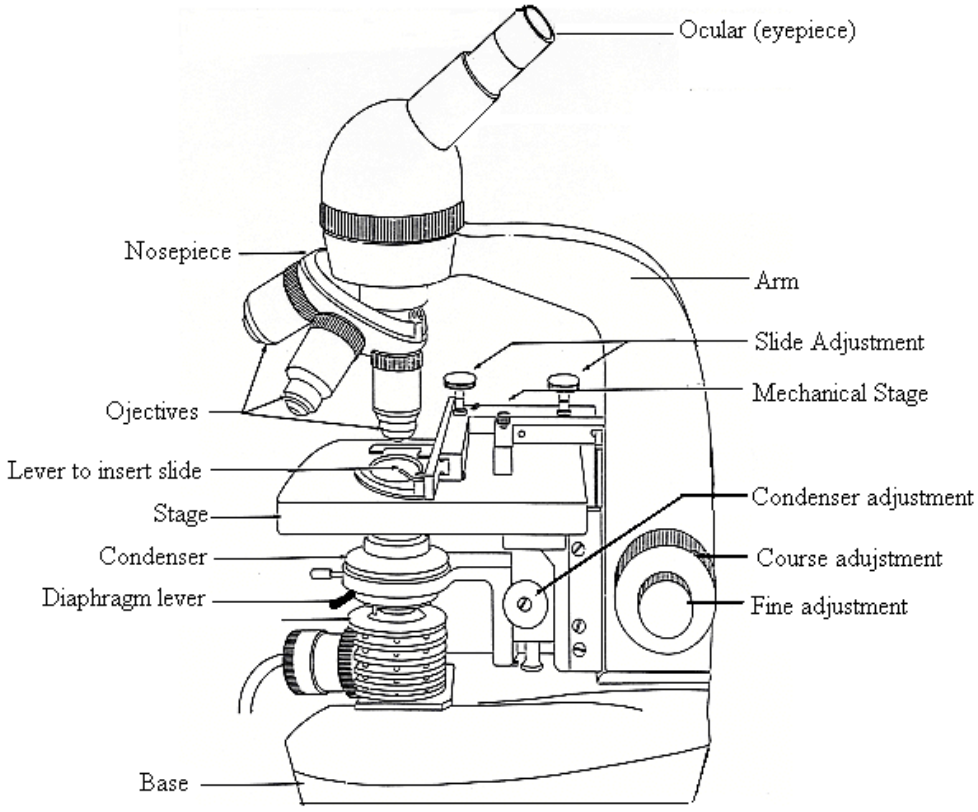


Figure 1. A typical compound light microscope used in many biology labs.

Note each of the following features of the microscope and there individual uses to allow you to take full advantage of the use of a microscope.

- ***Ocular or Eyepiece***—contains a lens that magnifies the specimen (usually 10x)
- ***Revolving Nosepiece***—used to change magnifying lenses (objectives)
- ***Objectives***—magnifying lenses usually 4x, 10x, 100x and 100x (oil immersion lens).
- ***Condenser***—adjustable device gathers the light rays from the light source and focuses them onto the specimen. The condenser should be raised to be as close to the stage as possible.
- ***Diaphragm***—this lever controls the amount of light shown onto the specimen
- ***Coarse and Fine Focus Knobs***—used to focus the specimen. The course focus knob is only used with the 4x (low power) objective.

Magnification and Resolution

In using the microscope it is important to know how much you are magnifying an object. To compute the ***total magnification*** of any specimen being viewed multiply the power of the eyepiece (ocular lens) by the power of the objective lens being used. For example, if the eyepiece magnifies 10x and the objective lens magnifies 40x, then 10 x 40 gives a total magnification of 400x.

The compound microscope has certain limitations. Although the level of magnification is almost limitless, the resolution (or resolving power) is not. ***Resolution*** is the ability to discriminate two objects close together as being separate. The human eye can resolve objects about 100 µm apart (note: 1 µm = 1 micrometer = 1 millionth of a meter). Under ideal conditions the compound microscope has a resolution of 0.2 µm, about 500 times the resolving power of the human eye. Objects closer than 0.2 µm are seen as a single fused image.

Resolving power is determined by the amount and physical properties of the visible light that enters the microscope. In general, the greater the amount of light delivered to the objective lens, the greater the resolution. The size of the objective lens aperture (opening) decreases with increasing magnification, allowing less light to enter the objective lens. *Thus, it is often necessary to increase the light intensity at the higher magnifications.*

Depth Perception and the Microscope

Any microscopic object viewed has depth as well as length and width. While the lens of your eye fully adjusts to focus on an object being viewed and provides you with a three dimensional interpretation, the lenses of a microscope are focused mechanically and can only "see" in two dimensions, length and width. For example, if the specimen you are examining has three layers of cells, you will only be able to focus on one cell layer at a time. *In order to perceive the relative depth of your specimen use the fine adjustment to focus through the different planes* (i.e. the three cell layers) individually to build a three-dimensional picture or interpretation of your specimen.

The Field of View and Estimating the Size of Specimens

When you view an object under the microscope you will observe that it lies inside a circular field of view. Each different magnification has a different sized field of view. If you determine the diameter of the field of view you can estimate the size of an object seen in that field. As you increase the magnification, the field of view (and diameter) gets proportionately smaller. As a consequence, a critter that appears small under scanning power may appear large under high power. The actual size of the critter did not change, only the space in which you placed it for viewing.

Refer to part B of the procedure on page 7 for a discussion on how to determine the diameter of the field of view and how to estimate the size of a specimen viewed with a microscope.

The Oil Immersion Lens

Although the oil immersion lens (100x) when used properly offers the ability to view objects at high magnification (1000x), the objects viewed in this lab exercise do not warrant its use. As its name implies, an oil immersion lens requires a drop of immersion oil to be in contact between the lens and the slide for the lens to function effectively. Since immersion oil has the same refractive index as glass, it prevents the scattering of light as light passes from the glass slide to the objective lens (also made of glass). ***Poor resolution is the result if the immersion lens is used without oil*** since light will be bent (and thus scattered) as it passes from the slide to air, and then through the objective because air and glass bend light differently as a result of having different refractive indexes.

Care of the Microscope

Your microscope is an expensive instrument that must be given proper care. Always follow these general instructions when using a microscope.

1. Carry the microscope with both hands, one hand under the base, and the other on the arm. When getting ready to put the microscope away, always return it to the low power or scanning power setting.
2. When setting the microscope on a table, always keep it away from the edge.
3. It is generally best to clear your lab table of items that are not being used.
4. The lenses of the microscope cost almost as much as all of the other parts together. Never clean them with anything other than lens paper. Paper towels and other paper tissues will scratch the lens.
5. *Please inform the instructor or the biology lab technician of any microscope damage or irregularity in its operation as soon as possible. Do not return a faulty microscope without first informing the instructor or lab tec*h.
6. You are responsible for the microscope while using it—treat it with care!

How to Use a Compound Light Microscope

Be familiar with the following procedures outlining the correct usage of the microscope before coming to lab. The steps that follow should be observed in this lab exercise and all other lab activities in this and other courses/lab experiences.

1. **Place the low-power objective in position** (if not already in position). In changing from one objective to another, you will hear a *click* when the objective is set in proper position.
2. **Make certain that the lenses are clean.** Dirty lenses will cause a blurring or fogging of the image. The high power and ocular lenses are the lenses that most often get dirty.

How to clean microscope lenses: Place a drop of lens cleaning fluid on a piece of *lens paper*. Clean lens with a *gentle* circular motion, then dry with a *fresh* piece of lens paper. Always use lens paper for cleaning! Any other material (including Kimwipes) may scratch the lens.

3. **Check the Preliminary Lighting**
   a. Plug in the electrical cord and turn and turn on the substage light with the light switch located on the base of the microscope
   b. Position the ***condenser*** as high as it will go by turning the substage adjustment. This provides for a maximum of light. *The condenser should be as close to the stage as possible*.
   c. Adjust the iris diaphragm by means of the lever beneath the condenser, which is below the stage of the microscope. Unless you are looking at a specimen that does not let much light through, it is best to start with the diaphragm closed as much as possible.
   d. When looking through the ocular you should see a white circular field of view that is *evenly* illuminated like a full moon.


Adjusting the condenser to be as close to the stage as possible

Which objective should you begin your observations with?
- If you need to scan the slide to find the location of a specimen use the low power objective (4x) with its larger field of view.
- If you have a pretty good idea where the specimen is located on the slide it is O.K. to start with the medium power objective (10x).
- Because of the danger of damaging these lenses and their very small field of view, *never* begin microscopic examinations with the high power (40x) or the oil immersion (100x) objectives.
- ***See the page 3 for information concerning the oil immersion lens***.

4. **Place the slide on the stage for viewing at scanning or low power.**
   a. Make certain that the low power objective (4x) or the medium power objective (10x) is clicked properly in place.
   b. Using the spring loaded lever of the mechanical stage insert a properly prepared slide (see the pictures below) on the stage.
   c. Using the knobs of the mechanical stage move the object to be viewed on the slide *directly* above the condenser.


Place the slide on the stage, and keep in place with the slide holder.

5. **Focus: Proper Focusing Technique for low and medium Power**

   a. Viewing the stage from the side, use the *coarse adjustment knob to raise the stage* until the stop is reached that will prevent further movement of the stage.
   b. Looking through the eyepieces *lower the stage slowly by turning the coarse adjustment knob* away from you until the object is in focus. It should take *less* than a quarter of a turn to bring the image into focus.
   c. Use the ***fine adjustment*** to bring the object into sharp focus.
   d. Adjust the amount of light with the ***iris diaphragm*** and intensity of light with the condenser for optimum viewing. Too much or too little light adversely affects the quality of the image viewed!

Raise the stage using the coarse adjustment knob.

Tips:

- When it is difficult to find a specimen to focus on (e.g. when examining cheek cells), bring the edge of the cover slip into the center of the field of view, and then try focusing on the edge. Then search the slide for the desired specimen.
- Reduce the light intensity to aid in the observation of viewing clear/transparent objects such as amoeba or cheek cells.

Use the ***fine adjustment*** knob bring an object into sharp focus.

6. **Increasing Magnification: Switching from Low or Medium to a Higher Power**
   a. First, be sure the object that you want to view at a higher magnification is in the *center* of the field of view and *sharply* focused under *low* power.
   b. Switch to high power. Watch from the side to make sure that the objective lens does not touch the slide. Since your microscope is ***parfocal***, the object should be in focus, or almost in focus. ***Parfocal***—means that little refocusing is needed when moving from one lens to another. Only *fine* adjustment may be required. If properly focused at low power, and the slide is prepared correctly (i.e. the specimen is *thin* and flattened by a coverslip), you should be able to switch automatically from low power to high without fear of having the high power objective lens scraping or touching the slide.

7. **Re-Focus with the Fine Adjustment under High Power**
   a. **Only use the fine adjustment at high power!** To avoid damaging the lens, *never* use the coarse adjustment when the high-power objective is in place.
   b. Adjust the amount and intensity of light for optimum viewing. The amount of light may need to be increased since less light passes through the objective at higher magnification.
   c. The ***working distance*** is the distance between the specimen viewed and the objective lens of the microscope. As you increase magnification the working distance becomes less and less. The objective will be almost touching the cover slip when properly focused at high power.

If the image viewed at high power is not sharp try the following:

- The object may not have been focused properly at low power. Repeat steps 4-6.
- Something may be dirty and needs to be cleaned. Common culprits include the high power objective, ocular, cover slip, and/or slide.

8. **Remove the slide from the stage**
   a. Switch the objective to low power. Removing a slide while under high power may scratch the lens.
   b. Lower the stage using the coarse adjustment.
   c. Remove the slide from the stage:
   - **Disposal of Wet Mounts: Discard** the cover slip (plastic coverslips in the trash, glass coverslips in the broken glass container at the front of the lab), rinse the slide at a sink, dry thoroughly with a paper towel and then return the slide to the proper container on lab supply table in the middle of the lab.
   - **Prepared slides:** Return to their proper location within the plastic slide container on the lab supply table in the middle of the lab.

Preparing a Wet Mount

1. Place a drop of water on a clean slide with a dropper.
2. Put the object in the water drop.
3. Lower one edge of the coverslip to the edge of the water drop as shown in the illustration (Figure 2). Lower the coverslip slowly to avoid air bubbles. A gentle tapping will usually remove any bubbles that may be present. Blot any excess water with a paper towel. More water can be added with a dropper at the edge of the coverslip. Do not let your specimen dry out.

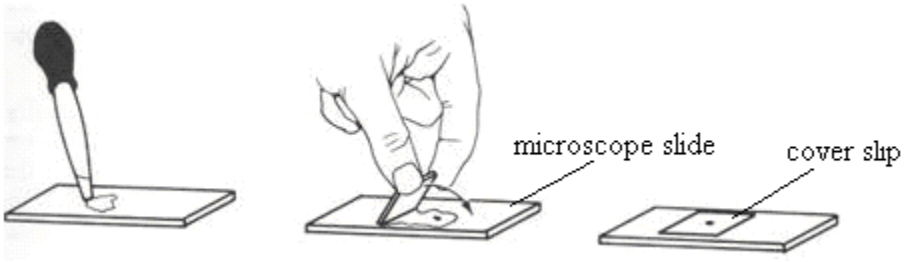


Figure 2. Wet Mount Preparation

Procedure

Important !!

Answer the questions on that start of page 4 of the lab 2 report sheet as you carryout the procedures outlined below. Perform procedures that can only be done in the lab *before* doing procedures or answering questions that can be performed outside of the biology laboratory!

Part A. Determining the Total Magnification of each Objective Lens

To be able to compare data with other scientists, microscopists need to know the structure and function of the various cellular components. Reliability of size relationships is critical. The following exercise is designed to give you information about the total magnification of each objective lens of the compound light microscope.

Materials Needed for Part A: Compound microscope (1 per person)

1. To determine the total magnification of the low, medium, high, and oil immersion fields of view you need to locate the numbers (integers) inscribed on the eyepiece and each of the objective lenses**.** To compute the ***total magnification*** multiply the power of the eyepiece (ocular lens) by the power of the objective lens being used. Record the data in the Summary Chart (#1 of the report sheet on page 4).

Part B. Determining the Diameter of the Field of View

Background Information

It is often important for a biologist to know the size of the object being observed under the microscope. A simple method is described below to obtain estimates of the size of microscopic objects (e.g. cells). This method enables you to estimate of the size of an object by comparing it with the diameter of the field of view. Knowing the diameter of the field of view for each objective lens, you can compare the size of the specimen against the known field diameter and make a reasonable estimate of the size of the specimen. To do this, you must:

- use a metric ruler to measure the diameter of the field-of-view at *low power* in millimeters and micrometers
- know the total magnification for each of the other objective lenses.

The metric ruler (fig. 3) is divided into millimeters, with

10 mm = 1 cm (centimeter). The unit of length that is commonly used for microscopic objects is the ***micrometer***. The prefix ***micro-*** is given the symbol of the Greek letter for the letter "m", μ. A micrometer (μm) is equal to one-millionth of a meter. More appropriately, for microscopic objects a micrometer is equal to one-thousandth of a millimeter (0.001 mm.), or ***1000 μm = 1 mm***.

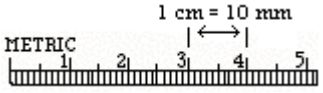
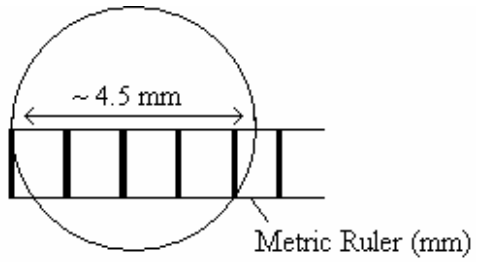


Figure 3. Metric Ruler

The field diameter for low power is illustrated in Figure 4, below.

Figure 4. The field diameter at *low power* is easily determined with the use of a metric ruler calibrated in millimeters. The field diameter at all other magnifications can then be calculated once that at low power is known.



Materials Needed for Part B: Plastic metric ruler and a compound microscope (1 per person)

Procedure for Part B—Determining the Field Diameter.

To measure the field diameter, do the following:

1. With the low power objective (4x) in position, place the millimeter ruler on the center of the stage so that the graduated edge of the metric ruler is across the *midline (diameter) of the field of view*. Bring the ruler into focus. Since very little light passes through the ruler, in order to see the ruler clearly you may need to *open the diaphragm and/or adjust the light dimmer switch to allow more light to pass through*.
2. Line up the one of the millimeter lines with the left side of the circular field of view. Be particularly careful that the *edge* of the ruler is across the *middle* of the field of view. At low power (total magnification of 40x) should look like Figure 4.
3. Count the number of millimeters included from one side of the field to the opposite side. If the right side of the field of view does not coincide with one of the lines, you will have to estimate to a fraction of a millimeter. If done properly you should get a field diameter of about 4.5 mm. Record the diameter in both *millimeters and micrometers* in the Summary Chart (#1 on page 4 of the report sheet). Recall: One millimeter equals 1000 micrometer: ***1 mm = 1000 μm***
4. Turn the 10x objective (medium power) into place and fine adjust. Estimate the diameter of the field of view at medium power—it should be between 1 and 2 mm. To get an accurate value, ***calculate the field diameter in millimeters and micrometers*** by the following method and then record your results in the Summary Chart (#1 on page 4 of the report sheet).

   How to Calculate Field Diameter:

   Just as with a camera, there is an inverse relationship between magnification and field size: field size *decreases* by a proportional amount as magnification *increases*. Likewise, field size *increases* by a proportional amount as magnification *decreases*. This is expressed mathematically as:

$$higher\ power\ field\ diameter = lower\ power\ field\ diamter\ x\ \left[\frac{lower\ power\ magnification}{higher\ power\ magnification}\right]$$

   For example, if the field diameter at 100x is 1.8 mm, then you would calculate the field diameter at 1000x as follows:

$$\text{Field Diameter at 1000x} = \left[1.8\ \text{mm}\right] \text{x} \left[\frac{100}{1000}\right] = 0.18\ \text{mm} = 180\ \mu\text{m}$$

5. Now turn the 40x objective (high power) into place. You should observe the field diameter is less than 1 mm. ***Calculate the field diameter in millimeters and micrometers at high power and for oil immersion***, and record your results in the Summary Chart (#1 on page 4 of the report sheet).

Part C. Observation of the Letter "a"

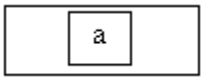
In this exercise you will learn to use the microscope to examine a familiar object, a self-prepared slide of the letter "a". Practice adjusting your microscope to become proficient in locating a specimen, focusing clearly, and adjusting the light for optimum viewing.

Materials Needed for Part C

Letter "a" cut from a Newspaper
Microscope slide and cover slip
Dropper bottle of water
Compound light microscope (1 per person)

Procedure

1. Use scissors to cut out a *lower case* letter "a" from the newspaper and place it right side up on a clean glass slide.
2. Cover the letter "a" with a clean cover slip. See the figure below.



3. Using an eyedropper, place a drop of water on the edge of the cover slip where it touches the glass slide. The water should be sucked under the slide if done properly.
4. Turn on the microscope and place the slide on the stage; making sure the "a" is right side up in the normal reading position as seem with the naked eye—see the figure above). Using the course focus knob and low power, move the stage down until the "a" can be seen clearly.
5. Answer questions 2-12 on pages 4 and 5 of the report sheet on the report sheet as you observe the letter "a" with the low, medium and high power objectives of the compound microscope.

Part D. Observations of Cells under the Compound Microscope

In the circles provided on the report sheet, make neat and accurate labeled drawings of the cells listed below. *Your sketches should be accurate enough so that an informed individual could identify the cells or organisms from your drawings*.

1. Human Cheek Cells

Materials Needed for Part 1

Compound microscope
Clean microscope slide and cover slip
Flat-tipped toothpick
Ringer's solution (Physiological saline)
Methylene Blue stain
Autoclave bag for the class for used toothpicks

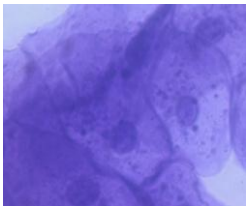
Procedure

1. Place a drop of Ringer's solution in the center of the slide. Using the flat end of the toothpick, *gently* scrape the inner lining of your cheek. Agitate the end of the toothpick containing the cheek scrapings in the drop of saline.
2. Add a tiny drop of **methylene blue stain** to the preparation and stir again with the toothpick. Cheek epithelial cells are nearly transparent and thus difficult to see without the stain, which colors the nuclei of the cells and makes them look much darker than the cytoplasm. **Discard the used toothpick in the disposable autoclave bag provided at the front table**.

3. Add a cover slip and observe under low and then under high power.

Tips:

Although the cells form a solid sheet of cells in your mouth, the scraping of the toothpick probably caused the cells to separate from each other. Try to find a cluster of two or three cells whose shapes have not been totally distorted. Avoid observing clumps of cells that show little cellular detail.



A Clump of Cheek Cells

Human Cheek Cell

4. In the appropriate space on your report sheet, use a *sharp pencil* to make an *accurate sketch* of a cluster of **two or three cells as viewed at high power** (or one cell if 2 or 3 cells can't be found together).
5. Estimate and record the approximate *size* of a single epithelial cell in *micrometers,* μm.
6. Identify on individual cells and *neatly label* on your sketch the following structures: **Plasma Membrane, Cytoplasm, and Nucleus**

2. Filamentous algae wet mount: *Spirogyra*

Materials

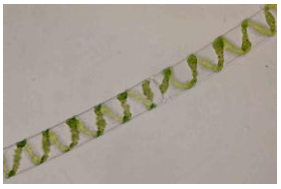
Clean microscope slide and coverslip, filamentous algae (*spirogyra),* and compound microscope

Procedure

1. Prepare a wet mount of *spirogyra*: Use a pipette or a dissecting needle to place a sample of the freshwater algae, *Spirogyra,* on a clean slide, cover with coverslip, and observe with low and high power.

Tip:

Use a dissecting needle to straighten and untangle the strands of *Spirogyra* on your microscope slide—the goal is to observe as clearly as possible a single strand of spirogyra and the individual cells that it consists of.



A single strand of spirogyra

2. In the appropriate space on your report sheet, use a *sharp pencil* to make an *accurate sketch* of *two or three* consecutive cells of *Spirogyra* as viewed *at high power*.
3. Estimate and *record next on your drawing* the approximate *length and width* (in micrometers, μm) of a single cell.
4. Identify and ***neatly label*** on your sketch the following structures. Use your textbook as a reference if needed.
   - **Cell Wall:** Often coated with slime in filamentous algae such as *Spirogyra*.
   - **Cytoplasm:** the internal aqueous environment inside the cell.
   - **Chloroplast:** In *Spirogyra,* it appears as a long green spiral band that runs the length of the cell.
   - **Nucleus:** Embedded within the cytoplasm of *Spirogyra;* Very light in color and therefore difficult to find if the diaphragm is opened too wide and/or the condenser is not adjusted properly.

3. Two Different Types of Microscopic Organisms of Your Choice

Materials

Clean microscope slide and coverslip, pond water and other cultures of microorganisms, and compound microscope

Procedure

1. Prepare a wet mount of pond water or of other organisms provided by your instructor and observe with low- and high-power.
2. In the appropriate space on your report sheet draw at least *two different kinds of organisms of your choice* (preferably, protists—don't sketch vegetation!) as viewed at the high power or at the highest magnification possible that allows you to view the whole organism.
3. *Neatly label all cell parts possible.*
4. *Estimate the length and width (or diameter) of this organism in µm*. Record these dimensions on your drawing.
5. *Use the identification keys in the lab to identify the kingdom (and phylum, if possible) that the organism belongs to.*