

LABORATORY MANUAL

Sylvia S. Mader

HUMAN BIOLOGY

SIXTH EDITION



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HUMAN

B I O L O G Y

SIXTH EDITION

Sylvia S. Mader
with contributions by
Nancy Jo Segsworth
Capilano College



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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

To the Instructor

The laboratory exercises in this manual are coordinated with *Human Biology*, a text that has two primary functions: (1) to understand how the human body works and (2) to understand the relationship of humans to other living things in the biosphere.

This laboratory manual can be adapted to a variety of course orientations and designs. There are a sufficient number of laboratories to permit a choice of activities over the length of the course. Many activities may be performed as demonstrations rather than as student activities, thereby shortening the time required to cover a particular concept.

The Exercises

All exercises have been tested for student interest, preparation time, estimated time of completion, and feasibility. The following features are particularly appreciated by adopters.

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 material, and in the review. This organization will help students better understand the goals of each laboratory session.

Self-contained Content

Each laboratory contains all the background information necessary to understand the concepts being studied and to answer the questions asked. This feature will reduce student frustration and increase learning.

Human Emphasis

All laboratories have a human emphasis, which has been strengthened in this edition of the manual. Each laboratory was revised to increase an emphasis on human beings.

Student Activities

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

Live Materials

Although students work with living material during some part of almost all laboratories, the exercises are designed to be completed within one laboratory session. This facilitates the use of the manual in multiple-section courses.

Laboratory Safety

Laboratory safety is of prime importance and the listing on page ix will assist instructors in their efforts to make the laboratory experience a safe one.

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 3: Cell Structure and Function is better organized, particularly the portion concerning diffusion and osmosis. There is a new experiment regarding diffusion across the plasma membrane.

Laboratory 14: Human Development is a new laboratory that pertains only to human development.

Laboratory 15: Mitosis and Meiosis now has a better emphasis on spermatogenesis and oogenesis.

Laboratory 16: Human Genetics has been rewritten to include a simulation exercise on nondisjunction.

Laboratory 18: Evidences of Evolution has a better emphasis on human evolution.

Laboratory Resource Guide

The *Laboratory Resource Guide* is essential for instructors and laboratory assistants and is available free to adopters of 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 the 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. If your text is *Human Biology*, by Sylvia S. Mader, see the text *chapter reference* in the table of contents at the beginning of the *Laboratory Manual*.

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.

Laboratory Review

Each laboratory ends with approximately ten short-answer questions that will help you determine if you have accomplished the objectives for the laboratory. The answers to these questions are found in Appendix B.

Student Feedback

If you have any 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 agree to follow them and all other instructions given by the instructor.

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.
5. 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 their 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).

Table 1.1 Metric Units of Measurement

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: Length

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, $1 \mu\text{m} =$ _____ mm, and $1 \text{ nm} =$ _____ μm . Therefore, $1 \text{ mm} =$ _____ $\mu\text{m} =$ _____ nm.
2. Measure the diameter of the circle shown to the nearest millimeter. This circle is _____ mm = _____ $\mu\text{m} =$ _____ nm.
3. Obtain a meter stick. 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 meter stick 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 meter stick and the method shown in Figure 1.1 to measure the length of two long bones from a disarticulated human skeleton. Lay the meter stick flat on the lab table. Place a long bone next to the meter stick between two pieces of cardboard (each about $10 \text{ cm} \times 30 \text{ cm}$), which are held upright at right angles to the stick. The narrow end of each piece of cardboard should touch the meter stick. 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, then 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.
5. Record the length of two bones. One bone: _____ cm = _____ mm. Another bone: _____ cm = _____ mm.

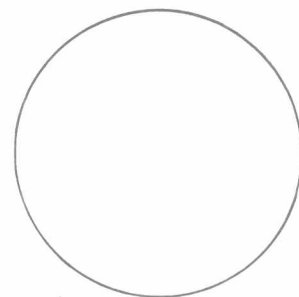
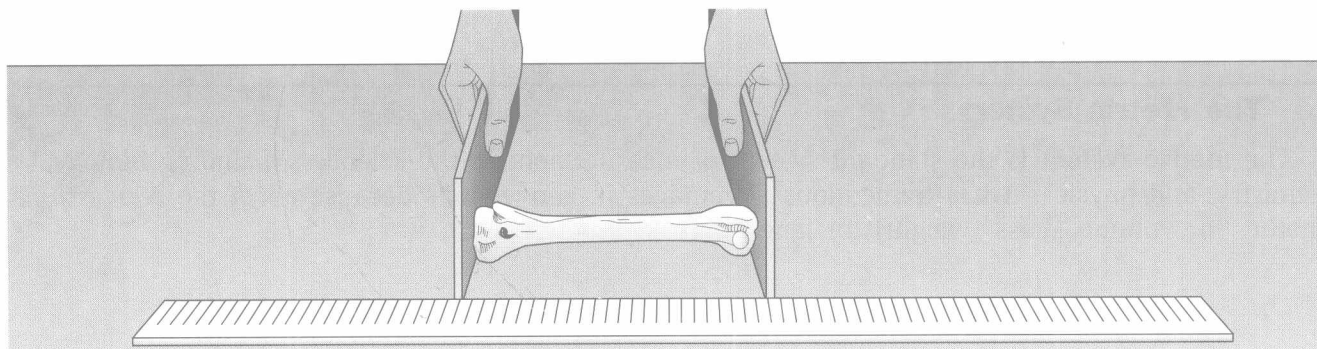


Figure 1.1 Measurement of a long bone.

This figure shows how to measure a long bone using a meter stick.



Temperature

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

Experimental Procedure: Temperature

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.
2. To convert from the Fahrenheit to the Celsius scale, use the following equation:

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

A room temperature of 68°F is what temperature on the Celsius scale? _____

3. Of the following, record any two of the temperatures in your lab environment. In each case, allow the end bulb of the Celsius 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 = _____ °C
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 g = _____ mg; 0.2 g = _____ mg; and 2 mg = _____ g.

Experimental Procedure: Weight

1. Use a balance scale to measure the weight of a wooden block small enough to hold in the palm of your hand.
2. On a triple-beam balance, make sure that the balance registers zero by moving all indicator slides to zero and adjusting the zeroing knob if necessary. Place the block in the center of the pan at the left-hand end of the scale.
3. Measure the weight of the block to the tenth of a gram. The weight of the wooden block is _____ g = _____ mg.
4. 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 liter = _____ ml.

Experimental Procedure: Volume

1. 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 cm^3) by multiplying: length \times width \times depth = _____ cm^3 . 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. 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 that you weighed in the previous Experimental Procedure? _____

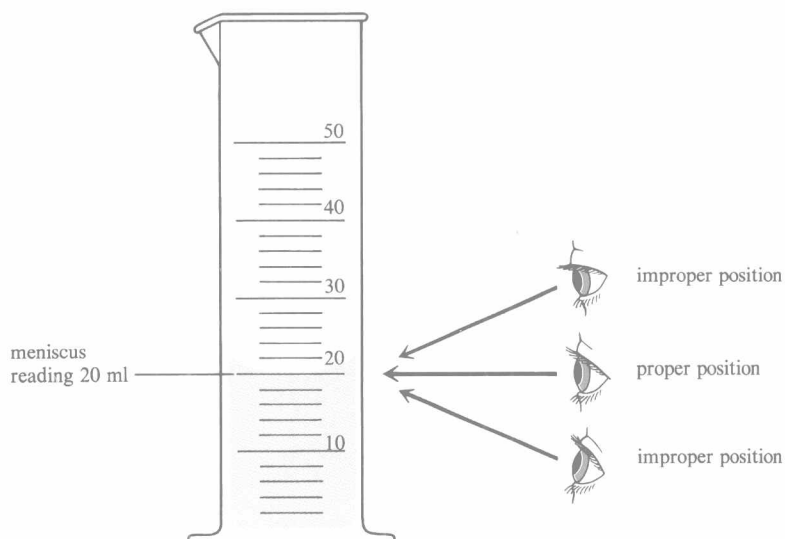
Perform this operation. The item, _____, has a volume of _____ ml.

5. 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? _____

Figure 1.2 Meniscus.

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.

Light Microscopes

Light microscopes use light rays that are magnified and focused by means of lenses. The **binocular dissecting microscope** (stereomicroscope) 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.

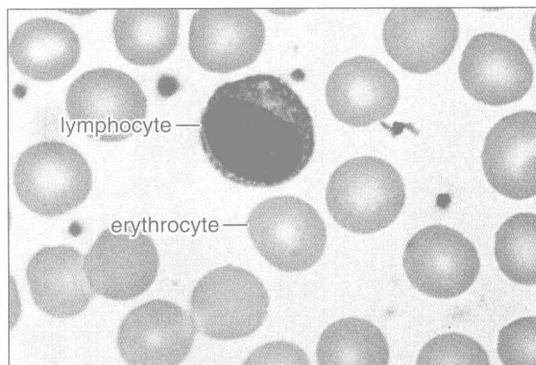
Electron Microscopes

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 three-dimensional surface 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 in that it gives a two-dimensional, planar view of a cell. 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.

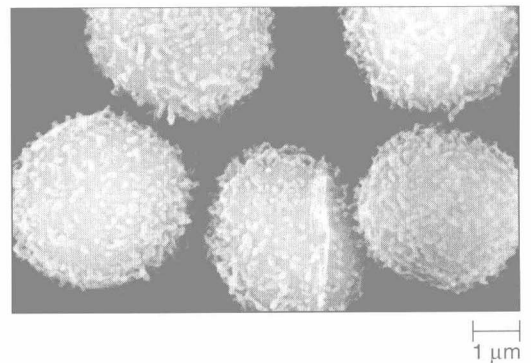
Figure 1.3

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

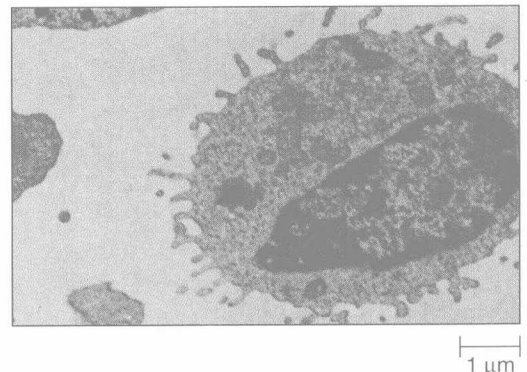
b.: Courtesy Kessel, R.G., and Kardon, R.H., *Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy*. 1979. W.H. Freeman and Company.



a. Image by compound light microscope



b. Image by scanning electron microscope



c. Image by transmission electron microscope

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 microscope's ability to show detail, or its 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 0.1 nm 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.

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 \times the one below it.)

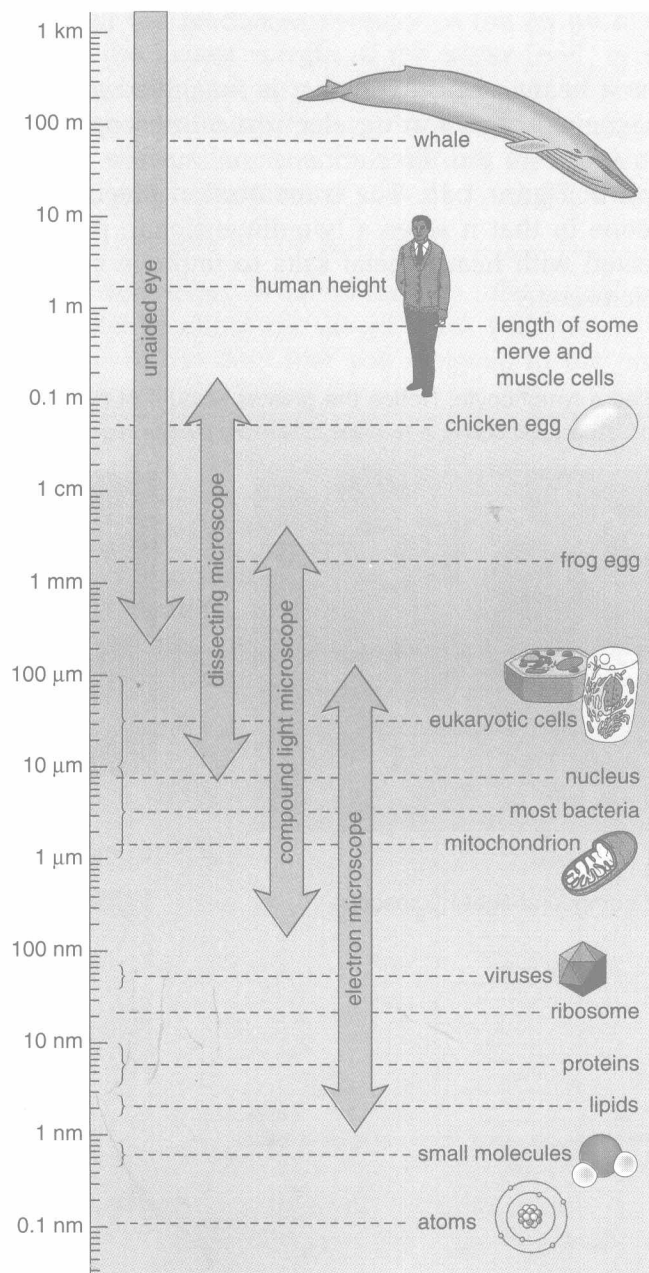


Table 1.2 Comparison of the Compound Light Microscope and Electron Microscope

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 \cong 200 nm	3. Resolution \cong 0.1 nm
4. Magnifies to 2,000 \times	4. Magnifies to 100,000 \times
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

Conclusions

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:

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

1.3 Use of the Compound Light Microscope

Identification of Parts

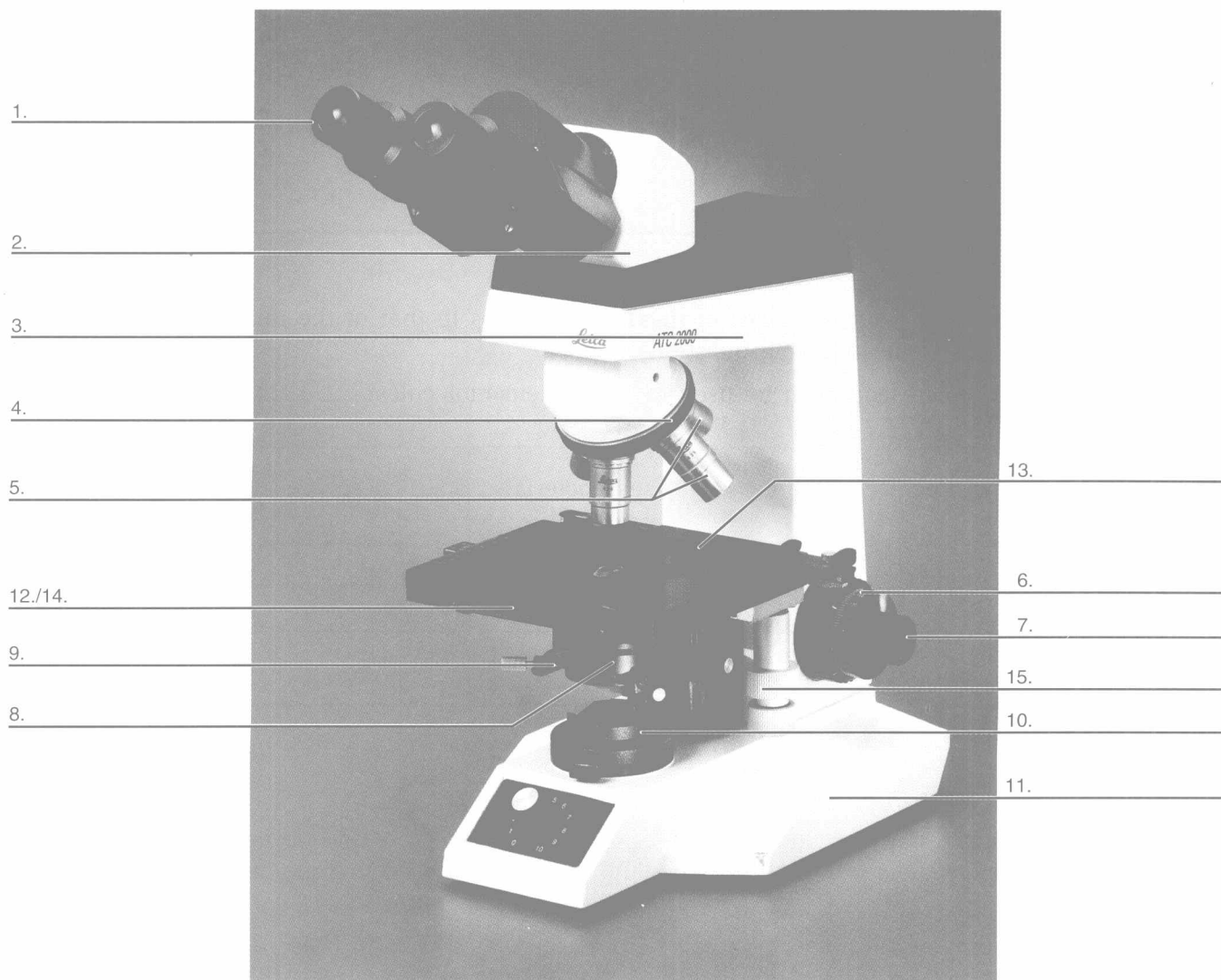
After your instructor has explained how to carry a microscope, obtain one from the cabinet and place it securely on the table. Identify the following parts on your microscope, and label them in Figure 1.5.

1. **Eyeiece (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:** This is the shortest of the objective lenses and is used to scan the whole slide. The magnification is stamped on the housing of the lens. It is a number followed by an \times . What is the magnifying power of the scanning lens on your microscope? _____
 - b. **Low-power objective:** This lens is longer than the scanning lens and is used to view objects in greater detail. What is the magnifying power of this lens on your microscope? _____
 - c. **High-power objective:** If your microscope has three objective lenses, this lens will be the longest. It is used to view an object in even greater detail. What is the magnifying power of the high-power objective lens on your microscope? _____
 - d. **Oil immersion objective** (on microscopes with four objective lenses): Holds a 95 \times (to 100 \times) 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.

Figure 1.5 Compound light microscope.

Compound light microscope with binocular head and mechanical stage

Courtesy of Olympus America, Inc., Precision Instrument Division.



6. **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. **Condensor:** Lens system below the stage used to focus the beam of light on the object to be viewed.
9. **Diaphragm or diaphragm control lever:** Controls amount of illumination used to view the object.
10. **Light source:** An attached lamp that directs a beam of light up through the object.
11. **Base:** The flat surface of the microscope that rests on the table.
12. **Stage:** Holds and supports microscope slides.
13. **Stage clips:** Hold slides in place on the stage.
14. **Mechanical stage** (optional): A moveable stage that aids in the accurate positioning of the slide. Does your microscope have a mechanical stage? _____
15. **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.