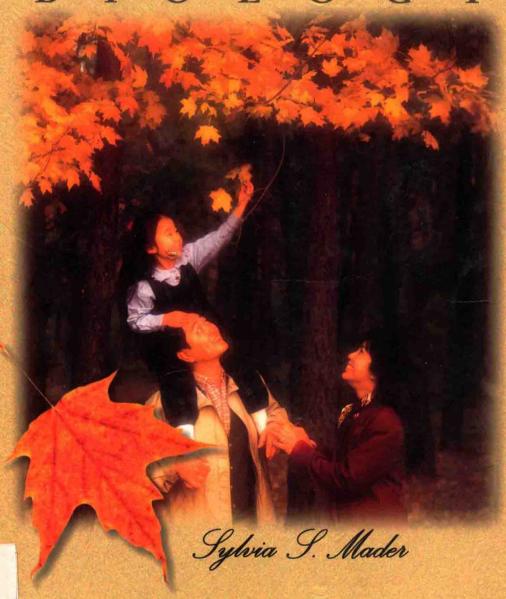
Laboratory Manual

B I O L O G Y



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LABORATORY MANUAL

Fifth Edition

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B I O L O G Y

Sylvia S. Mader



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Preface

The laboratory exercises in the *Laboratory Manual to accompany Human Biology* are coordinated with *Human Biology*, a biology text that emphasizes the application of biological knowledge to the concerns of humans. The manual is as practical as the textbook it accompanies. The exercises are tested for student appeal, ease of preparation, and educational value. They can be performed easily in most laboratory settings, and they adequately provide an opportunity for students to actively learn laboratory skills and the scientific process.

The laboratory manual has two appendices. Appendix A reviews the metric system and Appendix B provides answers to the laboratory review questions. Also included are practical examination answer sheets.

New to this Edition

Integrated Opening

Each laboratory begins with a list of learning objectives that are organized according to the major sections of the laboratory. The major sections of the laboratory are numbered on the opening page, in the laboratory text material, and in the review. This organization will help students better understand the goals of each laboratory session.

Materials List

In the previous edition, each laboratory began with a materials list. Now instructors should consult the *Resource Guide* for materials required and for all information needed to successfully complete each exercise.

Student Activities

A color bar is used to designate student activities and experimental procedures. An icon appears whenever a procedure requires a period of time before results can be viewed. Sequentially numbered steps now guide students as they perform an activity.

New Laboratories

All laboratories have been revised. New introductory material, student activities, and illustrations appear throughout the manual. The laboratories listed below are new or have been significantly revised.

Laboratory 13 Nervous System and Senses

contains material that was in two separate laboratories in the previous edition. Observation of the brain and spinal cord and the eye and ear are retained. Experimental procedures regarding reflexes and the senses are also found in this laboratory.

Laboratory 15 Mitosis and Melosis

has been revised to include simulation activities

Laboratory 17 Molecular Genetics

now begins with simulation activities that will allow all instructors to include this laboratory in the course syllabus.

To the Instructor

You will find this laboratory manual to be both flexible and practical. In addition, it is accompanied by a Laboratory Resource Guide.

Flexible Handling of Exercises

The author has taken several measures that enable instructors to modify the laboratory experience to suit a particular course. The number of exercises per laboratory is sufficient to permit a choice of student activities. Some instructors may prefer to demonstrate particular exercises rather than having students do them.

Practicality of Manual

Students work with living material during some part of each laboratory. In only rare instances is it necessary to continue an exercise the following week. The instructions are easy to follow, and the exercises require only simple materials that are available in most laboratories or from general supply houses. Furthermore, the exercises are designed so that students in successive laboratory sessions can do the same lab on any particular day.

Laboratory Resource Guide

The Laboratory Resource Guide is designed to assist instructors as they prepare for each laboratory. It has a cumulative list of all materials required that will be helpful when ordering supplies for the course. Each laboratory is divided into two parts: the *Materials* section, which gives preparation information, and the *Exercises* section, which gives expected results and answers to the questions in the laboratory manual.

To the Student

Special care has been taken in preparing the *Laboratory Manual for Human Biology* so that you will *enjoy* the laboratory experience as you *learn* from it. The instructions and discussion are written clearly so you can understand the material while working through it. Student aids are designed to help you focus on important aspects of each exercise.

Student Learning Aids

Student learning aids are carefully integrated throughout this manual. The *learning objectives* set the goals of each laboratory session and help you review the material for a laboratory practical or any other kind of exam. In this edition, the major topics are numbered, and the learning objectives are grouped according to these topics. The section numbering is used in the text material and in the laboratory review questions. This system allows students to study the chapter in terms of the objectives presented.

The *introduction* establishes the rationale for coming work and reviews much of the necessary background information required for comprehension of upcoming experiments. *Color bars* bring attention to exercises that require your active participation by highlighting Observations and Experimental Procedures, and an icon indicates a timed experiment. Throughout, *space* is provided for recording answers to questions and the results of investigations and experiments. Each laboratory ends with a set of review questions covering the day's work.

Appendices at the end of the book provide useful information on the metric system and answers to review questions. Practical examination answer sheets are also provided.

Laboratory Preparation

Read each exercise before coming to the laboratory. *Study* the introductory material and the experimental procedures. If necessary, to obtain a better understanding *read* the corresponding chapter in your text.

Explanations and Conclusions

Throughout the laboratory you are often asked to formulate explanations or conclusions. To do so, you will need to synthesize information from a variety of sources, including the following:

- 1. Your experimental results and/or the results of other groups in the class. If your data are different from other groups in your class, do not erase your answer; add the other answer in parentheses.
- 2. Your knowledge of underlying principles. Obtain this information from the laboratory introduction or the appropriate section of the laboratory and the corresponding chapter of your text.
- 3. Your understanding of how the experiment was conducted and/or the materials that were used. *Note:* Ingredients can be contaminated or procedures incorrectly followed, resulting in reactions that seem inappropriate. If this occurs, consult with other students and your instructor to see if you should repeat the experiment.

In the end, be sure you are truly writing an explanation or conclusion and not just giving a restatement of the observations made.

Color Bars and Icon

Throughout the chapter, the following color bars and icon are used to assist you:

- **Observation**—An activity in which you observe models or slides and make identifications or draw conclusions.
- **Experimental Procedure**—An activity in which a series of laboratory steps is followed to achieve a learning objective.
- Time—Allow the designated amount of time for this activity. Start these activities at the beginning of the laboratory, proceed to other activities, and return to these when the designated time is up.

Student and Instructor Feedback

If you have suggestions for how this laboratory manual could be improved, you can send your comments to

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Laboratory Safety

Many of the reagents (chemicals) and some equipment in a biology laboratory are potentially dangerous. Following rules of laboratory safety and using common sense throughout the course will enhance the learning experience by increasing the student's confidence in his or her ability to safely use chemicals and equipment.

The following rules of laboratory safety should be studied and should become a habit.

- 1. Wear safety glasses or goggles during exercises in which glassware and solutions are heated, or during which dangerous fumes may be present, creating possible hazard to eyes or contact lenses.
- 2. Assume that all reagents are poisonous and act accordingly. Read labels on chemical bottles for safety precautions and know the nature of the chemical you are using. If chemicals come into contact with skin, wash immediately with water.

3. DO NOT

- a. ingest any reagents;
- b. eat, drink, or smoke in the laboratory. Toxic material may be present, and some chemicals are flammable;
- c. carry reagent bottles around the room;
- d. pipette anything by mouth;
- e. put chemicals in sink or trash unless instructed to do so;
- f. pour chemicals back into containers unless instructed to do so;
- g. operate any equipment until you are instructed in its use.

4. DO

- a. keep work area neat, clean, and organized. Ask your instructor for assistance in cleaning up broken glassware and spills. Wash hands, desk area, and glassware at the end of each exercise and/or before leaving the laboratory.
- b. stopper all reagent bottles when not in use. Immediately wash reagents off yourself and your clothing if they spill on you, and immediately inform the instructor. If you accidentally get any reagent in your mouth, rinse mouth thoroughly and immediately inform your instructor.
- c. handle hot glassware with test-tube clamp or tongs. Use caution when using heat, especially when heating chemicals. Do not leave a turned-on hot plate or a lit Bunsen burner unattended; do not turn on a hot plate or a Bunsen burner near a gas tank or cylinder; do not move a turned-on hot plate or lit Bunsen burner; keep hair and loose clothing well away from turned-on hot plate or a lit Bunsen burner; make certain gas jets are off when Bunsen burner is not in use. Use proper ventilation and hoods when instructed.
- d. use extra care when working with scalpels, knives, and glass tubing.
- e. wear clothing that, if damaged, would not be a serious loss, or use aprons or laboratory coats, since some chemicals may damage fabrics.
- f. wear shoes as protection against broken glass or spillage that may not have been adequately cleaned up.
- g. be familiar with the experiments you will be doing before coming to the laboratory. This will increase your understanding, enjoyment, and safety during exercises. *Confusion is dangerous*. Completely follow the procedure set forth by the instructor.
- h. note the location of emergency equipment such as a first-aid kit, eyewash bottle, fire extinguisher, switch for ceiling showers, fire blanket(s), sand bucket, and telephone (911). Report all accidents immediately.
- i. report any condition that appears unsafe or hazardous to the instructor.

I understand the safety rules as presented above, and the instructor.	agree to follow them and all other instructions given by
Name	Date

Laboratory Class and Time

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1

Use of the Light Microscope

Learning Objectives

Students should be able to

1.1 The Metric System

1. state and use metric units of measure for length, temperature, weight, and volume.

1.2 Microscopy

2. describe three differences between the compound light microscope and the electron microscope.

1.3 Use of the Compound Light Microscope

- 3. name and give the function of the basic parts of the compound light microscope.
- 4. list, in proper order, the steps used for bringing an object into focus with the compound light microscope.
- describe how the slide of the letter e provides information on the inversion of the image in the compound light microscope.
- 6. calculate the diameter of the field and the total magnification for both the low- and high-power lens systems.
- 7. explain how the slide of colored threads provides information on the depth of field.

1.4 Microscopic Observations

- 8. name the three kinds of cells studied in this exercise.
- 9. state two differences between onion epidermal cells and human epithelial cells.

1.5 Binocular Dissecting Microscope (Stereomicroscope)

10. identify the parts and tell how to focus the binocular dissecting microscope.

Introduction

Comprehending the relative size of objects and the structures within them aids in understanding the complicated organization within biological systems. Biologists use the **metric system** of measurement. This laboratory emphasizes the metric units used to indicate the size of cells and cell structures.

This laboratory also examines the features, functions, and use of the **compound light microscope** and the **binocular dissecting microscope** (stereomicroscope). **Scanning** and **transmission electron microscopes** are explained, and various micrographs appear throughout these exercises. The compound light microscope is used to examine cells of three different organisms.

1.1 The Metric System

The **metric system** is the standard system of measurement in the sciences, including biology, chemistry, and physics. It has tremendous advantages in conversions because all of the conversions, whether for volume, mass (weight), or length, are in units of ten.

Length

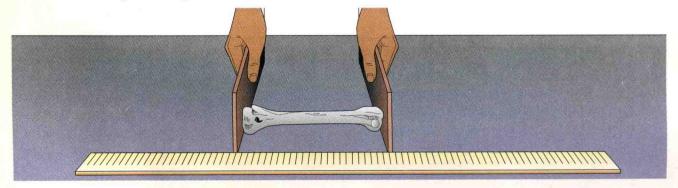
Metric units of length measurement studied in this laboratory include the **nanometer** (nm), **micrometer** (µm), **millimeter** (mm), **centimeter** (cm), and **meter** (m) (Table 1.1).

·····································	Units of Measureme		
Unit	Value	Value (Exponent)	Relative Size
Meter (m)	100 cm	10 ⁰ m	Largest
Centimeter (cm)	0.01 m	10 ⁻² m	
Millimeter (mm)	0.1 cm	10 ⁻³ m	
Micrometer (µm)	0.001 mm	10 ⁻⁶ m	
Nanometer (nm)	0.001 μm	10 ⁻⁹ m	Smallest

Experimental Procedure

- 1. Obtain a small ruler marked in centimeters and millimeters. How many centimeters are represented? _____ One centimeter equals how many millimeters? ____ To express the size of small objects, such as cell contents, biologists use even smaller units of the metric system than those on the ruler. These units are the micrometer (μm) and the nanometer (nm). According to Table 1.1, 1 μm = ____ mm, and 1 nm = ____ μm. Therefore, 1 mm = ____ nm.
- **2.** Measure the diameter of the circle shown to the nearest millimeter. This circle is mm = um = nm.
- **3.** Obtain a meterstick. On one side, find the numbers 1 through 39, which denote inches. One meter equals 39.37 inches; therefore, 1 meter is roughly equivalent to 1 yard. Turn the meterstick over, and observe the metric subdivisions. How many centimeters are in a meter? _____ How many millimeters are in a meter? _____ The prefix *milli* means
- 4. Use the meterstick and the method shown in Figure 1.1 to measure the length of two long bones from a disarticulated human skeleton. Lay the meterstick flat on the lab table. Place a long bone next to the meterstick between two pieces of cardboard (each about 10 cm × 30 cm), which are held upright at right angles to the stick. The narrow end of each piece of cardboard should touch the meterstick. The length between the cards is the length of the bone in centimeters. For example, if the bone measures from the 22 cm mark to the 50 cm mark, the length of the bone is _____ cm. If the bone measures from the 22 cm mark to midway between the 50 cm and 51 cm marks, its length in millimeters is ____ mm or ____ cm. Record the length of two bones. One bone: ____ cm = ____ mm. Another bone: ____ mm.

Figure 1.1
How to measure a long bone using a meterstick.



Temperature

Appendix A shows two temperature scales: the Fahrenheit (F) and centigrade (Celsius, C) scales. Science has adopted the centigrade scale.

Ex	ne	rim	en	tal	Pro	ced	ure
	\sim		C	cui		CCU	uic

- 1. Study the two scales in Appendix A, and complete the following information:

 - a. Water freezes at either _____ °F or ____ °C.b. Water boils at either ____ °F or ____ °C.
- To convert from the Fahrenheit to the centigrade scale, use the following equation:

$$^{\circ}C = 5/9 (^{\circ}F - 32^{\circ})$$

A room temperature of 70°F is what temperature on the centigrade scale?

3. Of the following, record any two of the temperatures in your lab environment. In each case, allow the end bulb of the centigrade thermometer to remain in or on the sample for one minute.

Room temperature = °C

Surface of your skin = $_$ °C

Cold tap water in a 50 ml beaker = ____ °C

Hot tap water in a 50 ml beaker =

Ice water = °C

Weight

Two metric units of weight are the gram (g) and the milligram (mg). A paper clip weighs about 1 g, which equals 1,000 mg. $2 \text{ g} = ____ \text{mg}$; $0.2 \text{ g} = ___ \text{mg}$; and $2 \text{ mg} = ___ \text{g}$.

Experimental Procedure

- 1. Use a balance scale to measure the weight of a wooden block small enough to hold in the palm of your hand. Place the block in the center of the pan at the left-hand end of the scale. Slide the indicator that measures grams from the zero mark toward the right. If you are using a triple-beam balance, which has two additional scales—one to measure 10 gram increments and the other to measure 100 gram increments—set these indicators to zero.
- 2. Measure the weight of the block to the tenth of a gram. The weight of the wooden block is
- Measure the weight of an item that is small enough to fit inside the opening of a 50 ml graduated cylinder. The item, a _____ _____, is _____ g = ____ mg.

Volume

Two metric units of volume are the **liter** (l) and the **milliliter** (ml). One l = ml.

Experimental Procedure

 Volume measurements can be related to those of length. For example, measure the wooden block, used in the previous Experimental Procedure, along its edges with a millimeter ruler to get its length, width, and depth.

Length = _____ cm Width = ____ cm Depth = ____ cm

The volume, or space, occupied by the wooden block can be expressed in cubic centimeters (cc or cm³) by multiplying: length × width × depth = _____ cm³. For purposes of this experimental procedure, 1 cubic centimeter equals 1 milliliter; therefore, the wooden block has a volume of _____ ml.

- 2. In the biology laboratory, volume usually is measured directly in liters or milliliters with appropriate measuring devices. For example, use a 50 ml graduated cylinder to add 20 ml of water to a test tube. First, fill the graduated cylinder to the 20 ml mark. To do this properly, you have to make sure that the lowest margin of the water level, or the **meniscus** (Fig. 1.2), is at the 20 ml mark. Place your eye directly parallel to the level of the meniscus, and adjust the water level until the meniscus is at the 20 ml mark. A dropper bottle filled with water can help you do this. A large, blank, white index card held behind the cylinder also can help you to see the scale more clearly. Now, pour the 20 ml of water into the test tube.
- 3. How could you find the total volume of the test tube? _

Now perform this suggested operation. What is the test tube's total volume? __

4. How could you determine how many drops from the pipette of the dropper bottle equal 1 ml?

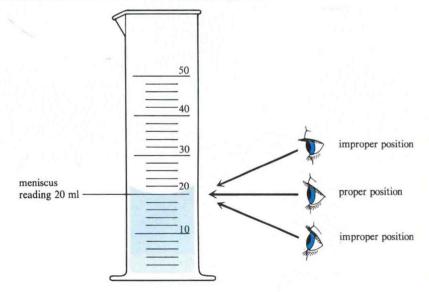
Now perform this suggested operation. How many drops from the pipette of the dropper bottle equal 1 ml? _____ Some pipettes are graduated and can be filled to a certain level as a way to measure volume directly. Your instructor will demonstrate this. Are pipettes customarily used to measure large or small volumes? ____

5. Fill a 50 ml graduated cylinder to about the 20 ml mark with water. How can you use this setup to calculate the volume of the item in the Experimental Procedure that was small enough to fit inside the opening of a 50 ml graduated cylinder?

Perform this operation. The item, _____, has a volume of _____ ml.

Figure 1.2

The proper way to view the meniscus.



1.2 Microscopy

Because biological objects can be very small, a microscope is often used to view them. Many kinds of instruments, ranging from the hand lens to the electron microscope, are effective magnifying devices. A short description of two kinds of light microscopes and two kinds of electron microscopes follows. Your instructor also may discuss the phase-contrast, dark-field, reflecting, polarizing, X-ray, fluorescent, and ultraviolet microscopes.

Light Microscopes

Light microscopes use light rays that are magnified and focused by means of lenses. The **binocular dissecting microscope** is designed to study entire objects in three dimensions at low magnification. The **compound light microscope** is used for examining small objects or thinly sliced sections of objects under magnification that is higher than that of the dissecting light microscope. Illumination is from below, and the light passes through clear sections but does not pass through opaque sections. To improve contrast, the microscopist uses stains or dyes that bind to cellular structures and absorb light. Figure 1.3a is a **photomicrograph**, a photograph of an image produced by a compound light microscope.

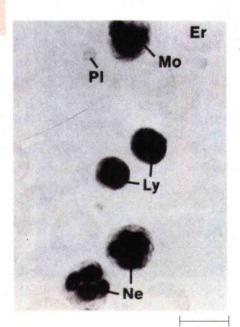
Electron Microscopes

Electron microscopes use a beam of electrons that is magnified and focused on a photographic plate by means of electromagnets. The **scanning electron microscope** is analogous to the dissecting light microscope. It gives an image of the surface and dimensions of an object, as is apparent from the electron micrograph in Figure 1.3b. The **transmission electron microscope** is analogous to the compound light microscope. The object is ultra-thinly sliced and treated with heavy metal salts to improve contrast. Figure 1.3c is an image produced by this type of microscope.

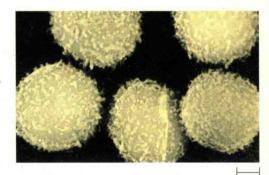
Figure 1.3
Images of a white blood cell, called a lymphocyte. Notice the greater amount of detail in (c) compared to (a).

a: Courtesy Kessel, R. G., and Kardon, R. H., Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy. 1979. W. H. Freeman and Company.

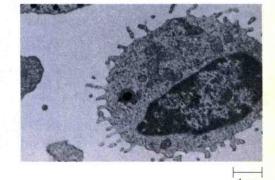
Key: Ly = lymphocyte; Ne = neutrophil; Pl = platelet; Mo = monocyte; Er = erythrocyte



a. Image by compound light microscope



b. Image by scanning electron microscope



c. Image by transmission electron microscope

Figure 1.4 compares the visual ranges of these microscopes to that of the unaided human eye. Use this figure to answer the following questions:

- 1. Which type of microscope can be used to see protein molecules? ______
- 2. Which types of microscopes are used to see bacteria?
- 3. What items could be viewed by both the unaided eye and the dissecting microscope?

Figure 1.4

Visual ranges of microscopes as compared to the range of the unaided eye. (This scale is logarithmic. Each line [or unit] is 10× the one below it.)

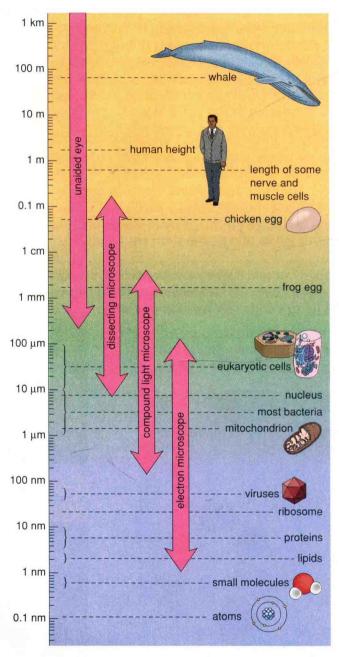


Figure 1.4 indicates that much smaller objects can be viewed with electron microscopes than with the compound light microscope. The images in Figure 1.3 also demonstrate this. The difference between these two types of microscopes, however, is not simply a matter of magnification; it also is the electron microscopes' ability to show detail, or their resolving power. *Resolution* is the ability to discriminate between two points; a compound light microscope allows you to see two separate points as long as they are 200 nm apart; an electron microscope allows you to see two separate points even if they are only 0.1nm apart. The use of electrons rather than light gives electron microscopes a much greater resolving power. Table 1.2 lists several differences between the compound light microscope and the electron microscope.

Compound Light Microscope	Electron Microscope		
1. Glass lenses	1. Electromagnetic lenses		
2. Illumination by visible light	2. Illumination due to beam of electrons		
3. Resolution ≅ 200 nm	3. Resolution ≅ 0.1 nm		
4. Magnifies to 2,000×	4. Magnifies to 100,000×		
5. Costs up to tens of thousands of dollars	5. Costs up to hundreds of thousands of dollars		
6. Specimen may be living or dead	6. Specimen must be dead		

1.3 Use of the Compound Light Microscope

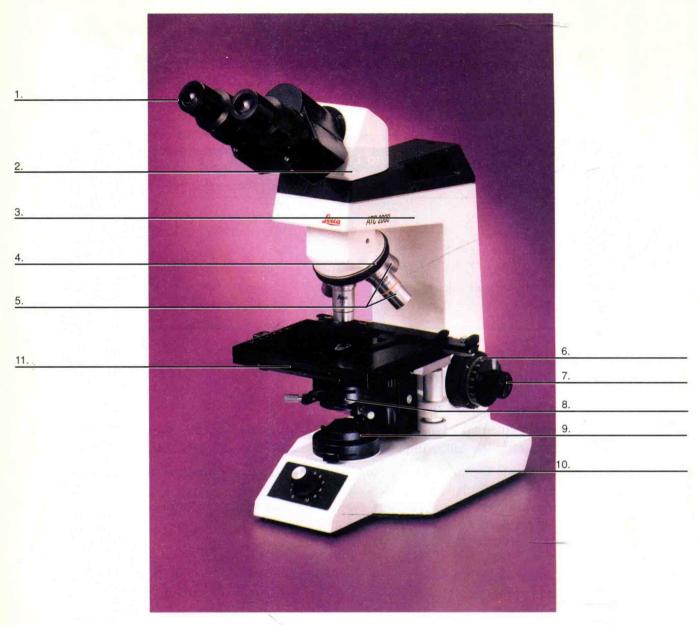
Identification of Parts

After your instructor has explained how to carry a microscope, obtain one from the cabinet and place it securely on the table. Identify the following parts on your microscope, and label them in Figure 1.5.

- 1. **Eyepiece (ocular lens):** Topmost series of lenses through which an object is viewed. What is the magnifying power of the ocular lens on your microscope?
- 2. Body tube: Holds nosepiece at one end and eyepiece at the other end; conducts light rays.
- 3. Arm: Supports upper parts and provides carrying handle.
- 4. Nosepiece: Revolving device that holds objectives.
- **5. Objectives** (objective lenses): Individual objectives are not identifiable in Figure 1.5.
 - **a. Scanning power objective:** Holds 4× lens used to view the whole slide. Does your microscope have a scanning power objective? ______ If yes, what is its magnifying power? _____
 - **b. Low-power objective:** Holds 10× lens used to view the object in greater detail. What is the magnifying power of this lens on your microscope?
 - **c. High-power objective:** Holds $40 \times (43 \times \text{ or } 45 \times)$ lens used to view the object in even greater detail. What is the magnifying power of this lens on your microscope?
 - d. Oil immersion objective (on microscopes with four objective lens): Holds a 95× (to 100×) lens and is used in conjunction with immersion oil to view objects with the greatest magnification. Does your microscope have an oil immersion objective?

 If this lens is available, your instructor will discuss its use when the lens is needed.
- Coarse-adjustment knob: Knob used to bring object into approximate focus; used only with low-power objective.
- 7. Fine-adjustment knob: Knob used to bring object into final focus.
- 8. Diaphragm or diaphragm control lever: Controls amount of illumination used to view the object.
- 9. Light source: An attached lamp that directs a beam of light up through the object.

Figure 1.5
Compound light microscope.



Compound light microscope with binocular head and mechanical stage

- **10. Base:** The flat surface of the microscope that rests on the table.
- 11. Stage: Holds and supports microscope slides.
- **12. Stage clips:** Hold slides in place on the stage.
- **13. Mechanical stage** (optional): A moveable stage that aids in the accurate positioning of the slide. Does your microscope have a mechanical stage?
- **14. Mechanical stage control knobs** (optional): Two knobs that are usually located below the stage. One knob controls forward/reverse movement, and the other controls right/left movement.

Focusing the Microscope—Lowest Power

- 1. With the coarse-adjustment knob, lower the stage or raise the nosepiece until it stops. This will maximize the distance between the stage and the objective lens.
- 2. Turn the nosepiece so that the *lowest* power lens is in straight alignment over the stage. Always begin focusing with the *lowest* power objective lens $(4 \times [\text{scanning}] \text{ or } 10 \times [\text{low power}])$.
- **3.** Place a slide of the letter *e* on the stage, and stabilize it with the clips. (If your microscope has a mechanical stage, pinch the spring of the slide arms on the stage, and insert the slide.) Center the *e* as best you can on the stage. (If your microscope has a mechanical stage, use the two control knobs located below the stage to center the *e*.)
- **4.** Again, be sure that the lowest power objective is in place. Then, as you look from the side, decrease the distance between the stage and the tip of the objective lens until the lens comes to an automatic stop or is no closer than 3 mm above the slide.
- 5. While looking into the eyepiece, rotate the diaphragm (or diaphragm lever) to give the maximum amount of light.
- **6.** Slowly increase the distance between the stage and the objective lens, using the coarse-adjustment knob, until the object—in this case, the letter *e*—comes into view, or focus.
- 7. Once the object is seen, you may need to adjust the amount of light. To increase or decrease the contrast, rotate the diaphragm slightly.
- **8.** Use the fine-adjustment knob to sharpen the focus if necessary. Practice having both eyes open when looking through the eyepiece, as it greatly reduces eyestrain.

Inversion

Inversion refers to the fact that a microscopic image is upside down and reversed.

Exi	oer	imen	tal F	roc	edure

1.	In the space provided here, through the eyepiece).	draw the letter e	as it appears of	n the slide (look	from the side, not
	1	<u> </u>	2		

1.	2.
	Nach de la State de la Colonia

- **2.** Next to this, draw the letter e as it appears when you look through the eyepiece.
- 3. What differences do you notice?
- **4.** Inversion refers to the image being upside down and reversed. Move the slide to the right. Which way does the image appear to move? ______

Focusing the Microscope—Higher Powers

Compound light microscopes are **parfocal**; that is, once the object is in focus with lowest power, it should also be almost in focus with the higher power.

- 1. Bring the object into focus under the lowest power by following the instructions in the previous section.
- 2. Make sure that the letter *e* is centered in the field of the lowest objective.