Darrell Vodopich & Randall Moore

BIOLOGY

Laboratory Manual

Third Edition

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THIRD EDITION

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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PREFACE

We designed this laboratory manual for an introductory biology course. The experiments and procedures are simple and easy to perform and are especially appropriate for large classes. Each exercise includes many photographs, traditional topics, and experiments that work. Procedures within each exercise are numerous and discrete so that an exercise can be tailored to the needs of the students, the style of the instructor, and the facilities available.

TO THE STUDENT

We hope this manual is an interesting guide to many areas of biology. As you survey these areas you'll probably spend equal amounts of time observing and experimenting. Don't hesitate to go beyond the observations that we've outlined—your future success as a scientist depends on your ability to search for and notice things that others may overlook. Now is the time to develop this ability with a mixture of hard work and relaxed observation. If you have fun, learning will come easily. And remember that this manual also is designed with your instructors in mind. Go to them often with questions—their experience is a valuable tool that you should use as you work.

TO THE INSTRUCTORS

This manual's straightforward approach emphasizes experiments and activities that optimize students' investment of time and your investment of supplies, equipment, and preparation. Simple and straightforward experiments can be the most effective if you interpret the work in depth. Most experiments can be conducted easily by a student in 3 hours. Terminology, structures, photographs, and concepts are limited to those the student can readily observe and understand. In each exercise we have included a few activities requiring a greater investment of effort if resources are available, but omitting them will not detract from the objectives.

This manual functions best with an instructor's guidance—it is not an "autotutorial" system. We've tried to guide students from observations to conclusions, and to make the transition to biological principles. But discussion and interaction between student and instructor are major components of a successful laboratory. Be sure to examine the "Questions for Further Thought and Study" in each exercise. We hope they will help you expand students' perceptions that each exercise has broad applications to their world.

THE THIRD EDITION

All exercises have been thoroughly reviewed and revised for this third edition, and six new exercises have been added. New experiments involving electrophoresis, chromatography, human biology, and population growth provide current topics investigated with contemporary and traditional techniques. We've also added an appendix on dissection of fetal pig. The order of laboratory exercises reflects their treatment in *Biology, ed. 3* by Peter Raven and George Johnson. However, all exercises are self-contained and compatible with virtually all modern textbooks on general biology.

We've added two sections of color photographs with detailed captions describing adaptations of plants and animals. We chose this pictorial essay because strategies and adaptations in whole organisms integrate many of the "pieces" of biology investigated in individual exercises. The exercises that survey the kindgoms emphasize continuity. These photographs illustrate diversity and the incredible strategies that organisms use to survive

and reproduce. Many evolutionary themes can be discussed and based on diversity of adaptations that we've illustrated.

Although photographs and line drawings have been enhanced in the third edition, we have retained photographs that depict what students actually see through their microscopes. These photographs were taken with preparations commonly available through biological supply houses.

The questions in each exercise have been revised to (1) direct observations of organisms and experiments, (2) record experimental results, and (3) broaden the context and application of observations. The questions have been placed in the margin to avoid interruption of the text and procedures. Their reiteration at the end of each exercise provides an organized page for efficient review by the student and evaluation by the instructor.

An *Instructor's Manual for Lab Preparation* is available from Mosby–Year Book, Inc. It contains helpful comments for instructors on setting up and performing the experiments covered in this laboratory manual. This revised *Instructor's Manual* now includes expanded descriptions of experimental preparations and specific catalog numbers for purchase of prepared slides and other supplies.

Darrell Vodopich
Randy Moore

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THE MICROSCOPE
Basic Skills of Light Microscopy

OBJECTIVES

By the end of this exercise you should:

1. Be able to identify and state the function of the primary parts of a compound and stereoscopic microscope.
2. Be familiar with basic skills of light microscopy, including how to (a) carry a microscope, (b) focus a microscope, (c) prepare a wet mount, (d) determine the magnification and size of the field of view, and (e) determine the depth of field.

Many organisms and biological structures are too small to be seen with the unaided eye. 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 inch = 25.4 mm), meaning that our eyes can distinguish two points 0.1 mm apart. A good quality 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. 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, since it was used to formulate the cell theory and study structure at the cellular level. The light microscope is the most fundamental and important tool of most biologists.

THE COMPOUND LIGHT-MICROSCOPE

Study and learn the parts of the typical compound light-microscope shown in Fig. 1-1. 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 concentrates light on the specimen. It usually consists of a light source, condenser lens, and iris diaphragm. The **light source** is a light-bulb located 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** is located immediately below the specimen. It focuses light from the light source onto the specimen. Just below the condenser is the **iris diaphragm,** which is a knurled ring or lever that can be opened and closed to regulate the amount of light reaching the specimen. When the iris diaphragm is open, the image will be bright, and when closed it 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 3-4 lenses

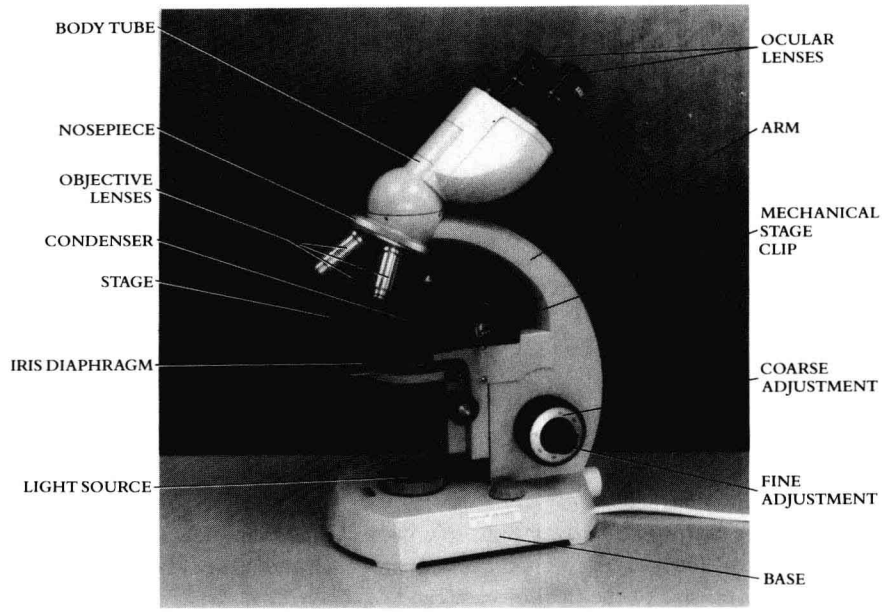

Fig. 1-1 Compound microscope.

mounted on a revolving nosepiece. Each objective is actually a series of several lenses that magnify the image, improve resolution, and correct aberrations in the image. The magnifying power of each objective is etched on the side of the lens (e.g., 4×, 10×, or 43×). The **ocular** is the lens that you look through. Microscopes with one ocular are **monocular** microscopes, and those with two are **binocular** microscopes. The 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 body tubes and inclined oculars, the body tube contains mirrors and a prism that redirects light to the oculars. The **stage** secures the glass slide on which the specimen is mounted.

Viewing and Recording System

The viewing and recording system converts radiation to a viewable and/or permanent image. It 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 resolving power of light microscopes has not improved significantly during the last century, construction and design of light microscopes has improved with newer models. For example, built-in light sources have replaced adjustable mirrors in the illuminating system, and lenses are made of much better glass than they were in the past. Your lab instructor will review with you the parts (and their functions) of the microscopes you will use in lab.

After familiarizing yourself with the parts of a microscope, you're now ready for some hands-on experience with the instrument.

PROCEDURE: 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.
2. Clean all of the microscope's lenses with lens paper (**NOTE:** Do not use paper towels or Kimwipes, since they can scratch the lenses). Do not remove the oculars or any other parts from the body tube of the microscope.
3. Plug in the microscope and turn on the light source.
4. If it isn't already in position, rotate the nosepiece until the "low power" (i.e., 4× or 10×) objective is in line with the body tube. You'll feel the objective

click into place when it is properly positioned. *Always begin examining slides with the low-power objective.*

5. 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 under low magnification.
6. Rotate the coarse-adjustment knob clockwise to move the objective within 1 cm of the stage (1 cm = 0.4 inches). If your microscope is binocular, adjust the distance between the oculars to match your interpupillary distance (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.
7. Place a microscope slide of newsprint of the letter "e" on the horizontal stage so that the "e" is directly below the low-power objective lens and is right-side-up.
8. Look through the microscope and focus on the "e" by rotating the coarse adjustment knob counterclockwise (i.e., raising the objective lens). If you don't see an image, the "e" is probably off-center. Recheck to be sure that the "e" is located directly below the objective lens and that you can see a spot of light surrounding the "e."
9. Adjust the iris diaphragm so that the brightness of the transmitted light provides the best view.
10. Focus up and down to achieve the crispest image possible.

Magnification

PROCEDURE: DETERMINE MAGNIFICATION

1. Estimate the magnification of the "e" by looking at the magnified image 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 1-1.
3. Calculate and record in Table 1-1 the total magnification for each objective using the following formula:

Total Magnification of image = (Magnification of objective lens) × (Magnification of ocular)

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 normal.

4. Slowly rotate the high-power (i.e., 43×) objective into place. Be sure that the objective does not touch the slide! If the objective does not rotate into place without contacting the slide, do not force it. Ask your lab instructor to help you. After the high power objective has been rotated into place you should notice that the image remains somewhat in focus. Most light microscopes are **parfocal,** meaning that the image will remain nearly focused after the high-power objective lens is in place. Most light microscopes are also **parcentered,** meaning that the image will remain centered after the high-power 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 slightly changes the specimen-to-objective distance, and therefore makes it easy to fine-focus the image.
7. Compare the size of the image under high magnification with the image under low magnification.

Determining the Size of the Field of View

The **field of view** is the area that you can see through the ocular and objective (Fig. 1-2). Knowing the size of the field of view is important, since you can use it to determine the approximate size of an object you are examining. The field of view can be measured with ruled **micrometers** (Fig. 1-3). An **ocular micrometer** is a small glass disk with uniformly

QUESTION 1

a. While viewing 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? Up or down?

c. What happens to the brightness of the view when you go from low to high power?

CAUTION:
Never use the coarse adjustment knob to fine-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?

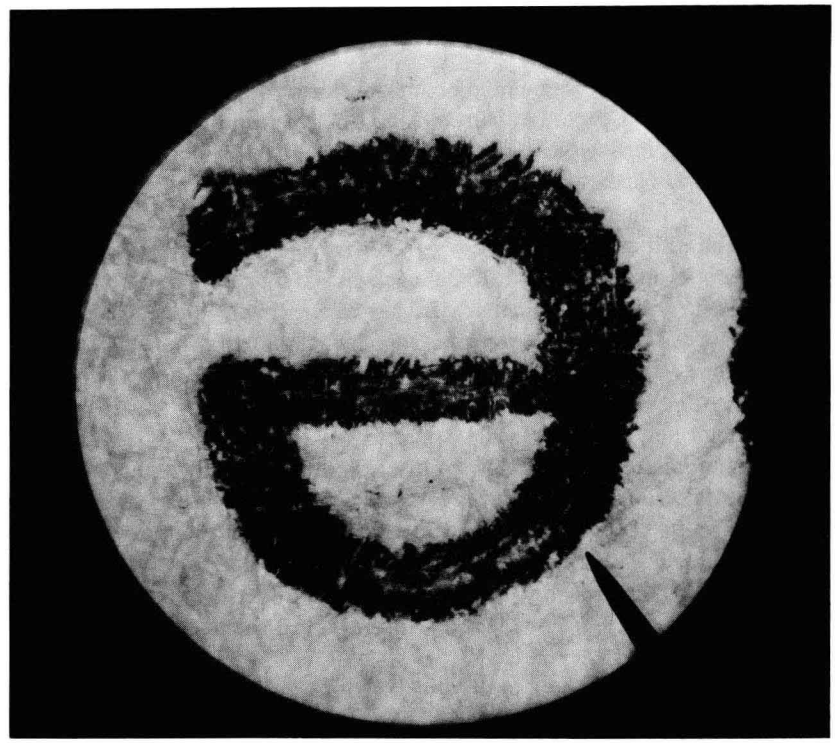

Fig. 1-2 The circular field of view of a compound microscope.

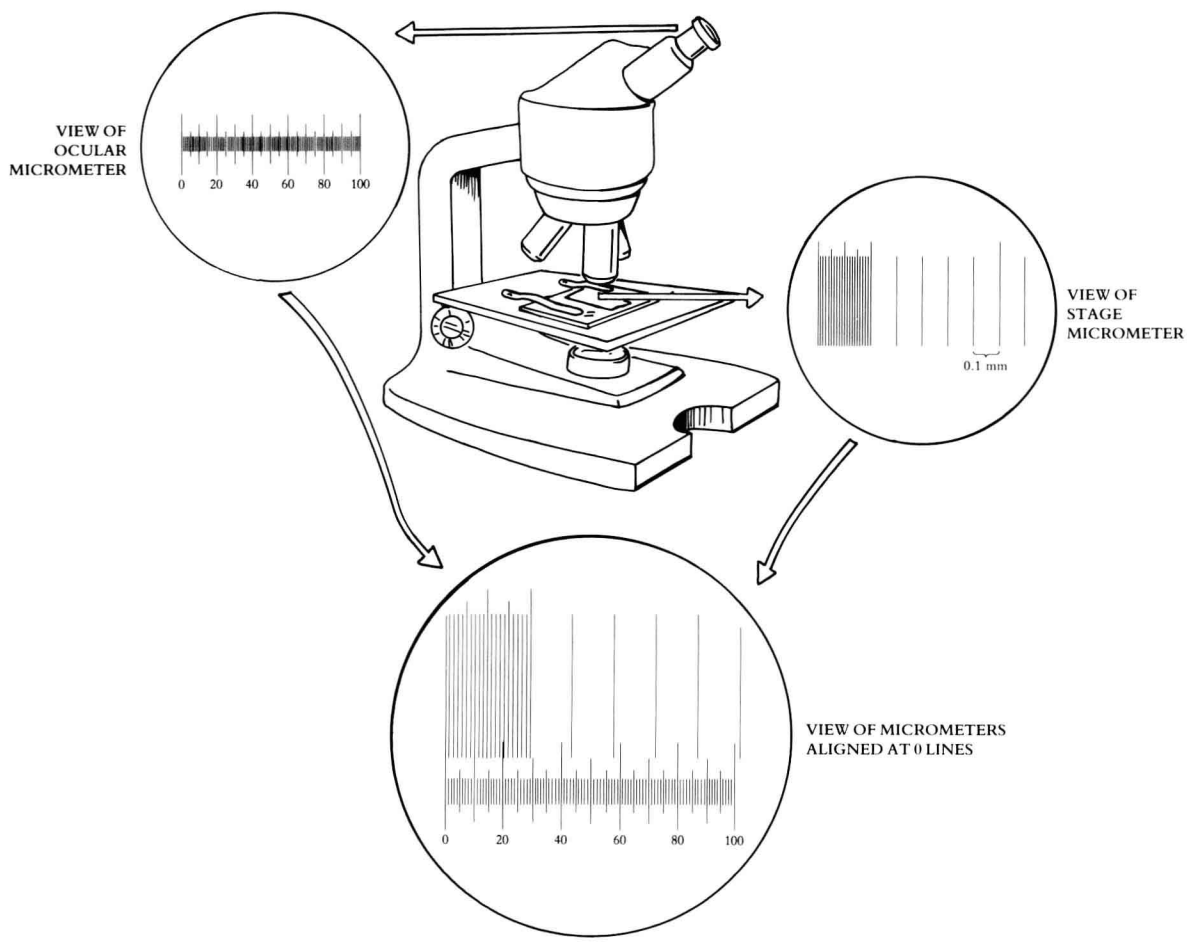

Fig. 1-3 Stage and ocular micrometers.

spaced lines etched at *unknown* intervals. An ocular micrometer is inserted in an ocular, and the distance between its lines is calibrated against a standard ruler called a **stage micrometer.** A stage micrometer is a glass slide having uniformly spaced lines etched at *known* intervals.

Examine the demonstration of a microscope equipped with ocular and stage micrometers, and use the following procedure to determine the diameter of the field of view with each objective lens.

PROCEDURE: DETERMINE SIZE OF FIELD OF VIEW USING OCULAR AND STAGE MICROMETERS

1. Rotate the ocular until the lines of the ocular micrometer parallel those of the stage micrometer (Fig. 1-3).
2. Align lines at the left edges of the two micrometers by moving the stage micrometer (Fig. 1-3).
3. Count how many spaces on the stage micrometer fit precisely in a given number of spaces on the ocular micrometer. For example, 10 spaces on the ocular micrometer may be exactly the same width as 8 spaces on the stage micrometer.
4. Calculate the distance in mm between lines of the ocular micrometer. Since the smallest space on a stage micrometer = 0.01 mm, then

   $$10 \text{ spaces on the ocular} = N \text{ spaces on the "stage"} \times 0.01 \text{ mm}$$

   Therefore,

   $$1 \text{ space on the ocular} = (N \text{ spaces on the stage} \times 0.01 \text{ mm})/10$$

   For example, if 10 spaces on the ocular equal 8 spaces on the stage, then

   $$1 \text{ ocular space} = (8 \times 0.01 \text{ mm})/10 = 0.008 \text{ mm} = 8 \ \mu\text{m}$$

   Therefore, if a specimen spans 8 spaces on your ocular micrometer with that objective in place, that specimen is 64 μm long.
5. Calibrate the ocular micrometer for each objective on your microscope. Record in Table 1-1 the diameter of the field of view for each objective.
6. Use this information to determine the area of the circular field of view with the following formula:

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

7. Record your calculated FOV areas in Table 1-1.

PROCEDURE: DETERMINE SIZE OF FIELD OF VIEW USING A TRANSPARENT RULER (An Alternate Procedure)

1. Obtain a clear plastic ruler with a metric scale.
2. Place the ruler on the stage of your microscope and rotate the nosepiece to the objective of lowest magnification.
3. Focus with the coarse adjustment and then fine adjustment until the metric markings on the ruler are clear.

QUESTION 3

a. Which provides the largest field of view, the low or high-power objective?

b. How much more area can you see with the 4× objective than with the 43× objective?

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

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

Table 1-1 TOTAL MAGNIFICATIONS AND AREAS OF FIELD OF VIEW (FOV) FOR THREE OBJECTIVES

OBJECTIVE MAGNIFICATION	OCULAR MAGNIFICATION	TOTAL MAGNIFICATION	FOV DIAMETER (mm)	FOV AREA (mm^2)
_____×				
_____×				
_____×				

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 1-1 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:

   (**diameter** FOV for low power obj.) × (**mag.** for low power obj.) =
   (**diameter** FOV for higher power obj.) × (**mag.** for higher power obj.)

7. Calculate and record in Table 1-1 the diameters of the field of view for the medium and high magnifications.
8. Calculate and record in Table 1-1 the circular area of the field of view for the three magnifications by using the formula below.

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

Depth of Field

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

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 provides the greatest depth of field, high or low power?

PROCEDURE: DETERMINE DEPTH OF 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. Remember that the order of the threads will not be the same on all slides.
3. Re-examine the threads using the high-power objective lens.

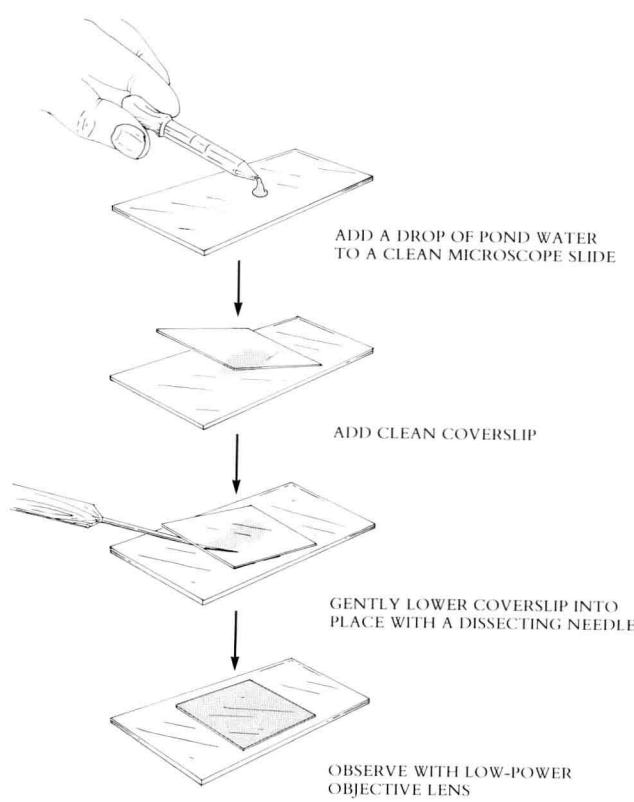

Fig. 1-4 Preparing a wet mount.

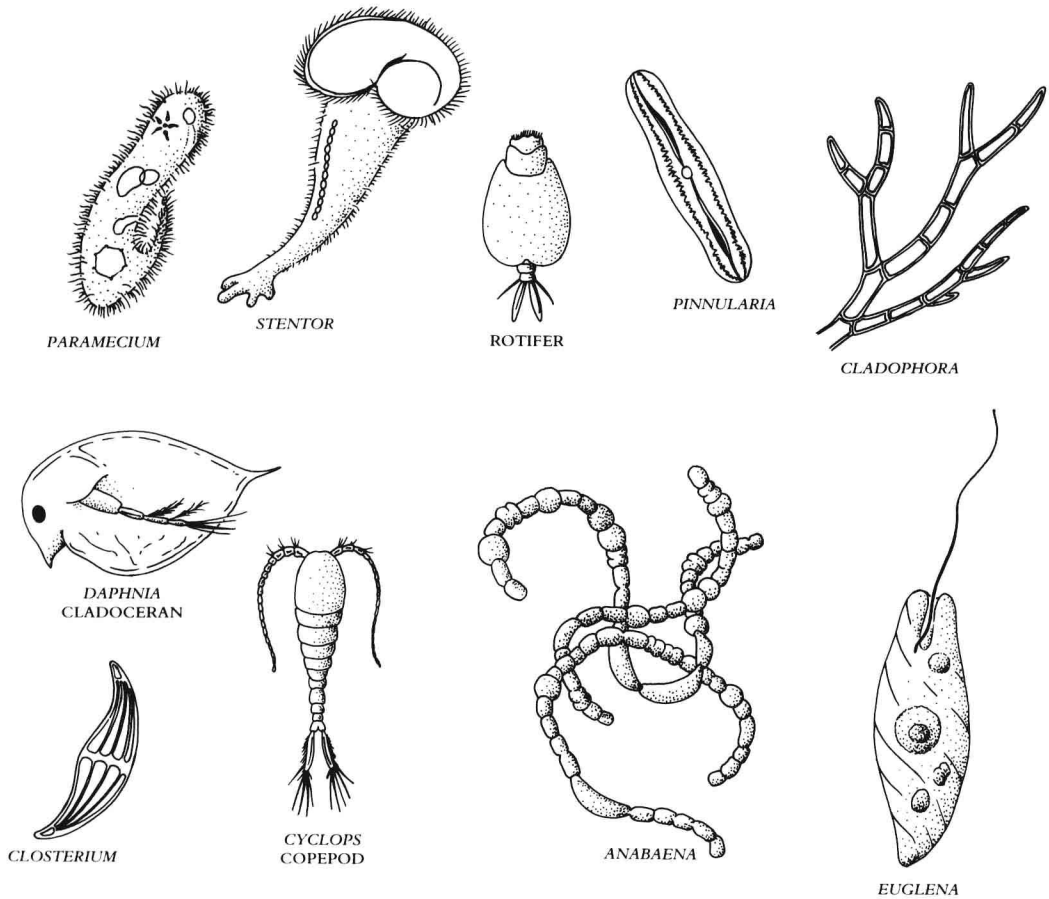

Fig. 1-5 Common organisms found in pond water.

Preparing a Wet Mount of a Biological Specimen

PROCEDURE: PREPARE A WET MOUNT OF A BIOLOGICAL SPECIMEN

1. Place a drop of pond water on a clean microscope slide.
2. Place the edge of a clean coverslip at one edge of the drop and slowly lower the coverslip onto the drop so that no air bubbles are trapped (Fig. 1-4—your lab instructor will demonstrate this technique). This fresh preparation is called a **wet mount** and can be viewed with your microscope.
3. Examine your preparation of pond water, and sketch the organisms that you see. Fig. 1-5 will help you identify some of these organisms. Don't mistake air bubbles for organisms! Air bubbles appear as uniformly round structures with dark, thick borders.
4. Prepare a wet mount of some newly-hatched brine shrimp *(Artemia)* and their eggs. Use your calculations for the diameter of the field of view to estimate the length of the shrimp.

QUESTION 5

a. Why is it important to put a coverslip over the drop of water when preparing a wet mount?

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

PRACTICE

For practice using your microscope, 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 details. Rather, use this exercise to familiarize yourself with the microscope. 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, replace the plastic dust cover, and store the microscope in its cabinet.

THE DISSECTING (STEREOSCOPIC) MICROSCOPE

A **dissecting (stereoscopic) microscope** offers some advantages over a compound microscope. A compound microscope is characterized by high magnifications and excellent resolution. However, 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. In contrast, a dissecting microscope is used to view objects that are opaque or too large to see with a compound microscope. Unlike compound microscopes, a dissecting microscope provides a larger working distance than does a compound microscope. This distance is usually several centimeters (compared to one 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, dissecting microscopes use a light source above the specimen, and the image is formed from reflected light.

Dissecting microscopes are always binocular (Fig. 1-6). 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 counteracted by lower resolution and magnification than a compound microscope. Most dissecting microscopes magnify 4-50×.

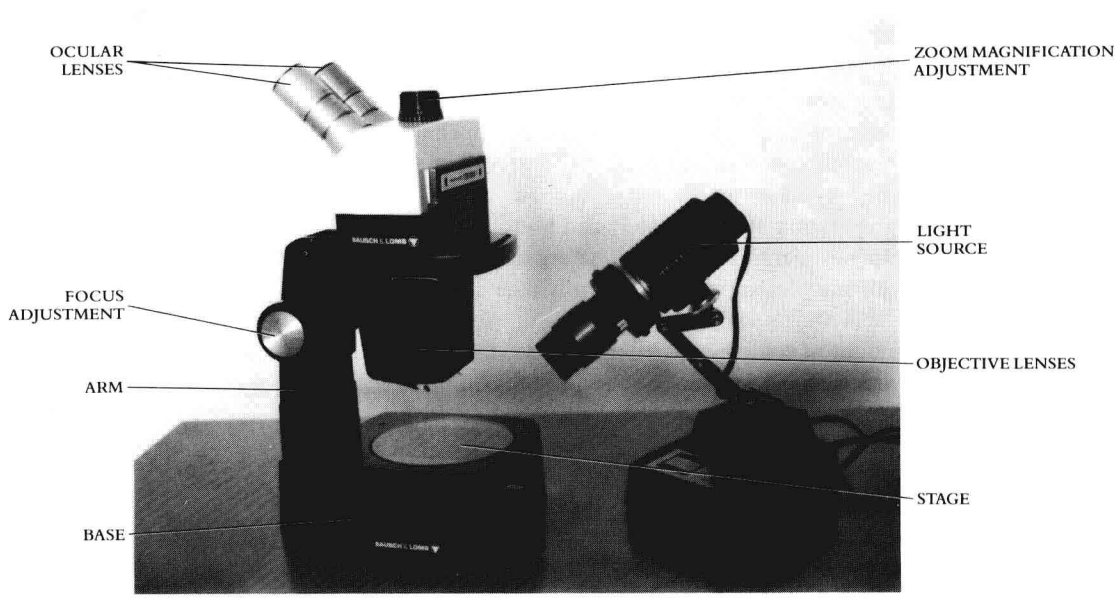

Fig. 1-6 Stereoscopic microscope.

PROCEDURE: USE A DISSECTING MICROSCOPE

1. Carry your microscope to your desk and clean its lenses in the same manner as for the compound microscope.
2. Use Fig. 1-6 to familiarize yourself with the parts of your microscope.
3. Use your dissecting microscope to examine the organisms available in lab. Sketch some of these organisms.
4. Use a rule to measure the diameter of the field of view with your dissecting microscope.

QUESTION 6

a. What is the area of the field of view when you use the low-power of your dissecting microscope?

b. What is the area when you use the highest-power objective?

A COMPARISON OF COMPOUND AND DISSECTING MICROSCOPES

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

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

RECAP OF ACTIVITIES

1. Use and examine the parts of a compound microscope.
2. Determine magnification.
3. Measure the size of field of view.
4. Determine depth of field.
5. Prepare a wet mount of a biological specimen.
6. Use a dissecting microscope.
7. Compare the features of a dissecting versus compound microscope.

QUESTIONS FOR FURTHER THOUGHT AND STUDY

1. What is the function of each major part of a compound and dissecting microscope?
2. What are the advantages of knowing the diameter of the field of view at a given magnification?
3. Why must specimens viewed with the compound microscope be thin? Why are they sometimes stained with dyes?
4. What are the advantages and disadvantages of using a compound microscope as compared to a stereoscopic microscope?
5. Why is depth of field important for studying biological structures?