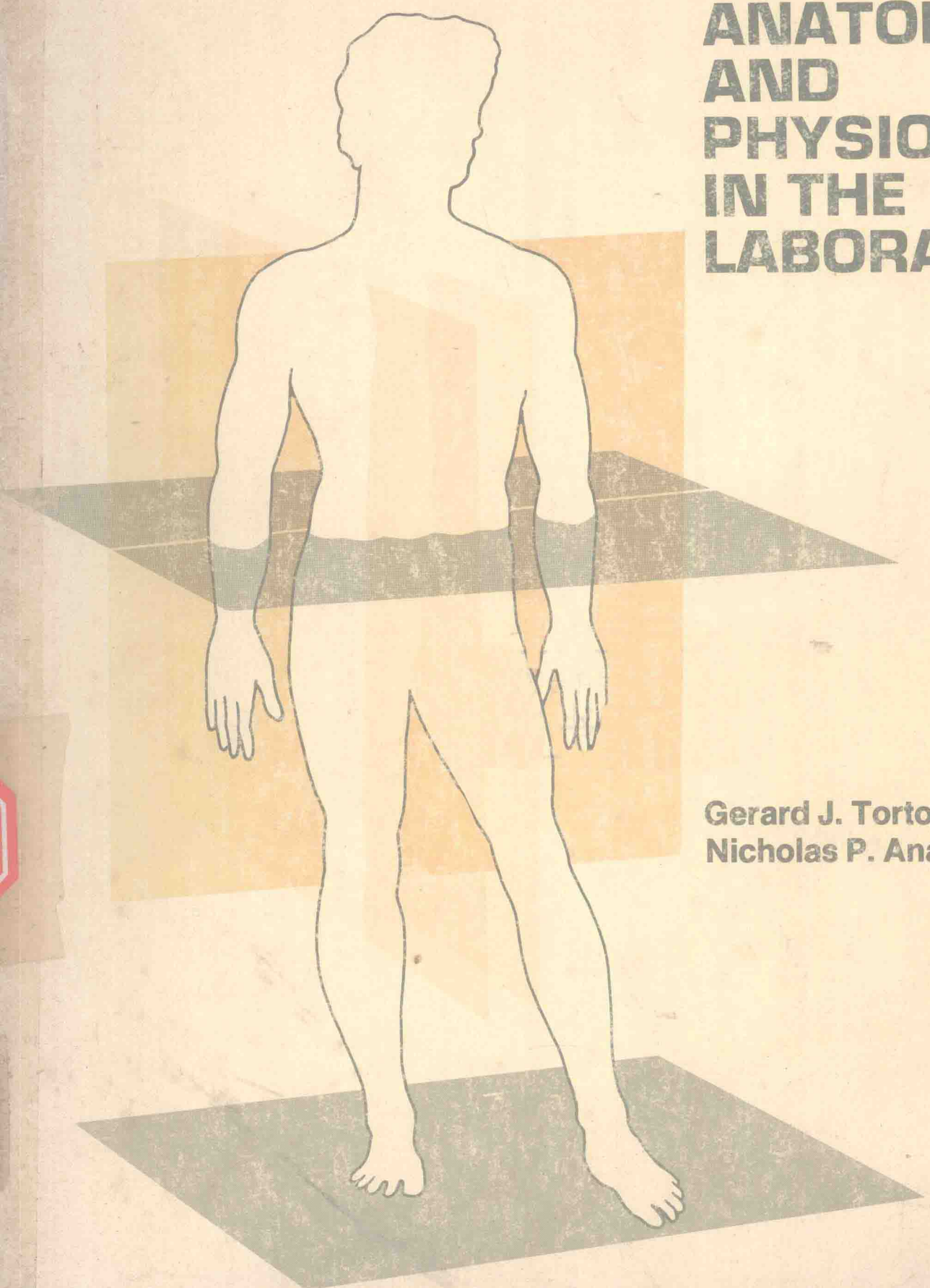
PRINCIPLES OF ANATOMY AND PHYSIOLOGY IN THE LABORATORY



Gerard J. Tortora
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PRINCIPLES OF ANATOMY AND PHYSIOLOGY IN THE LABORATORY

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Burgess Publishing Company • Minneapolis, Minnesota

Printed in the United States of America
ISBN 0-8087-2046-5

10 9 8 7 6 5 4 3

PREFACE

Principles of Anatomy and Physiology in the Laboratory has been prepared to accompany the authors' *Principles of Anatomy and Physiology* textbook, which was designed for use in introductory courses. However, we have provided a comprehensive and flexible sequence of modules, and this Manual may readily be used with other anatomy and physiology textbooks as well.

The Manual contains numerous modules for the introductory course. Many of the modules can be completed in a single laboratory period. In some instances, several modules can be completed in a single period. In other cases, a double laboratory period may be devoted to one module. We feel that such a design provides the instructor with considerable flexibility in selecting appropriate modules, varied sequences, and specific quantities of work that best suit his needs and are within the limits of practical considerations. Moreover, such a design affords utilization of the Manual in either one-semester or one-year courses in anatomy and physiology.

Each module is a self-contained unit consisting of three principal divisions. The *OBJECTIVES* provide both the student and instructor with the desired outcomes of the module. The *CONCLUSIONS* are a series of questions designed to review and apply the principles and concepts studied in the module. The *STUDENT ACTIVITY* is designed to help the student reinforce one or more of the concepts under investigation.

Throughout the Manual, space is provided for students to answer questions. In addition, there is space for students to make drawings and label diagrams. There are also many Tables for recording data.

We would like to emphasize that the Manual is both a laboratory workbook and a study guide. The laboratory modules require students to make microscopic examinations and evaluations of cells and tissues, to observe and interpret chemical reactions, to record data, to make gross examinations of organs and systems, and to conduct physiological experiments and interpret and apply the results of the experiments. The study modules have been carefully developed to reinforce lecture and laboratory work, and to introduce clinical applications.

This Manual is distinctive in its use of clinical applications, its comprehensiveness, its flexibility, and the quality of its illustrations. The Manual contains 44 separate modules that cover virtually every aspect of the human body that might be treated in an introductory course. In this regard, we feel that the instructor is less burdened with preparation since all he has to do is select the appropriate modules from the Manual without having to supplement his syllabus from several sources. This

Manual also provides the instructor with a great deal of flexibility regarding assignments. Many of the modules have been specifically designed so that the instructor, at his discretion, may assign a module to be completed out of class to supplement lecture or laboratory work. These assignments require the student to research specific areas of study. Or, the instructor may wish to assign some modules in class for evaluation. The authors' textbook is clinically oriented and, accordingly, the Manual is also clinically oriented. We feel that most laboratory work in anatomy and physiology courses neglects the clinical aspects. We have, therefore, incorporated many modules that deal only with clinical applications. Here again, the instructor may assign these modules as out-of-class projects or use them as a means of evaluation. We are especially pleased with the outstanding quality of the illustrations in the modules. They have been reproduced from the authors' textbook. We hope that the use of these illustrations will greatly assist the student to cross-refer to the illustrations in the textbook should the instructor decide to use the authors' textbook.

Several appendixes have been included. These are Appendix A, Preparations of Specimens for Experimentation, Appendix B, Use of Physiologic Equipment, and Appendix C, Metric Units of Length and Some English Equivalents.

A complimentary *Teachers' Guide* is offered for instructors who use *Principles of Anatomy and Physiology in the Laboratory*. It is designed to assist in the selection of appropriate audio-visual materials and other materials required to complete each module, to present instructional concepts, and to provide students with correct responses to questions asked in the modules.

Gerard J. Tortora
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CHEMICALS IN EYE

RAPID TREATMENT IS VITAL!
Use large volumes of water over eyeball until medical help is available.

Eye-wash fountain KNOW IT'S LOCATION

Eye-wash bottle KNOW IT'S LOCATION

Use water from any source

ALKALINE MATERIALS IN THE EYE ARE MOST DANGEROUS

SAFETY SHOWER

For chemical spills or fire victim. Operate by pulling down ring. Area near shower should always be clear.

Alternate method. Remove clothing from area affected.

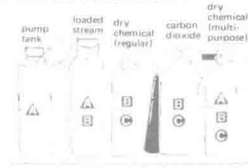
EXTINGUISHING A FIRE

Using a Fire Extinguisher
1. know its location
2. remove from mounting
3. pull pin
4. squeeze lever
5. discharge at base of flame
6. report use/recharge

Use sand to extinguish burning metals.

Small fires may be smothered with a book or cloth.

TYPES OF FIRE EXTINGUISHERS



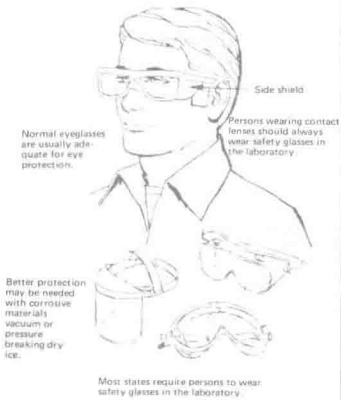
A	YES	YES	NO	NO	YES
B	NO	YES	YES	YES	YES
C	NO	NO	YES	YES	YES

For ordinary combustibles. Cloth . . . wood . . . paper.

For flammable liquids. Oil . . . grease . . . gasoline.

For use on live electrical equipment.

EYE PROTECTION



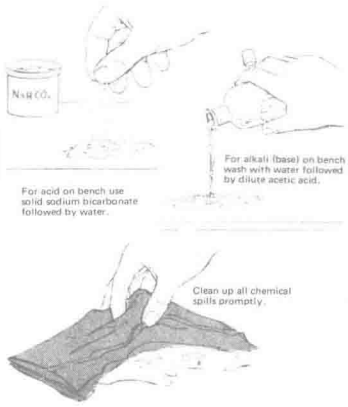
Normal eyeglasses are usually adequate for eye protection.

Persons wearing contact lenses should always wear safety glasses in the laboratory.

Better protection may be needed with corrosive materials vacuum or pressure breaking dry ice.

Most states require persons to wear safety glasses in the laboratory.

ACID/ALKALI SPILLS

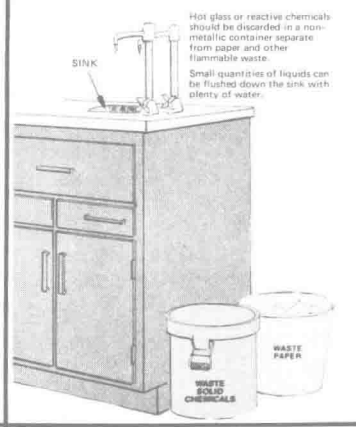


For alkali (base) on bench wash with water followed by dilute acetic acid.

For acid on bench use solid sodium bicarbonate followed by water.

Clean up all chemical spills promptly.

WASTE DISPOSAL



Hot glass or reactive chemicals should be discarded in a non-metallic container separate from paper and other flammable waste.

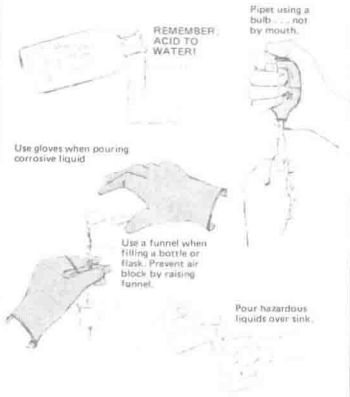
Small quantities of liquids can be flushed down the sink with plenty of water.

CARRYING CHEMICALS AND EQUIPMENT

Carry long apparatus, such as tubing or burets, in an upright position close to the body.

Grasp bottles firmly with both hands and hold them close to the body. Do not carry bottles by the neck.

TRANSFERRING LIQUIDS



Pipet using a bulb . . . not by mouth.

Use gloves when pouring corrosive liquid

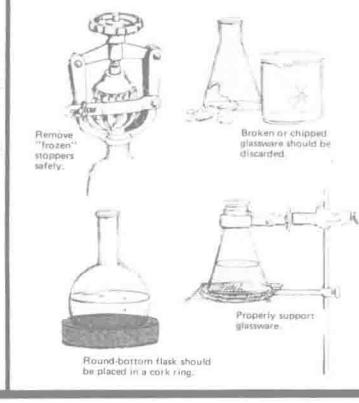
Use a funnel when filling a bottle or flask. Prevent air block by raising funnel.

Pour hazardous liquids over sink.

LABORATORY CONDUCT

Fooling around in the laboratory can be dangerous.

WORKING WITH GLASSWARE



Remove "frozen" stoppers safely.

Broken or chipped glassware should be discarded.

Properly support glassware.

Round-bottom flask should be placed in a cork ring.

ADDITIONAL RULES FOR SAFETY IN THE LABORATORY

No smoking.

No food or beverages.

No running.

Know location of exits.

Keep aisles clear . . . put coats and books in designated areas.

Do not leave experiment unattended.

Extinguish burners when away from desk.

ALWAYS BE PREPARED TO HELP A FELLOW STUDENT IN AN EMERGENCY.

(Courtesy Science Related Materials, Janesville, Wis. 53545.)

CONTENTS

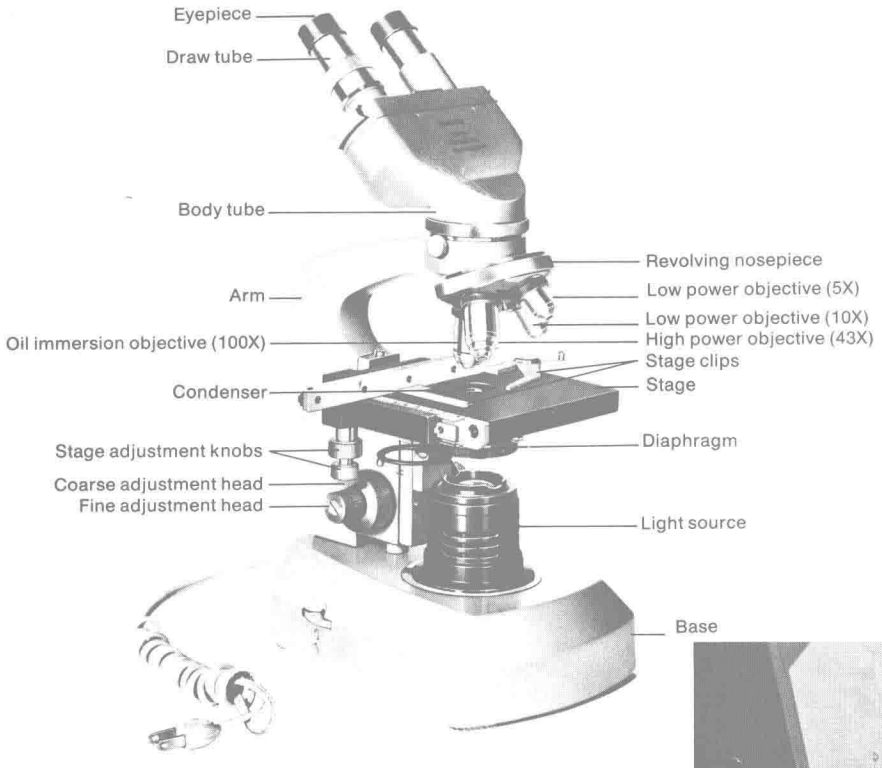
MODULE 1
MICROSCOPY

OBJECTIVES

1. To identify the different parts of the compound microscope
2. To learn the proper use and care of the compound microscope
3. To calculate the approximate sizes of materials under investigation
4. To compare the resolution of a photomicrograph and an electron micrograph
5. To contrast the principles employed in light microscopy and electron microscopy

One of the most important instruments that you will use in your anatomy and physiology course is the compound microscope. With this instrument, objects too small to be seen clearly with the naked eye can become highly magnified and their minute details will be revealed. This delicate and expensive instrument must be cared for properly, if you are to be completely successful in your understanding of it, and if you wish to make accurate observations of the various specimens you examine. In this module you will also be introduced to some of the principles employed in electron microscopy.

There is little doubt that the single most important instrument that led to the systematic study of cells has been the **compound microscope.** This instrument, initially developed by the Dutch lens maker Zacharias Janssen at the close of the sixteenth century, has undergone considerable modification over the years. A photograph of a modern compound microscope is shown in Figure 1-1a. Any optical instrument is limited in the structural details it can make visible by its resolution. The *resolution* of a microscope is the ability of a microscope to distinguish the smallest distance between 2 points on an object. Resolution is determined, among other factors, by the wavelength of radiation used to illuminate the object. The smaller the wavelength, the smaller the limit of resolution and the more structural detail is visible. Since the source of radiation for the compound microscope is light and since light has a relatively long wavelength, the smallest objects that can be seen in detail are about 0.3 micrometers (μm). Parts smaller than 0.3 μm cannot be resolved with the light microscope. One *micrometer,* symbolized μm, is a microscopic unit of measurement equal to 1/1,000 millimeter (mm.) or about 1/25,000 inch. A photomicrograph of ciliated epithelium is shown in Figure 1-2a. A *photomicrograph* is a photograph of an object taken under a light microscope.



(a)

(b)

Figure 1–1. Modern microscopes. (a) Modern compound light microscope. Since microscopes of this kind use light, they cannot resolve structures smaller than 0.3 micrometer. This limitation is the result of the relatively long length of visible light waves. (b) Modern electron microscope. Instead of light, this instrument uses a beam of electrons. Since the wavelengths of electrons are about 1/100,000 that of the normal wavelength of white light, an electron microscope can magnify objects up to 200,000 times (a: Courtesy of Tasco Sales, Inc., Miami, Fla., b: Courtesy of RCA Laboratories, Princeton, N.J.).

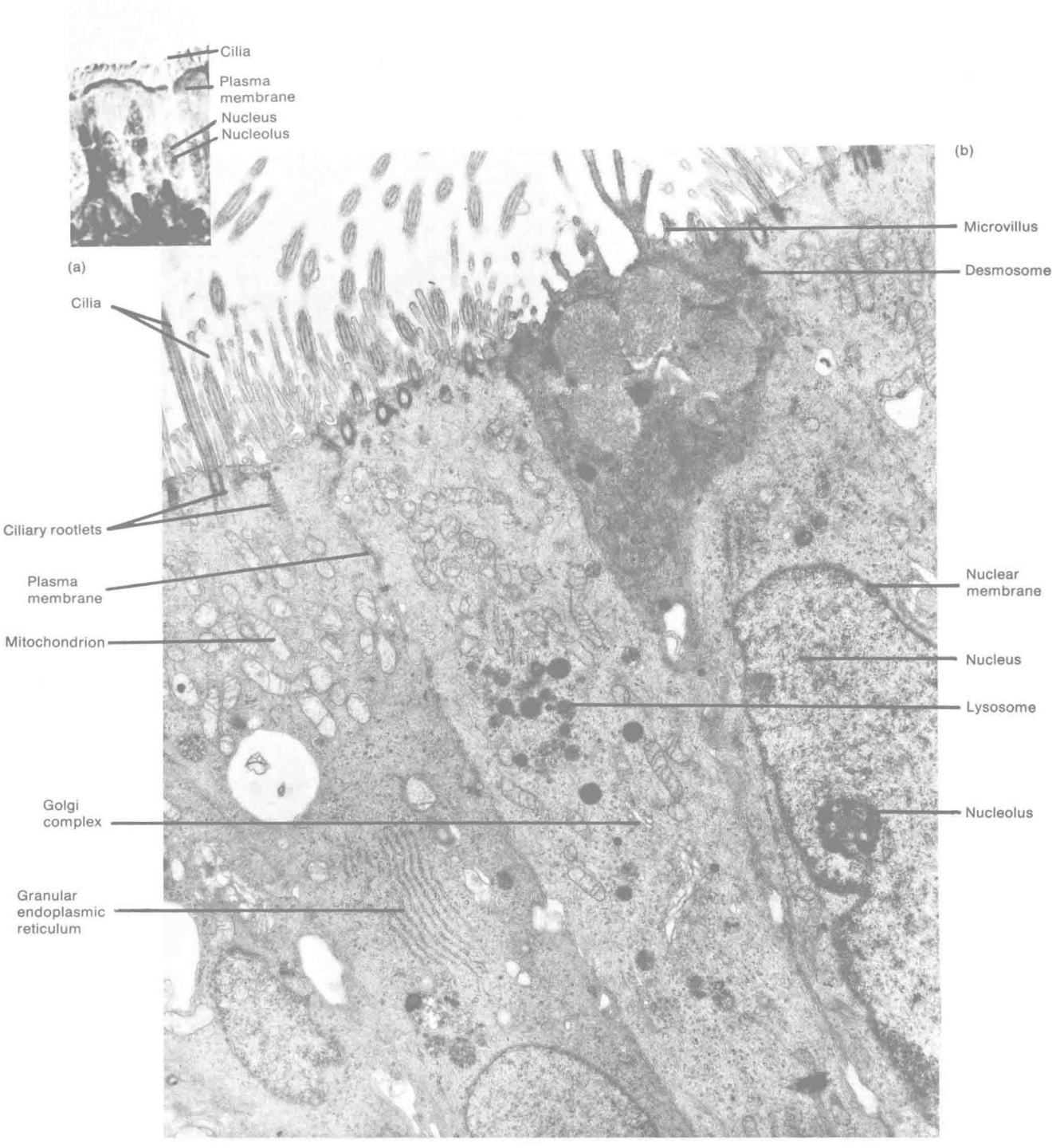


Figure 1–2. Comparison between the resolution of a light microscope and an electron microscope. (a) Photomicrograph of ciliated epithelial cells, 450X. Note the cilia at the surfaces of the cells, the plasma membrane between cells, the nucleus, and the nucleolus inside the nucleus. (b) Electron micrograph of ciliated epithelial cells, 54,000X. The resolution of the electron microscope becomes readily apparent. Note the cilia and their rootlets, the plasma membrane, the nucleus, and the nucleolus. In addition, observe the mitochondrion, granular endoplasmic reticulum, Golgi complex, lysosome, microvillus, and desmosome. These structures are rendered visible by the resolution of the electron microscope (a: Courtesy of Carolina Biological Supply Company, Burlington, N.C. b: Courtesy of Dr. John A. Terzakis, Lenox Hill Hospital, New York, N.Y.).

Many parts of cells are much smaller than 0.3 μm, and if they are to be seen in any detail another kind of microscope must be used. This instrument, which came into use in the 1940s, is called an **electron microscope** (Figure 1-1b). Instead of light, an electron microscope employs high electrical voltages to drive a beam of electrons. As a result, an electron microscope increases resolution because the wavelength of electrons is much smaller than that of visible light (about 1/100,000 of visible light). The present limit of resolution is approximately 3Å. One *angstrom*, symbolized Å, is equal to 1/10,000 of a micrometer, or 1/250,000,000 of an inch. Whereas maximum magnifications with the light microscope are 1,000-2,000X, an electron microscope can magnify an object up to 200,000X.

A listing of commonly used metric units of length is presented in Appendix C.

When using a light microscope, the observer looks directly at the object. In the electron microscope, however, the observer either views a fluorescent screen which lights up as the beam of electrons hits it, or the image is directed onto a photographic emulsion (film or glass plate) which is then developed, enlarged, and examined. The resulting photograph is called an *electron micrograph* (Figure 1-2b). If you compare the photomicrograph of the ciliated epithelium in Figure 1-2a with the electron micrograph of the ciliated epithelium in Figure 1-2b, you will see considerable differences in resolution.

PART I THE COMPOUND MICROSCOPE

IDENTIFICATION OF THE PARTS OF THE MICROSCOPE

The microscope should be carefully carried from the cabinet to your desk by placing one hand around the arm and the other hand firmly under the base. Gently place it on your desk, directly in front of you, with the arm facing you. Locate the following parts on the microscope, and as each is discussed, label Figure 1-3. Although different microscopes vary somewhat in design, they consist of the same basic parts.

1. *Base*—the heavy U-shaped structure upon which the microscope rests.
2. *Body tube*—the main cylindrical part.
3. *Arm*—angular or curved part of the frame.
4. *Inclination joint*—some microscopes have a movable hinge allowing the instrument to be tilted to a comfortable viewing position.
5. *Stage*—a platform upon which slides or other material to be studied is placed. The opening in the center allows light to pass from below through the material being examined.
6. *Stage (spring) clips*—two clips mounted on the stage which hold slides to be examined securely in place.
7. *Mirror*—found in some microscopes below the stage, functioning to direct light from its source through the stage opening and through the lenses. If the light source is built in, then no mirror is found.
8. *Diaphragm*—located beneath the stage functioning to regulate light intensity passing through the lenses to the observer's eyes. One of 2 types of diaphragms are usually used. An *iris diaphragm*, as found in cameras, is a series of sliding leaves which vary the size of the opening. Another type, a *disc diaphragm*, consists of a plate with a graded series of holes any of which may be rotated into position.
9. *Condenser*—a lens that controls the light-beam size that is located beneath the stage opening.
10. *Condenser adjustment knob*—a knob that functions to raise and lower the condenser for optimum viewing. When it is in its highest position it allows full illumination.
11. *Coarse adjustment knob*—when it is turned, it functions to raise and lower the body tube for focusing the microscope.
12. *Fine adjustment knob*—usually found below the coarse adjustment and used for fine or final focusing. Some microscopes have both coarse and fine adjustments combined into one.
13. *Nosepiece*—a plate at the bottom of the body tube which is usually circular.

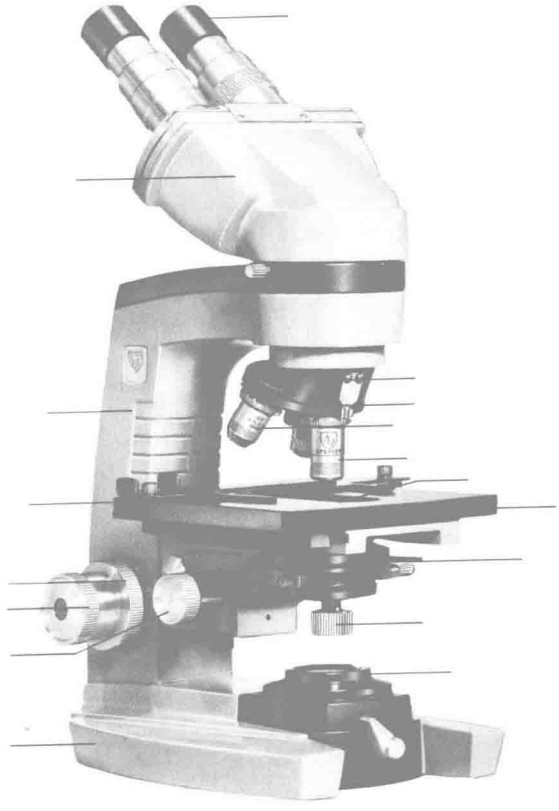


Figure 1–3. Parts of a compound light microscope (Courtesy of American Optical Corporation, Buffalo, N.Y.).

14. *Revolving nosepiece*—the lower, movable part of the nosepiece that contains the various objective lenses.
15. *Scanning objective*—a lens, marked 5X on most instruments.
16. *Low power objective*—a lens, marked 10X on most instruments.
17. *High power objective*—a lens, marked 43X or 45X on most instruments.
18. *Oil-immersion objective*—a lens marked 100X on most instruments, and distinguished by an etched red circle (special instructions for this objective are discussed later).
19. *Ocular (eyepiece)*—a lens that is removable, at the top of the body tube, marked 10X on most microscopes.

RULES OF MICROSCOPY

There are certain basic rules of microscopy that must be observed at all times, in order to obtain maximum efficiency and provide proper care for your microscope.

1. All parts of the microscope must be kept clean, especially the lenses of the ocular, objectives, condenser, and mirror. Only the special lens paper that is provided should be used, and never paper towels or cloths since these tend to scratch the delicate glass surfaces. When using lens paper, do not use the same area on the paper for cleaning the lens. As you go across the lens, change the position of the paper each time.
2. Do not permit the objectives to get wet, especially when observing a wet mount (described later). These preparations must always be examined by using a cover slip or the image becomes distorted.

3. Your instructor should be consulted if any mechanical or optical difficulties arise. *Do not try to solve these problems yourself.*
4. It is important that you keep *both* eyes open at all times while observing materials through the microscope. At the start this will be difficult but with practice it becomes a natural procedure. This technique will help you to draw and observe microscopic specimens without moving your head. Only your eyes will move.
5. The low power objective should always be used first to locate an object; then, if necessary, switch to a higher power.
6. If you are using the high power objectives, never focus with the coarse adjustment. The distance between these objectives and the slide is very small and you may break the cover glass and the slide and scratch the lens system.
7. When looking through the microscope, never focus downward. By observing from one side you can see that the objectives do not make contact with the cover slip and slide.
8. Make sure that you raise the body tube before placing a slide on the stage or before removing a slide.
9. At the end of a laboratory session when the microscope is to be returned to the cabinet, leave it with the scanning or low power objective aligned, the diaphragm open, and the condenser raised to its highest fixed position.

SETTING UP THE MICROSCOPE

1. The microscope is placed on the table with the arm toward you, and with the back of the base at least one inch from the edge of the table.
2. Position yourself and the microscope so that you can look into the ocular comfortably.
3. Wipe the objectives, the top lens of the eyepiece, the condenser, and the mirror with lens paper. This sequence should be the most delicate and the least dirty lens is cleaned first. Xylol or ethanol may be applied to the lens paper in order to remove grease and oil from the lenses and microscope slides.
4. Position the low power objective in line with the body tube. When it is in its proper place, it will click. Lower the body tube with the coarse adjustment until the bottom of the lens is approximately ¼ inch from the stage.
5. The minimum amount of light is then admitted by opening the diaphragm if it is an iris, or turning the disc to its largest opening.
6. Place your eye to the eyepiece (ocular), and adjust the concave surface of the mirror to the light source.
7. The mirror is then adjusted until a uniform circle of light appears without any shadows. This is called the *microscopic field* and the microscope is ready for use.

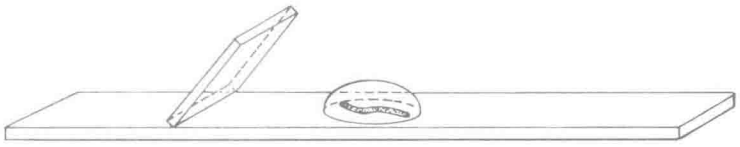
PREPARING MATERIALS FOR STUDY

The materials for microscopic examination can be prepared in one of 2 basic ways: (1) the temporary (wet) mount or (2) the permanent mount. Whichever method is used, the material to be studied must be thin enough for light to pass through it.

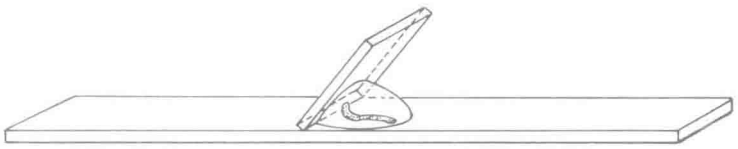
1. *Temporary (wet) mount.* In this method, the preparation will be studied for only a short period of time. This mount is prepared as follows (Figure 1-4):
   a. A small drop of water is placed near the center of a clean glass microscope slide.
   b. Then a *very small* amount of the material to be examined is placed in this water, making sure that the liquid covers the material.
   c. A glass or plastic cover slip is then held at a 45° angle, contacting the drop of water, and gradually allowed to fall into place over the material. When the cover slip is dropped from too flat an angle, many air bubbles are produced, interfering with accurate observations.

Step 1. A drop of water is placed on a clean microscope slide.

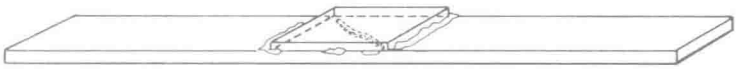
Step 2. The object is added to the drop of water.



Step 3. Cover slip is held at a 45° angle against slide.



Step 4. Cover slip is moved against drop of water at same angle.



Step 5. Cover slip is dropped slowly over water.

Figure 1–4. Preparing a wet mount (Modified from Lawrence S. Dillon and William A. Cooper, *A Laboratory Survey of Biology,* 2nd ed., 1969, The Macmillan Company, New York, N.Y.).

2. *Permanent mount* (optional). This method is a tedious, time-consuming process that is used for slides that will be examined on many different occasions, weeks or months apart.
   a. The material is killed by placing it in an FAA solution (10 ml. of 40% formaldehyde, 50 ml. of 95% ethanol, 2 ml. of glacial acetic acid, and 40 ml. of water).
   b. The killed material is then dehydrated by passing it through a number of grades of alcohol. Gradations of 50, 70, 85, 95, and 100 percent are usually used.
   c. The dehydrated material is then imbedded in a block of paraffin wax.
   d. A microtome is then used to cut very thin sections of the material which are then mounted on a microscope slide with an adhesive.
   e. The paraffin is then dissolved with an organic solvent (toluene or xylene), and the slide is passed back down the graded alcohols to 50 percent.
   f. The slide is then passed through certain specific dye solutions; the material is dehydrated once again.
   g. The cover slip is added and sealed to the slide with resin.

USING THE MICROSCOPE

1. Raise the body tube to its highest fixed position, using the coarse adjustment.
2. Make a temporary mount using a single letter of newsprint, or use a slide that has been specially prepared with a letter, usually the letter "e." If you prepare such a slide, cut a single letter—"a," "b," or "e"—from the smallest print available and place this letter in the correct position to be read with the naked eye.
3. Place the slide on the stage, making sure that the letter is centered over the opening in the stage, directly over the condenser. Secure the slide in place with the stage clips.

4. The low power objective should be in line with the body tube and the objective should be about ¼ inch from the cover slip.
5. The body tube should then be lowered as far as it will go, while watching it from the side, taking care not to touch the slide. The tube will reach an automatic stop which prevents the low power objective from hitting the slide.
6. While looking through the eyepiece, the coarse adjustment knob is turned counterclockwise, raising the body tube. Watch for the material to suddenly appear in the microscopic field. If it is in proper focus, the low power objective is about ½ inch above the slide. When focusing, always *raise* the body tube.
7. The fine adjustment is used to complete the focusing, using a counterclockwise motion once again.
8. Compare the position of the letter as originally seen with its appearance under the microscope. How has the position of the letter been changed?
9. While looking at the slide through the ocular, change the position of the slide by either moving it with your thumbs, or using the mechanical stage knobs, if the microscope is equipped with them. This exercise is to teach you to quickly and efficiently move your material in various directions. In which direction does the letter move when you move the slide to the left?
   This is called "scanning" a slide and will be useful in the examination of living material or examining slides where the object to be observed is not immediately centered under the tube.
   Make a drawing of the letter as it appears under low power in the space provided on page 9.
10. Change your magnification from low to high power by carrying out the following steps:
    a. Place the letter in the center of the field under low power. This is important because you are now focusing on a smaller area of the microscopic field.
    b. Increase your illumination.
    c. The letter should be in focus, and if the microscope is *parfocal*, ("parfocal" means that when clear focus has been attained using any objective at random, revolving the nosepiece results in a change in magnification but the specimen is still in focus), the high power objective can be switched into line with the body tube without changing focus. If it is not completely in focus after switching the lens, a slight turn of the fine adjustment knob will complete it.
    d. If your microscope is not parfocal, observe the stage from one side and carefully switch the high power objective in line with the body tube.
    e. While still observing from the side and using the coarse adjustment, *carefully* lower the objective until it almost touches the slide.
    f. Look through the ocular and focus up slowly. Finish focusing by turning the fine adjustment toward you.
    g. If your microscope has an oil-immersion objective, special procedures must be followed. A drop of special *immersion oil* is placed on the microscope slide, and this objective is lowered until it just contacts the oil. If you have a parfocal microscope, you do not have to raise or lower the objectives. For example, if you are using the high power objective and the specimen is in focus, just switch the high power objective out of line with the body tube. Then add the oil, switch the oil immersion objective into position, and the specimen should be in focus. The same holds true when you switch from low power to high power. The special light-transmitting properties of the oil are such that light is concentrated at one tiny spot, permitting the use of extra powerful objectives in a relatively narrow field of vision. This objective is extremely close to the slide being examined, so extra precautions must be taken by *never focusing downward* while you are looking through the eyepiece. Whenever you finish working with immersion oil be sure to saturate a piece of lens paper with xylol or alcohol and clean the oil immersion objective and the slide if it is to be used again.