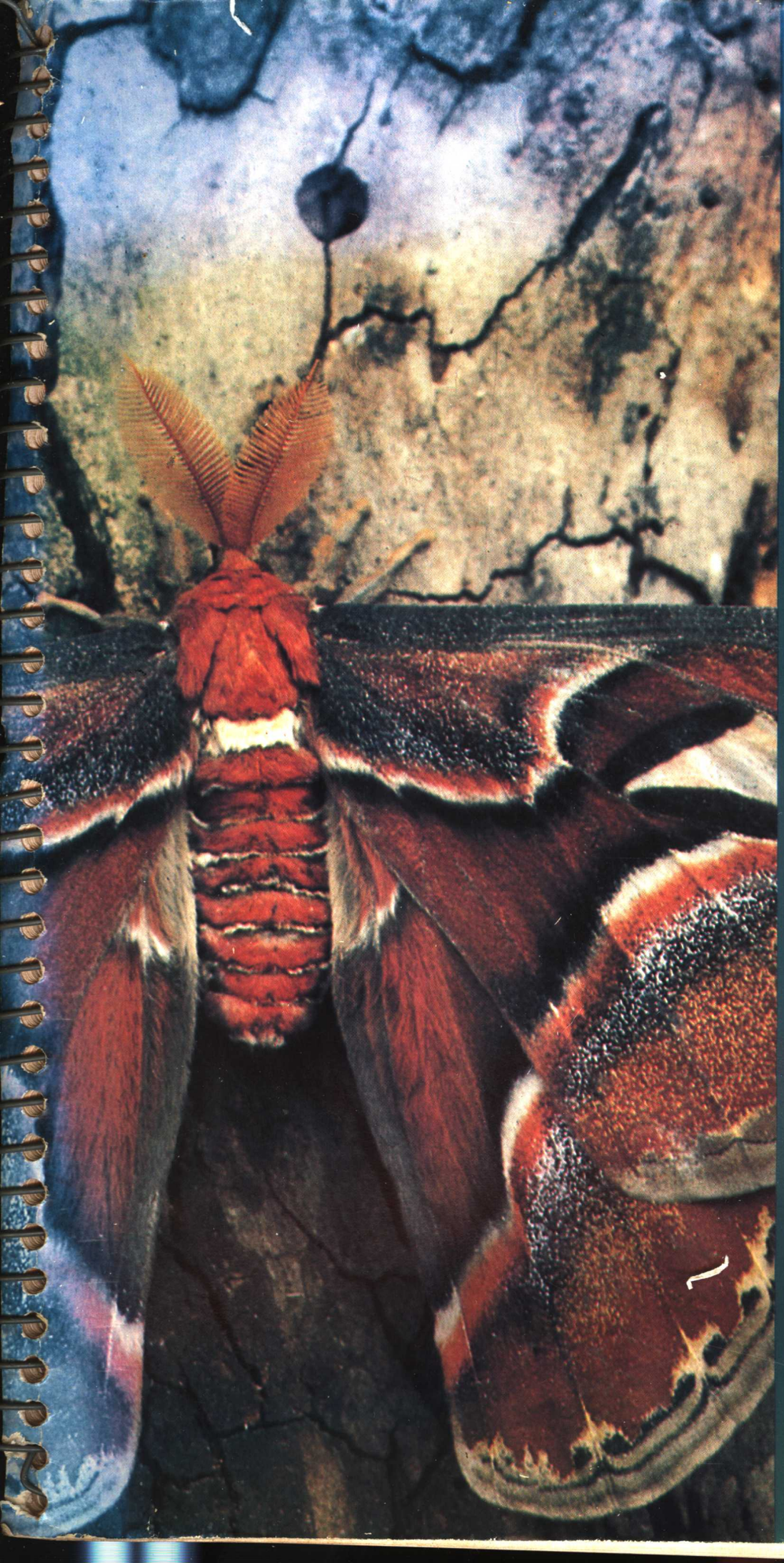

Inquiry into Life

Laboratory Manual

Fourth Edition

Sylvia S. Mader

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Preface

The laboratory exercises in this manual are coordinated with *Inquiry into Life,* a general biology text that covers the entire field of biology and emphasizes the application of biological knowledge to the concerns of humans and their relationships with other organisms. This laboratory manual may also be coordinated with other texts, as is frequently done.

The manual is as practical as the textbook it accompanies. The exercises have been tested for student appeal, ease of preparation, and educational value. They can be performed easily in most laboratory settings, and instructors can be confident that the exercises provide opportunities for their students to actively pursue important biological knowledge and skills.

TO THE INSTRUCTOR

Flexible Handling

To enable an instructor to design laboratory sessions that suit the particular orientation of a given course, the author has taken several measures. There are a sufficient number of exercises to permit a choice of activities over the length of a course. Many laboratories contain optional activities that can be used if time permits. Some instructors may prefer to utilize particular activities as demonstrations rather than student exercises.

Practicality

The author made a deliberate effort to provide that students work with living material during some part of each laboratory. Even so, in only a few 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 can be found in most laboratories or general supply houses. Furthermore, the exercises are designed so that they may be performed in a number of successive laboratory sessions if that is desirable or necessary.

Instructional Aids

To help the instructor prepare for the laboratories, each one begins with a list of materials and equipment. The items listed are generally quite simple, readily available, and easily used by students. Additional lab preparation instructions, located in the Appendix, further assist with planning the day's work. This section contains information on sources of supplies, solution preparation, and apparatus setup as well as troubleshooting recommendations. At the back of the manual are some laboratory practical examination answer sheets that can be removed and used when desired.

To the Student

A number of student aids focus on important aspects of each exercise. They will help you to organize the inquiry process for your laboratories.

Each laboratory begins with a list of learning objectives that define the goals of the laboratory session. You should find them useful as you review for a laboratory practical or any other kind of examination. Then comes an introduction that establishes the rationale for the coming work and provides background information you need for the upcoming experiments. Boldface type indicates important vocabulary, and italics indicates specific directives for the exercise. Throughout, you will find spaces for recording your answers to the questions and the results of your investigations and experiments.

Each laboratory ends with a set of review questions covering the day's work. To provide instant feedback, the answers to the questions are provided at the end of each set of questions. The Appendix contains a review of the metric system and the classification system for organisms used by the author. All of these aids have been designed with your needs in mind.

ADDITIONAL AIDS

Student Study Guide

To ensure coordination with the text, the author also wrote the *Student Study Guide* that accompanies *Inquiry into Life.* For each text chapter, there is a study guide chapter, which includes a listing of behavioral objectives, pretest, study exercises, and a posttest. At the end of each study guide chapter, the student will find answers to all of the questions so that immediate feedback is provided.

Instructor's Manual

The *Instructor's Manual* is designed to assist instructors as they plan and prepare for classes using *Inquiry into Life.* Suggestions for possible course organizations for semester and quarter systems are offered, as are suggestions for alternative sequencing of chapters. An outline and a general discussion give the overall rationale for each chapter. A large number of objective test questions and several essay questions are also provided for each chapter, as is a list of suggested films. The appendix gives the addresses of film suppliers including some that supply films for a moderate fee or free of charge.

Contents

Laboratory 1 Use of the Light Microscope

Materials*

Prepared slides of letter *e* and colored threads
Millimeter rulers
Slides and coverslips
Toothpicks
Onion
Methylene blue or IKI
Scalpels
Pond water or live *Paramecium*
Methyl cellulose (protoslo)

Learning Objectives

Each student should be able to

1. state the linear units of measurement in the metric system and convert one unit to another;
2. name and give the function of 12 basic parts of a light microscope;
3. list three major characteristics of microscopes and explain how these vary in an electron microscope and light microscope;
4. describe how the slide of the letter *e* and the slide of threads provide information on the inversion and depth of field, respectively, of a light microscope;
5. calculate the diameter of field and total magnification of high and low power;
6. list, in the proper order, steps to be used for bringing an object into focus with the light microscope;
7. identify the three types of cells studied in this exercise and state three differences between plant and animal cells.

Introduction

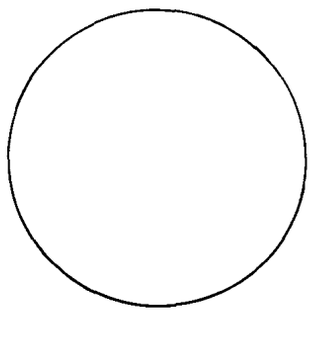
Metric System**

All linear measurements in the laboratory will be stated in the metric system. Obtain a ruler marked in centimeters and millimeters on one side, and inches and its divisions on the other side. Compare one side to the other. One inch equals how many centimeters? _____ 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 nanometer (nm). According to figure 1.1, one millimeter = _____ micrometers = _____ nanometers. According to table 1.1, one micrometer = _____ mm and one nanometer = _____ μm.

To demonstrate that you understand the relationship of one metric unit to another, as described in figure 1.1 and table 1.1, *fill in* the blanks below.

1.5 mm = _____ μm 1000 nm = _____ μm
0.25 mm = _____ μm 1,500 μm = _____ mm
55 nm = _____ μm 7.5 μm = _____ nm
1.5 cm = _____ mm = _____ μm
5,000 nm = _____ μm = _____ mm _____ cm



Measure the diameter of this circle to the nearest millimeter.

This circle is _____ mm; _____ μm; _____ nm.

*See Appendix.
**Linear measurements only are considered in this laboratory. Other types of measurements are reviewed in the Appendix.

Figure 1.1 Life-size ruler. The relationship of nanometers to micrometers to millimeters is indicated.

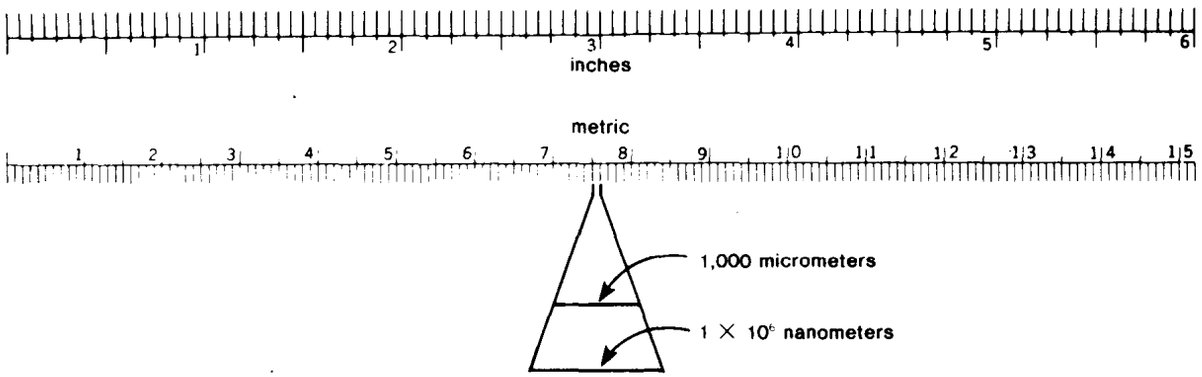


Table 1.1 Units of measurement

Unit	Symbol
Centimeter	cm = 0.4 inch
Millimeter	mm = 0.1 cm
Micrometer	μm = 0.001 mm
Nanometer	nm = 0.001 μm

Microscopes

Because biological objects can be very small (fig. 1.2) it is often necessary to use a microscope to view them. Many kinds of instruments serve as effective magnifying devices, ranging from the hand lens to the electron microscope. A short description of two kinds of light microscopes and of the electron microscope follows. Your instructor may wish also to discuss the phase contrast, dark field, reflecting, polarizing, X-ray, fluorescence, and UV microscopes.

Light Microscope

Light microscopes use light rays that are magnified and focused by means of lenses.

The *dissecting microscope* is designed to study entire objects in three dimensions at low magnification.

The *compound light microscope* is used for examination of small or thinly sliced cross sections or longitudinal sections of objects under magnification that is higher than that of the dissecting 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 light microscope.

Electron Microscope

Electron microscopes use a beam of electrons that are 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 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 thinly sliced and treated with heavy metal salts to improve contrast. Figure 1.3c is an image produced by this type of microscope.

Figure 1.2 compares the visual range of these microscopes to the naked eye. According to this figure, what is the smallest object that could be detected by the

1. eye _____
2. dissecting light microscope _____
3. compound light microscope _____
4. electron microscope _____

Figure 1.2 Visual range of microscopes as compared to the range of the naked eye. Originally in Anderson, P., and Mader, S.: *General Biology*. Dubuque, Iowa: Kendall/Hunt Publishing Company, 1973.

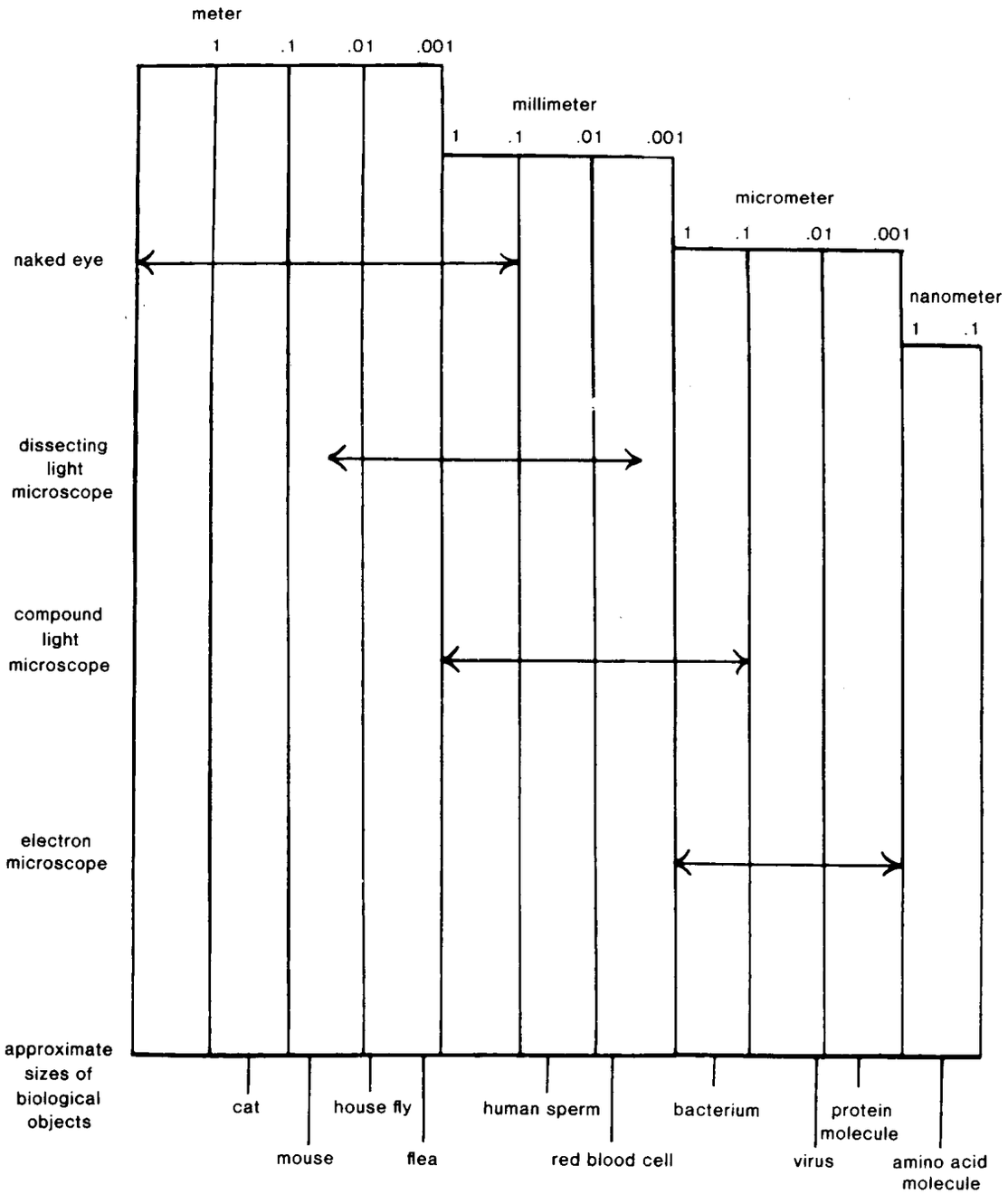


Figure 1.3 Images of a white blood cell, called a *lymphocyte,* produced by *(a)* compound light microscope, *(b)* scanning electron microscope, and *(c)* transmission electron microscope. Notice the greater amount of detail in *(c)* compared to *(a).*

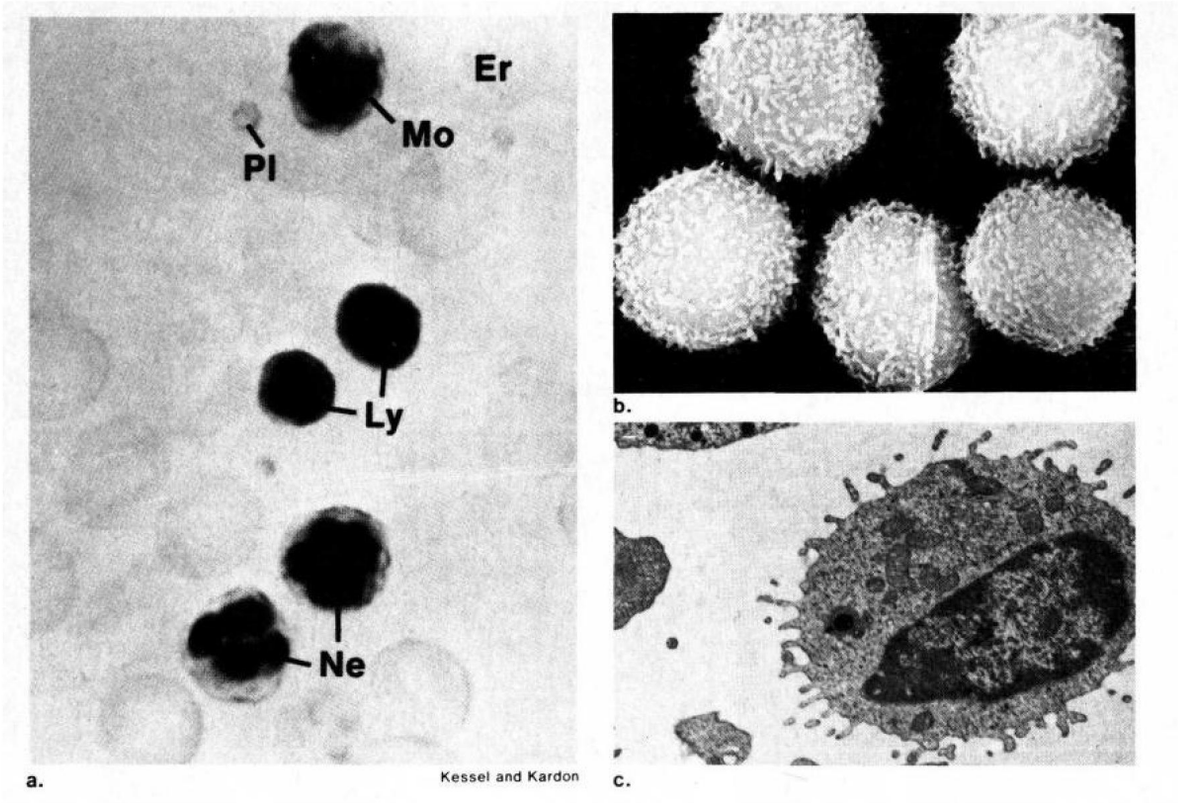


Table 1.2 A comparison of a light and an electron microscope

Light	Electron
1. Glass lenses	1. Electromagnetic lenses
2. Illumination by light of many wavelengths	2. Illumination due to beam of electrons of one wavelength
3. Resolution $\simeq$ 0.1 μm	3. Resolution $\simeq$ 1 nm
4. Magnifies to 2,000×	4. Magnifies to 100,000×
5. Cost: $50-several $1,000	5. Cost: $20,000-$250,000
6. Specimen may be living or dead	6. Specimen must be dead

It is obvious from figure 1.2 that the electron microscope allows one to view much smaller objects than the light microscope does. Figure 1.3 demonstrates this visually also. The difference between these two instruments is not simply a matter of magnification; it also has to do with the electron microscope's ability to show detail, or the *resolving power* of the microscope. The use of electrons rather than light gives the electron microscope a much greater resolving power. Table 1.2 lists several differences between the compound light microscope and the electron microscope.

Use of the Compound Light Microscope

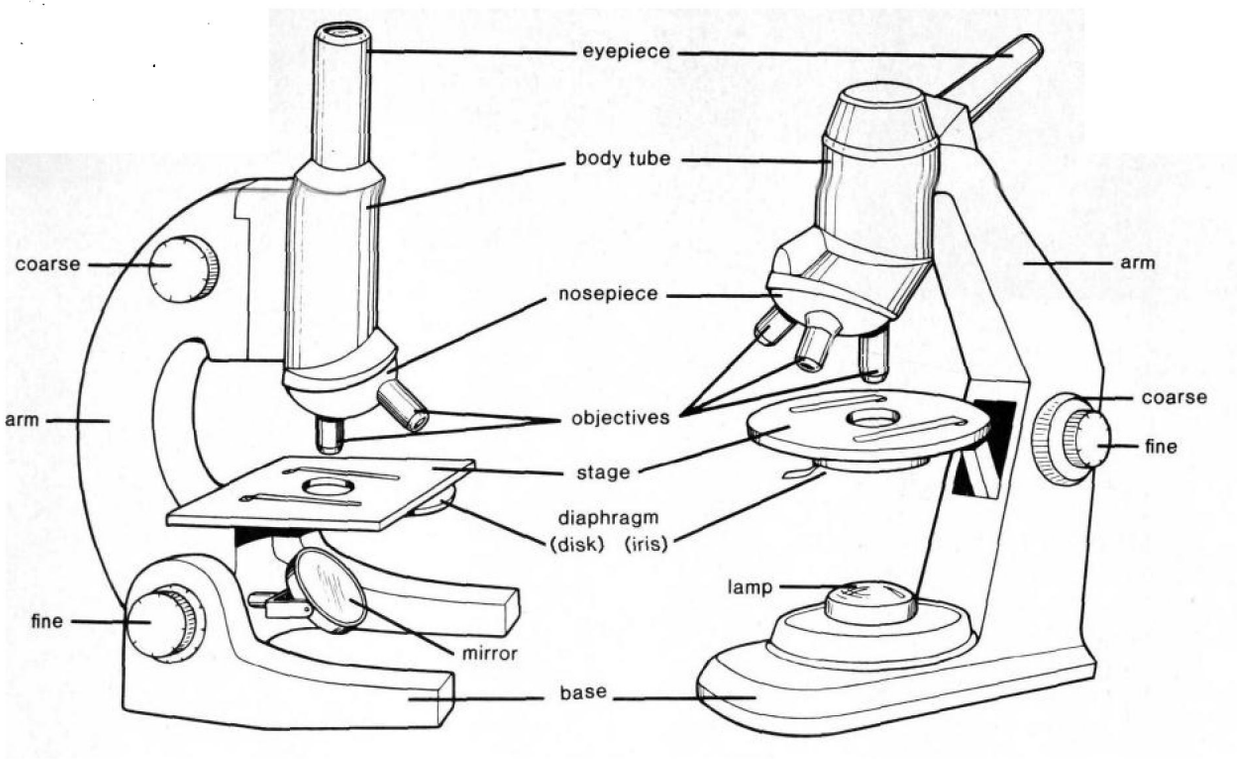
Identification of Parts

After your instructor has told you how to carry the microscope, *obtain* one from the cabinet and place it securely on the table. *Identify* the following parts with the help of figure 1.4.

Eyepiece (ocular lens): Topmost series of lenses through which an object is viewed

Body tube: Holds nosepiece at one end and eyepiece at the other end; conducts light rays

Figure 1.4 Comparison of two basic types of light microscopes.



Arm: Supports upper parts and provides carrying handle

Nosepiece: Revolving device that holds objectives

Scanning power objective: Holds 4× lens used to view the whole slide

Low power objective: Holds 10× lens used to view the object in greater detail

High power objective: Holds 43× lens used to view the object in even greater detail

Stage: Holds and supports microscope slides

Coarse adjustment knob: Knob used to bring object into approximate focus; used only with low power objective

Fine adjustment knob: Knob used to bring object into final adjustment

Diaphragm: Controls amount of illumination used to view the object

Light source: Either a mirror that reflects light or an attached lamp that directs a beam of light up through the object

Focusing the Microscope—Low Power

1. With the *coarse adjustment knob, raise* the nosepiece until it stops.
2. *Place* a slide of the letter *e* on the stage and stabilize it with the clips. *Center* the *e* as best you can on the stage.
3. Make sure the 10× objective is in place; then *as you look from the side decrease* the distance between the stage and the nosepiece until the nosepiece is no closer than ⅛ in. above the slide.
4. Looking into the ocular, *rotate* the diaphragm to give the maximum amount of light.
5. Slowly *increase* the distance between the stage and the nosepiece using the coarse adjustment knob until the object—in this case, the letter *e*—comes into view or focus.
6. Once the object is seen, it may be necessary to adjust the amount of light. To create shadows, rotate the diaphragm slightly.
7. Use the fine adjustment knob to sharpen focus if necessary.

Inversion

1. In the space provided here, *draw* the letter *e* as it appears on the slide (look from the side, not through the eyepiece).
2. Next to this, *draw* the letter *e* as it appears when you look through the eyepiece.

1.	2.

What differences do you notice? Inversion refers to the fact that the image is not only inverted but it is also reversed.

Move the slide to the right. Which way does the image appear to move? _____

Focusing the Microscope—High Power

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

1. Bring object into focus under low power by following previous instructions (p. 5).
2. *Make sure* the letter *e* is centered on the stage beneath the low power objective.
3. *Move* the high power objective into place by turning the nosepiece.
4. If any adjustment is needed, use only the *FINE* adjustment knob. Use only the fine adjustment knob with high power. On your drawing of the letter *e* as it was observed with low power, *draw* a circle around the portion of the letter that you are now seeing with high power magnification. When you have finished your observations of this slide (or any slide), *rotate* the nosepiece until the low power objective clicks in place and then *remove* the slide from the stage.
5. The following rules should also be observed:
   a. Have both eyes open when looking through the eyepiece.
   b. The low power, or scanning, objective should be in position both at the beginning and the end of use.
   c. Use only lens paper for cleaning the lens.
   d. Do not tilt the microscope.
   e. Keep the stage clean and dry to prevent rust and corrosion.
   f. Do not remove parts of the microscope.
   g. Keep the microscope dust free by covering.
   h. Report any malfunctioning.

Total Magnification

Total magnification is calculated by multiplying the magnification of the ocular lens by the magnification of the objective lens. What is the total magnification of

	Ocular Lens		Objective Lens		Total
Low power:	_____	×	_____	=	_____
High power:	_____	×	_____	=	_____

Diameter of Field

Low Power

Place a clear plastic ruler across the stage so that the inner edge of the ruler is visible as a vertical line along the diameter of the low power field. Be sure you are looking at the millimeter side of the ruler. *Estimate* the number of millimeters, to tenths, that you see along the field and note this here: _____ . *Change* this figure to micrometers: _____ . This is the diameter of field for low power.

High Power

First Method

Figure the fractional difference between low power magnification and high power magnification of your microscope. (If your low power objective is 10X and your high power objective is 40X, then the fractional difference is 4.) *Divide* this into the diameter of the field for low power: _____ . This is the diameter of field for high power.

Second Method

Show that you get exactly the same results by using the following formula to calculate the diameter of field for high power:

$$\text{HPD} = \text{LPD} \times \frac{\text{LPM}}{\text{HPM}} = \_\_\_\_\_$$

HPD = high power diameter of field
LPD = low power diameter of field
HPM = high power magnification
LPM = low power magnification

In comparing low power to high power, which has a larger field and allows you to see more of the object? _____ Which has a smaller field, but magnifies to a greater extent? _____

Depth of Focus

Obtain a slide with three or four colored threads mounted together, or make a slide by crossing a blond hair with a brown hair (using the directions that follow for making a wet mount). With low power, *find* a point where the threads or hairs cross. Slowly *focus* up and down. *Notice* that when one thread or hair is in focus, the other seems blurred. Determine the order of the threads or hairs and *complete* table 1.3.

Table 1.3 Order of threads

Depth	Thread color
Top	
Middle	
Bottom	

The vertical distance that remains in focus at one time is called the **depth of focus.** *Switch* to high power and *notice* that the depth of focus is more shallow with high power than with low power. If you make constant use of the fine adjustment knob when viewing a slide with high power, you will get an idea of the specimen's three-dimensional form. It would be possible, for example, to reconstruct the three-dimensional structure in figure 1.5 from sections *1, 2,* and *3* in the figure.

Figure 1.5 A demonstration of how focusing at depths (1), (2), and (3) would produce three different images that could be used to reconstruct the original three-dimensional structure. (From Dolphin, Warren D., *Laboratory Manual* to accompany *Biology*. © 1983 Wm. C. Brown Publishers, Dubuque, Iowa. All Rights Reserved. Reprinted by permission)

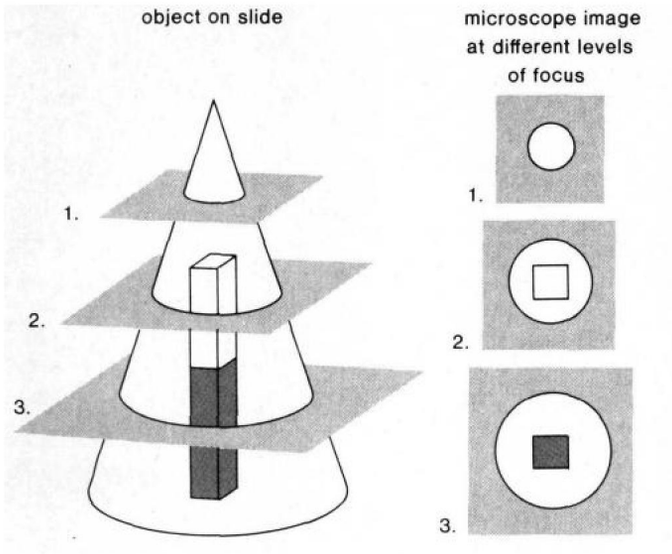
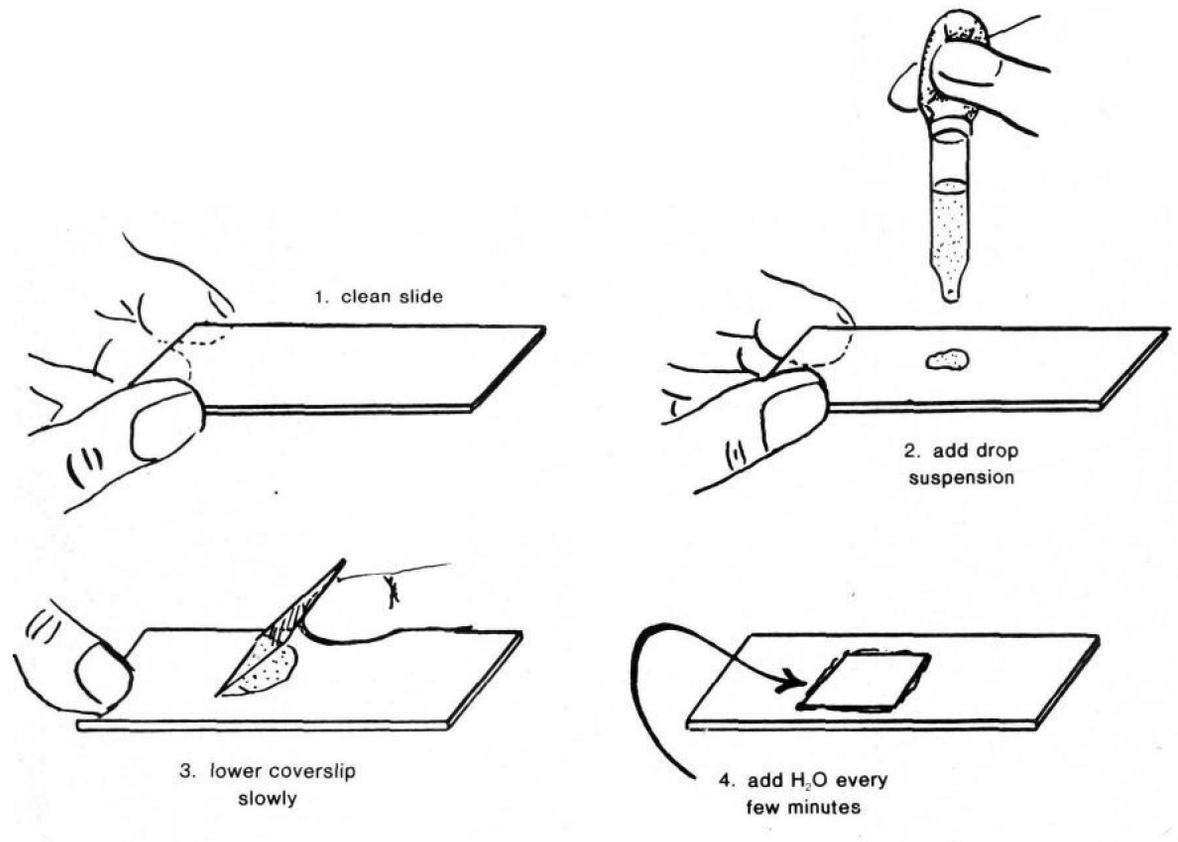


Figure 1.6 Preparation of wet mount. (Originally in Anderson, P., and Mader, S.: *General Biology*. Dubuque, Iowa: Kendall/Hunt Publishing Company, 1973)



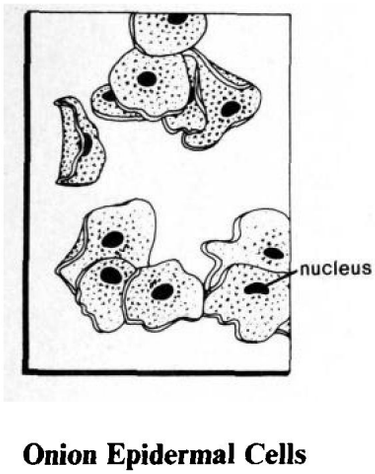
Observations

Often it is necessary to prepare a specimen for observation. In such cases, the object should always be viewed as a **wet mount**. A wet mount is prepared by placing a drop of liquid on a slide or, if the material is dry, by placing it directly on the slide and adding a drop of water or stain. The mount is then covered with a coverslip, as illustrated in figure 1.6.

Human Epidermal Cells

Gently *scrape* the inside of your cheek with a clean, flat toothpick and *place* the scrapings on a clean dry slide. *Add* a drop of very weak methylene blue or IKI and *cover* with a cover slip. *Observe* under the microscope. *Locate* the nucleus, a central round body in each cell (fig. 1.7).

Figure 1.7 Cheek cells. (Originally in Anderson, P., and Mader, S.: *General Biology.* Dubuque, Iowa: Kendall/Hunt Publishing Company, 1973)



Onion Epidermal Cells

With a scalpel, *strip* a small, thin transparent layer of cells from the *inside* of an onion leaf. *Place* it gently on a clean glass slide and *add* a drop of methylene blue. *Cover* with a cover slip and observe. *Locate* the cell wall and the nucleus near the cell wall (fig. 1.8). *Note* some obvious differences between the plant cell and the animal cell and list them in table 1.4.

Estimate how many onion cells stretch across the low power field. _____ Knowing the diameter of the field as calculated above, decide the length of each onion cell. _____

Figure 1.8 Onion cells. (Originally in Anderson, P., and Mader, S.: *General Biology.* Dubuque, Iowa: Kendall/Hunt Publishing Company, 1973)

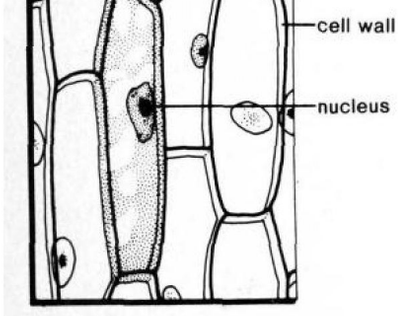


Table 1.4 Differences between plant and animal cells

Differences	Plant cell	Animal cell
Shape		
Cell wall		
Other		

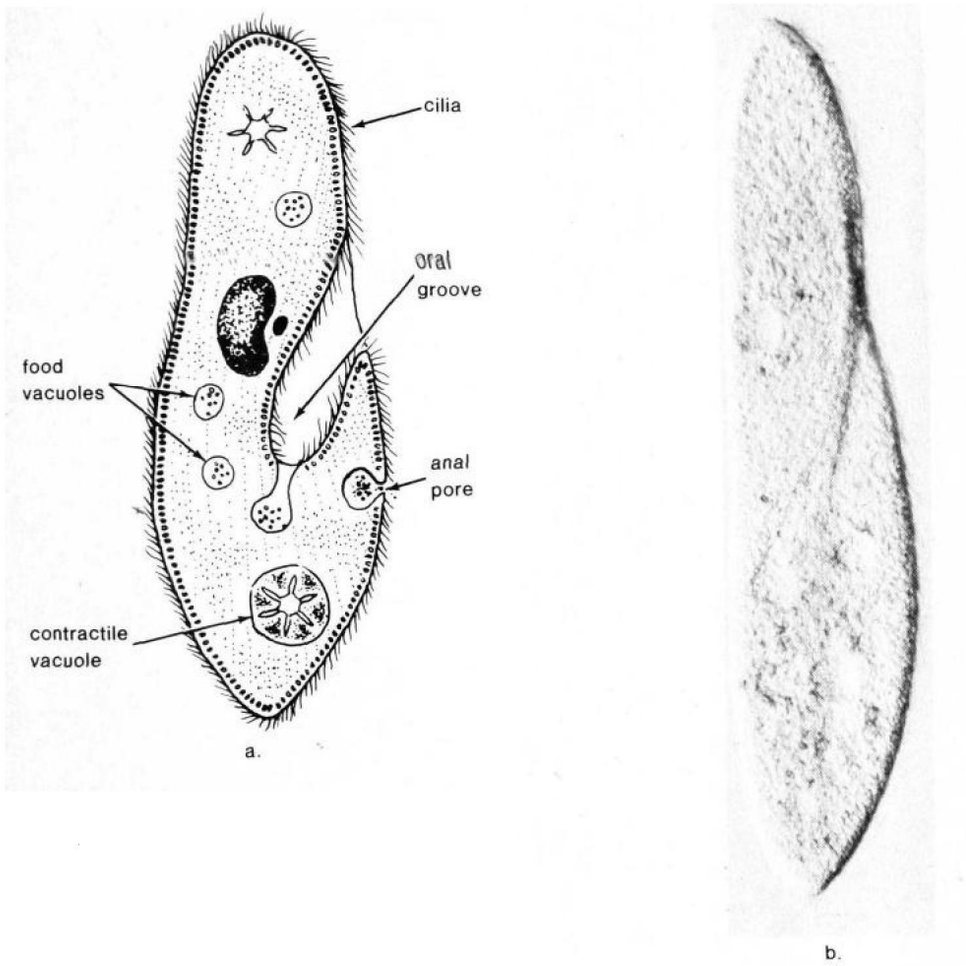
Pond Water

Examination of pond water will serve as a critical test of your ability to observe objects with the microscope and to utilize depth of focus. *Make* a wet mount of pond water by taking a drop from the bottom of the container. To locate any organisms present, *scan* the slide: start at the upper left-hand corner and *move* the slide forward and back as you work across the slide from the left to the right. Use the pictorial guides provided by your instructor to assist you in identifying the organisms present. Be sure to use both low and high power and to focus up and down with the fine adjustment knob.

Paramecium

Examination of *Paramecium* can serve as an alternate if pond water is not available. *Make* a wet mount of *Paramecium* by using a drop of the culture from the bottom of the container and add a drop of methyl cellulose (protoslo) on a slide. The protoslo slows the organism's swimming. Mix thoroughly and add a coverslip. To locate the *Paramecium*, *scan* the slide: start at the upper left-hand corner and *move* the slide forward and back as you work across the slide from the left to the right. The *Paramecium* may be at the edge of the slide because it shows an aversion to the protoslo. Use figure 1.9 to help you identify structural details. Be sure to use both low and high power and to focus up and down with the fine adjustment knob.

Figure 1.9 a. Diagrammatic representation of *Paramecium*. b. Photomicrograph of *Paramecium*. (a: From Reynolds, William W., *Laboratory Manual for Man, Nature, and Society*. © 1975 William C. Brown Publishers, Dubuque, Iowa. All Rights Reserved. Reprinted by permission)



Laboratory Review 1

_____ 1. Which objective should always be in place, both when beginning to use the microscope and when putting it away?

_____ 2. Which objective is in focus at the greatest and safest distance from the slide?

_____ 3. A total magnification of 100× requires the use of the 10× ocular lens with which objective?

_____ 4. If the letter *e* is moved to the right, in what direction does it appear to move in the field of view?

_____ 5. If the letter *e* is placed on the stage in this position *(upright)*, in what position does it appear in the field of view?

_____ 6. A microscope is called compound when it has more than one set of _____ .

_____ 7. What part of a microscope regulates the amount of light?

_____ 8. What word is used to indicate that if the object is in focus at low power, it will also be in focus with high power?

_____ 9. What part of the microscope is used if some focusing adjustment is needed with high power?

_____ 10. Is as much of the letter *e* visible with high power as with low power?

_____ 11. If the diameter of field is 1600 micrometers with low power, then what is the diameter of field at high power (400×)?

_____ 12. If the thread layers are red, brown, green from top to bottom, then which layer will appear first if the student is using the microscope properly?

Answers to Laboratory Review 1

1. low power; **2.** low power; **3.** low power; **4.** to the left; **5.** ə; **6.** lenses; **7.** diaphragm; **8.** parfocal; **9.** fine adjustment knob; **10.** no; **11.** 400 micrometers; **12.** green

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Paul, C. 1972. *Principles of biological science.* Dubuque, Iowa: Kendall/Hunt Publishing Company.