

LABORATORY 1: INTRODUCTION TO LABORATORY TECHNIQUES

I. INTRODUCTION

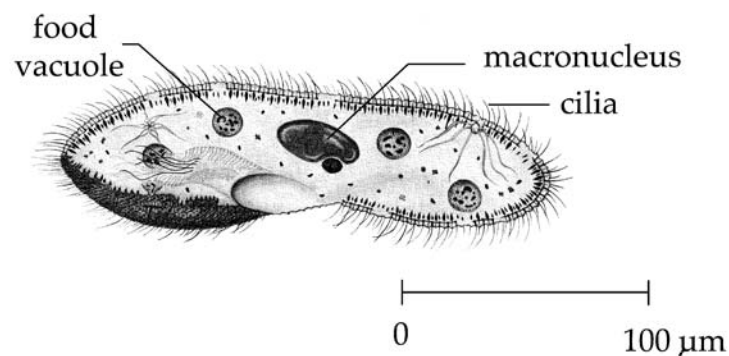
For this and subsequent labs:

YOU WILL NEED:

1. A notebook with a hard back and good quality **unlined** paper. You will not be allowed to do the laboratory without this basic equipment.
2. Hard and soft pencils, and an eraser. Some people use pencil crayons (colored pencils).

Here are some ground rules for your laboratory notebook. Other points will be added as we go along.

1. Everything is recorded directly into your notebook. Notes and labels may be added later, but your **DRAWINGS SHOULD NOT BE ALTERED UNLESS YOU HAVE THE SPECIMEN TO REFER TO IN FRONT OF YOU.**
2. Drawings are made in pencil; labels and notes may also be in pencil.
3. Drawings are labeled by using a single straight-line pointer (no arrowheads) carried well out from the drawing. There should be no writing within the drawing. All writing on a page should be on a horizontal plane and should be right side up.
4. Please include a scale bar so it is possible to tell the size of the specimen. See Figure 1 below.



Paramecium 400x
(single cell)
(Kingdom Protista)

Figure 1: Sample lab notebook drawing

After this lab you should be able to:

- a. Prepare a wet mount of some living material.
- b. Set up the microscope so that a wet mount slide can be clearly seen under both low and high power magnifications.
- c. Determine the approximate size of an organism using the micrometer under any objective of a microscope.
- d. Determine the total magnification of the microscope at low, medium, and high power.

II. MICROSCOPES

Biology is the study of life. Initially biology consisted almost entirely of observations made with the unaided eye. Unaided, the average person can distinguish two points as being separate if there is a distance of at least 0.1 mm separating the points. This is known as **resolution**. With the invention of simple lenses and microscopes, smaller organisms and cellular structures could be studied. Stains and other techniques furthered these studies.

The microscope contains two separate but interacting systems. There is a system of **illuminating** the specimen and an additional system of **lenses** that produce and magnify an image of the object being examined. The dissecting microscope is useful for examination of larger specimens or organs, while the compound microscope is useful for looking at sections of tissues and individual cells.

A. The Dissecting Microscope

You will be using this tool in most of the laboratories. It provides an intermediate range of magnification that allows you to see the relative positions of organs in small animals, for example. Since it uses light coming at an angle to the specimen rather than transmitted light, it allows appreciation of three-dimensions. Also, it provides a less stressful environment for living specimens so you can watch natural movements.

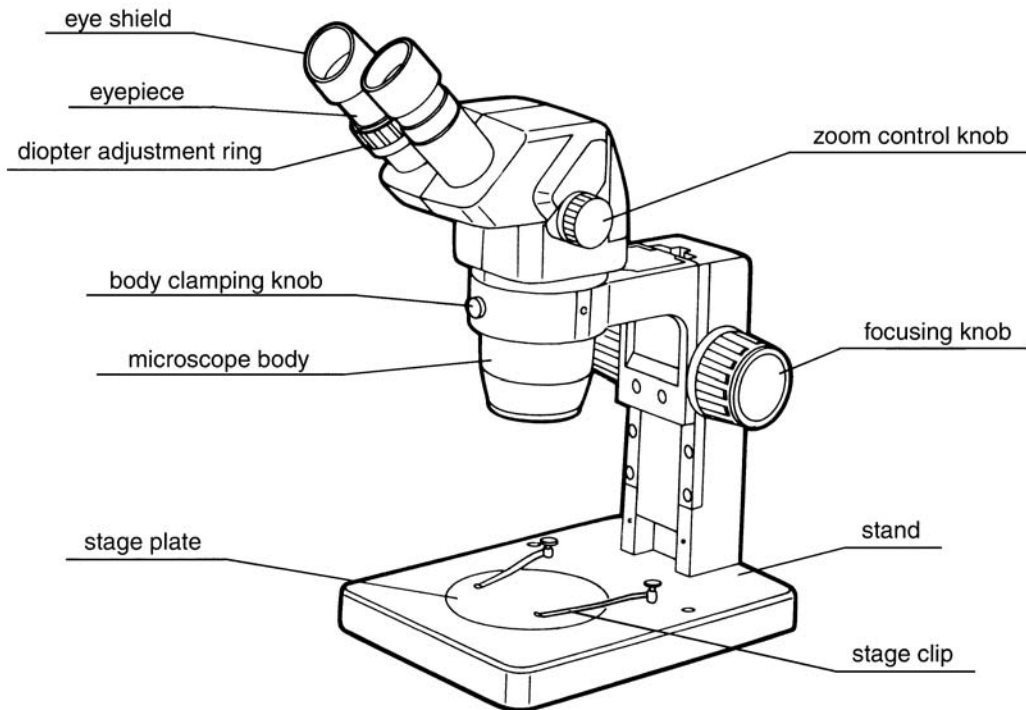


Figure 2: The dissecting microscope

1. Our dissecting microscopes have continuous focus. Turn the zoom control knob to change magnification.
2. Use bench lamps to illuminate your specimen; experiment with angle and closeness of the lamp so as to best reveal the characters of interest.
3. All wet specimens should be examined under water. Be sure that water covers the whole specimen; otherwise you will get lots of confusing surface reflections.
4. Usually you will move the dish containing the specimen under the microscope to find points of interest. Find small organisms under the lowest power, and then increase magnification. Move the dish very gently if you are watching live organisms.

B. The Compound Microscope

The compound microscope is one of the most useful tools in biology. It is important that you become familiar with it and competent in its use since this instrument is essential for proper examination of much of the material you will study in biology.

Once you have acquainted yourself with the microscope and its workings, develop your microscopist skills by examining the various specimens available. Refer back to these notes as we use the microscope in different ways during the course.

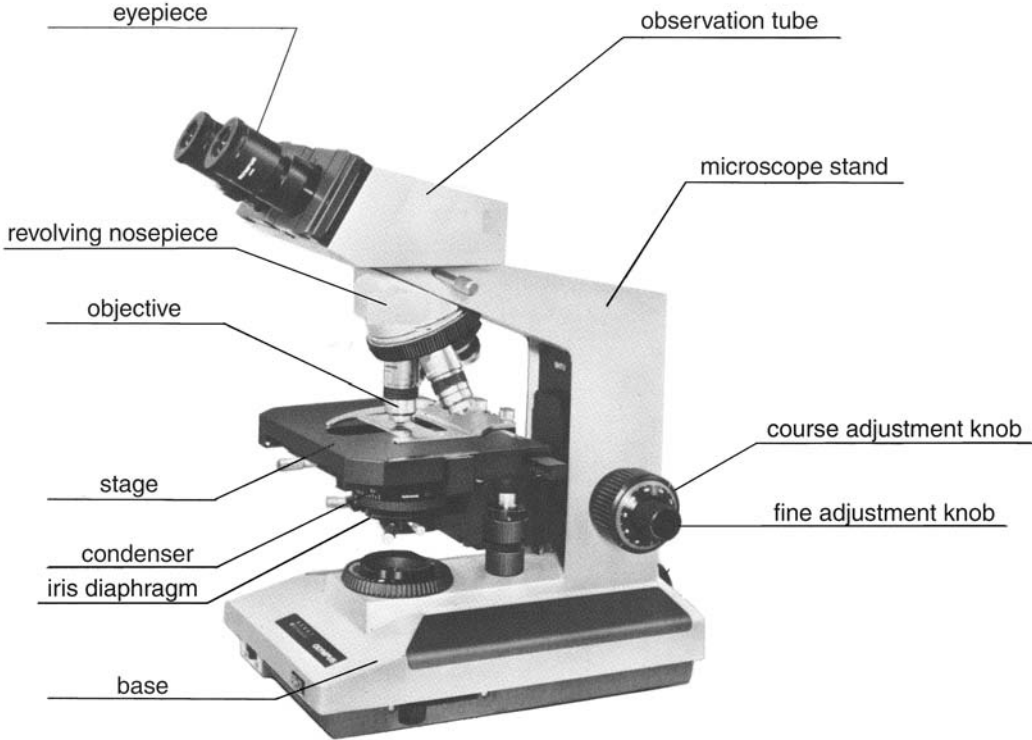


Figure 3: The compound microscope

1. PARTS OF THE MICROSCOPE.

Stage:	Specimens are usually mounted on a glass slide first, and then positioned over the centrally located hole in the stage. The slide may be held firmly in place either by two clips on the stage or by the mechanical stage that some microscopes have.
Revolving nosepiece:	The nosepiece, located at the lower end of the body tube, holds the objectives. Turn it gently by means of the revolving nosepiece. When an objective is in proper alignment below the body tube you will hear a click.
Objectives:	Each objective contains a magnifying lens. Your microscope has a very low power, an intermediate power and a high power. The magnifying power is marked on the objective.
Ocular:	The ocular or eyepiece also contains a magnifying lens and may be removed as a unit from the top of the body tube. The eyepiece of most microscopes has a magnification of 10X. It may be equipped with a pointer. Total magnification of the specimen is the ocular magnification times the objective magnification.
Light source:	The light source is located below the stage and serves to direct light through the material being examined. The sliding switch serves to control the intensity of the light.
Condenser:	The condenser contains another set of lenses and is located below the stage. It focuses light onto the material being studied. Notice the adjustment knob by which the condenser may be raised and lowered. The condenser should usually be adjusted so that it is very close to the stage.
Iris diaphragm:	The iris diaphragm consists of metal diaphragm plates and a lever at the side of the condenser regulates the size of the diaphragm opening and thus the amount of light that enters the microscope. Each time you examine a slide, the diaphragm opening (aperture) should be adjusted to obtain maximum contrast (usually almost closed). As the magnification of the objective lens increases, the circle of light must be adjusted in order for the microscope to produce distinguishable images; hence, the aperture must be increased.
Coarse focus adjustment:	The body tube can be moved rapidly up or down by means of the large knob located near the top of the body tube in most microscopes. COARSE ADJUST SHOULD BE USED ONLY WITH THE LOW POWER OBJECTIVE, NEVER WITH THE HIGH POWER OBJECTIVE!
Fine focus adjustment:	Fine adjustments of the level of the body tube can be made with this smaller knob on the side of the tube. After initial focusing with the coarse adjustment, sharp focus is obtained by using the fine adjustment.

2. CARE OF THE MICROSCOPE.

The microscope is a delicate and expensive instrument. Always carry the microscope with two hands, one hand on the arm or stand and the other supporting the base.

The lenses of the eyepiece, objectives and condenser should be cleaned before and after using the microscope. Use only the lens paper provided, as other materials will scratch the lenses. If fluids are spilled on any part of the microscope they should be cleaned away immediately. If oil immersion is used care should be taken to remove all traces of oil from the objective.

Before putting away the microscope turn off the lamp, return the low power objective to the center position and remove the slide from the stage. Wind the cord, cover the microscope with the plastic cover, and return it to the cabinet.

3. USING THE MICROSCOPE.

Familiarize yourself with the parts of the microscope and their function. Look at the labeled parts in the above diagram and correlate them with the instrument in front of you. Begin by working from the bottom of the microscope. After you are comfortable with the location and purpose of the controls, continue. Effective use of the microscope is dependent on proper illumination; following the next steps will give you the best viewing conditions.

a. Critical Illumination

1. Lower your stage all of the way and rotate into place the low power objective by rotating the nosepiece using the knurled edges, not the objective lenses.
2. Obtain a slide of yellow parachute cloth. Clean the slide and place it on the stage such that the cloth is over the hole in the center of the stage.
3. Watching from the side, lower the objective to within 1 cm of the stage.
4. To set up critical illumination, **close the iris diaphragm** as far as it will go and rotate the condenser knob until the **condenser is at its uppermost position**.
5. Turn on the lamp, adjusting the light output to a comfortable setting.
6. Bring the fibers into focus using the coarse then the fine adjustment knobs. (If you are not able to get the fibers into focus you may have the slide upside down.)
7. **Lower the condenser** slowly until you see granularity come into focus superimposed on the image of the nylon cloth. You are seeing the granularity of the glass diffuser covering the light source. Now **raise the condenser slightly** until the granularity just becomes no longer visible. This is the optimal condenser setting.
8. The last step is adjusting the iris diaphragm. Open and close the iris diaphragm slowly, watching the changing quality of the image of the fibers. When the iris is fully closed, contrast is maximum chance for "false lines" is the greatest due to imperfections in the condenser, but when the iris is

fully opened the "washout" of the image takes over. Find a position, **iris slightly opened**, where the image is clear bright, detailed and sharp. This is the optimal iris diaphragm setting.

9. You have now achieved critical illumination!

b. Preparing a Wet Mount

Making a wet mount is a quick, temporary way of preparing material for observation under the microscope.

1. Clean and dry a slide and cover slip thoroughly.
2. Place 1 drop of water containing organisms onto the slide, or place 1 drop of water on the slide, then add the specimen.
3. Lower the cover slip slowly to an angle of 45° so that one side of it touches the drop.
4. Allow the drop to spread along the edge of the cover slip; then slowly and carefully complete lowering the cover slip. This will prevent air bubbles from being trapped under the cover slip.
5. To avoid crushing larger organisms such as protozoans, you may need to support the cover slip with Vaseline. A small dab of Vaseline may be placed under each corner of the cover slip or a ridge of Vaseline may be placed under each edge of the cover slip. (Your G.S.I. will demonstrate this upon request.) Another way to prevent crushing of specimen is to use a coverslip bridge, and the

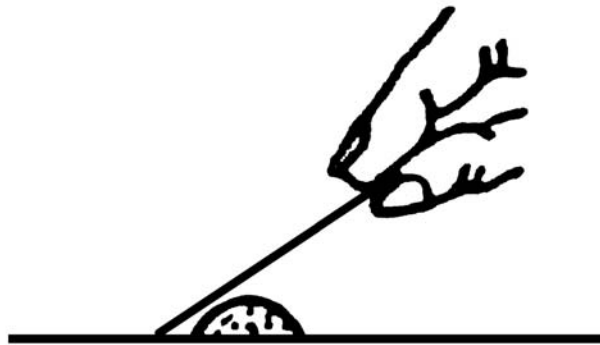


Figure 4: Technique for preparing a wet mount

6. To keep the slide from drying out, place a fresh drop of water at the edge of the cover slip about every 15 minutes; or completely seal the cover slip with Vaseline.

c. Staining a Wet Mount

1. Place a drop of the desired stain at one edge of the cover slip.
2. Hold a piece of Kimwipe close to the opposite edge of the cover slip. The Kimwipe will absorb water from under the cover slip, drawing the dye across the slide.

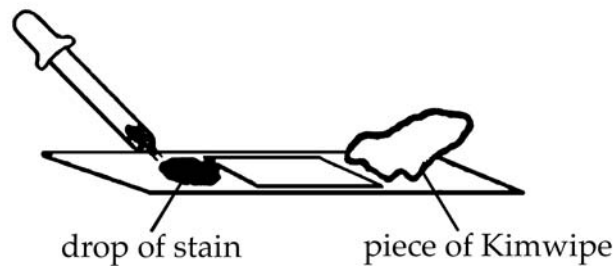


Figure 5: Technique for staining a wet mount

d. Examination of Material

1. Center the slide to be examined on the stage.
2. Move the condenser up as far as it will go. Turn the nosepiece so that the 10X objective is centered above the stage aperture.
3. Follow the above steps for critical illumination.
4. Slowly raise the body tube with the coarse adjustment until an image comes into focus. If the subject is quite small, focus on the edge of the cover slip first, and then locate the organism.
5. Focus on the material as sharply as you can with the fine adjustment. Regulate the light intensity with the iris diaphragm. Greater contrast can often be obtained by reducing the light intensity. This is especially true for clear, unstained organisms and tissues.
6. Switch to high power if needed. The objectives on your microscope are parfocal. This means they are all approximately in focus when the nosepiece is changing from one objective that has been focused to another; only a slight movement of the fine adjustment knob is necessary to restore proper focus. Always locate an object and find the approximate focus under low power first before using the other objectives.
7. When working with the high power objective you can obtain an idea of the 3-D structure of an object by carefully focusing up and down with the fine adjustment.
8. You will avoid eyestrain if you learn to keep both eyes open while looking through the microscope.

e. Magnification and Scale

When you examine an object and change from low to high power, you will immediately realize that you see a much smaller portion of that object at any one time under high power than low power. The area that can actually be seen with a particular objective is called the actual field. The size of the actual field decreases as the magnification increases.

Notice the MICROMETER, the lined image superimposed on your field of view. The micrometer allows you to measure objects viewed under the microscope by counting dashes, just like using a ruler. However, the size of the micrometer doesn't change as the size of the actual field decreases when you change objectives (see last paragraph). Therefore, the distance between dashes is different under each objective as follows:

OBJECTIVE	DISTANCE BETWEEN EACH DASH
4X	25 μM
10X	10 μM
40X	2.5 μM
100X	1 μM

QUESTION #1 Using the micrometer, give the diameter in millimeters of the actual field of your microscope using the 10X objective: _____. With the 40X objective: _____

The total magnification of a microscope is calculated by multiplying the magnification of the objective lens by the magnification of the ocular lens.

QUESTION #2 How many times is an object magnified when observed with the low power objective? _____
 With the high power objective? _____

Observe some prepared slides of biological material provided by your GSI. Record what you see through drawings and notes. Be sure to use scale bars.

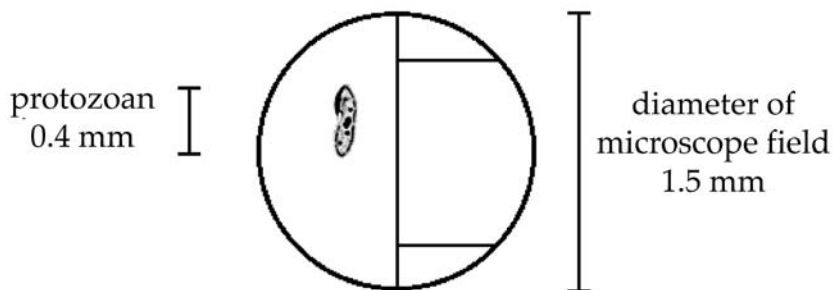


Figure 7: **Microscope field under 10X objective**

III. LABORATORY EXERCISE - Prokaryotic Cells (Kingdom Prokaryota)

1. Bacteria (for this exercise, work in pairs): You will be preparing cultures of bacteria to observe next week.
 - a. Obtain three petri dishes containing sterile nutrient agar from your GSI. Label all plates with your name, the date, and experiment number.

Plate #1 Expose the plate to the air for 20 minutes.

Plate #2 Open a plate and breathe on the agar three times before closing it.

Plate #3 Do not open the third plate. This will serve as your **control**.

Observe the plates at the next laboratory period. Bacterial colonies look like shiny, smooth "blobs" which may be colored. Each colony consists of many individual bacteria that were produced by **fission** from one original bacterium. Fungi may also be growing on your culture plates. These are distinguishable from bacterial colonies in that they appear "fuzzy" and filamentous rather than smooth and shiny.

QUESTION #3 What is the purpose of the "control" petri dish that was not exposed to breath or air?

- b. Look at the specially prepared bacterial slides showing one type of bacterium. Draw and label what you see. Be sure to include a scale bar in this and all drawings.
2. Cyanobacteria
 - a. Observe *Chroococcus*, a common inhabitant in soil that tends to remain moist for long periods. It is a colonial form. Characteristically, small groups of cells are embedded in a gelatinous matrix.
 - b. *Oscillatoria* is a filamentous form and its name derives from its ability to move through oscillations when in contact with solid surfaces.