LABORATORY

Observations and Measurements: The Microscope

OVERVIEW

The success of a scientific experiment depends upon several factors: the problem must be well defined, the variables must be identified, and the experimental techniques and equipment used must be appropriate for the method of inquiry.

Understanding and properly applying the methods of scientific inquiry require that you become proficient at observing and recording data accurately. To do this, you need to be familiar with the types of instruments used for experimental work and with proper sampling techniques. During this laboratory period you will learn about the use and care of the **compound microscope** and the **dissecting microscope.** You will prepare living materials for observation and you will learn to use the microscope to measure the size of cells.

STUDENT PREPARATION

Prepare for this laboratory by reading the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency. Review Laboratory I, "Science—A Process."

✓ EXERCISE A | Identifying the Parts of the Compound Microscope

The simplest example of a microscope is a double convex lens of the type that is used as a magnifying glass. In the late 1500s, two Dutch spectacle makers developed the compound microscope. Their device had two convex lenses placed at either end of a tube and was capable of magnifying an object to 10 times (10×) its actual size. Today, developments in microscopy provide scientists with a wide selection of instruments with which to view the smallest organisms and even the components of individual cells. These microscopes range in complexity from the relatively simple models you will use in the laboratory today to highly sophisticated scanning and transmission electron microscopes.

Objectives

- ☐ Locate the optical and mechanical parts of the compound microscope.
- ☐ Discuss the function of each part of the compound microscope.

Procedure

To use the microscope properly, you must first be familiar with the care of this expensive and delicate instrument. Keep the following precautions in mind:

- Always carry the microscope in an upright position. Use one hand to grasp the arm of the microscope; use the other to support the base. The eyepiece (ocular lens) slides into the body tube and could fall out if the microscope is tilted.
- Never place the microscope close to the edge of the lab table or counter. Be sure to place the electrical cord out of the way and not in a position where it could catch and drag the microscope to the floor.
- Use only lens paper for cleaning the lenses. Using your fingers, handkerchief, or other materials could smudge or damage the lenses.
- When you are finished with your observations, turn off the illuminator and rotate the low-power objective into viewing position. Never put a microscope away with the high-power objective in the viewing position.

1. Obtain a compound microscope from your instructor.
2. Study the **optical** system of the microscope, familiarizing yourself with the location and function of each part. The letters in Figure 1A-1 correspond to the parts described below. After locating each part on the diagram, write the name of the part in the lettered space on Figure 1A-1, and identify that part on your own microscope.

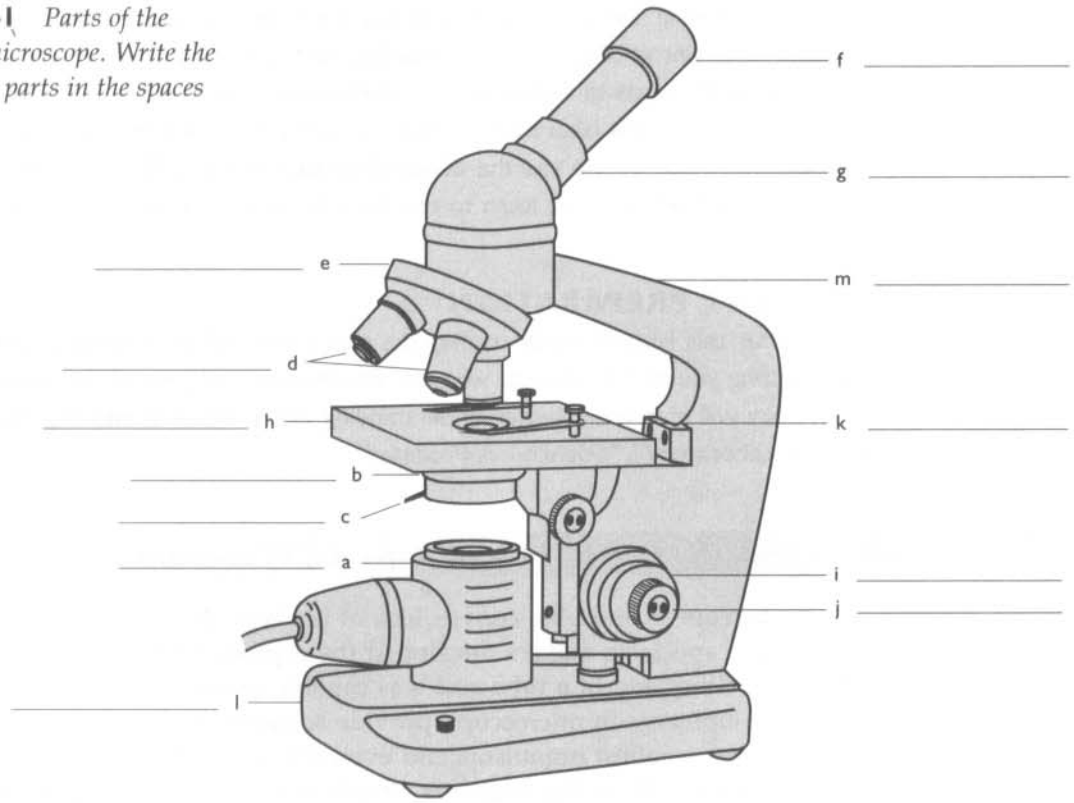

Figure 1A-1 *Parts of the compound microscope. Write the names of the parts in the spaces provided.*

a Light source May be built into the base with a lens that focuses light onto the lower condenser lens or may be a separate light that is focused onto the condenser lens by a mirror.

b Condenser Contains a system of lenses that focuses light on the object (Figure 1A-2). Some microscopes may not have a condenser, particularly if they do not have a built-in light source. Others have either a movable or a fixed condenser. If your microscope is equipped with a movable condenser, locate the knob that raises and lowers the condenser and circle it on the diagram.

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*Mechanical stage not shown.

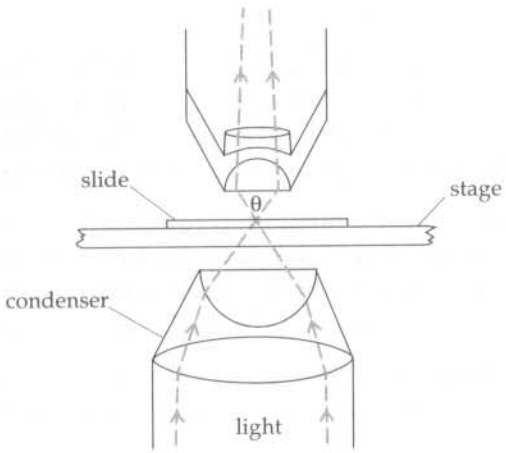


Figure 1A-2 *The microscope condenser focuses light onto the specimen on the microscope stage. Observe the point at which the dotted lines cross.*

c **Iris diaphragm** Used to adjust the amount of light striking the object. It can be opened or closed using the lever on the side of the condenser. On some microscopes this function is accomplished by a disk-aperture diaphragm. (Different-sized holes in the diaphragm are used to view objects at different magnifications.)

d **Objective lenses** Mounted on a revolving **nosepiece** or **turret e.** Most new microscopes are **parfocal;** that is, when an object is in focus with one lens, the lenses can be changed without completely losing focus. Each objective contains a complex lens system. The lens closest to the specimen produces the **magnification.** Magnification is indicated on the side of the objective (Figure 1A-3). The nosepiece usually holds the following objectives (check to see which of these are present on your microscope): scanning objective (4× magnification), low-power objective (10× magnification), high-power objective (40×, 43×, or 45× magnification), and oil-immersion objective (100 × magnification).

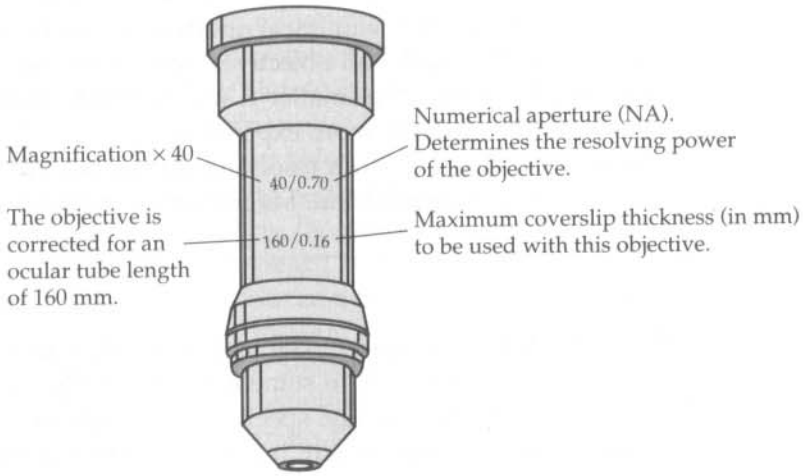


Figure 1A-3 *The power or magnification of an objective lens is engraved on the side of the objective.*

A second lens within the objective is responsible for limiting its **resolving power:** the ability to reveal detail, to distinguish two closely spaced objects as being two rather than one. The smaller the distance between two objects that can be distinguished from one another, the better is the resolving power of the instrument used to view the objects. The unaided human eye can distinguish (resolve) two objects when they are at least 0.1 mm apart, whereas with the light microscope, the human eye can distinguish two objects as separate when they are up to 1,000 times closer than that!

Resolving power (R) is dependent on three factors:

Angular aperture (θ) Examine Figure 1A-2. Note the cone of light entering the objective. The optimum value for θ is the angle that produces a cone of light whose diameter just matches the diameter of the objective. When angle θ is too small, the resolution is poor. One of the functions of the microscope's condenser is to produce the ideal angle of the cone of light.

Refractive index (n) The medium through which the light must travel will affect the shape of the cone of light and thus resolution. Air has a refractive index of $n = 1$. Oil has a greater refractive index than air ($n = 1.5$) and is often used to increase resolution of the microscope at higher powers by increasing the angle (θ) of the cone of light that passes into the objective.

Wavelength of light (λ) The shorter the wavelength of light, the greater is the resolution of the objective. The value of λ can be changed by using colored filters.

The value of R (resolving power) can be determined by the expression

$$R = \frac{\lambda}{2\,[n \sin (\tfrac{1}{2}\,\theta)]}$$

where

R = resolving power

λ = wavelength of light used

n = refractive index of the medium between the lens and object

θ = angular aperture of light cone

The expression $[n \sin (\tfrac{1}{2}\theta)]$ in the above equation is known as the **numerical aperture** (NA), and the equation can be rewritten as

$$R = \frac{\lambda}{2\,\mathrm{NA}}$$

Note that since λ is the only term in the equation expressed in units, the value of R will be expressed in the same units: nanometers (nm). Numerical aperture is a pure number: it is unitless. The numerical aperture is engraved on the side of all objectives next to the number indicating magnification (Figure 1A-3). The higher the NA value, the smaller R will be. And remember, the smaller R, the better will be the resolution of the objective (and the more expensive!).

As magnification increases, so does resolving power, but the relationship is not linear: resolution always increases less than magnification. Magnification without increased resolution is not advantageous for studying specimens. *a. Why?* _____

_____

f **Ocular lens** or **eyepiece** The lens you look through. It will usually magnify objects to 10 times their size (10×). In some cases, the body tube **g** can be rotated, making it easier for someone else to view the specimen without moving the entire microscope. If your microscope has one ocular, it is mononuclear. If there are two oculars, it is binocular.

3. Study the **mechanical system** in the same manner as you studied the optical system. Letters in Figure 1A-1 correspond to the parts described below. Continue to label the diagram and locate each part on your own microscope.

h **Stage** Holds the slide to be viewed. The stage can be moved vertically by turning the **coarse adjustment knob i** and the **fine adjustment knob j.** These are located in different places on different types of microscopes, either separately or together. Coarse adjustment is used for initial focusing of specimens at low power. Fine adjustment makes very slight changes, allowing precision focusing at higher power.

k **Stage clips** Hold the slide so that it can be moved by hand. If your microscope does not have stage clips, it will be equipped with a mechanical stage (not shown in Figure 1A-1). Adjustment knobs are used to move the slide in the horizontal plane, that is, side to side and toward and away from you.

l **Base** and **arm** **m** Important support parts of the microscope; these also allow for easy carrying.

EXERCISE B Using the Compound Microscope

Objectives

- ☐ Learn to use the compound microscope properly.
- ☐ Learn to position specimens properly and adjust the microscope for optimum use.
- ☐ Calculate the magnification of a specimen.
- ☐ Define "resolution" and explain how it applies to obtaining maximum clarity of image.

Procedure

To get maximum performance from the microscope, you will need to adjust the illumination and focus properly.

1. Place the microscope on the table with the ocular pointing toward you. If your microscope has a binocular head (two eyepieces), you will need to adjust the distance between the two oculars to match the distance between your eyes. There is an adjustment dial between the oculars. Turn the dial to change the interocular distance until the oculars are positioned in front of your eyes. When the adjustment is correct, you can comfortably see a single, round field of view.
2. Revolve the nosepiece until the scanning objective (4×) is in line with the body tube. You will hear (or feel) a click when the objective is properly engaged (otherwise you will see only your eyelashes against a black background).
3. Turn on the light switch and adjust the illumination. Higher-power objectives require more illumination. Adjust the *condenser* if it is movable. When using the 4× or 10× low-power objective, the upper lens of the condenser should be about 5 mm below the slide, but when using a 40× (high-power) objective lens, the upper lens of the condenser should be slightly below its uppermost position or about 2 mm below the slide.

   The best way to adjust the condenser is to begin by placing the sharpened tip of a pencil directly on top of the light source. Then, using the condenser adjustment knob, move the condenser up and down until the tip of the pencil is clearly in focus. The condenser should be readjusted with each objective, but the amount of adjustment needed will be slight.

   The iris diaphragm or disk-aperture diaphragm must also be adjusted to obtain the proper balance between contrast and resolution. The amount of light that enters the microscope affects both **contrast** (ability to distinguish something from its background) and **resolution** (ability to distinguish two points as separate). Best observation with the microscope occurs when neither contrast nor resolution is maximized. To adjust the iris diaphragm, remove the ocular lens (or one of the lenses, if the microscope is binocular) and use the diaphragm lever to adjust its leaves until they are just at the edge of the circle of light seen through the open ocular. Then, adjust the leaves so that they are approximately one-third of the way in toward the center of the circle of light. At this setting, the best balance exists between contrast and resolution. Replace the ocular lens before proceeding.
4. Use the coarse adjustment knob to adjust the stage *downward* before placing the slide on the microscope stage. (*Note:* If your microscope has a movable body tube rather than a movable stage, move the body tube *upward*.)

a. Which way did you have to turn the coarse adjustment knob (toward you or away from you) to increase the distance between the objective and the stage? _____

5. Obtain a slide of the letter **e** (or make a wet-mount slide: cut the letter **e** from an old newspaper, add a drop of water, and cover with a coverslip). Place the slide on the microscope stage, making sure that the coverslip is facing upward toward the objective. Hold the slide in place with stage clips or the mechanical stage. If your microscope is equipped with a mechanical stage, do *not* try to place the slide beneath the arms of the slide carrier. Pinch together the two metal extensions of the carrier and its arms will open to allow for insertion of the slide. Now position the slide so that the specimen is directly over the hole in the stage. You will need to do this by hand if your microscope is equipped with stage clips. To move the mechanical stage, use the two knobs beneath the right side of the stage. The upper knob moves the stage backward and forward. The lower knob moves the stage from side to side.

6. To adjust the focus, you will need to move the objective lens using the coarse and fine adjustment knobs. Watch from the side as you adjust the stage upward (or the body tube downward) until the objective lens is almost touching the slide. While doing this, observe the direction in which you are turning the coarse adjustment knob—toward you or away from you. With experience, you will develop a feel for which way to turn the knob. Take care not to bump the slide with the objective lens because this could damage both the objective lens and the slide.

7. While looking through the eyepiece, use the coarse adjustment knob to slowly move the stage downward away from the objective lens (or move the body tube upward away from the stage). When the specimen becomes visible, turn the fine adjustment knob slowly to sharpen the focus. If you are using a binocular microscope, you may have to adjust the focus for each of your eyes. Sometimes the visual acuity of our two eyes is different. After you think you have the letter **e** in focus, close your left eye and fine-tune the focus by turning either the coarse or fine adjustment knob. Once the picture has been sharpened, open your left eye and close your right eye. Turn the binocular focus knob of the left ocular tube until the object is in sharp focus. You can determine how much your specimen is magnified by multiplying the power of the objective lens by the power of the ocular lens. If you are using a binocular microscope, consider the power of only one of the ocular lenses.

Total magnification = ocular magnification × objective magnification

8. Move the slide to the right.

b. Which way does the letter **e** *move (as viewed through the microscope)?* _____ *c. Is it upside down?* _____ *Is it backwards?* _____ *(It is important to remember these spatial relations.)*

9. Now obtain a prepared slide of *Oscillatoria*. Place the slide on your microscope stage and repeat steps 1 through 8.

10. Rotate the nosepiece so that the 10× objective clicks into place. Again, check the space between the objective lens and your slide. The **working distance** (the space between the objective lens and the slide) decreases with higher-power objectives and increased magnification (Figure 1B-1). If your microscope is parfocal, you will probably be able to bring your specimen into focus by using only the fine adjustment. If you do need to use the coarse adjustment, remember: never turn the coarse adjustment while looking through the ocular. Always view from the side.

    The size of the **field of view** (the area you can see) varies inversely with magnification. The greater the magnification, the smaller is the field of view (Figure 1B-1).

11. Examine a slide of *Spirogyra* or *Oedogonium*. Count the number of cells you can see in one strand at 4× and 10×.

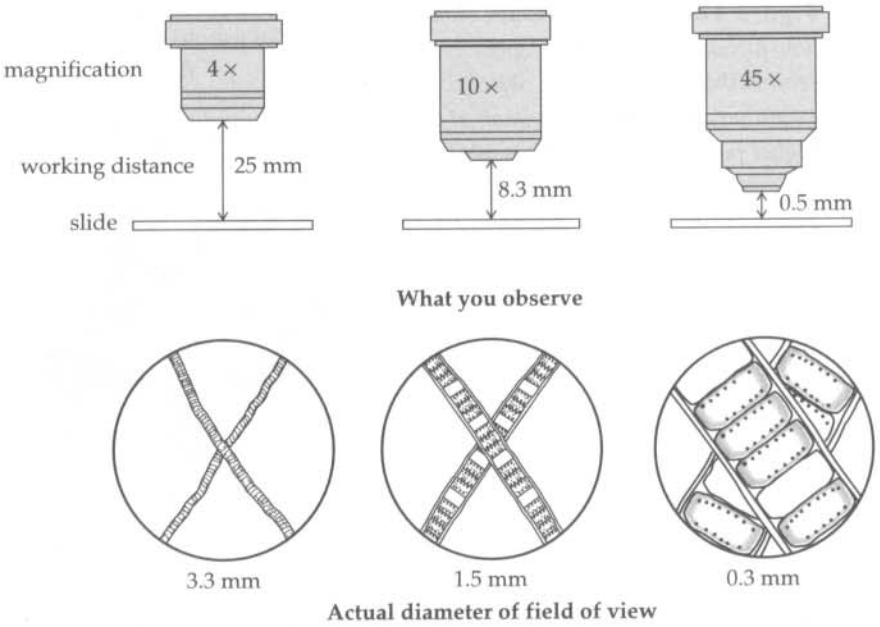


Figure 1B-1 *The field of view and the working distance change with magnification. (When each of these magnifications is used with a 10× ocular, the magnification is multiplied by 10.)*

d. What happened to your field of view as you changed from the 4× objective to the 10× objective?

_____

e. Examine Figure 1B-1. What can you say about the relationship between magnification and field of view? _____

12. Practice moving the slide until you can move it smoothly. Locate something in the field of view that can be followed as the slide is shifted. Attempt to move the object completely around the edge of the field without losing it (manipulations of this type will be particularly important when you view live organisms). Rotate the fine adjustment knob as you move the slide.

f. Do some parts of the specimen appear to be in focus when other parts are not? _____

If you said yes, you are probably correct. Your specimen is not of equal thickness in all places and the depth to which your microscope can focus (called the **depth of field**) is limited. The higher the power or magnification, the shallower is the depth of field. You can study thick objects by continually changing the fine focus, thereby bringing into focus different planes through the specimen. This allows you to "optically section" some materials (Figure 1B-2).

13. When you can operate the microscope successfully using 10× magnification, change to the 40× high-power objective. When using high power, the object to be viewed must be at the center of the field because the high-power objective magnifies only a small portion of the field of view observed under low power (Figure 1B-1).

14. Again, to avoid hitting the slide with the objective lens, watch from the side as you switch to the high-power objective. You may need to use the fine adjustment knob to focus the specimen. Remember: never use the coarse adjustment knob with higher power.

g. What is the total magnification of your specimen with the 40× objective in place? _____

15. On a separate sheet of paper, accurately draw what you observe at this magnification. Drawings should always be done in pencil and be labeled to indicate the name of the specimen and the total magnification.

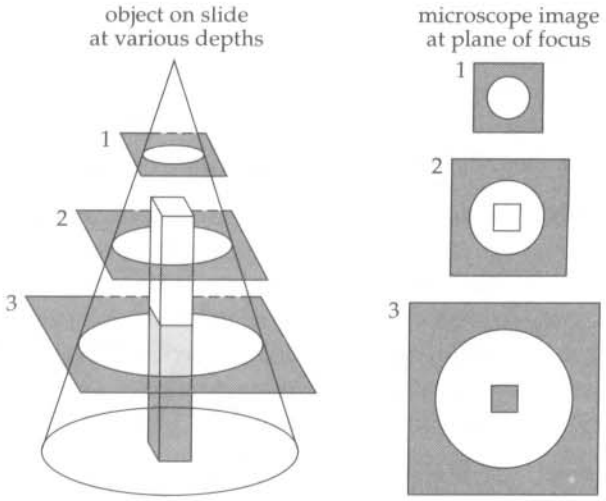


Figure 1B-2 *This schematic shows how focusing at different depths reveals the structure of an object. When viewing a thick specimen at a higher power (40× or 100×), you may use this optical sectioning process to examine the specimen's structure at various depths.*

Below are a few additional suggestions for using a microscope.

- Be certain that you have the proper amount of light.
- Try to keep both eyes open. This will take some practice on your part, but will be less tiring for your eyes.
- Always clean ocular and objective lenses with lens paper before use.
- If you wear glasses, remove them.
- Always begin by using a low-power objective lens to find the specimen. You can then turn to a higher power to make your observations.
- If you are having difficulty locating the specimen, use a systematic pattern to search the slide.
- If all else fails, ask your instructor for help.

EXERCISE C Preparing a Wet-Mount Slide

A wet-mount slide is a temporary preparation. Specimens are mounted in a drop of liquid and are covered with a coverslip.

Objectives

☐ Learn the proper technique for making a wet-mount slide.

Procedure

1. Place a drop of water in the center of a clean microscope slide. To the drop, add a torn piece of an *Elodea* leaf.
2. Place one edge of a coverslip at the edge of the water drop and gently lower it so that the water containing the specimen completely spreads out under the coverslip (Figure 1C-1). Take care not to trap bubbles under or around the specimen. Do not press down on the coverslip. If there is too much water, draw off the excess by touching the corner of a paper towel to the edge of the coverslip.
3. Examine your wet mount under low power and then under high power. Remember, care must be taken to avoid touching the coverslip with the objective lens. Not only will it move your specimen, it might break the coverslip or scratch the objective lens.

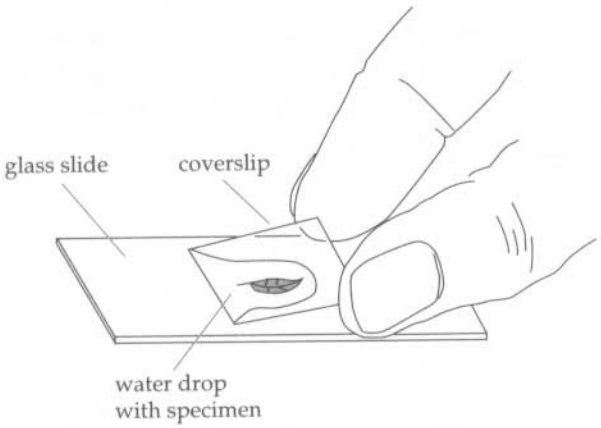


Figure 1C-1 *Technique for preparing a wet-mount slide.*

a. Give three reasons for using a coverslip when preparing a wet mount.

4. Use the 10× objective to focus the torn edge of the *Elodea* leaf. Notice that only part of the whole thickness of the leaf is in focus. Using the fine adjustment knob, focus on various planes throughout the thickness of the leaf. Note that the leaf is more than one cell layer thick.
5. Now switch to your 40× objective. You should notice that even less of the leaf's thickness is in focus at any one setting. What you have just accomplished is a demonstration of a principle of magnification that was discussed earlier: the higher the magnification, the shallower is the depth of field (depth of the area that is clearly focused).
6. With the *Elodea* leaf still in position, notice the movement of the little green bodies inside each cell (Figure 1C-2). These are chloroplasts, organelles responsible for photosynthesis in plant cells. The movement you observe is called **cyclosis,** or cytoplasmic streaming. As the cytoplasm moves around the large central vacuole, it carries with it dissolved substances as well as suspended organelles. Does cyclosis occur in the same direction in all cells?

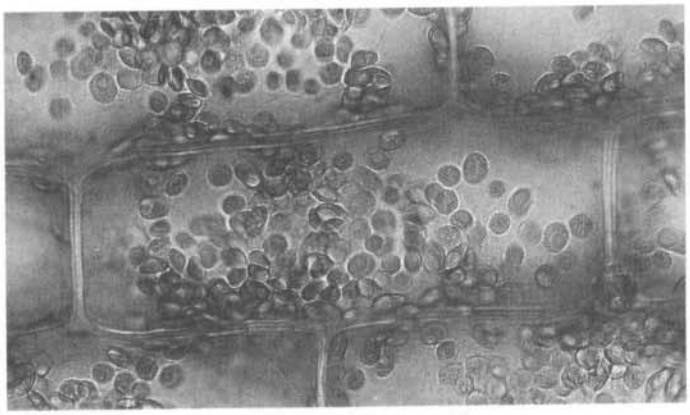


Figure 1C-2 *A cell of an* Elodea *leaf. The small round bodies in the cell are chloroplasts.*

b. What might be the practical advantage of cyclosis to the cells of the leaf?

7. Now replace the liquid around your specimen with a concentrated salt solution (Figure 1C-3). Place a drop of the concentrated salt solution at the edge of your *Elodea* wet mount. Draw the

solution under the coverslip by placing the corner of a paper towel at the opposite edge. Watch closely to see what happens to the cells of the leaf. You will probably see the cytoplasm shrink away from the cell walls. This phenomenon is called **plasmolysis.**

c. Does cyclosis continue or does it stop? _____

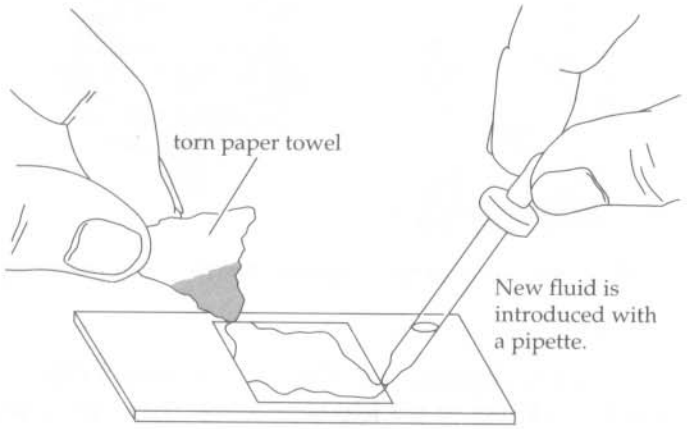


Figure 1C-3 *The solution under a coverslip can be changed without removing the coverslip by using a piece of torn paper towel to absorb the fluid at one corner edge of the coverslip, and introducing new solution with a pipette or eyedropper near the opposite corner.*

EXTENDING YOUR INVESTIGATION: MEASURING CYCLOSIS

In steps 6 and 7 of this exercise, you observed cyclosis. Do you think that environmental conditions can affect natural functions of cells, including such processes as cyclosis? *Does temperature affect cyclosis? Does light affect cyclosis?* Choose one of these questions and formulate a hypothesis to explore it. (Refer to Laboratory I, Science—A Process.)

HYPOTHESIS:

NULL HYPOTHESIS:

What do you **predict** will happen to the rate of cyclosis for the conditions you have chosen to investigate?

Identify the **independent variable** in this experiment.

Identify the **dependent variable** in this experiment.

Design an experimental procedure to test your hypothesis. (How might you measure cyclosis? You might consider the rate of chloroplast movement as a measure of cyclosis activity.)

PROCEDURE:

Now, determine the rate of cyclosis. (How many measurements should you make?)

RESULTS:

From your results, describe what happened to the rate of cyclosis given the environmental conditions you chose. (Graphing your data may make your results easier to interpret.)

Do your results support your hypothesis?

Your null hypothesis?

Was your prediction correct?

What do you **conclude** about the effects of varied environmental conditions on cyclosis?

8. If pond water, algal, or protozoan cultures are available, make wet mounts of these by placing one drop of culture medium on a clean slide and applying a coverslip. Some of the unicellular organisms you will see are protozoans, which may move very rapidly across the microscope's field. It may be necessary to slow these organisms by adding a drop of methyl cellulose (Protoslo) to the culture drop (mix with a toothpick).

   On a separate piece of paper, draw, in as much detail as possible, one or more of the organisms you observe and insert your drawing into the laboratory manual.

9. Turn off the microscope light, coil the cord as directed by your instructor, and return your specimens and microscope to the proper place.

✓ EXERCISE D Measuring the Size of Objects Using the Compound Microscope

The microscope can be used as a tool to gather quantitative data in addition to serving as an instrument for making qualitative observations.

Objectives

- ☐ Determine the diameter of the field of view for microscope objectives.
- ☐ Estimate the size of an object from the diameter of the field of view.
- ☐ Accurately determine the size of an object using an ocular micrometer.

Procedure

The size of objects viewed with the compound microscope can be estimated by first determining the diameter of the field of view (see Exercise B) for a particular microscope objective and then estimating the size of the specimen by comparing it with the total diameter of the field of view.

1. Place a transparent ruler across the field of view under scanning power and record the diameter in millimeters: _____ What is the diameter in micrometers? _____ The diameter of the field of view using the scanning objective (A) can be used to calculate the

diameter using any other objective (B) (*Recall:* total magnification = objective magnification × ocular magnification):

$$\frac{\text{Total magnification A}}{\text{Total magnification B}} \times \text{diameter A } (\mu\text{m}) = \text{diameter B } (\mu\text{m})$$

2. Calculate the diameters of the fields of view using the other objectives on your microscope.

Objective	Diameter (μm)

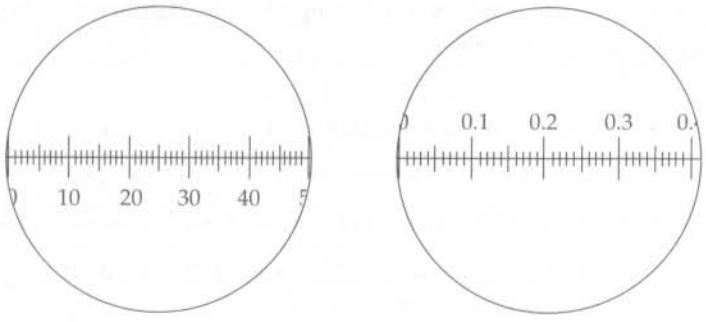
3. Obtain a prepared slide of *Nostoc,* a type of cyanobacterium. Estimate the length of one cell in micrometers (*Hint:* Use the diameter of the field of view to determine the length of a strand of cells, then divide by the number of cells): _____ μm

   a. If a cell measures 10 μm at 100×, what is the length at 200×? _____

4. To determine the diameter of the field of view more accurately, you can use an **ocular micrometer,** a glass disk with a scale etched into it. It is placed in the ocular of the microscope and is visible when you look through the ocular. The ocular micrometer appears as in Figure 1D-1a. Notice there are numbered divisions, but no units per division.

   Due to variations among microscopes, an ocular micrometer must be **calibrated** for the microscope with which it will be used. A **stage micrometer** is used to calibrate an ocular micrometer. The stage micrometer (Figure 1D-1b) looks like a microscope slide but has a standard scale etched into it. The smallest divisions are 0.01 mm in length. It is just like a tiny ruler!

Figure 1D-1 *Ocular micrometer and stage micrometer.*



(a) the ocular micrometer (b) the stage micrometer

You can calibrate an ocular micrometer as follows. Using your scanning objective (4×), look through the ocular containing the micrometer and focus on the stage micrometer. Now move your objective to low power (10×) and refocus on the micrometer using the fine adjustment knob. The two scales should appear to be superimposed on one another. Move the stage micrometer to match up its left end with the left end of the ocular micrometer

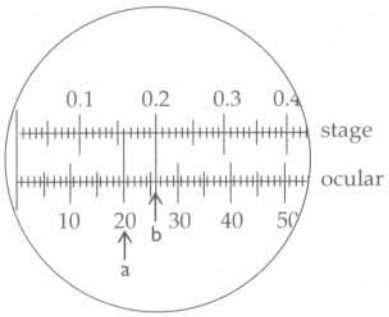


Figure 1D-2 *Ocular and stage micrometers are aligned for calibration. (For clarity, the stage and ocular micrometers are shown separated here; as you look through the ocular, the two scales should appear superimposed.)*

(Figure 1D-2). The actual distance between the lines of the ocular micrometer can be calculated by finding a line on the ocular scale that aligns exactly with a line on the stage micrometer scale.

Example In Figure 1D-2 observe that 20 spaces (**a**) on the ocular micrometer equal 0.16 mm and that 26 spaces (**b**) equal 0.2 mm. Therefore, 1 space on the ocular scale equals:

$$\frac{0.16}{20} \text{ or } \frac{0.20}{26} = 0.008 \text{ mm} = 8\ \mu\text{m}$$

Your instructor has already calibrated the ocular micrometer (using the 10× objective) in one of the microscopes on demonstration. Note that the value for a division varies for different objectives. *b. Why?* _____

You will need to recalibrate the ocular micrometer for each objective.

c. 10× objective: 1 division on the ocular micrometer = _____ *mm =* _____ *μm*

d. 43× objective: 1 division on the ocular micrometer = _____ *mm =* _____ *μm*

It is now possible to measure the size of a specimen by viewing it through an ocular containing an ocular micrometer that has been calibrated.

5. Remove the stage micrometer. Using low power, locate a filament of *Nostoc* and bring it into clear focus. Switch to high power and, as accurately as possible, count the number of ocular micrometer divisions from one end of a cell to the other or the number of cells that reach from one division to the next. (Each cell is approximately the same size.) For example, if you had a cell that fit between the 0 and the 25 on your ocular, you could then determine its length. If you had calibrated the ocular at 43× and each division was equal to 2 μm, then to determine the length of the cell you would multiply the number of divisions the cell covered by the measurement of each division: 25 × 2 μm = 50 μm.

   You determined that each division of the ocular micrometer equals _____ μm.

   Therefore, the length of one *Nostoc* cell = _____ μm. Show your work in the space below.

✓ EXERCISE E The Stereoscopic Dissecting Microscope

Most of the biological specimens you observe through the compound microscope are very thin and can almost be considered two-dimensional. Larger specimens, including whole organisms, can be viewed more easily using the **stereoscopic microscope.** Dissection is often done using this microscope. Thus, it is sometimes called a **dissecting microscope.**

The dissecting microscope is really two microscopes in one—each eyepiece has its own objective and ocular lenses. Each eye is presented with its own image. Human beings ordinarily see in three dimensions by virtue of receiving a slightly different image in each eye. This possibility is lost in the compound microscope with its single objective lens for both eyes, but is permitted by the design of the dissecting microscope.

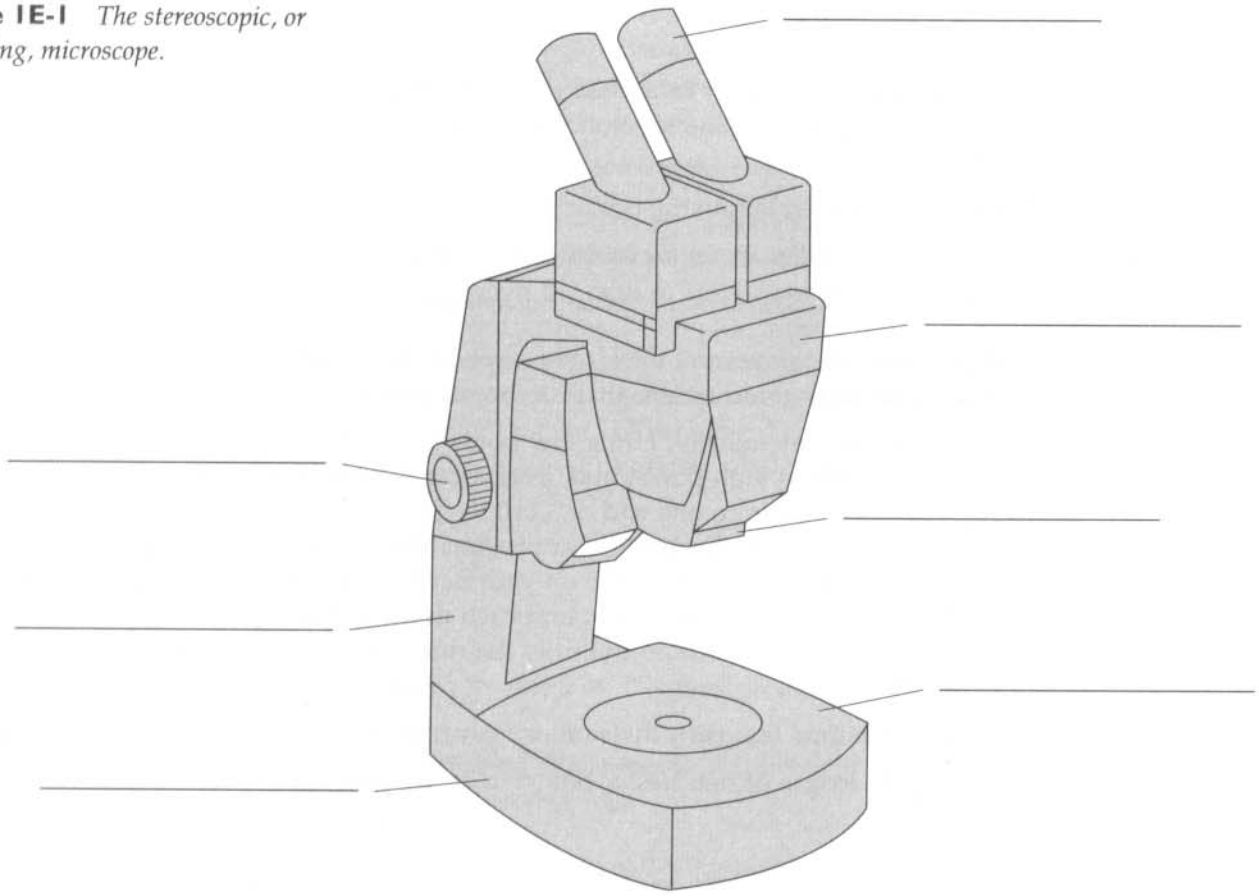
Objectives

- ☐ Locate and describe the function of each part of a dissecting microscope.
- ☐ Learn to use the dissecting microscope properly.

Procedure

1. Use your knowledge of the compound microscope to identify the parts of the dissecting microscope. Write the names of the parts on the lines provided in Figure 1E-1: **eyepiece** or **ocular, focus knob, body tube, arm, objective, stage,** and **base.**

Figure 1E-1 *The stereoscopic, or dissecting, microscope.*



2. Find the **magnification changer** on your microscope. Most, but not all, dissecting microscopes have this feature. If present, it will be located on the side or top of the microscope. Draw the magnification changer on Figure 1E-1. What final magnification can your dissecting microscope achieve?
3. The **light source** for your microscope may be a separate light. Light can be **transmitted** through a specimen on the microscope stage by proper adjustment of the mirror below the

stage. Light can be **reflected** from the surface of a specimen by positioning the light source above the specimen so that it shines downward onto the microscope stage. If your illuminator is built into the microscope, light will be adjusted by two switches, the **transmitted light** switch and the **reflected light** switch. Familiarize yourself with each of these and add them to Figure 1E-1.

4. Select a three-dimensional specimen from those provided. Center your specimen on the stage plate.
5. Grasp both ocular housings and move them together or apart to adjust the spacing until you are comfortable using both eyes simultaneously to view a single field.
6. Try using both reflected and transmitted light to illuminate your specimen.

   a. Which works better? _____ *Why?* _____

   _____

7. Use the coarse adjustment knob to focus. There is no fine adjustment knob on the stereoscopic microscope.
8. If possible, change to a higher magnification and refocus as necessary to view different portions of your specimen.
9. Sketch your specimen in the space below.

10. Turn off the microscope light, coil the cord as directed by your instructor, and return your specimens and microscope (and its light) to the proper place.

Laboratory Review Questions and Problems

1. What is the resultant total magnification of an object as seen through a microscope with 10× oculars and each of the following objectives? *a.* 4× _____ *b.* 10× _____ *c.* 43× _____

2. Describe the difference between magnification and resolution.

3. Which part of the microscope is most important in determining its resolving power? Why?

4. You are interested in purchasing a microscope. The salesperson shows you several microscopes, each with one of the following objectives. Which would you want to purchase?

(a) (b) (c)

5. Briefly describe the function of each of the following parts of the microscope.
Objective

Ocular

Iris diaphragm

6. In which direction do you turn the coarse focus adjustment knob to move the objective *away* from your slide? _____ In which direction do you turn the fine adjustment? _____

7. What is the purpose of the condenser on a microscope? Relate this to the angle θ and to the numerical aperture (NA) of the objective.

8. The resolution of a microscope objective depends on numerical aperture. Is the resolution better with a high NA or low NA?

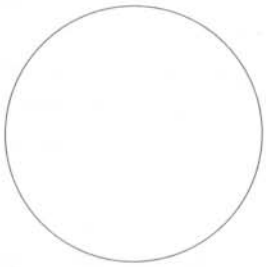
9. You are using a microscope with a 100× objective that has a numerical aperture of 0.71. You place a dot of oil ($n = 2$) on the coverslip of the slide you want to observe. What is the resolving power of the 100× objective if you are using light of wavelength 560 nm?

Which is preferable: a large or small R? _____ Why?

10. Suppose your 10× objective has an NA of 0.25 and you are using a green filter that limits the wavelength of light striking the objective to 560 nm. What is the resolving power for this objective? _____ What type of dimensions does your answer have? _____ This is the theoretical value for R. Suppose $\theta = 26°$. What would be the true resolution for your system? _____

11. What is meant by *field of view?* What will happen to the field of view for each resultant magnification as you change objectives from 4× to 10× to 43×?

12. Using your 4× objective, you measure the diameter of the field of view to be 3 mm. You notice that the length of a large letter **E** takes up half the width of the diameter of your field at 10×. The oculars on your microscope are 10×. What is the size of the letter **E**? Draw what this letter would look like through the microscope.



13. You use an ocular micrometer to measure the size of a cell, as seen below. The stage micrometer is divided into 0.1-mm units. Which of the following accurately represents the size of the cell? *a.* 3.2 μm; *b.* 350μm; *c.* 35 mm; *d.* 0.0035 mm.