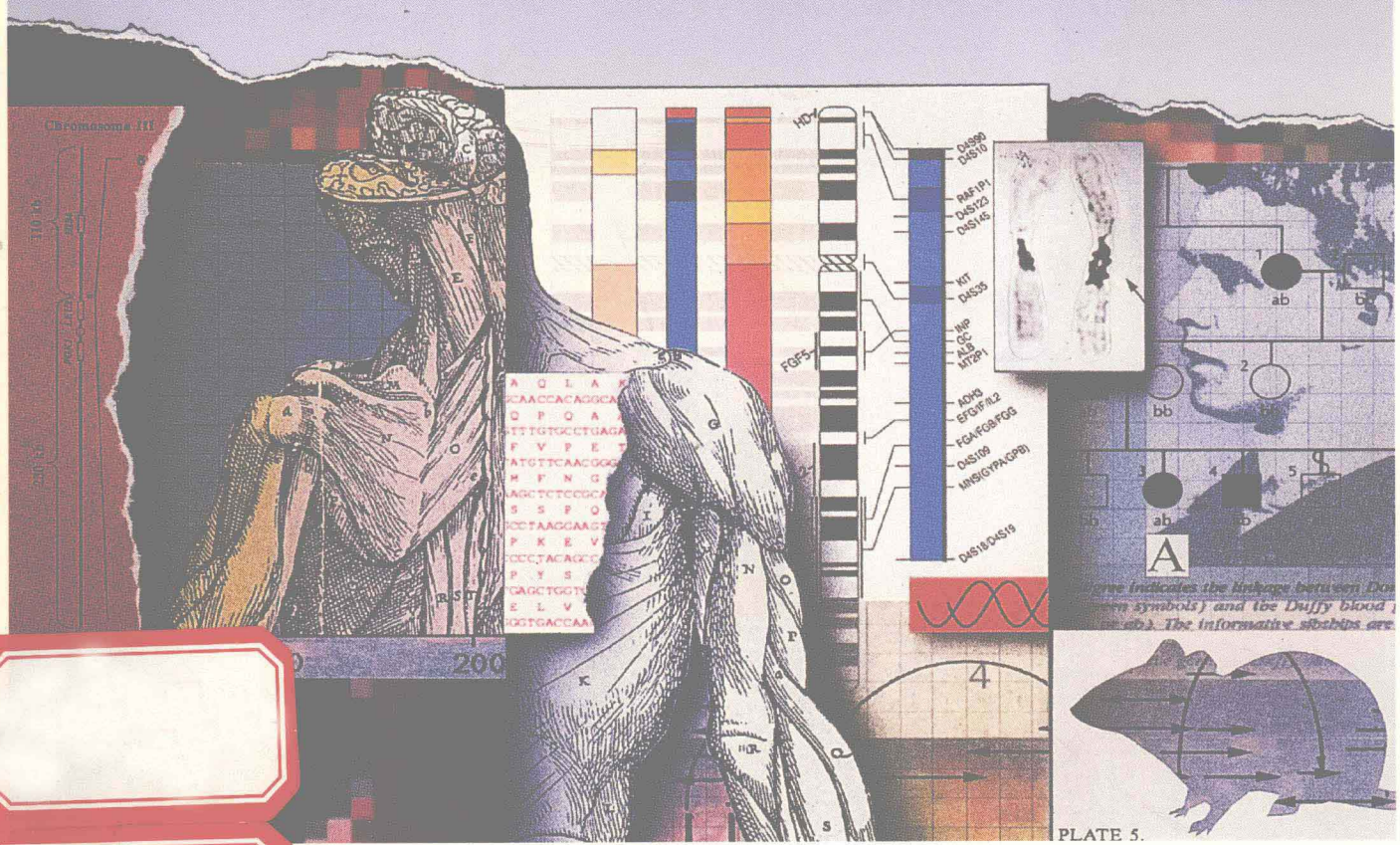
Fourth Edition

BIOLOGY
Laboratory Manual



Darrell S. Vodopich
Randy Moore

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Fourth Edition

BIOLOGY
Laboratory Manual

to accompany
BIOLOGY, 4/e

Darrell S. Vodopich
Baylor University

Randy Moore
University of Akron

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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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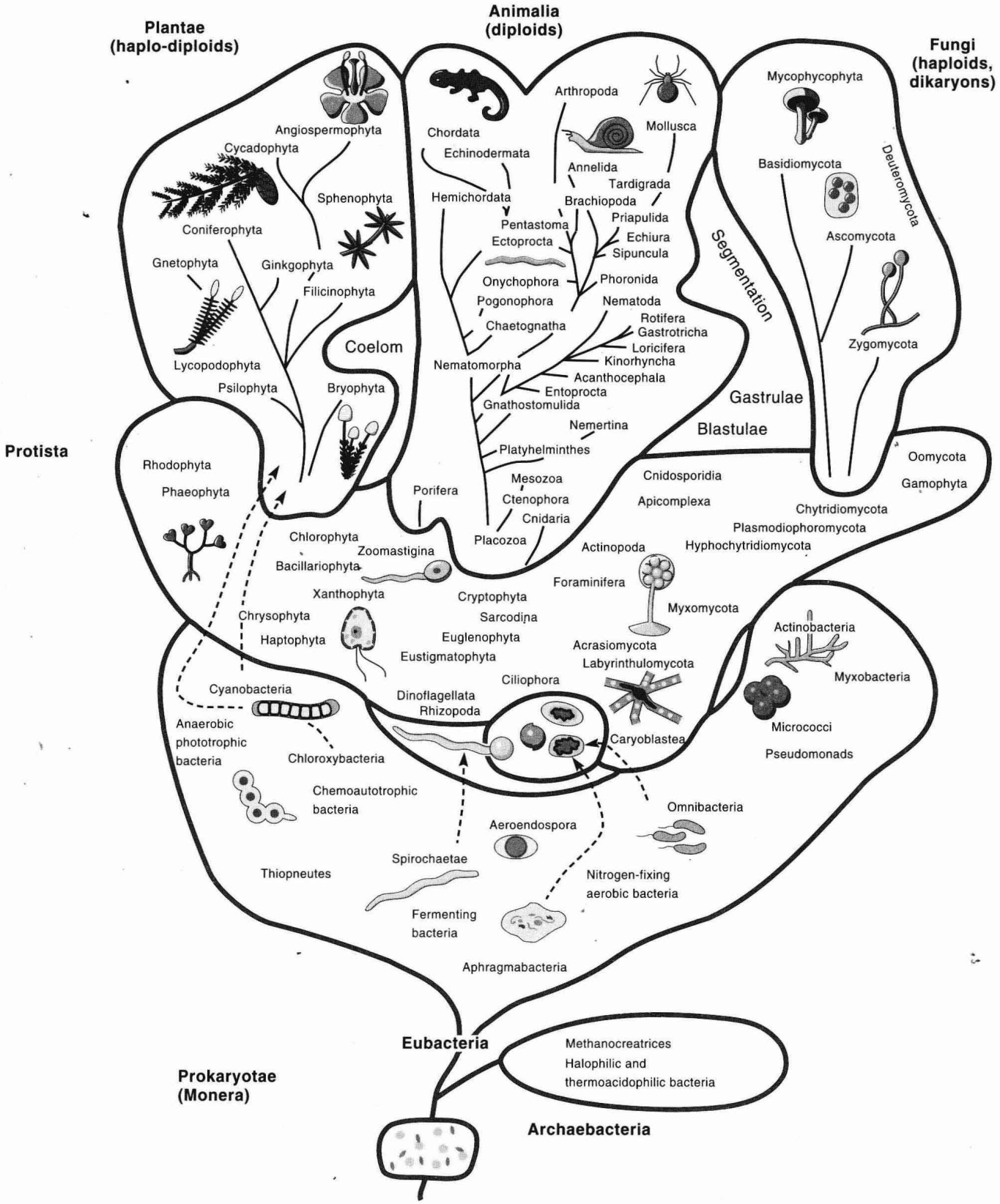

Classification and phylogeny of five kingdoms by Margulis and Schwartz

From *Five Kingdoms* 2/e by Margulis and Schwartz.

BIOLOGY
Laboratory Manual

Preface

We designed this laboratory manual for an introductory biology course with a broad survey of basic laboratory techniques. The experiments and procedures are simple, easy to perform, and especially appropriate for large classes. Few experiments require a second class meeting to complete the procedure. Each exercise includes many photographs, traditional topics, and experiments that work. Procedures within each exercise are numerous and discreet 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 seek and notice things that others may overlook. Now is the time to develop this ability with a mixture of hard work and relaxed observation. Have fun, and learning will come easily. Also, remember that this manual is designed with your instructors in mind as well. Go to them often with questions—their experience is a valuable tool that you should use as you work.

To the Instructor

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 done easily by a student in three 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, and is not an autotutorial system. We've tried to guide students from observations to conclusions, and to make the transition to biological principles. But discussions and interactions 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 Fourth Edition

All exercises have been thoroughly reviewed and revised. The following laboratory exercises are new to this fourth edition: Exercise 14, Human Evolution: Skull Examination; Exercise 15, Ecology: Diversity and Interaction in Plant Communities; Exercise 35, Human Biology: The Human Skeletal System; Exercise 36, Human Biology: Muscles and Muscle Contraction; and Exercise 44, Animal Behavior: Taxis, Kinesis, and Agonistic Behavior. These new investigations and experiments apply contemporary and traditional techniques to current topics. In addition to the 44 lab exercises, this manual offers five Optional Exercises: Exercise A, Separating Organic Compounds; Exercise B, Spectrophotometry; Exercise C, Bioassay; Exercise D, Dissection of a Fetal Pig; and Exercise E, Community Succession. A new appendix (Appendix II, A Classification of Organisms) appears in addition to Appendix I (Measurements in Science) and Appendix III (How to Write a Scientific Paper or Laboratory Report). The order of laboratory exercises reflects their treatment in *Biology*, fourth edition, by Peter H. Raven and George B. Johnson (see

the correlation table, page ix). However, all exercises are self-contained and compatible with any modern general biology textbook.

The illustration program has been completely revised and enhanced for this new edition. All exercises are in full color, and illustrations that explain procedures have been added for clearer instruction.

The exercises that survey the kingdoms emphasize continuity. The photographs illustrate diversity and the incredible strategies that organisms use to survive and reproduce. Many evolutionary themes and discussions can be based on the diversity of adaptations we've illustrated.

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. In addition to questions, many exercises contain a suggested topic for a writing assignment (Writing to Learn Biology) and a suggested experiment or hands-on procedure for work beyond the classroom (Doing Biology Yourself). These are especially appropriate for classes of advanced students.

A *Laboratory Resource Guide* is available from Wm. C. Brown Publishers and includes a complete list of all materials needed. It also contains helpful comments for instructors about setting up and doing the experiments covered in this laboratory manual. In addition, this revised resource guide now includes expanded descriptions of experimental preparations and specific catalog numbers for purchase of prepared slides and other supplies.

Darrell S. Vodopich
Randy Moore

Reviewers

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Suzanne Koptur	Florida International University
John Smarrelli	Loyola University
Roger Vasas	Becker College

Correlation Table

The following chapters in *Biology*, fourth edition, by Peter H. Raven and George B. Johnson, correlate with exercises in this laboratory manual.

Biology Chapter	Laboratory Exercise
1	Exercise 1 The Microscope: Basic Skills of Light Microscopy
5	Exercise 2 The Cell: Structure and Function
3	Exercise 3 Biologically Important Molecules: Carbohydrates, Proteins, Lipids, and Nucleic Acids
6, 7	Exercise 4 Diffusion and Osmosis: Passive Movement of Molecules in Biological Systems
6	Exercise 5 Cellular Membranes: Effects of Physical and Chemical Stress
8	Exercise 6 Enzymes: Factors Affecting the Rate of Activity
9	Exercise 7 Respiration: Aerobic and Anaerobic Oxidation of Organic Molecules
10	Exercise 8 Photosynthesis: Pigment Separation, Starch Production, and CO_2 Uptake
11	Exercise 9 Mitosis: Replication of Eukaryotic Cells
12	Exercise 10 Meiosis: Reduction Division and Gametogenesis
19	Exercise 11 Molecular Biology and Biotechnology: DNA Isolation and Bacterial Transformation
13	Exercise 12 Genetics: The Principles of Mendel
20	Exercise 13 Evolution: Natural Selection and Morphological Change in Green Algae
23	Exercise 14 Human Evolution: Skull Examination
25, 26	Exercise 15 Ecology: Diversity and Interaction in Plant Communities
24	Exercise 16 Population Growth: Limitations of the Environment

Biology Chapter	Laboratory Exercise
28	Exercise 17 Pollution: The Effects of Chemical, Thermal, and Acid Pollution
29, 30	Exercise 18 Survey of Bacteria: Kingdoms Archaebacteria and Eubacteria
31	Exercise 19 Survey of the Kingdom Protista: The Algae
31	Exercise 20 Survey of the Kingdom Protista: Protozoa and Slime Molds
32	Exercise 21 Survey of the Kingdom Fungi: Molds, Sac Fungi, Mushrooms, and Lichens
33	Exercise 22 Survey of the Plant Kingdom: Liverworts, Mosses, and Hornworts of Phyla Hepaticophyta, Bryophyta, and Anthocerophyta
33	Exercise 23 Survey of the Plant Kingdom: Seedless Vascular Plants of Phyla Pterophyta, Lycophyta, Psilophyta, and Sphenophyta
33	Exercise 24 Survey of the Plant Kingdom: Gymnosperms of Phyla Cycadophyta, Ginkgophyta, Coniferophyta, and Gnetophyta
34	Exercise 25 Survey of the Plant Kingdom: Angiosperms
35	Exercise 26 Plant Anatomy: Vegetative Structure of Vascular Plants
37	Exercise 27 Plant Physiology: Transpiration
36	Exercise 28 Plant Physiology: Tropisms, Nutrition, and Growth Regulators
38, 39	Exercise 29 Survey of the Animal Kingdom: Phyla Porifera and Cnidaria
39	Exercise 30 Survey of the Animal Kingdom: Phyla Platyhelminthes and Nematoda

Biology Chapter	Laboratory Exercise
40	Exercise 31 Survey of the Animal Kingdom: Phyla Mollusca and Annelida
41	Exercise 32 Survey of the Animal Kingdom: Phylum Arthropoda
41, 42	Exercise 33 Survey of the Animal Kingdom: Phyla Echinodermata, Hemichordata, and Chordata
43	Exercise 34 Vertebrate Animal Tissues: Epithelial, Connective, Muscular, and Nervous Tissues
44	Exercise 35 Human Biology: The Human Skeletal System
44	Exercise 36 Human Biology: Muscles and Muscle Contraction
46	Exercise 37 Human Biology: Breathing
46	Exercise 38 Human Biology: Circulation and Blood Pressure
48	Exercise 39 Human Biology: Sensory Perception
43	Exercise 40 Vertebrate Anatomy: External Features and Skeletal System of the Rat
43	Exercise 41 Vertebrate Anatomy: Muscles and Internal Organs of the Rat
43	Exercise 42 Vertebrate Anatomy: Urogenital and Circulatory Systems of the Rat
53	Exercise 43 Embryology: Comparative Morphologies and Strategies of Development
54	Exercise 44 Animal Behavior: Taxis, Kinesis, and Agonistic Behavior

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EXERCISE

1

The Microscope

Basic Skills of Light Microscopy

Objectives

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

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

Many organisms and biological structures are too small to be seen with the unaided eye (fig. 1.1). Biologists often use a light microscope to observe such specimens. A **light microscope** is a coordinated system of lenses arranged to produce an enlarged, focusable image of a specimen. A light microscope **magnifies** a specimen, meaning that it increases its apparent size. Magnification with a light microscope is usually accompanied by improved **resolution,** which is the ability to distinguish two points as separate points. Thus, the better the resolution, the sharper or crisper the image appears. The resolving power of the unaided eye is approximately 0.1 mm (1 in = 25.4 mm), meaning that our eyes can distinguish two points 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, because it was used to formulate the cell theory and study structure at the cellular level. Light microscopy has revealed a vast new world to the human eye and mind (fig. 1.2). Today, the light microscope is the most fundamental tool of many biologists.

The Compound Light Microscope

Study and learn the parts of the typical compound light microscope shown in figure 1.3. A light microscope has two, sometimes three, systems: an illuminating system, an imaging system, and possibly a viewing and recording system.

Illuminating System

The illuminating system, which concentrates light on the specimen, usually consists of a light source, condenser lens, and iris diaphragm. The **light source** is a 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,** located immediately below the specimen, 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; when closed, the image will be dim.

Imaging System

The imaging system improves resolution and magnifies the image. It consists of the objective and ocular (eyepiece) lenses and a body tube. The **objectives** are three or four lenses mounted on a revolving nosepiece. Each objective is actually a series of several lenses that

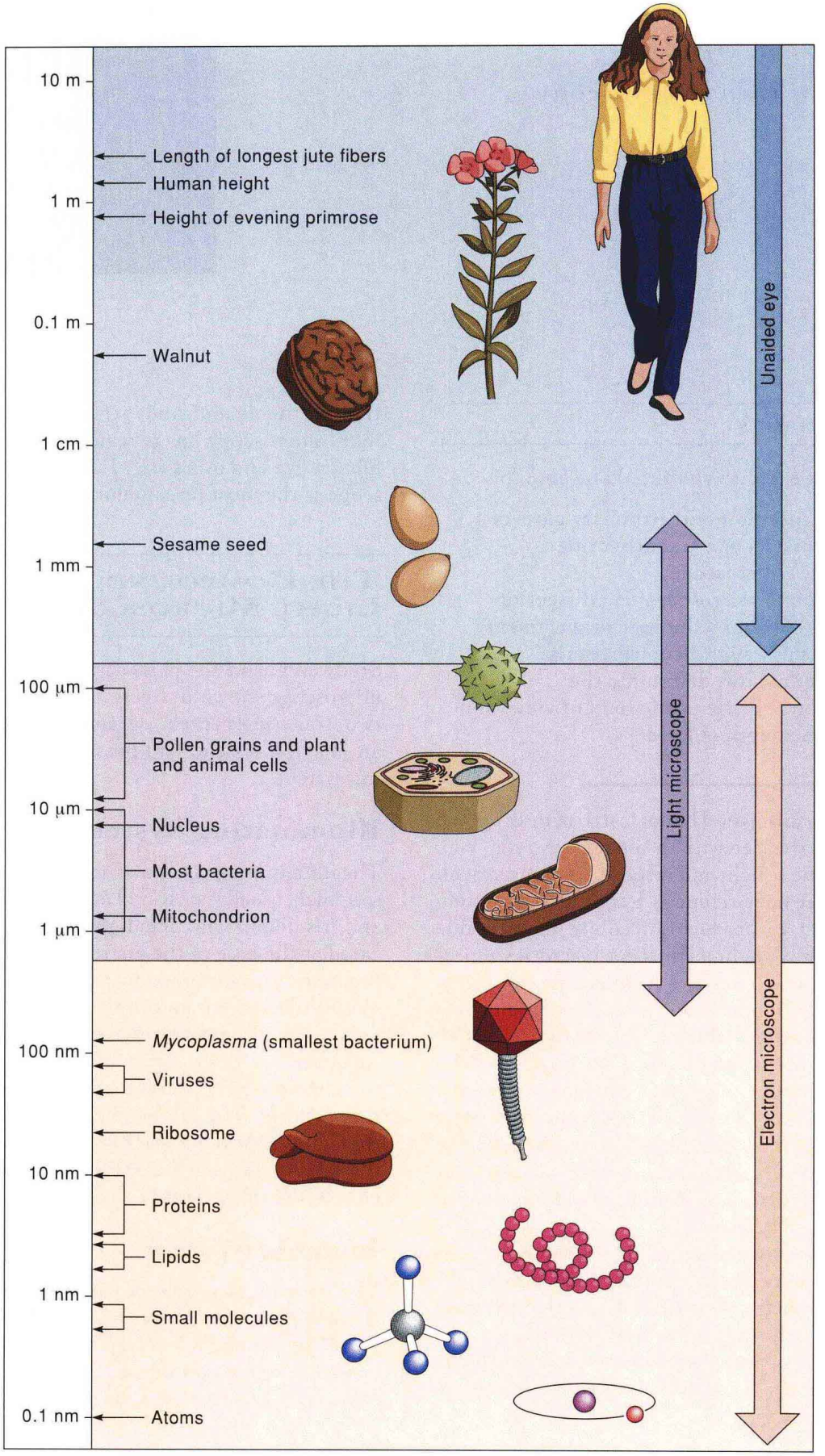

FIGURE 1.1

The relative sizes of cells and cellular parts, in metric units.

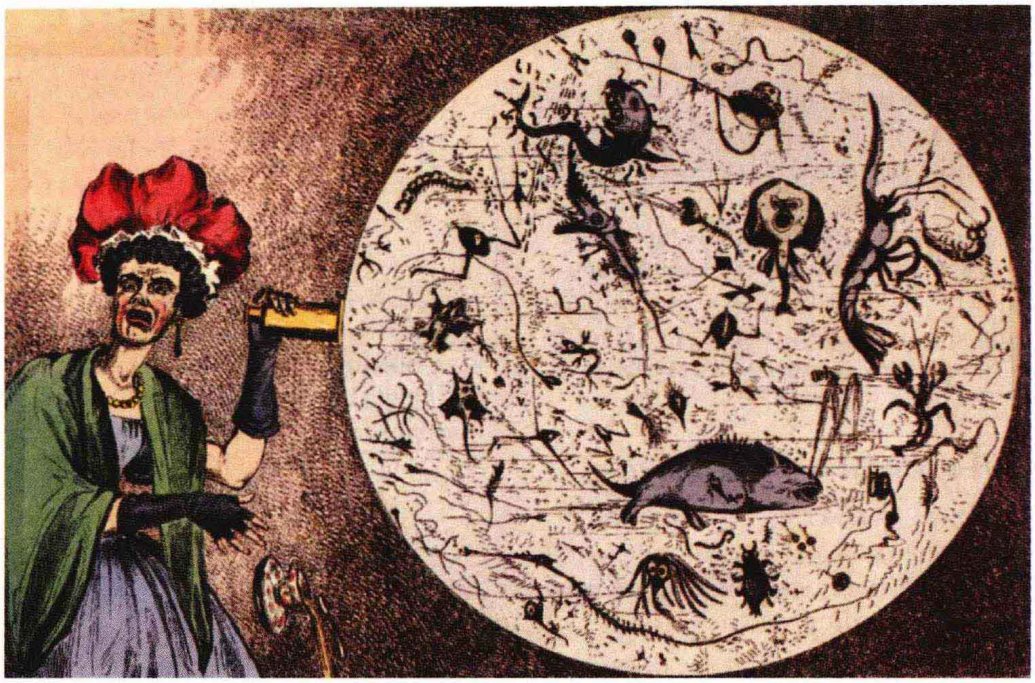

FIGURE 1.2

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

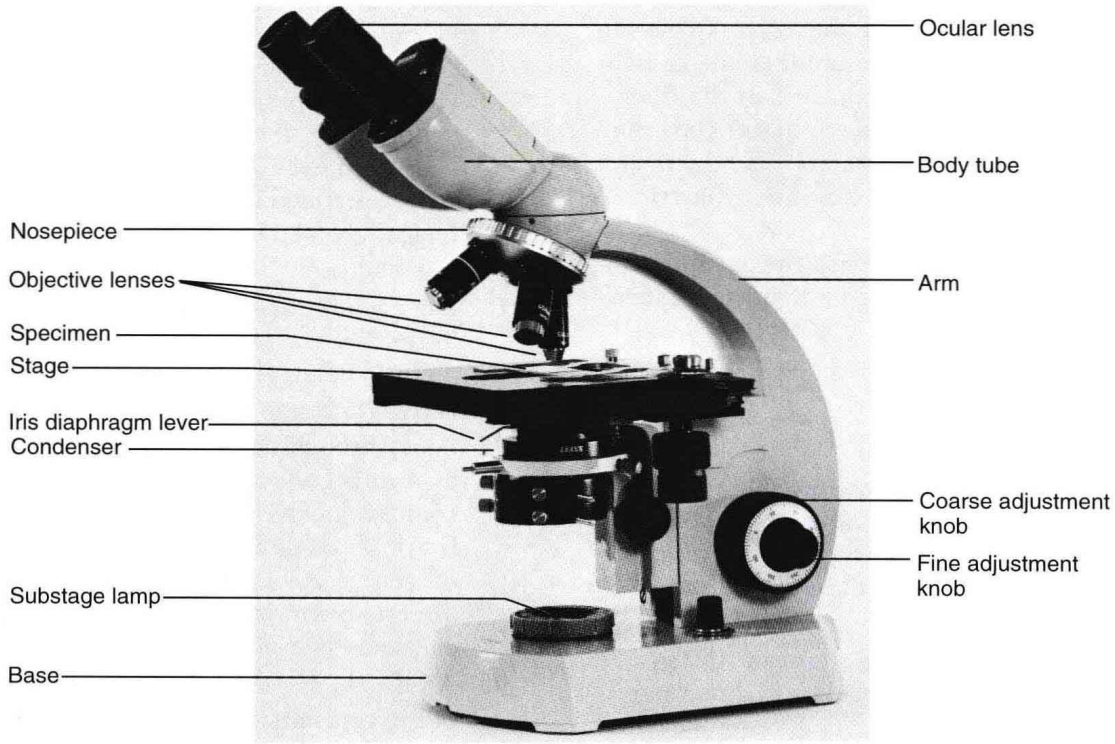

FIGURE 1.3

Major parts of a compound microscope.

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 ten 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 increased significantly during the last century, the 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 better glass than they were in the past. Your lab instructor will review with you the parts of the microscopes (and their functions) you will use in the lab.

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

Procedure 1.1

Use a compound microscope

1. Remove the microscope from its cabinet and carry it upright with one hand grasping the arm and your other hand supporting the microscope below its base. Place your microscope on the table in front of you.
2. Clean all of the microscope's lenses with lens paper. Do not remove the oculars or any other parts from the body tube of the microscope.

CAUTION

Do not use paper towels or Kimwipes; they can scratch the lenses.

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 positioned properly. 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 when you're viewing a specimen under low magnification.
6. Rotate the coarse adjustment knob clockwise to move the objective within 1 cm of the stage (1 cm = 0.4 in). 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 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.

Question 1

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

b. How does the image move when the slide is moved to the right or left? Up or down?

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

Magnification

Procedure 1.2

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 following this formula:

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

where

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

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

4. Slowly rotate the high-power (i.e., 43×) objective into place. Be sure that the objective does not touch the slide! If the objective does not rotate into place without touching the slide, do not force it; ask your lab instructor to help you. After the high-power objective is in place, you should notice that the image remains near 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 in the field of view 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 changes the specimen-to-objective distance slightly 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.

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?

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. 1.4). Knowing the size of the field of view is important because you can use it to determine the approximate size of an object you are examining. The field of view can be measured with ruled

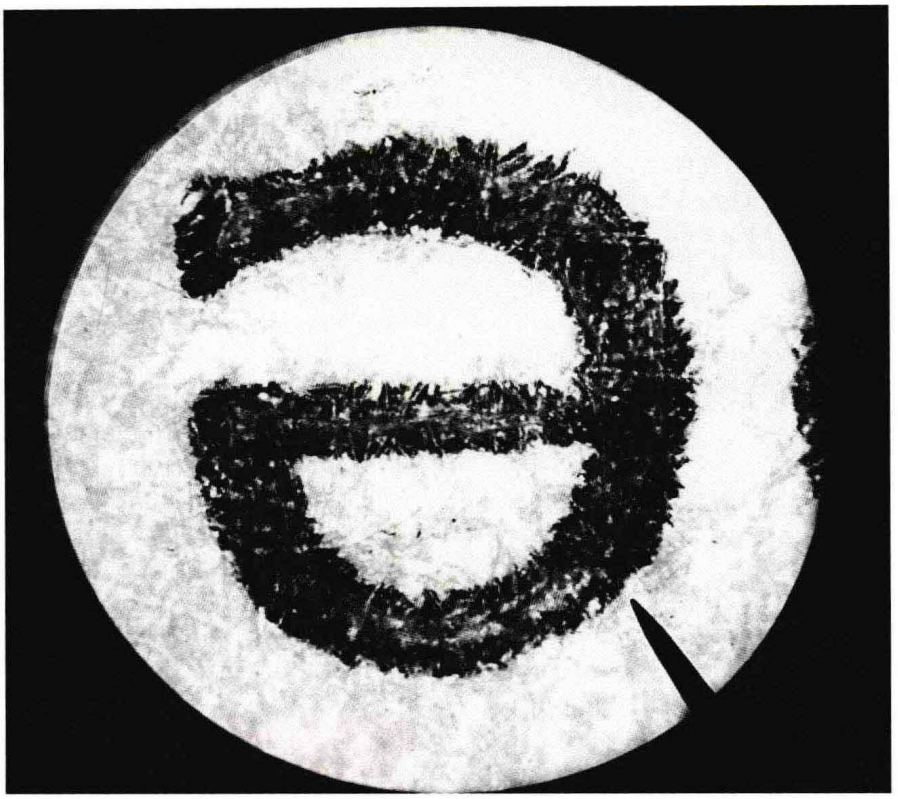

FIGURE 1.4

The circular, illuminated field of view of a compound microscope (40×).

micrometers (fig. 1.5). An **ocular micrometer** is a small glass disk with uniformly 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.

Procedure 1.3

Determine the size of the 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. 1.5).
2. Align lines at the left edges (0 lines) of the two micrometers by moving the stage micrometer (fig. 1.5).
3. Count how many spaces on the stage micrometer fit precisely in a given number of spaces on the ocular micrometer. Record the values below.

$$y \text{ ocular spaces} = x \text{ stage spaces}$$

y = _____

x = _____

Since the smallest space on a stage micrometer = 0.01 mm, then

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

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

$$y = 10$$
$$x = 8$$
$$10 \text{ ocular spaces (mm)} = 8 \text{ stage spaces} \times 0.01 \text{ mm}$$
$$1 \text{ ocular space (mm)} = 8/10 \times 0.01 \text{ mm}$$
$$1 \text{ ocular space (mm)} = 0.008 \text{ mm}$$
$$1 \text{ ocular space} = 8 \text{ µm}$$

Therefore, if a specimen spans eight spaces on your ocular micrometer with that objective in place, that specimen is 64 µm long.