PRINCIPLES OF BIOLOGY LABORATORY MANUAL

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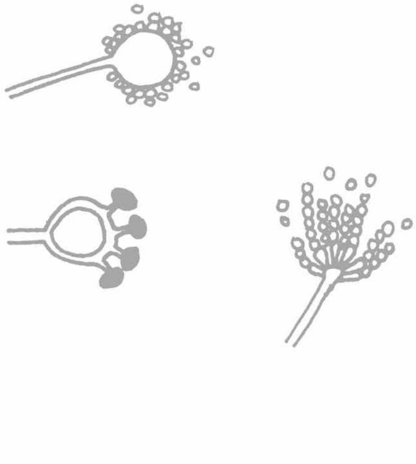

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Preface

The *Principles of Biology Laboratory Manual* is designed for use in colleges and universities to supplement an introductory general biology course for nonscience majors. Twenty two-hour laboratories are included. At Norwich University, where it has been used for the last four years, ten laboratory exercises and two examinations have been given per semester.

The exercises are designed to direct students through experimentation to conclusions that verify and enhance their understanding of basic biological principles. Anticipating marginal laboratory technique, experiments have been devised to succeed despite considerable error. Material usually covered in lecture is simplified and summarized in introductory sections and comparative tables. No expensive equipment is required, and expenditures for laboratory materials have been kept to a minimum.

The following is a brief synopsis of the 20 laboratory exercises:

(1) *Metric System and Microscope* provides an easy method for conversion from English to metric units of measurement. Students are prompted to think in metric terms. The microscope section introduces the theory behind and capabilities of various types of microscopes with emphasis on care and use of compound and dissecting microscopes.

(2) *Chemical Nature of Protoplasm* painlessly acquaints students with biochemistry. Working in groups, they determine the compositions of selected table foods using standard tests for mineral ions, carbohydrates, lipids and proteins.

(3) *Properties of Enzymes* stresses their very specific natures, and illustrates how enzymes are affected by factors including temperature, concentration and inhibitors.

(4) *Cell Structure* introduces techniques for making temporary slides and determining cell sizes. Students learn to recognize structural features of prokaryotic and eukaryotic plant and animal cells. Concepts of diffusion and osmosis are included.

(5) *Photosynthesis* highlights the roles of chlorophyll and accessory pigments in photosynthesis as it stresses the importance of light quality and intensity.

(6) *Respiration* fortifies concepts learned in the *Properties of Enzymes* Lab. Carbon dioxide production, oxygen consumption and activity of energy carriers are used to measure the rates of aerobic and anaerobic respiration under different environmental conditions.

(7) *Cell Division and Genetics* first reviews the phases of mitosis in plant and animal cells. Next, students are taught about gene expression while determining their own genotypes for various physical traits. Finally, they learn how genes are transmitted by creating hypothetical offspring.

(8–9) *Vertebrate Anatomy I and II* compare and contrast frog and human anatomy. External anatomy, skeletal structure, musculature and mouth structure are covered in Part I. Part II traces major internal organ systems through the preserved frog.

(10) *Vertebrate Physiology* illustrates spinal reflexes in living and decerebrated frogs, as well as blood flow in skin capillaries, heartbeat and internal organ systems.

(11) *Taxonomy* employs an uncomplicated classification table to make the biological classification system easier to understand. A simple dichotomous key is used to identify common tree genera.

(12) *Bacteria, Algae and Protozoans* features examples of bacteria, blue green algae, euglenoids, green algae, diatoms, brown algae, red algae and protozoans.

(13) *Fungi, Lichens and Nonflowering Plants* presents the slime molds, true fungi, liverworts, mosses, horsetails, club mosses, ferns and gymnosperms. Mosses and ferns provide illustrations for alternation of generations.

(14) *Flowering Plants* contrasts anatomical differences in monocot and dicot roots, stems, leaves, flowers and fruits, as well as the life cycle of the angiosperms.

(15) *Sponges, Coelenterates, Flatworms and Roundworms* introduces the more primitive members of the animal kingdom. A general dissection of *Ascaris* is included.

(16) *Segmented Worms and Mollusks* highlights three classes of annelids and four classes of mollusks. The earthworm and the clam are studied in detail.

(17) *Arthropods* acquaints students with representatives of five major classes and the various types of metamorphism. Dissections are performed on the crayfish and grasshopper.

(18) *Echinoderms and Chordates* provides examples of four classes of echinoderms, using the starfish for analyzation. Tunicates, lancelets and seven classes of vertebrates are covered in Phylum Chordata.

(19) *Evolution and Paleontology* provides a possible phylogenetic tree linking the 21 phyla covered previously. Summary tables of phylum characteristics help students determine the rationale behind its derivation. Various types of fossilization are displayed along with fossil organisms from modern and extinct phyla.

(20) *Ecology Field Trip* is an outdoor exercise which stresses the concepts of ecology through determination of environmental characteristics and vegetative and animal components of open field, young pine woods, hardwood forest and small stream ecosystems. Students observe previously studied organisms living in their natural habitats.

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LAUREN DAVIS HOWARD, Ph.D.

December 1979

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Exercise 1 — Metric System and Microscope

PART I. METRIC SYSTEM

Every day we use some form of measurement for length, area, volume, mass (weight) or temperature. The United States is the only major country still using the English System of yards, acres, gallons, pounds and degrees Fahrenheit. Other countries now use the *METRIC SYSTEM*, as do scientists throughout the world.

Table 1.1 lists a few of the more useful metric measures and their English equivalents. Table 1.2 lists some common English measures and their metric equivalents. Note that there seems to be no readily visible pattern to English measurements (12 inches = 1 foot; 3 feet = 1 yard; 5280 feet = 1 mile), but that the metric units are all based upon increments of ten (10 millimeters = 1 centimeter; 10 centimeters = 1 decimeter; 10 decimeters = 1 meter).

TABLE 1.1. CONVERSION OF METRIC UNITS INTO ENGLISH UNITS

Metric Unit	Abbr.	Metric Equivalent	English Equivalent
Kilometer	km	1000 m	0.62 mi
Meter	m	10 dm *or* 100 cm	1.09 yd
Decimeter	dm	10 cm *or* 0.1 m	3.94 in.
Centimeter	cm	10 mm *or* 0.01 m	0.394 in.
Millimeter	mm	1000 μ *or* 0.001 m	0.039 in.
Micron	μ	0.001 mm *or* 0.000001 m	0.00004 in.
Square Kilometer	km^2	100 ha	0.386 mi^2
Hectare	ha	10,000 m^2	2.47 a
Square Meter	m^2	10,000 cm^2	1.2 yd^2 *or* 10.8 ft^2
Square Centimeter	cm^2	100 mm^2 *or* 0.0001 m^2	0.155 $in.^2$
Liter	liter	1000 ml	1.06 qt
Milliliter	ml	0.001 liter	0.001 qt *or* 0.06 $in.^3$
Cubic Centimeter	cc		
Metric Ton	MT	1000 kg	0.984 tons
Kilogram	kg	1000 g	2.2 lb
Gram	g	1000 mg	0.035 oz
Milligram	mg	0.001 g	0.015 gr

It is important for you to learn to convert into metric units and eventually to think in metric units, since English units will not be used in this biology course. English units can be converted into metric units and vice versa by means of a simple *RATIO EQUATION*. On the left-hand side of the equation you place WHAT YOU ARE LOOKING FOR over WHAT YOU HAVE, and set this equal to the KNOWN RATIO OF THE TWO UNITS YOU ARE USING, which can be found in the conversion tables.

$$\frac{\text{WHAT YOU ARE LOOKING FOR (A units)}}{\text{WHAT YOU HAVE (B units)}} = \frac{\text{KNOWN NUMBER OF A units}}{\text{TO B units}}$$

Example 1: You have 50 feet of fencing and want to know if it will stretch across a space which is 15 yards long. You know from Table 1.2 that there are 3 feet in 1 yard.

TABLE 1.2. CONVERSION OF ENGLISH UNITS INTO METRIC UNITS

English Unit	Abbr.	English Equivalent	Metric Equivalent
Mile	mi	1760 yd *or* 5280 ft	1.609 km
Yard	yd	3 ft	0.914 m
Foot	ft	12 in.	30.5 cm
Inch	in.	0.083 ft	2.54 cm
Square Mile	mi^2	640 a	2.59 km^2
Acre	a	4840 yd^2 *or* 43,560 ft^2	0.405 ha *or* 4047 m^2
Square Yard	yd^2	9 ft^2 *or* 1296 $in.^2$	0.836 m^2
Square Foot	ft^2	144 $in.^2$	0.093 m^2
Square Inch	$in.^2$	0.007 ft^2	6.45 cm^2
Gallon	gal.	4 qt *or* 231 $in.^3$	3.785 liter
Quart	qt	2 pt *or* 57.75 $in.^3$	0.946 liter
Pint	pt	28.875 $in.^3$	0.473 liter
Ton	ton	2240 lb	1.016 MT
Pound	lb	16 oz	0.454 kg
Ounce	oz	2000 gr	28.35 g
Grain	gr	0.002 oz	0.065 g

You are looking for how many yards there are in 50 feet. In this case the "A units" are YARDS and the "B units" are FEET. The ratio equation can be set up as follows:

$$\frac{\text{? yards}}{\text{50 feet}} = \frac{\text{1 yard}}{\text{3 feet}}$$

Note that if yards are on the top of the left-hand side of the equation they must also be on the top of the right-hand side. The same is true for whatever units are on the bottom of the equation.

To solve the equation you must get "? yards" by itself on the left-hand side of the equation. You can do this by multiplying both sides of the equation by "50 feet." If you multiply both sides of an equation by the same number you will not change the value of the equation.

However, 50 feet/50 feet will cancel out on the left-hand side, and feet/feet will cancel out on the right-hand side.

$$\frac{50\text{ ft} \times ?\text{ yd}}{50\text{ ft}} = \frac{1\text{ yd} \times 50\text{ ft}}{3\text{ ft}} \text{ becomes: } ?\text{ yd} = \frac{1 \times 50}{3}\text{ yd}$$

$$?\text{ yd} = 16.7\text{ yd (more than enough fencing).}$$

Example 2: The width of this paper is 8.5 inches. Determine its width in centimeters. According to Table 1.2 there are 2.54 centimeters in 1 inch. With this information you can now set up the ratio equation, cross multiply and find the answer.

$$\frac{?\text{ cm}}{8.5\text{ in.}} = \frac{2.54\text{ cm}}{1\text{ in.}}$$

$$\frac{8.5\text{ in.} \times ?\text{ cm}}{8.5\text{ in.}} = \frac{2.54\text{ cm} \times 8.5\text{ in.}}{1\text{ in.}}$$

$$?\text{ cm} = \frac{2.54 \times 8.5}{1}\text{ cm}$$

$$?\text{ cm} = 21.6\text{ cm}$$

Example 3: What is the width of the paper in millimeters? *NOTE*: It is much easier to convert from one metric unit to another than from English to metric units! Since you just determined that the paper is 21.6 cm long, and you know from Table 1.1 that there are 10 millimeters in 1 centimeter, then:

$$\frac{?\text{ mm}}{21.6\text{ cm}} = \frac{10\text{ mm}}{1\text{ cm}}$$

$$\frac{21.6\text{ cm} \times ?\text{ mm}}{21.6\text{ cm}} = \frac{10\text{ mm} \times 21.6\text{ cm}}{1\text{ cm}}$$

$$?\text{ mm} = \frac{10 \times 21.6}{1}\text{ mm}$$

$$?\text{ mm} = 216\text{ mm}$$

Problems

(For problems 1 to 4 round your answers down to a single decimal place.)

(1) With a ruler measure the length of this paper in inches. __________in. Using the ratio equation determine its length in centimeters and millimeters. __________cm __________mm

(2) What is your approximate height in inches? __________in. Using the ratio equation determine your height in decimeters and meters. __________dm __________m

(3) What is your approximate weight in pounds? __________lb Using the ratio equation determine your weight in kilograms and grams. __________kg __________g

(4) Which would be less expensive, gasoline at 90¢/gal. or 25¢/liter?

(For problems 5 to 11 round your answers down to the nearest integer.)
YOU WILL BE HELD RESPONSIBLE FOR ALL THESE RATIOS!

(5) For each 10 miles you drive you travel about __________kilometers.

(6) For each 10 meters you run you move about __________yards.

(7) A 10 inch hotdog would be about __________centimeters long.

(8) A 10 hectare parcel of land would contain about __________acres.

(9) A 10 liter keg of beer would contain about __________quarts.

(10) Each time you lift 10 kilograms you lift about __________pounds.

(11) A 10 ounce bar of gold would weigh about __________grams.

(12) Draw a line corresponding to each of the following lengths:

1 inch

1 millimeter

1 centimeter

1 decimeter

In the metric system temperature is measured in degrees *CENTIGRADE*, a system based upon the freezing point and boiling point of water. The following formulas may be used to convert from Centigrade to Fahrenheit and vice versa.

To convert from Fahrenheit to Centigrade: $^{\circ}C = \frac{5 \times (^{\circ}F - 32)}{9}$

To convert from Centigrade to Fahrenheit: $^{\circ}F = \frac{9 \times {}^{\circ}C}{5} + 32$

(13) If water freezes at 32°F, what is its Centigrade equivalent? ________

(14) If water boils at 212°F, what is its Centigrade equivalent? ________

(15) Normal human body temperature is 98.6°F. What is its Centigrade equivalent?
____

(16) The optimum temperature for plant enzymes is 50°C. What is its Fahrenheit equivalent? ________

(17) In northern Vermont it often reaches −30°F. What is its Centigrade equivalent?
____

(18) Would 60°C be a comfortable temperature at which to live? ________

PART II. MICROSCOPE

(A) Microscope Theory

Microscopes are very important to biologists interested in the structure of living organisms. A good microscope provides both increased *MAGNIFICATION* (increases the apparent size of an object) and *RESOLUTION* (increases the clarity of the image).

The size of the object you see *(RETINAL IMAGE)* depends upon the *VISUAL ANGLE* from the object to the lens of your eye. As the visual angle becomes greater, magnification increases (Fig. 1.1).

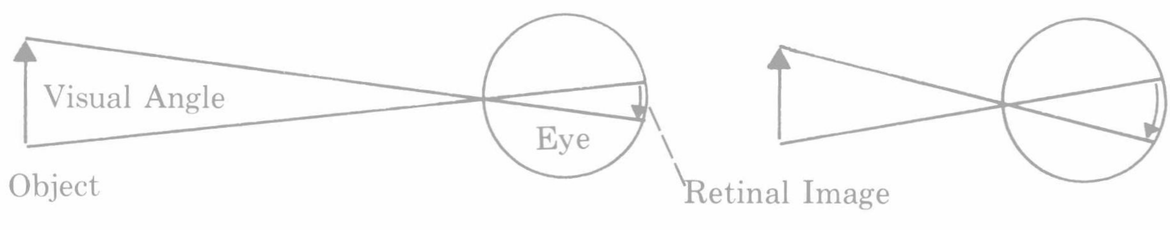


FIGURE 1.1

Resolution is the ability to distinguish between two closely spaced dots or lines. The unaided eye cannot separate points less than 0.1 mm apart. They appear as a single point because both images fall on the same cell in the retina of the eye.

Focus on the "Eye" circle with one of your eyes and hold the paper at arms length. Move the lab manual progressively closer to your eye until the paper almost touches.

Does the magnification continue to increase even as the paper approaches your eye? ____

Does the resolution continue to increase even as the paper approaches your eye? ________

(The resolution should increase to a certain point and then decrease, because the human eye cannot focus on objects closer than about 15 cm.)

Both magnification and resolution may be increased by use of a simple *HAND LENS*. A single lens can magnify objects about 20 times their actual sizes by increasing the visual angle. Your eye sees the *VIRTUAL IMAGE* (apparent size of the object) rather than the actual image (Fig. 1.2).

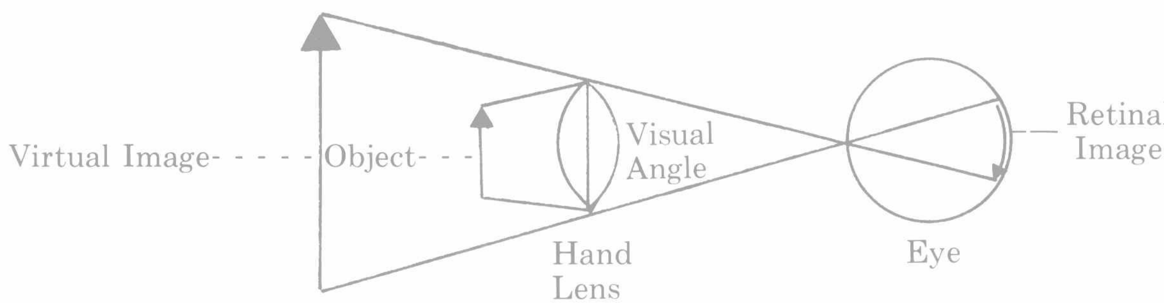


FIGURE 1.2

A *COMPOUND MICROSCOPE* has two lens systems and is capable of magnifying objects up to 1000 times their actual sizes. The *OBJECTIVE LENS SYSTEM* (near the object) produces an enlarged image. The *OCULAR LENS SYSTEM* (near the eye) magnifies the image produced by the objective. Notice from Fig. 1.3 that the use of two lens systems inverts the virtual image and the retinal image.

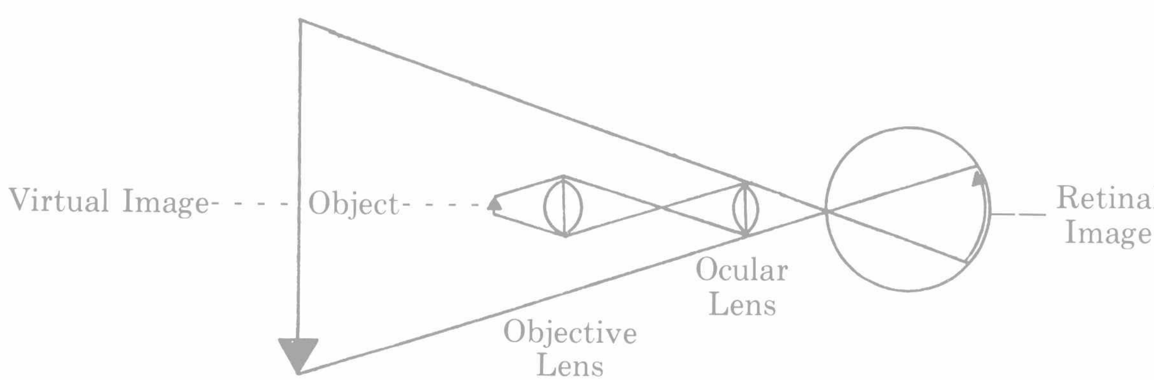


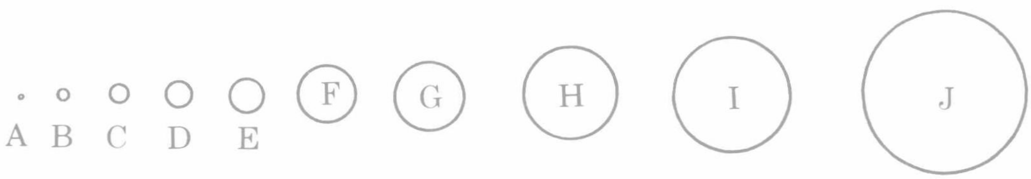
FIGURE 1.3

The *TOTAL MAGNIFICATION* of a compound microscope can be determined by multiplying the magnification capability of the objective lens by the magnification capability of the ocular lens. As can be seen in Table 1.3, as the total magnification increases the *DIAMETER OF THE FIELD OF VIEW* decreases. The resolution limit of a compound microscope is about 0.2 microns (0.0002 mm). It is ultimately limited by the shortest wave length of visible light.

TABLE 1.3

Objective in Use	Objective Magnification	Ocular Magnification	Total Magnification	Diameter of Field
Low Power	10×	10×	100×	1.80 mm
High Power	43×	10×	430×	0.46 mm
Oil Immersion*	97×	10×	970×	0.20 mm

*Your microscopes do not have oil immersion lenses.



The white area in which circle (excluding the black line) is approximately the same size as the field of view under low power? __________

The white area in which circle (excluding the black line) is approximately the same size as the field of view under high power? __________

An *ELECTRON MICROSCOPE* has a resolution limit of about 0.00001 microns, and is able to magnify objects up to 100,000 times their actual sizes. This is possible because an electron microscope uses a beam of electrons in place of a beam of light.

Each type of microscope has its benefits and limitations. A hand lens is easy to use outdoors, but it has limited enlargement capabilities. In the laboratory larger objects may be viewed under a *BINOCULAR DISSECTING MICROSCOPE*. This type of microscope can magnify objects from four to 30 times, and allows space to work beneath the objective lens. A compound microscope has better magnification and resolution, but can only be used with slides and coverslips. Only very small objects or thin slices of objects can be viewed. The electron microscope has the greatest magnification and resolution of all, but the specimens must be examined in a vacuum (so they must be dead) and it is often difficult to distinguish among real features, dust particles, stain particles, etc.

(B) Parts of the Compound Microscope

Identify the following parts of your microscope and label them on Fig. 1.4.

PART	FUNCTION
BASE	Supports the microscope.
LIGHT SOURCE	Illuminates the object to be studied.
IRIS DIAPHRAGM	Controls the light intensity on the object.
CONDENSER	Concentrates light on the object.
STAGE	Platform which supports the slide containing the object to be studied.
ARM	Carrying handle.
FOCUS ADJUSTMENT KNOBS	
COARSE ADJUSTMENT	Large inner knob which brings the virtual image into rough focus.
FINE ADJUSTMENT	Small outer knob which brings the virtual image into clear focus.
OBJECTIVE LENSES	Magnify the object to be studied.
LOW POWER OBJECTIVE	Magnifies the object ten times (10 ×).
HIGH POWER OBJECTIVE	Magnifies the object 43 times (43 ×).
OCULAR LENS	Magnifies the image produced by the objective lens ten times (10 ×).
REVOLVING NOSEPIECE	Holds the objective lenses and allows you to change directly from one objective to another without having to refocus.

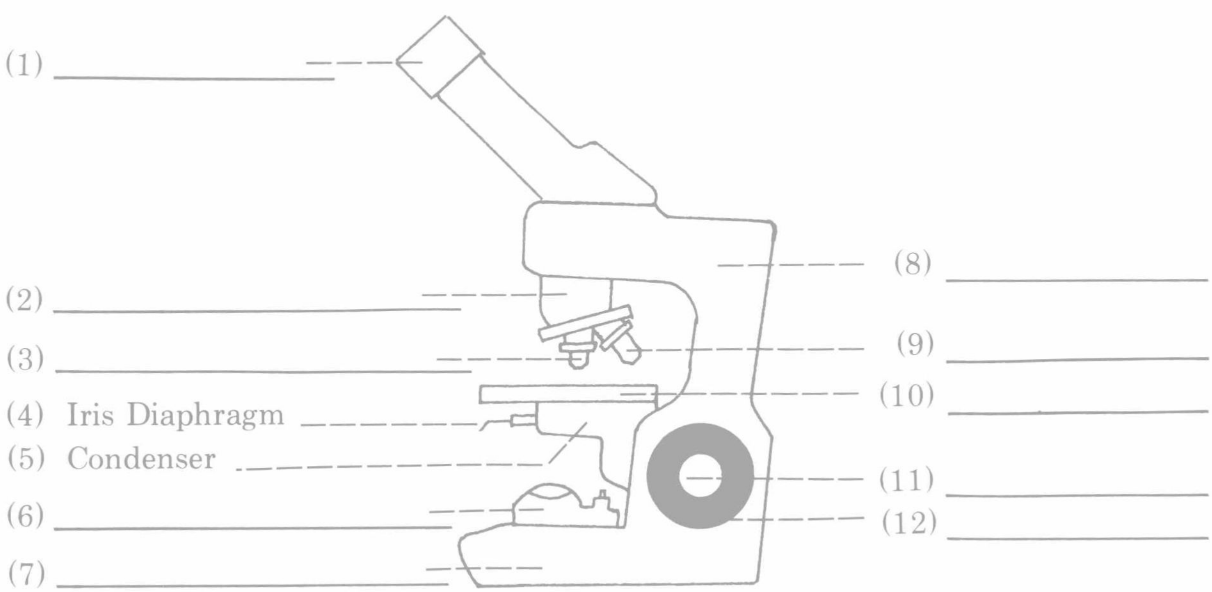


FIGURE 1.4

(C) Rules for Care of the Microscope

Always carry the microscope with *two hands* (one hand on the arm and the other under the base)!

Never turn the microscope sideways or upside down or remove a lens for any reason!

Never touch the lenses with anything but lens paper! To clean the lenses, simply breathe on them and wipe them gently with LENS PAPER.

Never examine a slide without a coverslip!

Always begin examination of a slide on LOW POWER (with the 10× objective in place), *never* on high power (with the 43× objective in place).

Always remove your slide when you are done and put the LOW POWER objective in place when you are through using the microscope.

Always cover the microscope before you put it away to prevent the accumulation of dust on the lenses.

(D) Rules for Use of the Microscope

(1) Insert the plug into an outlet and push down the light switch next to the light source.

(2) After making sure that the LOW POWER OBJECTIVE is in place, set the slide (with coverslip) on the stage and center the object over the light source.

(3) Looking from the side, turn the COARSE ADJUSTMENT until the LOW POWER OBJECTIVE is in its lowest possible position. Then, looking through the OCULAR LENS, focus upward with the COARSE ADJUSTMENT until a clear image appears.

(4) Turn the FINE ADJUSTMENT back and forth slowly until the image is sharp. This should not require more than a half a turn.

(5) After examining the object under low power, CENTER THE OBJECT EXACTLY IN THE FIELD OF VIEW and turn the HIGH POWER OBJECTIVE into position. The object should still be roughly in focus and only the FINE ADJUSTMENT should be used to improve the image. *(Never use the coarse adjustment when the high power objective is in place!)*

(6) Switch back to LOW POWER before removing your slide.

PROCEDURE

Students work individually.

(A) Letter e Slide.—Cut a *small e* out of a newspaper and prepare a *TEMPORARY SLIDE* by placing the *e* into a drop of water on a slide and covering it with a coverslip. Following the rules for use of the microscope, place the slide with the letter *e* onto the stage so that the *e* appears right side up to your unaided eye. Examine the *e* on LOW POWER.

Draw the *e* as you see it in the microscope:

Compare the image under the microscope with that seen by the naked eye. Is the image inverted from left to right? __________ Is it inverted from top to bottom? __________

When the slide is moved, is the apparent movement in the same direction as the actual movement? __________

To the unaided eye the ink of the letter *e* appears to be evenly spread. Is this also true for the microscope image? _________ Why is this so?

____

____

____

____

____

Determine the diameter of the letter *e* in millimeters without removing the slide from the microscope stage. You can do this by multiplying the known diameter of the field of view by the approximate distance across the field that the *e* extends (1/2, 2/3, 3/4, etc.). _________mm

Remove the slide and measure the letter *e* with a millimeter ruler. Was your previous determination correct? _________

How long is the letter *e* in microns? __________μ

(B) Pond Water Slide.—Prepare a temporary slide of some pond water by placing a drop from the bottom of the culture dish onto a slide and covering it with a coverslip. Observe the slide on LOW POWER. Many of the objects you see are *ARTIFACTS*. Artifacts, including air bubbles, dirt particles and scratches on the coverslip, are things you must learn to identify and avoid when you are using a compound microscope.

Identify and draw an AIR BUBBLE.

Draw and describe some PIECES OF DIRT.

Identify as many organisms as you can find in the pond water sample. Check the first few organisms with your instructor to make sure you are not looking at artifacts. The drawings in Fig. 1.5 may help you in your identifications.

(C) Binocular Dissecting Microscope Demonstration.—Learn how to focus a dissecting microscope and change from one power to another. You will be using these microscopes throughout the course.