

Microscopy and Diversity of Microorganisms

Goals of exercise

1. To learn the operating principles and proper use of a microscope.
2. To learn to distinguish and identify microorganisms that are classified as cyanobacteria, algae, protozoa, and microscopic animals.
3. To learn to make detailed critical observations of microorganisms that reveal the distinguishing properties of each.

Summary of exercise

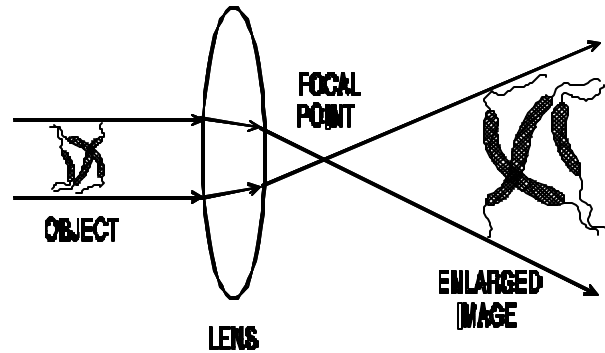
1. You will learn important terms and concepts that must be understood to properly use a microscope.
2. You will learn the name and function of the various parts of the compound microscope, and learn proper technique for using a microscope.
3. You will examine living specimens of microorganisms and make detailed observations and drawings that show their unique characteristics.

The microscope --the biologist's greatest and most powerful observational tool--has provided a view of nature that is otherwise hidden from the human eye. The existence of microorganisms, the most ubiquitous life form on Earth, remained unknown until the microscopic observations made by Antoni van Leeuwenhoek between 1660 and 1675. It was during this same time period that the cellular structure of plants and animals was first unveiled by Robert Hooke using a primitive compound microscope. Today, microscopes are the tools not only of biologists, but also of scientists working in fields ranging from electronics to chemistry. Yet, even in this age of electron microscopes allowing visualization of the very atomic structure of nature, light microscopes descending from those of vanLeeuwenhoek and Hooke retain central importance to biological research.

For the vast majority of biologists, the LIGHT MICROSCOPE remains the most useful type of microscope for routine observations. A light microscope functions by enlarging the image of an object illuminated by sunlight or an electric bulb. The enlargement results when the light rays carrying the image are bent as they pass through a glass LENS. The bending of light, which occurs as light waves pass through the interface between water and air, causes submerged objects to appear displaced from their actual positions. When the light rays pass through a convex-shaped lens they become FOCUSED at a position on the opposite side of the lens, and beyond this point create an enlarged inverted image of the object. The magnification of an image by a single lens is shown in Figure 1.

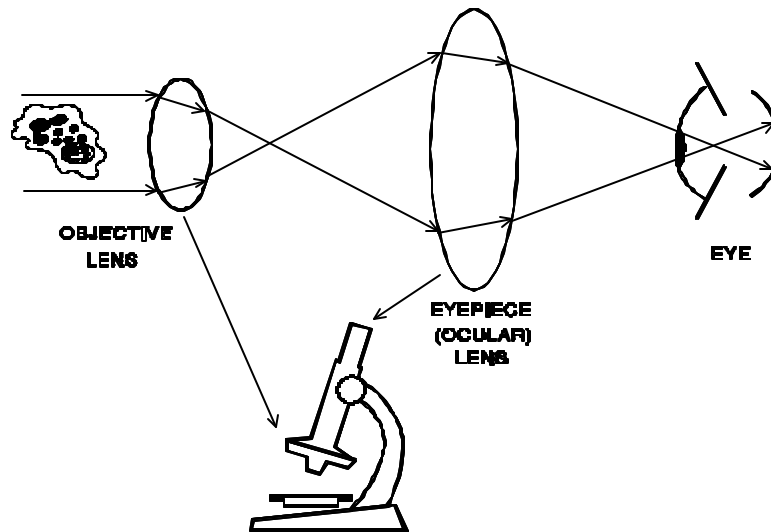
Figure 1. Magnification of an image by a single glass lens.

Notice that the enlarged image appears to be rotated 180°



During this lab period you will learn how to use a modern COMPOUND MICROSCOPE. In a compound microscope, the image passes through two lenses arranged in series. This provides much greater magnification than a single lens alone. Figure 2 shows the arrangement of lenses in a compound microscope.

Figure 2. Magnification of an image by a compound microscope.



Some important concepts

There are certain theoretical and practical aspects of light microscopy that must be understood to be able to properly use a microscope. The quality of a microscopic image is determined by the characteristics of the image referred to as MAGNIFICATION, RESOLUTION, BRIGHTNESS and CONTRAST. These aspects of the image must be properly adjusted on the microscope to obtain a good quality image and to minimize eye-strain while making microscopic observations.

MAGNIFICATION. The apparent increase in the size of an object viewed through a microscope is called "magnification". The total magnification of a microscope is the PRODUCT of the magnifications of the OBJECTIVE lens and the EYEPIECE lens. The total magnification of a microscope can be varied by rotating into place different objective lenses. The common objective lenses found on most compound microscopes are referred to as SCANNING (4x), LOW (10x), and HIGH (40 or 45x). The eyepiece commonly has a magnification of 10x. In Table 1 write in the magnification of the objectives and eyepiece of your microscope and calculate the total magnifications under each objective.

RESOLUTION. An essential function of all microscopes is to increase resolution as well as magnification. Resolution is the relative clarity of the microscopic image. Surprisingly, most of the technological advances in lens design have been directed toward improving resolution, rather than magnification. A lens that has high magnification but poor resolution will produce an image that is enlarged but too blurry and distorted to be of use. The resolution of the image is principally controlled using the COARSE AND FINE FOCUS KNOBS. However, other factors also affect the resolution of a microscope. In particular, the intensity of light, controlled by the SUBSTAGE DIAPHRAGM (located below the stage), has a dramatic effect upon the resolution of the image. Your ability to properly adjust the focus and illumination will determine the quality of the images that you view.

CONTRAST and BRIGHTNESS. Contrast is simply the difference in intensity between an object and its surroundings, and is most conveniently adjusted by use of the SUBSTAGE DIAPHRAGM located below the condenser lens. Decreasing the aperture of the diaphragm will increase the contrast, but decrease the brightness. For best image quality, the diaphragm must be adjusted to optimally balance the contrast and brightness of the image. As mentioned above, adjustment of the diaphragm will affect the resolution of the microscope. When students have problems locating or focusing on an object the most common problem is too little contrast (i.e., too much brightness) due to an improperly adjusted substage diaphragm. In general, the diaphragm should be nearly closed under the scanning (4X) objective, and opened to increase the brightness as you switch to higher power objectives.

WORKING DISTANCE and PARFOCAL. Working distance is the space between the upper surface of the slide and the front of the objective lens when the specimen is in focus. The working distance of the high magnification lens is quite small (less than a millimeter). Care must be exercised to avoid hitting the slide with the high power objective, which can damage the objective lens. The best way to avoid such an accident is to remember that the objective lenses on our microscopes are PARFOCAL. This means that when a specimen is in sharp focus under one objective lens, another objective lens can be rotated into place without hitting the slide and brought into focus with only minor adjustment of the fine focus knob. Never adjust the coarse focus knob when using the high power objective.

Steps to follow when using the microscope

It is very common for beginning students to blame the microscope for their inability to locate or focus on an object. In almost all cases the problem results from not properly (and patiently) following the correct steps in using the microscope. The following steps and helpful suggestions are provided to guide you in the steps that are necessary to find and view objects on a specimen slide.

1. Inspect your microscope before use. Make sure the microscope surfaces are clean of dust and lint. **YOU ARE RESPONSIBLE FOR THE PROPER HANDLING OF YOUR MICROSCOPE DURING THE LAB PERIOD. IF YOU FIND ANY DAMAGE TO THE MICROSCOPE UPON YOUR ARRIVAL IN LAB, YOU SHOULD REPORT IT IMMEDIATELY TO THE INSTRUCTOR.**
2. Always begin to locate a specimen with the scanning (4x) objective in position. Turn the coarse focus adjustment knob to place the 4x objective at its closest position to the stage. Note: the focusing knobs of some microscopes operate by moving the objective nosepiece, while others move the stage platform.
3. The light source (ILLUMINATOR) should be turned on. If your microscope has an adjustment for the light intensity on the base, turn it to position "8".
4. Close the sub-stage diaphragm to give minimum brightness. When initially locating a specimen under 4x power, high contrast is more important than brightness.
5. When using a permanently prepared slide, clean the top and bottom surface with a damp paper towel or Kimwipe. When preparing your own wet mount, be sure to use a clean microscope slide.
6. Holding the microscope slide between your fingers, visually locate the object to be viewed on the microscope slide. Place the microscope slide on the microscope stage, and center the object below the objective lens.
7. Bring the specimen into focus by SLOWLY moving the objective lens with the coarse focus knob. If the object is already visible, you can focus using the fine focus knob.
8. The specimen should be brought into sharp focus by adjusting the substage diaphragm and the fine focus adjustment knob.
9. IF YOU CANNOT LOCATE THE SPECIMEN, visually check to see that the slide is positioned correctly. Next, try closing the SUBSTAGE DIAPHRAGM further to increase contrast. If still nothing, then move the slide over to another area, and repeat the process from the beginning.
10. To view a specimen under the low power (10x) objective, first move the slide so that an object is centered in the field of view. Next, while watching from the side, rotate the low power objective into

viewing position. Since these objectives are parfocal, only minor adjustment of the focus will be necessary to bring the specimen into sharp focus. The substage diaphragm should be adjusted after changing objectives to obtain the best contrast and resolution.

11. To view a specimen under the high power lens, repeat the above procedures, and rotate the high power lens into position. Remember, NEVER ADJUST THE COARSE FOCUS KNOB WHEN USING THE HIGH POWER OBJECTIVE.

12. Before removing a microscope slide from the stage, always first rotate the lowest power objective into position!

Cleaning of microscope lenses

A dirty lens will cause distortion of a microscopic image. Dirt or a smudge can occur on the eyepiece, objective, condenser lenses, or the microscope slide itself. It is usually possible to identify the location of the dirt or smudge by following a simple procedure:

1. Rotate the eyepiece. If the dirt is on the ocular, it will rotate also.
2. Change the objective lens. If the distortion disappears, then the objective lens is dirty.
3. If the above steps fail, remove the slide and check the condenser lens and the microscope slide.

UTMOST CARE MUST BE USED WHEN CLEANING LENSES, AND YOU SHOULD ASK FOR THE INSTRUCTOR'S HELP BEFORE DOING SO. Only special "lens paper" provided in the lab should be used for this purpose since the glass of which lenses are made is relatively soft and is easily scratched.

Steps in putting away the microscope

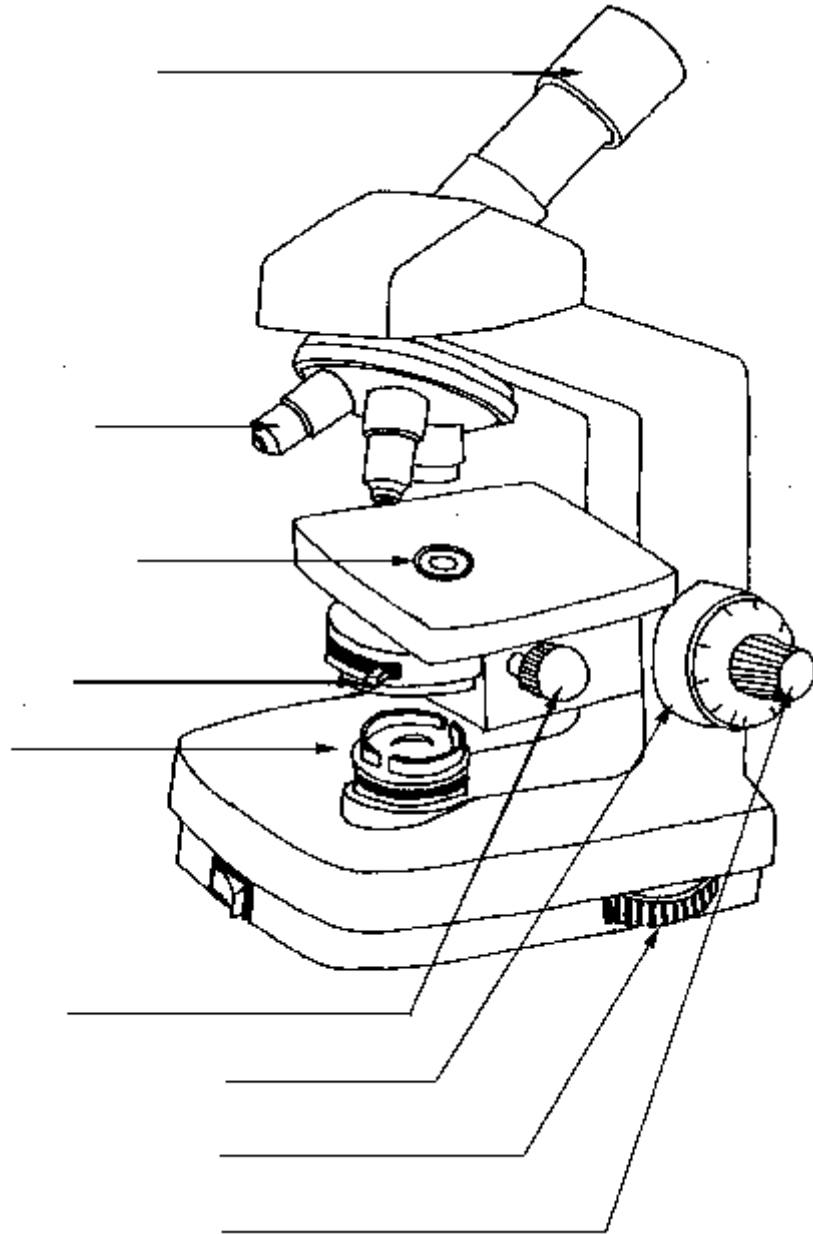
1. Rotate the scanning (4x) objective into position.
2. Remove the slide.
3. Clean the microscope surfaces free of water and dirt.
4. Only clean the lenses if they are known to be dirty, and then only using lens paper.
5. Reposition the slide hold-down clamps on the microscope stage.
6. Place the microscope back in its proper place in the cabinet.

Report any damage to your microscope to the instructor. **YOU ARE RESPONSIBLE FOR ANY DAMAGE THAT OCCURS TO YOUR MICROSCOPE DUE TO CARELESSNESS OR IMPROPER USE.**

Names: _____

Label the Parts of the Microscope

Label the following components of a compound microscope: fine focus adjustment knob, coarse focus adjustment knob, illuminator, illuminator adjustment knob, objective lens, ocular lens, condenser lens, condenser adjustment knob, and substage diaphragm.



Observations of microorganisms

Preparing a "wet mount"

1. Place a small sample of the microorganism culture in the center of a clean microscope slide. If the sample you will be observing is dry (e.g., a fungal culture), then add a drop of distilled water to the slide in which the sample can be suspended.
2. Add a cover slide over the specimen. There should not be so much liquid that the cover slide will "slosh" around over the microscope slide.
3. General description of the different organisms that you will be observing are provided below. Make DETAILED DRAWINGS of the different organisms in the space provided.

1. Algae: Kingdom Protista

Algae, along with protozoa, fungi, animals and plants are referred to as EUKARYOTES. Eukaryotes differ from PROKARYOTES (such as cyanobacteria) in that the cells are typically much larger, and contain visible intracellular structures (such as a nucleus and chloroplasts) within the cell cytoplasm. Many of the microorganisms that you will observe are "single-celled" organisms, i.e., the entire organism consists of a single cell that can live independently of other cells. *Euglena* is an example of a single-celled alga. Sometimes the cells are joined together in groups of cells called a COLONY. Colonies can occur as long chains of cells called a FILAMENT (e.g., *Spirogyra*), as a symmetrical, hollow ball (e.g., *Volvox*), or as an irregular mass.

Algae are green because they carry on photosynthesis and contain the pigment chlorophyll in internal structures called chloroplasts. Note that the CHLOROPLAST in *Spirogyra* has a fascinating spiral shape. Look for the faint cell walls that separate individual cells of the filament. *Volvox*, one of the most beautiful of algae, occurs as a COLONY of individual cells arranged in a large hollow ball. The cells possess hair-like "flagella" that beat in unison to propel the colony through the water.

Examine the following organisms. For *Spirogyra*, label an individual cell (identify the end-wall that separates adjoining cells), the spiral chloroplast, and nucleus. For *Volvox*, label an individual cell, and an internal mass of replicating cells (if present).

Euglena

Volvox

Spirogyra

2. Protozoa: Kingdom Protista

Organisms to examine: *Paramecium* and a mixed culture of protozoa

Protozoa are single-cellular eukaryotes that are also classified in the kingdom Protista; however protozoa are NEVER photosynthetic (and never green). Many protozoa are predators that actively hunt other types of microorganisms. Protozoan cells are among the largest and structurally most complex of all single-cellular organisms. Many protozoa, such as *Paramecium*, are covered with hair-like structures called CILIA (similar to but smaller than flagella) that aid in movement of the cell. Note that protozoa are also eukaryotes, and many large internal structures are obvious in the cytoplasm. A key containing diagrams of the types of protozoa found in the "mixed culture" will be provided in the lab.

Identify and make drawings of at least 2 types of protozoa (other than *Paramecium*) present in the mixed culture.

Paramecium

2 other organisms from mixed culture
(use the key provided to identify these)

3. Bacteria: Kingdom Monera

All PROKARYOTIC organisms are called bacteria and are placed in the kingdom Monera. These very small cells are believed to be the most primitive life form. One group of bacteria, called CYANOBACTERIA, are photosynthetic. *Oscillatoria* is a cyanobacterium that occurs as long filaments which can be easily observed. How can cyanobacteria be distinguished from algae? For one thing, the individual cells are much smaller than those of most algae. Even when using the high power objective of the microscope, the INDIVIDUAL CELLS comprising the *Oscillatoria* filament are difficult to discern. Furthermore, a careful analysis will show the *Oscillatoria* lack internal organelles such as chloroplasts. In order to better compare algae with cyanobacteria, PLACE A SAMPLE OF *Spirogyra* and *Oscillatoria* TOGETHER ON THE SAME SLIDE AND OBSERVE THE DIFFERENCES IN THEIR SIZE AND APPEARANCE. Cyanobacteria are sometimes called "blue-green algae" due to their typical color. Compare the color of the *Oscillatoria* to one of the types of algae.

A. Examine *Oscillatoria* under the 40x objective. Make a drawing and label an individual cell.

B. Place a sample of *Oscillatoria* and *Spirogyra* together on a single microscope slide and view under the 40 x objective. Make a simplified diagram that compares the relative sizes of these organisms. Label an individual cell in each.

4. Microscopic animals: Kingdom Animalia

Many small animals can be found in ponds and oceans. Many of these animals are visible to the unaided eye, but observation of the structural details of these organisms requires use of a microscope. Unlike single-celled organisms, the bodies of animals contain many different cell-types that perform specialized functions. However, the cells are so small that you will not be able to see them individually. The *Daphnia* provided are an example of a very small animal. Note that the individual animal can be seen in the original culture flask (squinting helps). When examined under the microscope (4x or 10x objective) the remarkable structural complexity of these animals can be seen. The body possesses appendages that aid in swimming and gathering food. Interestingly, this organism is actually related to crustaceans such as crabs and lobsters. A ROTIFER is also a multicellular organism, even though it is no larger than many types of protozoa. Note that it has a distinct mouth opening and a clearly discernable internal digestive system. Draw the following organisms:

Daphnia

Rotifer

Table 1. Characteristics of lenses of your microscope.

Objective	Objective Magnification	Ocular magnification	Total magnification
Scanning			
Low		same as above	
High		same as above	

Table 2. Comparison of Characteristics of Microorganisms .

Organism	Cell Structure Eukaryotic or Prokaryotic	Kingdom	Photosynthetic Yes, No or Sometimes
Algae			
Protozoa			
Bacteria			
Microscopic animals			

1. Which of the following is a characteristic of all organisms referred to as "prokaryotic?"
- A. are photosynthetic
 - B. cytoplasm contains organelles
 - C. have a "filamentous" cell shape
 - D. cytoplasm lacks internal organelles
 - E. are visible to the unaided eye

2. How do protozoa move?

Match the following terms with the optical property that they describe.

Term

Optical property

3. Resolution ____

A. The distance between the objective lens and the microscope slide.

4. Magnification ____

B. The apparent increase in size of the object.

5. Working distance ____

C. The actual size of the object on a microscope slide.

D. The difference in brightness between an object and the background.

E. The relative clarity of a microscopic image.

6. Why are the objectives of a microscopes said to be "parfocal"?

7. Suppose that you began to focus on a microscope slide, but had difficulty locating the specimen. You noticed that the image appeared very bright. Which component of the microscope should you adjust?

8. When you are finished using a microscope during a laboratory exercise, you should turn off the illuminator, and rotate the 40x objective into place.

A. True

B. False