EXERCISE

3

The Microscope

Basic Skills of Light Microscopy

Learning Objectives

By the end of this exercise you should be able to:

1. Identify and explain the functions of the primary parts of a compound microscope and dissecting (stereoscopic) microscope.
2. Carry and focus a microscope properly.
3. Use a compound microscope and dissecting microscope to examine biological specimens.
4. Prepare a wet mount, determine the magnification and size of the field of view, and determine the depth of field.

Please visit connect.mheducation.com to review online resources tailored to this lab.

Many organisms and biological structures are too small to be seen with the unaided eye (fig. 3.1). Biologists often use a light microscope to observe such specimens. A **light microscope** is a coordinated system of lenses arranged to produce an enlarged, focusable image of a specimen. A light microscope **magnifies** a specimen, meaning that it increases its apparent size. Magnification with a light microscope is usually accompanied by improved **resolution,** which is the ability to distinguish two points as separate points. Thus, the better the resolution, the sharper or crisper the image appears. The resolving power of the unaided eye is approximately 0.1 mm (1 in = 25.4 mm), meaning that our eyes can distinguish two points that are 0.1 mm apart. A light microscope, used properly, can improve resolution as much as 1000-fold (i.e., to 0.1 μm).

The ability to discern detail also depends on **contrast,** which is the difference between the lightest and darkest parts of an image. Therefore, many specimens examined with a light microscope are stained with artificial dyes that increase contrast and make the specimen more visible.

The invention of the light microscope was profoundly important to biology because it was used to formulate the cell theory and study biological structure at the cellular level. Light microscopy has revealed a vast new world to the human eye and mind (fig. 3.2). Today, the light microscope is the most fundamental tool of many biologists.

THE COMPOUND LIGHT MICROSCOPE

Study and learn the parts of the typical compound light microscope shown in figure 3.3. A light microscope has two, sometimes three, systems: an illuminating system, an imaging system, and possibly a viewing and recording system.

Illuminating System

The illuminating system, which concentrates light on the specimen, usually consists of a light source, condenser lens, and iris diaphragm. The **light source** is a lightbulb located

Caring for Your Microscope

Microscopes are powerful tools for understanding biology. However, they're also expensive and fragile and require special care. When you use your microscope, always do the following to ensure optimal performance and care:

- Always carry your microscope upright with both hands—one hand under the base and the other around the microscope's arm (fig. 3.3).
- Always begin by cleaning the ocular and objective lenses with lens paper.
- Always start your examinations with the low-power objective in place.
- If you shift to the high-power objective, rotate the objective into place carefully. Never force the objective lens into place. If the objective lens contacts the slide, stop and restart your examination with the low-power objective lens.
- After shifting to the high-power objective, always use only the fine adjustment to focus the image.
- When you've completed your work with the microscope, clean the lenses with lens paper, wrap the electrical cord securely around the microscope's arm, and return your microscope to its storage area.

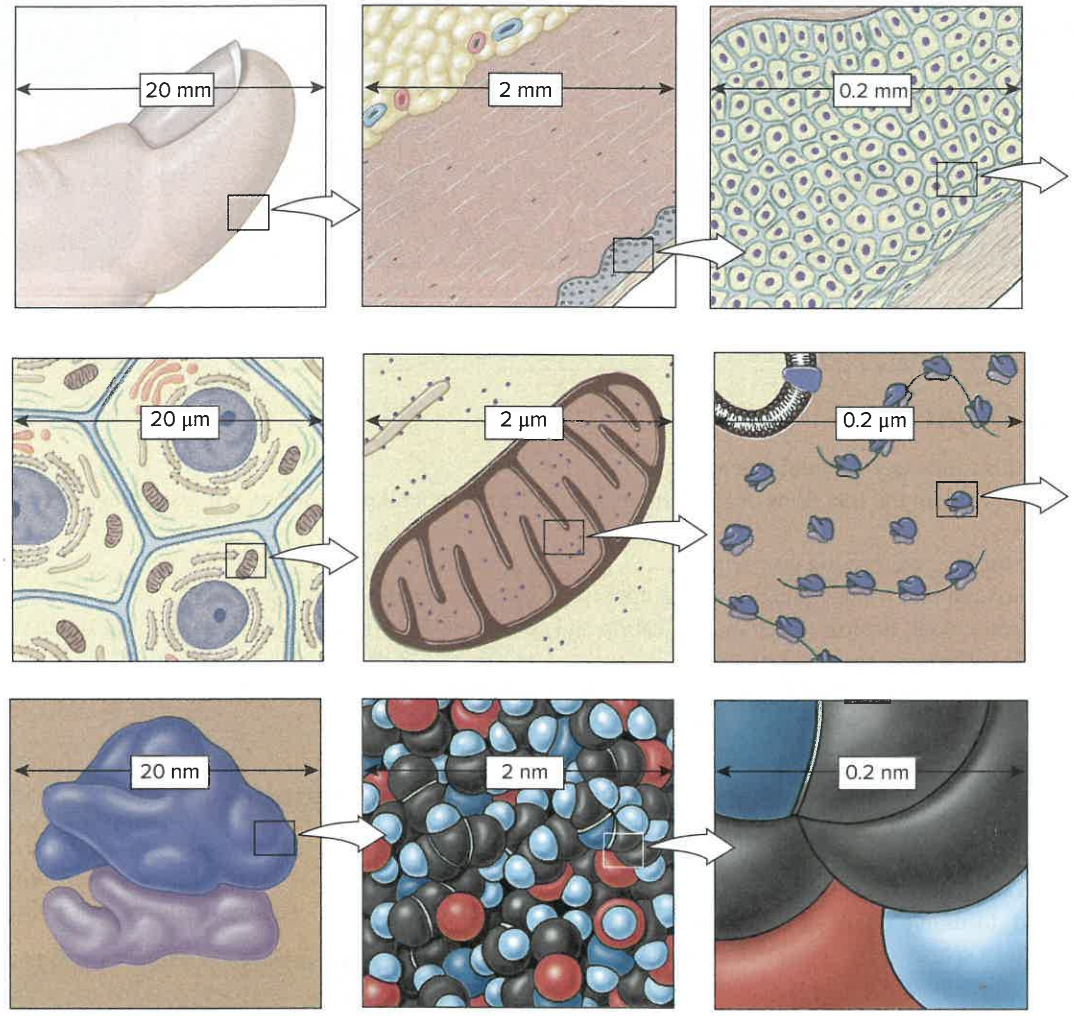


Figure 3.1 The size of cells and their contents. This diagram shows the size of human skin cells, organelles, and molecules. In general, the diameter of a human skin cell is about 20 micrometers (µm), of a mitochondrion is 2 µm, of a ribosome is 20 nanometers (nm), of a protein molecule is 2 nm, and of an atom is 0.2 nm.

at the base of the microscope. The light source illuminates the specimen by passing light through a thin, almost transparent part of the specimen. The **condenser lens,** located immediately below the specimen, focuses light from the light source onto the specimen. Just below the condenser is the **condenser iris diaphragm,** a knurled ring or lever that can be opened and closed to regulate the amount of light reaching the specimen. When the condenser iris diaphragm is open, the image will be bright; when closed, the image will be dim.

Imaging System

The imaging system improves resolution and magnifies the image. It consists of the objective and ocular (eyepiece) lenses and a body tube. The objectives are three or four lenses mounted on a revolving nosepiece. Each objective is a series of several lenses that magnify the image, improve resolution, and correct aberrations in the image. The most common configuration for student microscopes includes four objectives: low magnification (4×), medium magnification (10×), high magnification (40×), and oil immersion (100×). Using the oil immersion objective requires special instructions, as explained in Exercise 24 to study bacteria. To avoid damaging your microscope, do not use the oil immersion objective during this exercise.

The magnifying power of each objective is etched on the side of the lens (e.g., 4×). The **ocular** is the lens that you look through. Microscopes with one ocular are **monocular** microscopes, and those with two are **binocular** microscopes. Oculars usually magnify the image 10 times. The **body tube** is a metal casing through which light passes to the oculars. In microscopes with bent bodytubes and inclined oculars, the body tube contains mirrors and a prism that redirect light to the oculars. The **stage** secures the glass slide on which the specimen is mounted.

Figure 3.2 "Egad, I thought it was tea, but I see I've been drinking a blooming micro-zoo!" says this horrified, proper 19th-century London woman when she used a microscope to examine her tea. People were shocked to learn that there is an active, living world too small for us to see.

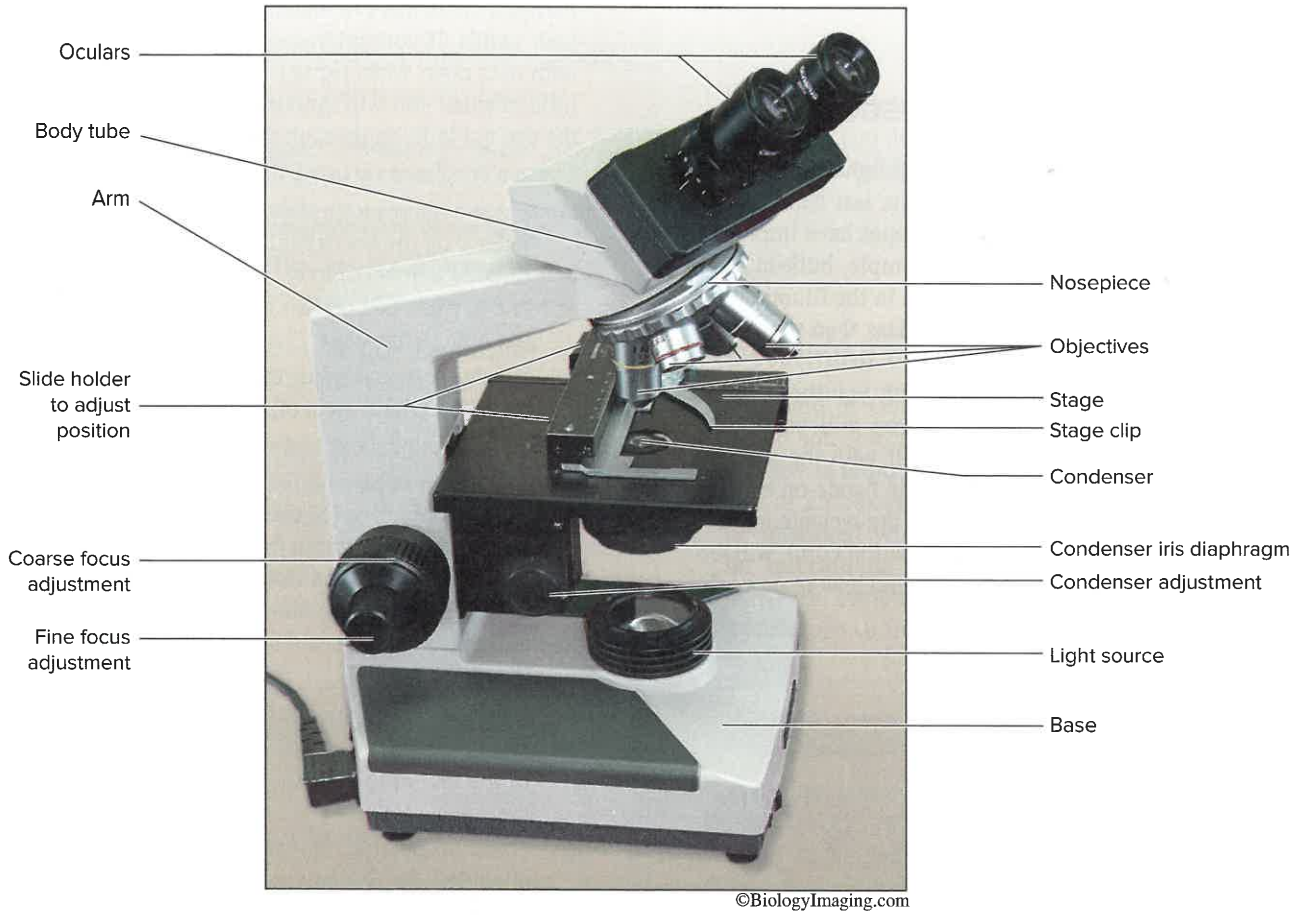


Figure 3.3 Major parts of a compound light microscope.

A Summary of How to Use a Compound Light Microscope

1. Place the specimen on the microscope's stage.
2. Rotate the low-power objective into place. Center the specimen below the objective.
3. Look through the oculars while using the coarse adjustment to focus on the specimen. Center the area of the specimen that you want to examine.
4. Slowly rotate the high-power objective into place. Look through the oculars while you use the fine adjustment to focus on the specimen.
5. If you "lose" your specimen when you switch from low power to high power, retrace the previous steps, paying special attention to placing the specimen in the center of the field of view.

Viewing and Recording System

The viewing and recording system, if present, converts radiation to a viewable and/or permanent image. The viewing and recording system usually consists of a camera or video screen. Most student microscopes do not have viewing and recording systems.

USING A COMPOUND MICROSCOPE

Although the maximum magnification of light microscopes has not increased significantly during the last century, the construction and design of light microscopes have improved the resolution of newer models. For example, built-in light sources have replaced adjustable mirrors in the illuminating system, and lenses are made of better glass than they were in the past.

Your lab instructor will review with you the parts of the microscopes (and their functions) you will use in the lab (fig. 3.3). After familiarizing yourself with the parts of a microscope, you're now ready for some hands-on experience with the instrument. The videos at the website associated with this manual (connect.mheducation.com) will be especially useful for helping you understand how to properly use your microscope.

Procedure 3.1 Use a compound microscope

1. Remove the microscope from its cabinet and carry it upright with one hand grasping the arm and your other hand supporting the microscope below its base. Place your microscope on the table in front of you.

Do not use paper towels or Kimwipes to clean the lenses of your microscope; they can scratch the lenses. Clean the lenses only with lens paper.

2. Plug in the microscope and turn on the light source.
3. If it isn't already in position, rotate the nosepiece until the lowest-power (4×) objective is in line with the light source. (The 4× objective is often called the "scanning objective" because it enables users to scan large areas of a specimen.) You'll feel the objective click into place when it is positioned properly. *Always begin examining slides with the lowest-power objective.*
4. Locate the coarse adjustment knob on the side of the microscope. Depending on the type of microscope that you're using, the coarse adjustment knob moves either the nosepiece (with its objectives) or the stage to focus the lenses on the specimen. Only a partial turn of the coarse adjustment knob moves the stage or nosepiece a relatively large distance. *The coarse adjustment should only be used when you're viewing a specimen with the 4× or 10× objective lens.*
5. If your microscope is binocular, adjust the distance between the oculars to match the distance between your pupils. If your microscope is monocular, keep both eyes open when using the microscope. After a little practice you will ignore the image received by the eye not looking through the ocular.
6. Focus a specimen by using the following steps:
   a. Place a microscope slide of newsprint of the letter *e* on the horizontal stage so that the *e* is directly below the lowest-power objective lens and is right side up. It should be centered over the hole in the stage.
   b. Rotate the coarse adjustment knob to move the objective within 1 cm of the stage (1 cm = 0.4 in).
   c. Look through the oculars with both eyes open.
   d. Rotate the coarse adjustment knob (i.e., raising the objective lens or lowering the stage) until the *e* comes into focus. If you don't see an image, the *e* is probably off center. Be sure that the *e* is directly below the objective lens and that you can see a spot of light surrounding the *e*.
   e. Focus up and down to achieve the crispest image.
   f. Adjust the condenser iris diaphragm so that the brightness of the transmitted light provides the best view.
   g. Observe the letter, then rotate the nosepiece to align the 10× objective to finish your observation. Do not use the oil immersion objective.

Question 1

a. As you view the letter *e*, how is it oriented? Upside down or right side up?

b. How does the image move when the slide is moved to the right or left? Toward you or away from you?

c. What happens to the brightness of the view when you go from 4× to 10×?

Magnification

Procedure 3.2 Determine magnification

1. Estimate the magnification of the *e* by looking at the magnified image on lowest magnification (4×), and then at the *e* without using the microscope.
2. Examine each objective and record the magnifications of the objectives and oculars of your microscope in table 3.1.
3. Calculate and record in table 3.1 the total magnification for each objective following this formula:

$$\mathbf{Mag_{Tot} = Mag_{Obj} \times Mag_{Ocu}}$$

where

Mag_{Tot} = total magnification of the image
Mag_{Obj} = magnification of the objective lens
Mag_{Ocu} = magnification of the ocular lens

For example, if you're viewing the specimen with a 4× objective lens and a 10× ocular, the total magnification of the image is 4 × 10 = 40×. That is, the specimen appears 40 times larger than it is.

4. Slowly rotate the high-power (i.e., 40×) objective into place. *Be sure that the objective does not touch the slide!* If the objective does not rotate into place without touching the slide, do not force it; ask your lab instructor to help you. After the 40× objective is in place, you should notice that the image remains near focus and that the field-of-view has gotten smaller. Most light microscopes are **parfocal,** meaning that the image will remain nearly focused after the 40× objective lens is moved into place. Most light microscopes are also **parcentered,** meaning that the image will remain centered in the field of view after the 40× objective lens is in place.
5. You may need to readjust the iris diaphragm because the high-magnification objective allows less light to pass through to the ocular.
6. To fine-focus the image, locate the **fine adjustment knob** on the side of the microscope. Turning this knob changes the specimen-to-objective distance slightly and therefore makes it easy to fine-focus the image. **Use only the fine adjustment when using the 40× (or higher) objective.**

Never use the coarse adjustment knob to focus an image on high power.

Question 2

a. How many times is the image of the *e* magnified when viewed through the high-power objective?

b. If you didn't already know what you were looking at, could you determine at this magnification that you were looking at a letter *e*? How?

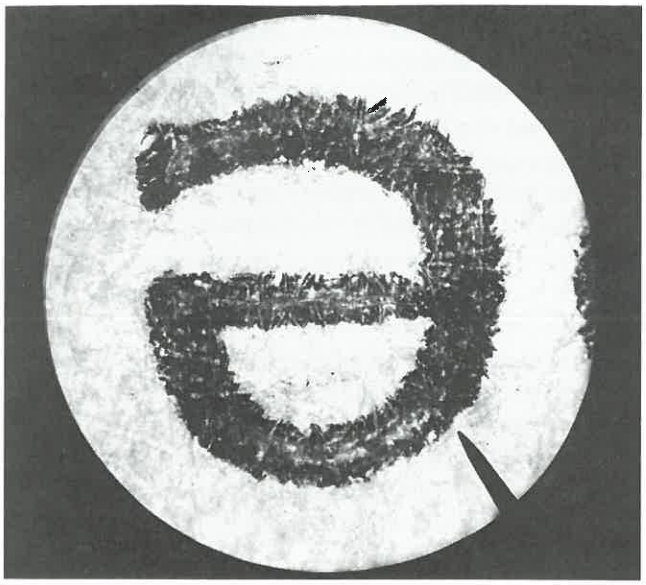
Determine the Size of the Field of View

The **field of view** is the area that you can see through the ocular and objective (fig. 3.4). Knowing the size of the field of view is important because you can use it to estimate the size of an object you are examining. The field of view can be measured with ruled **micrometers** (fig. 3.5). An **ocular micrometer** is a small glass disk with thin lines numbered and etched in a row. It was put into an ocular on your microscope so that the lines superimpose on the image and

Table 3.1

Total Magnifications and Areas of Field of View (FOV) for Three Objectives

Objective Power	Objective Magnification	×	Ocular Magnification	=	Total Magnification	FOV Diameter (mm)	FOV Area (mm²)	Measurement (mm) for 1 Ocular Space
4×	_____	×	_____	=	_____	_____	_____	_____
10×	_____	×	_____	=	_____	_____	_____	_____
40×	_____	×	_____	=	_____	_____	_____	_____



©BiologyImaging.com

Figure 3.4 The circular, illuminated field of view of a compound light microscope. Shown here is the letter *e* from newsprint that is magnified 40 times (i.e., 40×).

allow you to measure the specimen. Before you can use the micrometer you must determine for each magnification the apparent distance between the lines on the ocular micrometer. This means that you must calibrate the ocular micrometer by comparing its lines to those lines on a standard ruler called a **stage micrometer.** A stage micrometer is a glass slide having precisely spaced lines etched at known intervals.

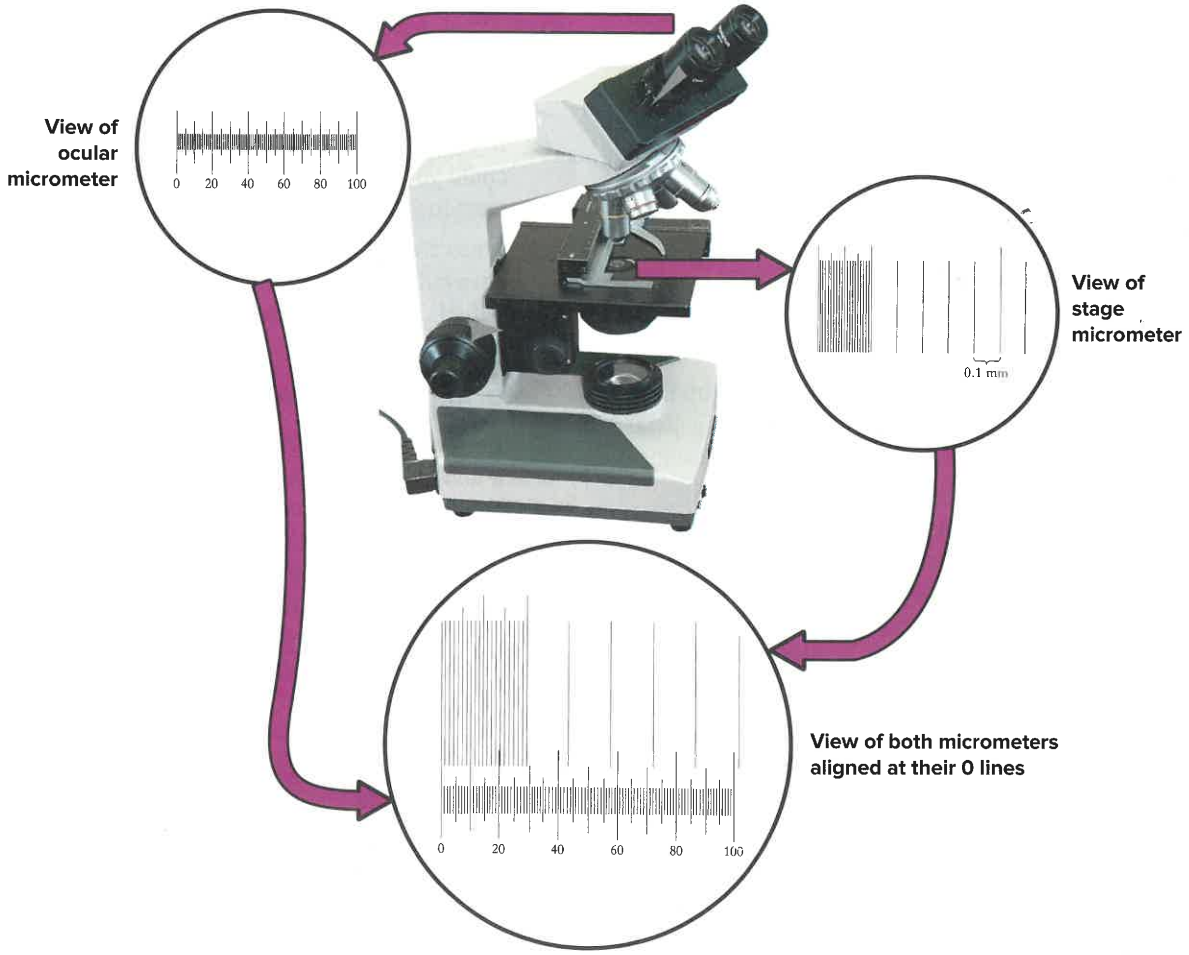
Procedure 3.3 Use a stage micrometer to calibrate the ocular micrometer, and determine the size of the field of view

1. Rotate the ocular until the lines of the ocular micrometer parallel those of the stage micrometer (fig. 3.5).
2. Align lines at the left edges (0 lines) of the two micrometers by moving the stage micrometer (fig. 3.5).
3. Count how many spaces on the stage micrometer fit precisely in a given number of spaces on the ocular micrometer. Record the values here.

y ocular spaces = x stage spaces

$y =$ _____

$x =$ _____



©BiologyImaging.com

Figure 3.5 Stage and ocular micrometers. Micrometers are used to calibrate microscopes and measure the size of specimens.

The smallest space on a stage micrometer = 0.01 mm, so

$$y \text{ ocular spaces (mm)} = x \text{ stage spaces} \times 0.01$$

$$1 \text{ ocular space (mm)} = (x/y) \times 0.01$$

4. Calculate the distance in millimeters between lines of the ocular micrometer. For example, if the length of 10 spaces on the ocular micrometer equals the length of seven spaces on the stage micrometer, then

$$y = 10$$

$$x = 7$$

$$10 \text{ ocular spaces (mm)} = 7 \text{ stage spaces} \times 0.01 \text{ mm}$$

$$1 \text{ ocular space (mm)} = (7 \times 0.01 \text{ mm})/10$$

$$1 \text{ ocular space (mm)} = 0.007 \text{ mm}$$

$$1 \text{ ocular space} = 7 \text{ μm}$$

Therefore, if a specimen spans eight spaces on your ocular micrometer with that objective in place, that specimen is 56 μm long.

5. Calibrate the ocular micrometer for each objective on your microscope. Record in table 3.1 the diameter of the field of view (FOV) for each objective. Also record for each objective lens in table 3.1 the measurement (mm) for 1 ocular space. You can use this information in future labs as you measure the sizes of organisms and their parts.
6. Calculate the radius, which is half the diameter.
7. Use this information to determine the area of the circular field of view with the following formula:

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

8. Record your calculated FOV areas in table 3.1.

Alternate Procedure 3.3 Use a transparent ruler to determine the size of the field of view

1. Obtain a clear plastic ruler with a metric scale.
2. Place the ruler on the stage and under the stage clips of your microscope. If your microscope has a mechanical stage, ask your instructor how to place the ruler to avoid damage. *Carefully rotate the nosepiece to the objective of lowest magnification.*
3. Slowly focus with the coarse adjustment, and then with the fine adjustment, until the metric markings on the ruler are clear.
4. Align the ruler to measure the diameter of the circular field of view. The space between each line on the ruler should represent a 1-mm interval.
5. Record in table 3.1 the diameter of this low-magnification field of view. Also calculate the radius, which is half the diameter.
6. The ruler cannot be used to measure the diameters of the field of view at medium and high magnifications because the markings are too far apart. Therefore, these diameters must be calculated using the following formula:

$$\text{FOV}_{\text{low}} \times \text{Mag}_{\text{low}} = \text{FOV}_{\text{high}} \times \text{Mag}_{\text{high}}$$

where

FOV_{low} = **diameter of the field of view of the low-power objective**

Mag_{low} = **magnification of the low-power objective (Be consistent and use the magnification of the objective, not total magnification.)**

FOV_{high} = **diameter of the field of view of the high-power objective**

Mag_{high} = **magnification of the high-power objective**

For example, if 3.0 mm is the diameter of the field of view for a 4× low-power objective, then what is the diameter of the field of view of the 40× high-power objective?

$$3.0 \text{ mm} \times 4 = \text{FOV}_{\text{high}} \times 40$$
$$0.30 \text{ mm} = \text{FOV}_{\text{high}}$$

7. Calculate and record in table 3.1 the diameters of the field of view for the 10× and 40× magnifications.
8. Calculate and record in table 3.1 the circular area of the field of view for the three magnifications by using the following formula.

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

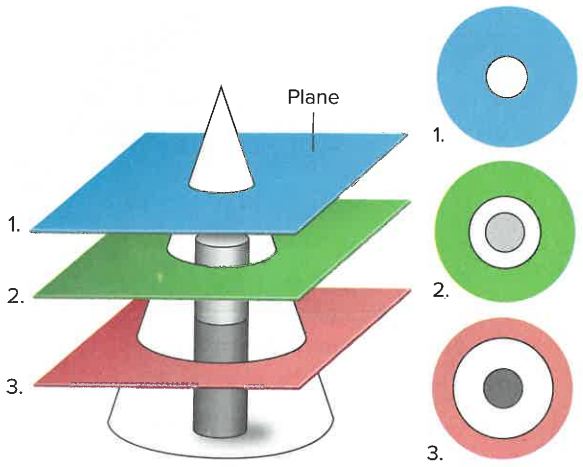
Question 3

a. Which provides the largest field of view, the 10× or 40× objective?

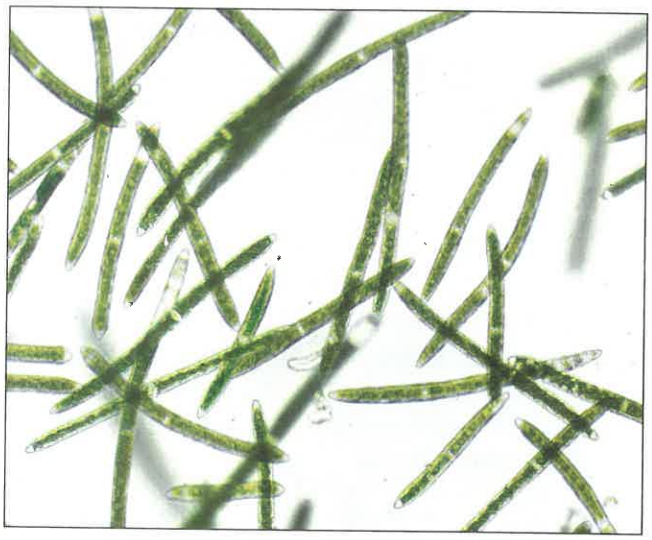
b. How much more area can you see with the 4× objective than with the 40× objective?

c. Why is it more difficult to locate an object starting with the high-power objective than with the low-power objective?

d. Which objective should you use to initially locate the specimen? Why?



(a)



(b) ©BiologyImaging.com

Figure 3.6 How focusing at different planes of a specimen would produce three different images. (*a*) Focusing up and down when you view a specimen can help you to understand its three-dimensional structure. (*b*) A thin depth of field is apparent in this 100× image of cells of *Closterium*, a green alga. The upper and lower layers of cells are out of focus, while the midlayer of cells is within the thin depth of field and is clearly focused.

Determine the Depth of Field

Depth of field is the thickness of the object in sharp focus (fig. 3.6). Depth of field varies with different objectives and magnifications.

Procedure 3.4 Determine the depth of the field of view

1. Using the low-power objective, examine a prepared slide of three colored threads mounted on top of each other.
2. Focus up and down and try to determine the order of the threads from top to bottom. The order of the threads will not be the same on all slides.
3. Re-examine the threads using the high-power objective lens.

Question 4

a. Are all three colored threads in focus at low power?

b. Can all three threads be in focus at the same time using the high-power objective?

c. Which objective, high or low power, provides the greatest depth of field?

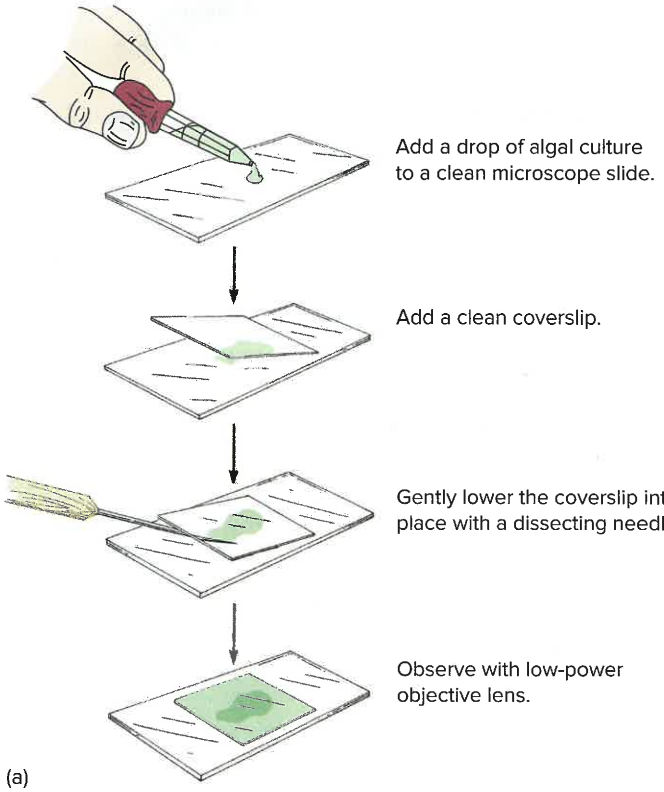
Preparing a Wet Mount of a Biological Specimen

Procedure 3.5 Prepare a wet mount of a biological specimen

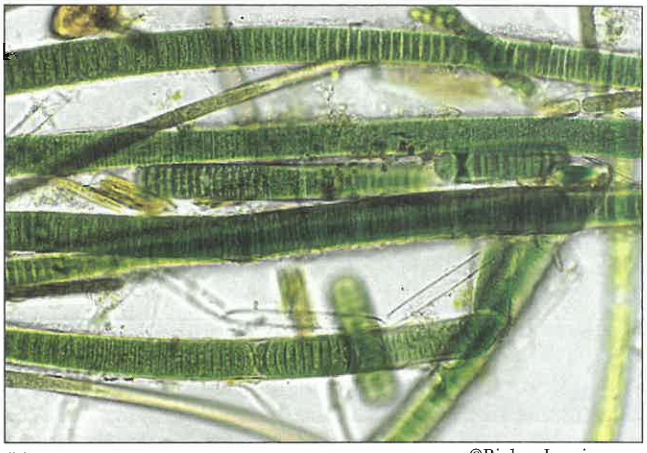
1. Place a drop of water containing algal cells from a culture labeled "Algae" on a clean microscope slide.
2. Place the edge of a clean coverslip at an edge of the drop at a 45° angle; then slowly lower the coverslip onto the drop so that no air bubbles are trapped (fig. 3.7). (Your instructor will demonstrate this technique.) The coverslip holds the specimen in place and prevents the lens of an objective from contacting the water and the specimen. This fresh preparation is called a **wet mount** and can be viewed with your microscope.
3. Experiment with various intensities of illumination. To do this, rotate the 4× objective into place and adjust the condenser iris diaphragm to produce the least illumination. Observe the image; note its clarity, contrast, and color. Repeat these observations with at least four different levels of illumination. The fourth level should have the diaphragm completely open.
4. Repeat step 3 for the 10× and 40× objectives.

Question 5

a. Is the image always best with the highest illumination?



(a)



(b) ©BiologyImaging.com

Figure 3.7 (*a*) Preparing a wet mount of a biological specimen. (*b*) A wet mount of pond water will often include the common cyanobacterium *Oscillatoria* (200×). See also figures 3.6 and 25.1–25.4.

b. Is the same level of illumination best for all magnifications?

c. Which magnifications require the most illumination for the best clarity and contrast?

5. Examine your preparation of algae, and sketch in the following field of view the organisms that you see. Don't mistake air bubbles for organisms! Air bubbles appear as uniformly round structures with dark, thick borders.

6. Prepare a wet mount of some newly hatched brine shrimp (*Artemia,* which are popularly referred to as "sea monkeys") and their eggs. Sketch in the following field of view what you see. Use your calculations for the diameter of the field of view to estimate the length of the shrimp.

Approximate length of the shrimp: _____

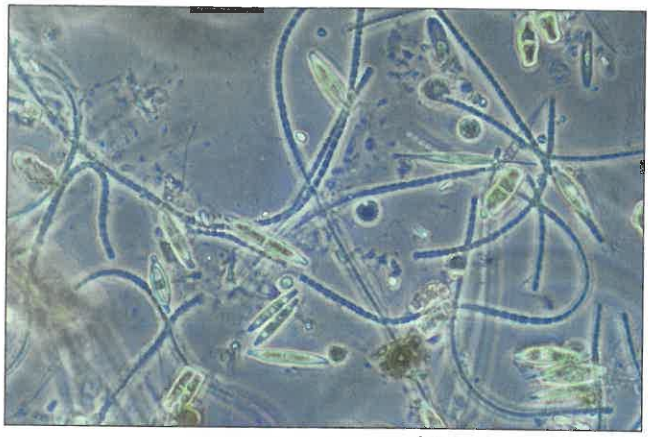
Question 6

a. Why is it important to put a coverslip over the drop of water when you prepare a wet mount? That is, what are the functions of a coverslip?

b. Approximately how long and wide is a brine shrimp?

Practice

For practice using your microscope, prepare some wet mounts of pond water or a hay infusion to view the diversity of protozoa and algae (fig. 3.8). If the protozoa are moving too fast for you to examine carefully, add a drop of methylcellulose (often sold commercially as Proto-Slo) to your sample. (The



©M. I. Walker/Science Source

Figure 3.8 The diversity of organisms in pond water (200×).

methylcellulose will slow the movement of the protozoa.) Also examine the prepared slides available in the lab. You'll examine these slides in more detail in the coming weeks, so don't worry about their contents. Rather, use this exercise to familiarize yourself with the microscope. Also prepare wet mounts of the cultures available in the lab and sketch the organisms that you see. When you've finished, turn off the light source, cover your microscope, and store the microscope in its cabinet.

THE DISSECTING (STEREOSCOPIC) MICROSCOPE

A **dissecting (stereoscopic) microscope** offers some advantages over a compound microscope. Although a compound microscope can produce high magnifications and excellent resolution, it has a small **working distance,** which is the distance between the objective lens and specimen. Therefore, it is difficult to manipulate a specimen while observing it with a compound microscope. Specimens that can be observed with a compound microscope are limited to those thin enough for light to pass through them. In contrast, a dissecting microscope is used to view objects that are opaque or too large to see with a compound microscope.

A dissecting microscope provides a much larger working distance than does a compound microscope. This distance is usually several centimeters (compared to a centimeter or less for a compound microscope), making it possible to dissect and manipulate most specimens. Also, most specimens for dissection are too thick to observe with transmitted light from a light source below the specimen. Therefore, many dissecting microscopes use a light source above the specimen; the image you see is formed from reflected light.

Dissecting microscopes are always binocular (fig. 3.9). Each ocular views the specimen at different angles through one or more objective lenses. This arrangement provides a three-dimensional image with a large depth of field. This is in contrast to the image in a compound microscope, which is basically two-dimensional. However, the advantages of a stereoscopic microscope are often offset by lower resolution and magnification than a compound microscope. Most dissecting microscopes have magnifications of 4× to 50×.

Procedure 3.6 Use a dissecting microscope

1. Carry the dissecting microscope to your desk by grasping the microscope's arm with one hand and placing your other hand under the microscope's base.
2. Use figure 3.9 to familiarize yourself with the parts of your microscope.
3. Use your dissecting microscope to examine the organisms available in the lab. Sketch some of these organisms.
4. Use a ruler to measure the diameter of the field of view with your dissecting microscope at several levels of magnification.

Question 7

a. What is the area of the field of view when you use the lowest magnification of your dissecting microscope? What about when you use the highest magnification?

b. Place a microscope slide of the letter *e* on the stage. As you view the letter *e,* how is it oriented?

c. How does the image through a dissecting microscope move when the specimen is moved to the right or left? Toward you or away from you?

d. How does the direction of illumination differ in dissecting as opposed to compound microscopes?

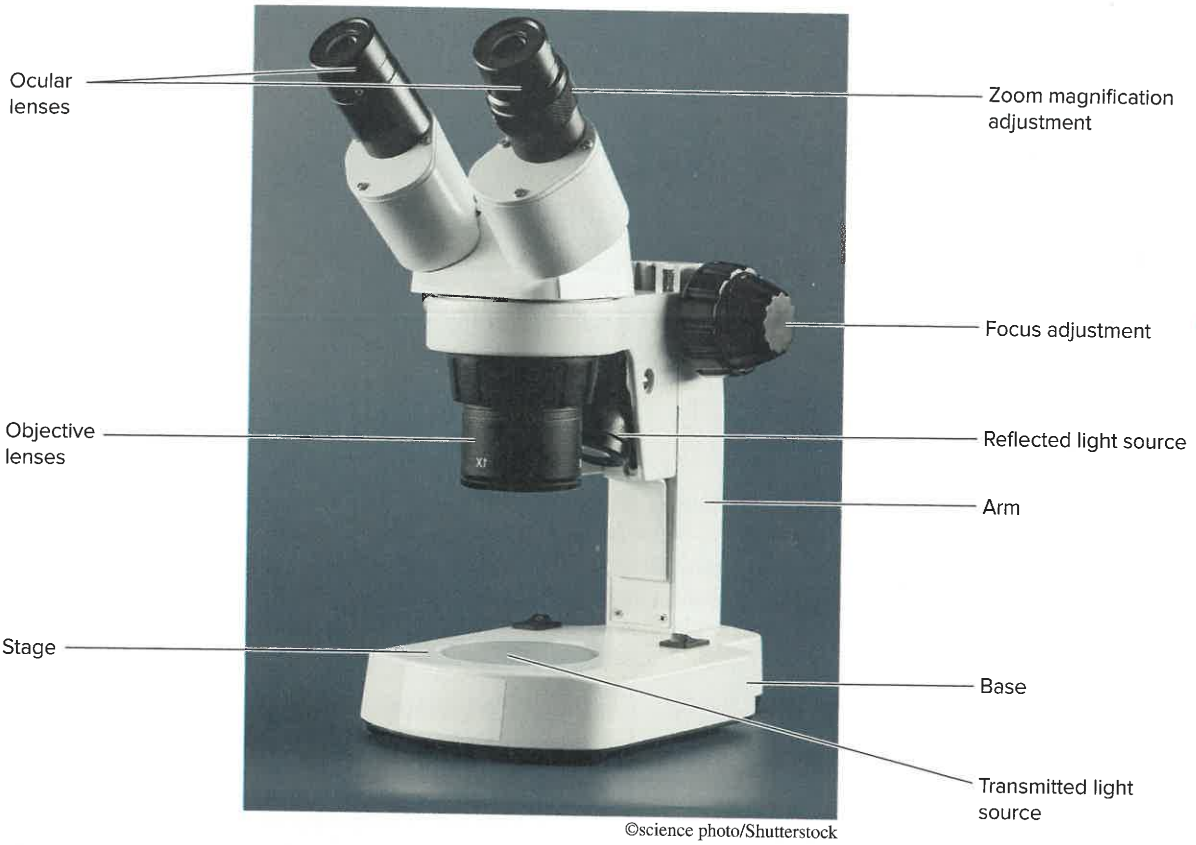
e. What are some examples of biological specimens that would be best examined with a dissecting microscope instead of a compound microscope?

A COMPARISON OF COMPOUND AND DISSECTING MICROSCOPES

Complete table 3.2 comparing magnification, resolution, size of the field of view, and depth of field of a dissecting microscope and a compound microscope. Use the terms *high, low,* or *same* to describe your comparisons.

Question 8

What other differences are there between compound and dissecting microscopes?



©science photo/Shutterstock

Figure 3.9 Major parts of a dissecting (stereoscopic) microscope.

Table 3.2

A Comparison of Dissecting and Compound Microscope

Characteristic	Dissecting Microscope	Compound Microscope
Magnification		
Resolution		
Size of field of view		
Depth of field		

INQUIRY-BASED LEARNING

What are the shapes, surface areas, and volumes of red blood cells?

Observation: Red blood cells, which are the most common type of blood cell, are used by vertebrates to deliver oxygen to body tissues. Red blood cells are filled with hemoglobin, which gives them their characteristic color.

Question: What are the shapes, surface areas, and volumes of red blood cells?

a. Establish a working lab group and obtain Inquiry-Based Learning Worksheet 3 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 3 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.