Laboratory Studies in Integrated Principles of ZOOLOGY

Eighth Edition

HICKMAN

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Preface

This laboratory manual is the outgrowth of the authors' many years of working directly with students in the zoology laboratory. Its objectives have always been clarity of expression, thoroughness of coverage, and convenience for both student and instructor. Perhaps most important of all is the expectation that through direct laboratory experience, students will learn to test basic biological principles, become familiar with standard techniques of animal study, and learn through dissection of selected specimens the distinctive anatomical features distinguishing each group of animals. Only in the laboratory can students come to understand the common architectural themes and adaptations that unite groups of animals.

Although this manual was written to accompany a particular textbook, *Integrated Principles of Zoology* (ed. 9), in a major's course in general zoology, it can easily be adapted for use with the shorter sister text, *Biology of Animals* (ed. 5), or any other introductory text and with a variety of course plans. By judicious selection of exercises it has long been used not only in full-year courses but also in many one-semester or one-term courses in vertebrate and invertebrate zoology and in one-semester introductory principles courses as well.

As in the other Hickman texts, there is an emphasis on biological principles and evolutionary relationships and advances that point the way to more highly organized phyla. Every effort has been made to give clear, lucid descriptions and instructions, and enough background material has been included to create interest in and understanding of the subject matter. Many illustrations are used to complement the written word.

This manual is more comprehensive than most manuals because we believe that students deserve more than a mere dissection guide. Students should have important concepts presented to them while the specimens which illustrate those concepts are before their eyes. This can be more effective than any number of descriptive lectures. We also believe that being able to name the anatomical parts of an organism is of limited value unless the functions and adaptations of those parts are also understood.

We emphasize without apology that this manual embraces more material than can be covered in a single zoology course. This is an asset to the instructor, who is thus free to make selections to fit his or her own or the department's needs and preferences, influenced, of course, by the time and materials available. For example, few zoology courses have time to cover all of the vertebrates included here; but whatever the choice, the instructor can be assured of complete and careful coverage of the subject. The fetal pig coverage is, we feel, the clearest and most practical to be found in any introductory guide on the market today. We prefer the fetal pig to the frog for its closer similarity to the human and for the opportunity it provides to study mammalian reproduction and fetal circulation.

Convenience for both student and instructor has always been a major consideration. Instructions have enough detail that students can work with a minimum of help from the instructor, thus freeing the instructor from lengthy introductory explanations. For the convenience of the instructor, a separate *Instructor's Handbook* has been prepared, giving lists of materials required for each exercise, suggestions for demonstrations and special student projects, notes on potential problems and pitfalls, and, for most exercises, a listing of appropriate references. The handbook ends with six appendixes, giving narcotizing and preservation methods, descriptions of special techniques, instructions for starting and maintaining aquariums and terrariums, and sources of living material and prepared microscope slides.

There are many aids for the student. Throughout the book, working instructions are clearly set off from descriptive material. Classifications, where appropriate, are included with the exercises. Function and physiology are explained along with anatomy. Topic headings help the student organize the material mentally. Metric tables and definitions are placed on the inside front and back covers for convenient use. Much of the new artwork has been designed to assist the student with difficult dissections.

The popular taxonomic chart, which has been completely redesigned and updated for this edition, can be glued into the back of the manual so that it can be unfolded for use, or it may be hung on the student's wall. In either case the student will find it a concisely and conveniently arranged review of the

animal phyla and an invaluable addendum to the text.

Traditionally there has been too little use of living materials in zoology laboratories. It is easy for the student, confronted with weeks of dissection, to forget that colorless, preserved specimens were once alive. This manual encourages the study, whenever possible, of living specimens in the laboratory. Each exercise, if appropriate, begins with a paragraph on the natural history and habitat of the animal to be studied, followed by a discussion of its behavior. Marine aquarium systems, so widely available now, can bring new life and excitement into the laboratory. For this reason we have stressed the use of living marine organisms. To observe the feeding action of a delicate sea anemone, the graceful movements of a nudibranch, or the movements of the sea star's podia on the side of the tank can reveal more to a student than volumes of reading material or hours of dissection. The addition of one or two "living rocks" to a marine tank will introduce a surprising assortment of strange and interesting marine forms. By seeing a wider variety of life than that presented by the type of specimen being studied, students come to appreciate the diversity of animal life and adaptive radiation within animal groups. Zoology comes alive for them.

THE REVISION—CHANGES AND ADDITIONS

Of the many changes and additions made in this eigth edition, most apparent to previous users will be the almost total reillustration of the vertebrate chapters (Chapters 19 through 23). The new illustrations were prepared by Dr. William C. Ober, who also reillustrated the invertebrate chapters for the previous edition. All new illustrations were made directly from dissections prepared by the senior author and the artist to produce artwork of the greatest possible accuracy. The text of all the vertebrate chapters was also thoroughly reworked and extensively rewritten.

In making revisions, we benefited from the reviews of several professional zoologists commissioned by the publisher as well as from the numerous unsolicited comments of scientific acquaintances and users of the manual who kindly took the time to write us with their suggestions. Following are the principal revisions and additions for this edition.

Part One is devoted to general principles: an exercise on the correct use of the microscope, and five exercises on cell biology, development, basic histology, and animal classification. Chapter 1, on the use of the microscope, has been revised to better explain how to obtain correct illumination and includes a new section, "Taking Control of Your Microscope," that summarizes correct use and care of the microscope. Exercise 2B now includes a new section describing the cell cycle. The introduction to Exercise 4A has been rewritten to clarify the distinction between mitosis and meiosis. Several sections of Chapter 5 have been reworked to improve clarity and in some cases to simplify the treatment.

Part Two comprises a survey of protozoan and animal phyla. Throughout Part Two we have included the derivation of specialized biological terms and genera where these terms and genera are first introduced. This is an important assistive device to the student in becoming familiar with the Latin and Greek roots from which technical terms are built. We have also spelled out the complete species binomial in the classification breakdown for each representative species in the exercises—this is done to repeatedly emphasize to students that the species is a binomial.

The introduction to Chapter 7 on the protozoa has been rewritten to explain the placement of protozoan phyla within the Kingdom Protista. In addition to reworking several sections in Exercises A through D, we have introduced a new physiological exercise (7E) that explains how to measure the effect of temperature on the activity of the ciliate *Stentor*. This is an instructive and enjoyable exercise for the students and requires only about 1 hour to complete. It also provides the student some experience in working with equations and graphical plots.

Other new invertebrate exercises or sections for this edition include a new exercise on the human blood fluke *Schistosoma* (Exercise 10B), a trematode parasite of enormous medical significance; an expanded description of the internal structure of the squid (Exercise 12C); and a new description of the honeybee sting (Exercise 16B). We also revised several of the behavioral observations described at the beginning of many of the invertebrate chapters and reworked some of the chapter introductions.

As already mentioned, the vertebrate Chapters 19 through 23 received the greatest attention in this revision. Two of the chapters are completely new: Chapter 21 on the Class Reptilia, with the painted turtle as the representative animal; and Chapter 22 on the Class Aves, with the pigeon as the representative animal. These new chapters round out the chordate treatment to embrace all chordate classes. Although few (if any) teaching programs will have time to treat all vertebrate representatives, all have the option of selecting sections from these chapters to put together their own synthesis suitable for the time available. One could, for example, select the fe-

tal pig for in-depth study of a vertebrate type, then offer a single additional laboratory that emphasizes the evolution of vertebrate morphology, using representative skeletons of the other vertebrate classes. All of the remaining vertebrate chapters have been extensively revised and all have been almost completely reillustrated.

Several important revisions were made in Part Three, which treats physiology, genetics, and ecology. Exercise 24B on the physiology of the myoneural junction, originally a student exercise, has been recast into a demonstration by the instructor because of the potential hazard presented by the handling of strychnine and curare. We also inserted an outline of safety procedures to be used in the study of human blood (Exercise 25A). Some instructors will be reluctant to use this exercise because of the fear surrounding the recent emergence of AIDS, but we feel that precisely because of this concern, students should learn that there is no hazard in working with human blood *provided that* accepted safety procedures are rigidly observed. These procedures are explained in greater detail in the *Instructor's Handbook.*

Exercise 28A, a new exercise contributed by James Munger of Boise State University, applies the scientific method to a study of population growth in flour beetles. This is an excellent project that can be started at the beginning of the term and completed with a report at term end. For those wishing to have their students gain experience in writing a full laboratory report for Exercise 28A, we have included suggestions for report organization on pg 112 of the *Instructor's Handbook.* This page can be photocopied and distributed to the students.

A new study of pond ecology (Exercise 28B) explains how to approach a field study and how to identify pond life using an illustrated key prepared by Deborah Kendall of Fort Lewis College. This study is applicable to almost any pond type found in North America.

ACKNOWLEDGMENTS

The authors were fortunate to have the comments of 11 reviewers engaged by the publishers to review the revised manual at the manuscript stage. These were zoology instructors who made countless suggestions and helped us to focus our objectives, all drawn from their collective experience in the teaching laboratory. These reviewers and the chapters they reviewed are as follows: William Bukovsan, SUNY at Oneonta (Chapters 19-26); Walter Diehl, Mississippi State University (Chapters 1-18); William Dyer, Southern Illinois University (Chapters 1-18); Charles L. Elliott, Eastern Kentucky University (Chapters 1-18); William R. Hawkins, Mt. San Antonio College (Chapters 19-26); Irwin R. Isquith, Fairleigh Dickinson University (Chapters 1-7); Barbara Marcum, Chico State University (Chapters 1-29); Edith C. Marsh, Angelo State University (Chapters 1-19); Robert Stiles, Samford University (Chapters 19-24); Donna M. Bruns Stockrahm, Moorhead State University (Chapter 23); and Barbara Taber, Southwest Missouri State University (Chapters 1-18). In addition to these 11, several scientists were interviewed by telephone for their comments. These include Frank Cliff, Chico State University; Kenneth Goodhue-McWilliams, California State University at Fullerton; Gaylen Eiben, Wartburg College; Reed Harris, James Madison University; Karen Haverman, Saddleback Community College; Jenna Hellack, Central State University at Edmond, OK; Don Hoyt, California Polytechnic State University at Pomona; Ronald Kirk, Indiana University—Purdue at Indianapolis; Eert Moed, Chicago State University; Larry Olson, Arkansas State University; and Grace Weingard, James Madison University.

As mentioned earlier, two zoologists became even more involved by actually creating much of the new Chapter 28 on the ecological relationships of animals. James C. Munger of Boise State University contributed the original draft for Exercise 28A, and Deborah M. Kendall of Fort Lewis College created the text of the key to common freshwater aquatic invertebrates of North America, which appears in Exercise 28B. Their contributions made possible the generation of two completely new ecological exercises, one tailored for the classroom and the other designed as a field study.

Three scientists took the time to write directly to us with their suggestions. These were Susan Grant, Holyoke Community College; Robert R. Glesener, Brevard College; and Robin Leech, University of Alberta. We thank them for their unsolicited participation in making this manual more correct and useful.

The authors were fortunate indeed that Mosby–Year Book engaged the talented editorial services of Cathleen E. Petree to direct us through this revision. She was a delight to work with, and her long experience in the publishing industry, scientific perspective, sense of humor, excellent judgment, and professionalism contributed immeasurably to the success of the revision. We also wish to thank Robert J. Callanan, our acquisitions editor; Gayle Morris and Shelia Walker, our production team; and all others at Mosby–Year Book who worked to bring this project to completion.

CLEVELAND P. HICKMAN, Jr.
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Contents

General Instructions

EQUIPMENT

Each student will need to supply the following equipment:

Laboratory manual and textbook
Dissecting kit containing scissors, forceps, scalpel, dissecting needles, pipette (medicine dropper), probe, and ruler, graduated in millimeters
Drawing pencils, 3H or 4H
Eraser, preferably kneaded rubber
Colored pencils—red, yellow, blue, and green
Box of cleansing tissues
Loose-leaf notebook for notes and corrected drawings

The department will furnish each student with all other supplies and equipment needed during the course.

AIM AND PURPOSE OF LABORATORY WORK

The zoology laboratory will provide your "hands-on" experience in zoology. It is the place where you will see, touch, hear, smell—but perhaps not taste—living organisms. You will become acquainted with the major animal groups, make dissections of preserved or anesthetized specimens to study how animals are constructed, ask questions about how animals and their parts function, and gain an appreciation of some of the architectural themes and adaptations that emphasize the unity of life.

GENERAL INSTRUCTIONS FOR LABORATORY WORK

Prepare For the Laboratory. Before coming to the laboratory, read the entire exercise to familiarize yourself with the subject matter and procedures. Read also the appropriate sections in your textbook. Good preparation can make the difference between a frustrating afternoon of confusion and mistakes and an experience that is pleasant, meaningful, and interesting.

Follow the Manual Instructions Carefully. It is your guide to exploring and understanding the organisms or functions you are investigating. Its instructions have been written with care and with you in mind, to help you do the work (1) in logical sequence, (2) with economy of time, and (3) to arouse a questioning attitude that will stimulate interest and curiosity.

Use Particular Care in Making Animal Dissections. A glossary of directional terms used in dissections will be found inside the front cover. The object in dissections is to separate or expose parts or organs so as to see their relationships. Working blindly without the manual instructions may result in the destruction of parts before you have had an opportunity to identify them. **Learn the functions** of all the organs you dissect.

Record Your Observations. Keep a personal record in a notebook of everything that is pertinent, including the laboratory instructor's preliminary instruction and all experimental observations. Do not record data on scraps of paper with the intention of recopying later; record directly into a notebook. The notes are for your own use in preparing the laboratory report later.

Take Care of Equipment. Glassware and other apparatus should be washed and dried after use. Metal instruments in particular should be thoroughly dried to prevent rust or corrosion. Put away all materials and equipment in their proper places at the end of the period.

TIPS ON MAKING DRAWINGS

You need not be an artist to make laboratory drawings. You do, however, need to be **observant.** Study your specimen carefully. Your simple line drawing is a record of your observations.

Before you draw, locate on the specimen all the structures or parts indicated in the manual instructions. Study their relationships to each other. Measure the specimen. Decide where the drawing should be placed and how much it must be enlarged or reduced to fit the page (read further for estimation of magnification). Leave ample space for labels.

When ready to draw, you may want first to rule in faint lines to represent the main axes, and then sketch the general outlines lightly. When you have the outlines you want, draw them in with firm dark lines, erasing unnecessary sketch lines. Then fill in

details. Do not make overlapping, fuzzy, indistinct, or unnecessary lines. Indicate differences in texture and color by stippling. Stipple deliberately, holding the pencil vertically and making a neat round dot each time you touch the paper. Placing the dots close together or farther apart will give a variety of shading. Avoid line shading unless you are very skilled. Use color only when asked for it in the directions.

Label the Drawing Completely. Print labels neatly in lower case letters and align them vertically and horizontally. Plan the labels so that there will be no crossed label lines. If there are to be many labels, center the drawing and label on both sides.

Indicate the magnification in size beneath the drawing, for instance, "×3" if the drawing is three times the length and width of the specimen. In the case of objects viewed through a microscope, indicate also the magnification at which you viewed the subject, for example, 430× (43× objective used with a 10× ocular).

Estimating the Magnification of a Drawing

A simple method for determining the magnification of a drawing is to find the ratio between the size of the drawing and the actual size of the object you have drawn. The magnification of the drawing can be expressed in the following formula:

$$\times = \frac{\text{Size of drawing}}{\text{Size of object}}$$

If your drawing of the specimen is 12 cm (120 mm) long, and you have estimated the specimen to be 0.8 mm long, then × = 120 ÷ 0.8, or 150. The drawing, then, is ×150, or 150 times the length of the object drawn.

This same formula will hold good whether the drawing is an enlargement or a reduction. If, for example, the specimen is 480 mm long, and the drawing is 120 mm, then × = 120/480, or ¼.

Statement on the Use of Living and Preserved Animals in the Zoology Laboratory

Congress has probably received more mail on the topic of animal research in universities and business firms than on any other subject. Do humans have the right to experiment on other living creatures to support their own medical, pharmaceutical, and commercial needs? A few years ago, Congress passed a series of amendments to the Federal Animal Welfare Act, a body of laws covering animal care in laboratories and other facilities. These amendments have become known as the three R's: reduction in the number of animals needed for research; refinement of techniques that might cause stress or suffering; and replacement of live animals with simulations or cell cultures whenever possible. As a result, the total number of animals used each year in research and in commercial product testing has declined steadily as scientists and businesses have become more concerned and more accountable. The animal rights movement, largely comprising vocal antivivisectionists, has helped to create an awareness of the needs of laboratory research animals and has stretched the resources and creativity of the researchers to discover cheaper and more humane alternatives to animal experimentation.

However, computers and cell cultures—the alternatives—can only simulate the effects on organismal systems of, for instance, drugs, when the principles are well acknowledged. When the principles are themselves being scrutinized and tested, computer modeling is insufficient. Nor can a movie or computer simulation match the visual and tactile comprehension of anatomical relationships provided by direct dissection of preserved or anesthetized animals. Medical and veterinarian progress depends on animal research. Every drug and every vaccine that you and your family have ever taken has first been tested on an animal. Animal research has wiped out smallpox and polio; has provided immunization against diseases previously common and often deadly, such as diphtheria, mumps, and rubella; has helped create treatments for cancer, diabetes, heart disease, and manic depression; and has been used in the development of surgical procedures such as heart surgery, blood transfusions, and cataract removal.

Animal research has also benefited other animals for veterinary cures. The vaccine for feline leukemia that could threaten the life of your cat, as well as the parvo vaccine given to your puppy, were first introduced to other cats and dogs. Many other vaccinations for serious animal diseases were developed through animal research; for example, rabies, distemper, anthrax, hepatitis, and tetanus.

The animal models used by the artist for the illustrations in the exercises of this laboratory manual, and the animals you will dissect in this laboratory course, were prepared for educational use following strict humane procedures. No endangered species have been used. No live higher organisms will be harmed or experimented on in this laboratory setting. Invertebrate animals that are to be dissected while alive are anesthetized before the procedure. The experiments selected are unoffensive, are respectful of the integrity of the animal's evolutionary contributions, and often require only close observation. The experiments closely follow the tenets of the scientific method, which cannot dictate ethical decisions but can provide the struc-

ture for common sense. Do not be wasteful. Share the animals with the other students as often as possible. At the same time, you are encouraged to observe the live animal in its natural setting and its relationships to other species, for only in this manner will you gain a full appreciation of the unique evolutionary position and special structure and systems of each animal.

PART ONE *Introduction to the Living Animal*

1 *The Microscope*

Exercise 1A: Compound Light Microscope

Parts and operation of the microscope
Getting acquainted with your microscope
Taking control of your microscope
Magnification in the microscope
How to measure size of microscopic objects
Exercises with the compound light microscope

Exercise 1B: Stereoscopic Dissecting Microscope

Exercises with the dissecting microscope

Exercise 1C: Electron Microscope

For a biologist the compound microscope is probably the most important tool ever invented. It is indispensable not only in biology but also in the fields of medicine, biochemistry, and geology; in industry; and even in crime detection and many hobbies. Yet even though the microscope is one of the most common tools in the biologist's laboratory, too frequently it is used without any effective understanding of its construction and operation. The results may be poor illumination, badly focused optics, and misleading interpretations of what is (barely) seen.

Both the compound microscope and the binocular dissecting microscope (stereoscopic microscope) will open up a whole new world for you if you make the effort to become proficient in their use. Learn their possibilities and use them to the greatest advantage. Take good care of the microscope. Microscopes are expensive instruments and, although they are sturdily built and will stand many years of use, they are precision instruments and are delicate enough to require careful treatment.

EXERCISE 1A

Compound Light Microscope

The compound light microscope may be either monocular or binocular, with either vertical or inclined oculars.

☞ Use both hands to carry a microscope. Grasp it firmly by the **arm** with one hand and support the **base** with the other. Carry it in a fully upright position.

Understanding the Parts and Operation of the Microscope

If you are not familiar with the parts of the microscope, please study Figs. 1-1 and 1-2.

The **image-forming optics** consist of (1) a set of **objectives** screwed into a **revolving nosepiece,** and (2) a **body tube,** or head, with one or two **oculars (eyepieces).**

Each **objective** is a complex set of tiny lenses that provide most of the magnification. Your microscope may have two, three, or four objectives, each with its magnification, or power, engraved on the side. For example, if the objective magnifies an object 10 times, the magnification is said to be 10 diameters and is commonly written simply as 10×. Most microscopes include a **scanning objective** (3.5× or 4.5×), a **low-power objective** (10×), and a **high-power objective** (40×, 43×, or 45×). Some microscopes also carry an **oil-immersion objective** (95×, 97×, or 100×), which must always be used with a

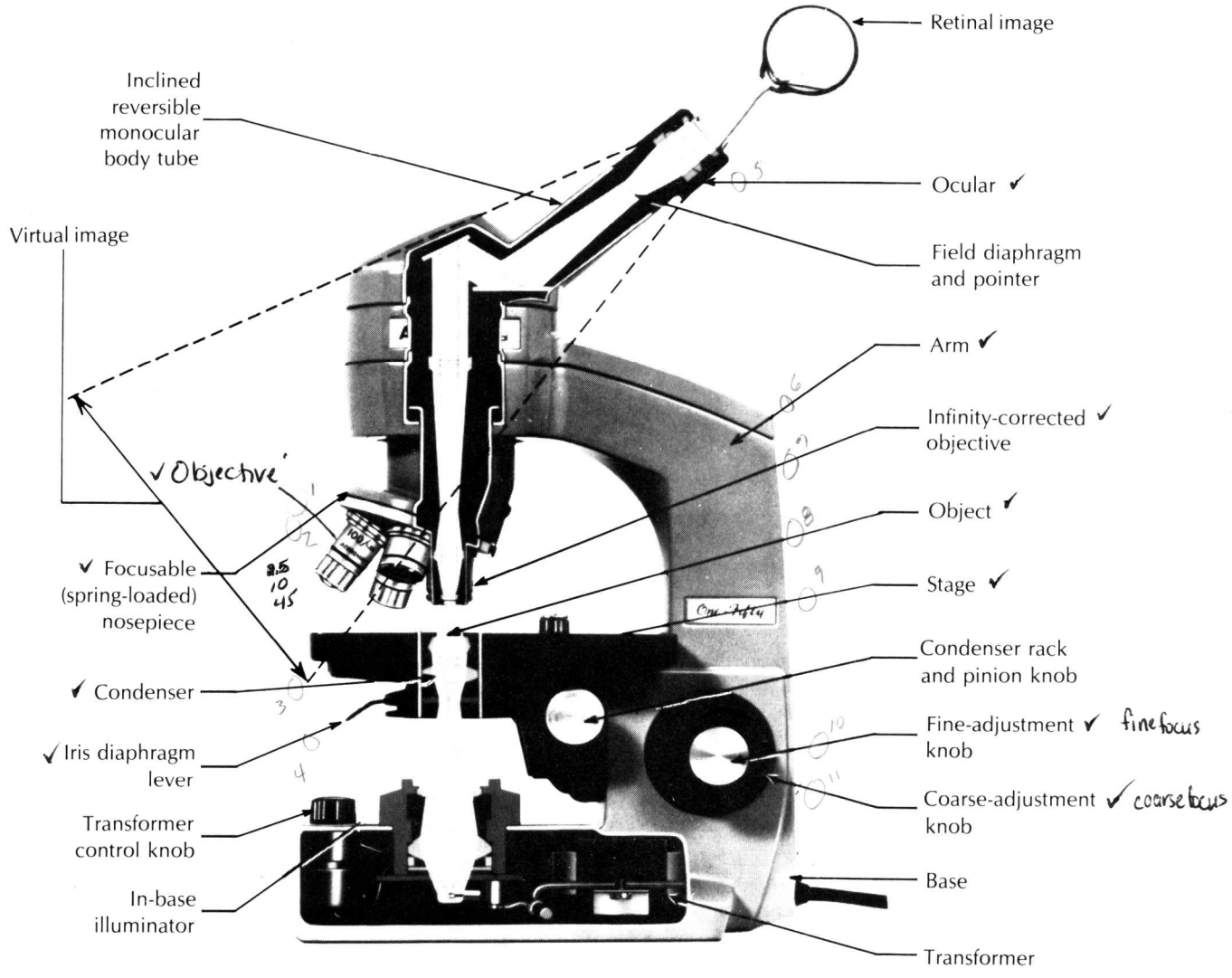

FIGURE 1-1 Optical and mechanical features of a compound microscope. From American Optical.

10 ×

magnification = magnification of oculars × magnification of objective

use 2.5 mag = 25 use 10 mag = 100 use 45 mage = 450

drop of oil to form a liquid bridge between itself and the surface of the slide being viewed.

☞ Revolve the nosepiece and note the clicking sound when an objective swings into place under the tube.

The lenses in the **ocular** further magnify the image formed by the objective. The ocular most often used is the 10×. A 6× or a 15× ocular may also be provided. Often a pointer is mounted into the ocular.

☞ If there is a pointer in your microscope, rotate the ocular and note the movement of the pointer.

The **stage** is the platform with clips to hold the slide in place. Some microscopes have a mechanical stage possessing knobs for moving the slide back and forth or up and down.

Directly beneath the stage of most microscopes is a **substage condenser,** a system of enclosed lenses that concentrates the light on the specimen above. The condenser may have a knob that allows you to move the condenser up and down.

Beneath the condenser, many microscopes have a built-in, low-voltage **substage illuminator.** Microscopes lacking a substage illuminator employ an adjustable **reflecting mirror** that reflects natural light or light from a microscope lamp up into the optical system. The **concave surface** of the mirror is used with natural light or with a separate microscope lamp when there is no condenser on the microscope. The

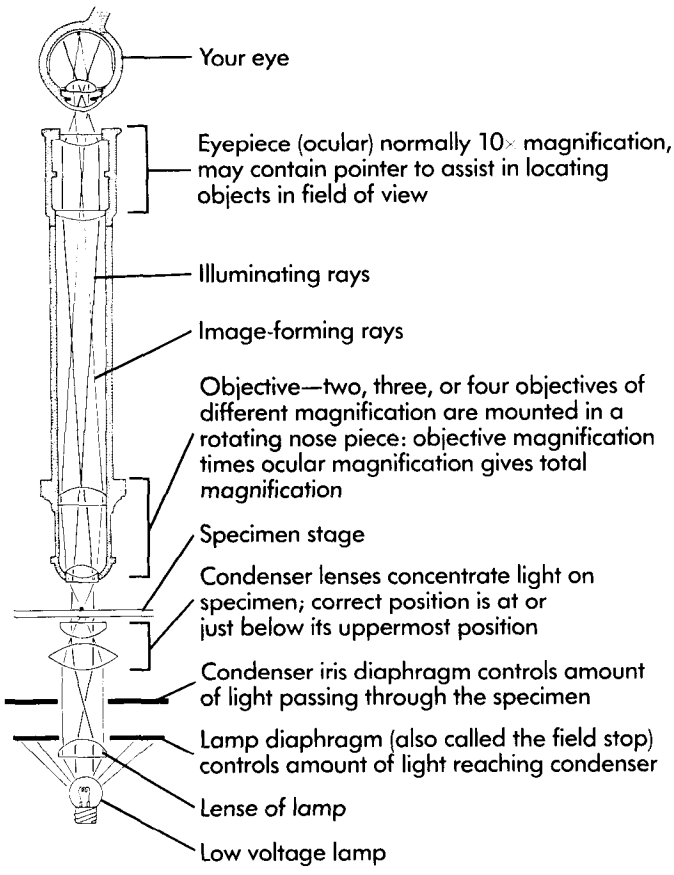

FIGURE 1-2 Optical path of light through a microscope.

plane (flat) surface of the mirror is used with a substage condenser.

☞ Turn on the substage illuminator. If your microscope lacks one, use a microscope lamp or position your microscope and mirror to take advantage of natural light. With the low-power objective in place, adjust the mirror to bring a bright, evenly distributed circle of light through the lens.

An adjustable **iris diaphragm** under the stage regulates the amount of light passing through the **aperture** of the stage. On some microscopes the light is regulated by revolving a **disc diaphragm**, which has holes of various sizes.

☞ Raise or lower the substage condenser to adjust the light to the desired intensity. Usually the best condenser position is near its uppermost limit, where it gives maximum illumination. Then close down the iris or disc diaphragm gradually until all glare is gone (but not too far; dark halos will appear around objects if you step down too much). You can check for the best diaphragm setting by removing the ocular and looking down the body tube. Adjust the diaphragm so that you have reduced the disc of light on the objective lens to about three-quarters of its fully illuminated setting. Replace the ocular lens.

The **body tube** is raised and lowered by two sets of adjustment knobs. The **coarse-adjustment knob** is for low-power work and for initial focusing. The **fine-adjustment knob** is for final adjustment and varying the plane of focus for viewing an object at different depths. Note: Microscopes with an inclined tube (like the one pictured in Fig. 1-1) are focused by **moving the stage** rather than the body tube up and down. If you are using an inclined microscope, read "lower the stage" when the directions say "raise the objective" in the following exercise. In either case the distance between objective lens and the object is increased.

☞ Turn the coarse-adjustment knob and note how it moves the body tube (or stage). Find out which way to turn the knob to raise and lower the tube.

Never use the coarse-adjustment knob when a high-power objective is in place. Turn the fine-adjustment knob. This moves the tube so slightly that you cannot detect it unless you are examining an object through the ocular. The fine-adjustment knob works the same as the coarse-adjustment knob. To focus downward, turn the knob in the same direction as you would to focus down with the coarse adjustment. Practice this. **Always use the fine adjustment when the high-power objective is in place.**

Getting Acquainted With Your Microscope

You need not wear glasses when using the microscope unless they correct a severe astigmatism. Nearsightedness and farsightedness can be corrected by adjusting the microscope.

First clean the lenses of both the ocular and the objectives by wiping them gently with a clean sheet of lens paper. Do this each time you use the microscope. **Never touch the lenses with anything except clean lens paper.** Do not remove an ocular or objective unless told to do so by the instructor. Dust the mirror with a cleansing tissue.

Keep both eyes open while using a monocular microscope. If this seems difficult at first, hold a piece of paper over one eye while viewing the object with the other. Should one eye become tired, shift to the other one.

How to Focus with Low Power. Turn the low-power (10×) objective until it clicks in place over the aperture. Adjust the condenser and iris diaphragm for optimal illumination as already described.

Obtain a slide containing the letter *e* (or *a*, *h*, or

k). Place it, coverslip up, on the stage with the letter centered under the objective lens. *While watching from the side,* lower the objective with the coarse adjustment until it is close to the slide surface.

Now look through the ocular and slowly raise the objective by turning the coarse adjustment toward you until the object on the slide is in sharp focus. Is the image upside down? Is it reversed; that is, does the left side of the letter appear on the right, and the right side on the left? On a separate sheet of paper, draw the letter as it appears.

Shift the slide very slightly to the right while viewing it through the ocular. In what direction does the image move? Move the slide away from you. What happens to the image? Turn the fine-adjustment knob toward and then away from you and observe the effect on the image.

How to Focus with High Power. Focus the object first with low power; then slowly rotate the high-power objective into position. If the microscope lenses have been constructed in a particular way by the manufacturer, the object in focus with low power will be nearly in focus under high power. Such lenses are said to be **parfocal** with respect to each other. Now turn the **fine-adjustment knob** to bring the specimen into sharp focus.

Never use the coarse adjustment while looking at an object under high power; you may ram the objective into the slide. This may damage the slide or ruin the lens of the objective.

If the microscope is not parfocal (your instructor will tell you), focus first with low power and then raise the tube by turning the coarse-adjustment knob one-half turn. **With your eye at the level of the stage** carefully swing the high-power objective into place, raising the tube a little further if the objective touches the slide. Now, **still watching the high-power objective from the side,** lower it slowly to about 1 mm from the cover glass. Then, **looking through the ocular,** raise the tube with the fine adjustment until the object is in focus. Do this several times to acquire skill in focusing.

If you cannot find anything at all with high power, it may be that, because of the small size of the highpower field, the object is not in the field. Turn back to lower power, adjust the slide so that the object is in the very center of your field of view, and then return to high power. If you still cannot find the object, it may be that it is too far out of focus to be seen. Use the fine-adjustment knob. You will have to learn by experience whether to rack (turn) up or down to bring the slide into sharp focus.

The light decreases when you switch to high power because the diameter of the high-power lens is so much smaller than that of the low-power lens. Adjust the light with the mirror and iris diaphragm. Do not tilt the microscope. Keep it vertical. This is particularly important with wet mounts.

Any object you see through the microscope, no matter how thin, has some depth, and you must view it at different focal planes to bring out all the details. To do this, **keep your hand on the fine adjustment** and constantly focus up and down. This is essential to really understand the nature of the material on a slide.

How to Use the Oil-Immersion Objective. Occasionally a project or demonstration exercise requires using an oil-immersion objective. This is an objective in the 90× to 100× range. The resolving power of this objective approaches the limit of the compound light microscope. This happens because the mean wavelength of visible light combined with the optical properties of the objective lens make points closer together than about 0.2 μm (microns) indistinguishable (a micron, also called a micrometer, is one thousandth of a millimeter). To deliver as much light as possible to the lens system, oil must be used to bridge the separation between the slide and the objective.

To use oil immersion, bring the specimen into focus first with the low-power and then with the high-power objective. Carefully center the point of interest in the field of view; then rotate the nosepiece to move the high-power objective off to one side. Place a single drop of immersion oil on the coverslip at the point where the objective will come into position. Now move the oil-immersion objective carefully into position, watching from the side to be certain that the lens clears the coverslip. The oil should now form a bridge between lens and coverslip. **Carefully** adjust the fine focus to bring the specimen into focus. Adjust the iris diaphragm or substage condenser to increase light as required.

When finished, clean the lens with a lens tissue wetted with xylol and then with a lens tissue wetted with Kodak Lens Cleaner. **Never** use alcohol, which will dissolve the cement around the lens system. If the lens is to be used again soon (within a day or two), it is best not to clean the lens face. Residual oil will not harm the lens unless it is allowed to harden over a long period without use.

Taking Control of Your Microscope

1. Proper lighting is the first requirement for happy microscopy. Too much light is as bad as too little (beginners usually tend to use too much). Never assume that the condenser and iris diaphragm were left correctly set by the student who last used your microscope. Carefully adjust both con-

denser and diaphragm for optimal lighting before proceeding with anything else. Transparent objects are often clearer in reduced light. Reduce light by closing down the iris diaphragm, **not** by lowering the substage condenser.

2. Focus with eyes relaxed. The image appears to your eye as though it were about 10 inches away, but the eye should be relaxed as though it were viewing an image in the distance. Imagine you are looking at a distant object rather than something at the end of the body tube. Look up periodically and train your eyes on something across the room. Then, if you keep your eyes relaxed, you should not have to readjust your focus very much when looking through the microscope. If you get a headache, the chances are you are trying to look *into* the microscope rather than *through* it.
3. If you are using a binocular microscope, it is important, (1) to adjust the microscope's interocular distance to match the distance between your own pupils, and, (2) to adjust the oculars for a sharp focus. If the focus is not sharp you may have to focus each eye separately. The microscope will have one fixed and one adjustable ocular. Adjust the focus for the fixed ocular first to suit that eye, then adjust the other ocular (usually by rotation) until the focus is sharp for both eyes.
4. Eye distance from the ocular(s) is important too: not too close or too far. Find the correct distance that affords a full view of the field, keep relaxed, hold your head steady, and enjoy the view.
5. Where's the dirt? If spots or smudges appear in the field of vision, it may be dirt on the ocular, on the slide, or on the objective. To find out which, first rotate the ocular; if the spots move the ocular needs to be cleaned (in laboratory, avoid using eye makeup, which may smear on the ocular surface). Move the slide; if the spots move with it, clean the slide. If after cleaning ocular and slide the spots persist, it is probably a dirty objective lens. Use only special lens paper to clean lenses. The slide may be cleaned very gently with a soft damp cloth or damp cleansing tissue. If after cleaning everything you still see spots or smudges, try moving the condenser up or down a little. Still not satisfied? You may need help from the assistant. Some people may see "floaters" that drift across the field of vision while viewing a brightly illuminated object. These are defraction images of red blood cell "ghosts" in the vitrous humor of the eye. While annoying at first, one can usually learn to ignore them.
6. When studying an object, especially when using high power, keep one hand on the fine adjustment and constantly focus up and down. This is the only way to see everything.
7. If the fine adjustment refuses to turn, the knob has reached its range limit. To correct this, give the fine-adjustment knob several turns in the opposite direction, and then refocus with the coarse adjustment.
8. Finally, be friendly to your microscope. It must be kept dust free, so return it **carefully** to its box or cupboard when finished with it. Before putting it away, put the low-power objective in place and raise the tube a little. Be sure not to leave a slide on the stage.

The prepared slides you will be using in the laboratory were made at the cost of great skill and patience and are quite expensive to buy. They are fragile and should be handled with great care. Your instructor may provide a demonstration of how slides are made (outlined in the Instructor's Handbook).

Magnification in the Microscope

How much your microscope will magnify depends on the power of the combination of lenses you are using. Your microscope is probably equipped with a 10× ocular, which magnifies the object 10 times in diameter. Other oculars may magnify 2×, 5×, or 20×. The objectives may be designated, respectively, 3.5× (scanning objective), 10× (low-power objective), and 45× (high-power objective). The total magnifying power is determined by multiplying the power of the objective by the power of the ocular. Examples of the magnification of certain combinations follow:

Ocular	Objective	Magnification
5×	3.5×	17.5 diameters
5×	10×	50 diameters
10×	3.5×	35 diameters
10×	10×	100 diameters
10×	45×	450 diameters
10×	90×	900 diameters

For handy reference, enter in the table above, at right, the total magnifying power for lens combinations on *your* microscope:

How to Measure Sizes of Microscopic Objects

It is often important to know the size of an organism or object you are viewing through the microscope because size may be a diagnostic characteristic. Or, if you are looking for a particular species of protozoan in a mixed culture and know that the species is usually about 400 μm long, it saves time to know just how large 400 μm will appear at either low or high power. Alternative methods for measuring object sizes are described below.

Magnifying powers for microscope number _____					
Magnification for:	**Ocular**		**Objective**		**Magnification**
Scanning lens	_____	×	_____	=	_____ diameters
Low-power lens	_____	×	_____	=	_____ diameters
High-power lens	_____	×	_____	=	_____ diameters
Oil-immersion lens	_____	×	_____	=	_____ diameters

Measuring Objects with Transparent Ruler Calibration. With this simple method, the viewer measures the diameter of the field of view and then estimates the proportion of the field occupied by the objects. This method is not as accurate as the alternative described in the next section, but often an approximation of size is all that the viewer requires.

☞ With the scanning objective in position, place a transparent ruler on the microscope stage and focus on its edge so that you can see the scale. Move the ruler right or left so that one of the vertical millimeter lines is just visible at the edge of the circular field of view. Count the number of millimeter lines spanning the field; you will probably have to estimate the last decimal fraction of a millimeter. Enter diameter in mm here _____. Now convert this figure to microns by multiplying by 1000. Enter this value here _____. This is the diameter in microns of the field for the scanning lens.

The diameters of the fields of view for your low-power and high-power objectives cannot be measured directly with the transparent ruler because of the high magnification. To calculate the field diameter of the low power objective, multiply the diameter of the scanning lens field by the magnifying power of the scanning objective and divide by the magnifying power of the low power objective:

$$\frac{\text{Magnification of scanning objective}}{\text{Magnification of low-power objective}} \times \text{Diameter of scanning objective field} = \text{Diameter of low-power field}$$

Make this calculation for your microscope and enter the value here _____. It usually ranges between 1.5 and 1.6 mm (1500 and 1600 μm).

Similarly, the diameter of the high-power field is determined as:

$$\frac{\text{Magnification of the low-power objective}}{\text{Magnification of the high-power objective}} \times \text{Diameter of low-power field} = \text{Diameter of high-power field}$$

Make this calculation for your microscope and enter the value here _____. It usually ranges between 0.36 and 0.42 mm (3600 and 4200 μm). Now enter these values in the table below for quick reference.

Diameters of fields of view for *microscope number* _____	
Scanning lens:	_____ microns
Low power lens:	_____ microns
High-power lens:	_____ microns

Measuring Objects with Ocular and Stage Micrometer Calibration. An **ocular micrometer** can be fitted into the microscope's ocular. It is a disc on which is engraved a scale of (usually) either 50 or 100 units. These units are arbitrary values that always appear the same distance apart no matter which objective is used in combination with the eyepiece. Therefore, the ocular micrometer cannot be used to measure objects until it has been calibrated with a **stage micrometer.**

The stage micrometer resembles an ordinary microscope slide but bears an engraved scale on its upper surface, usually 1 or 2 mm long, divided into 0.1 and 0.01 mm divisions.

Place the stage micrometer on the microscope stage and focus on the engraved scale with the low-power objective. Both scales should appear sharply defined. Rotate the eyepiece until the two scales are parallel. Now move the stage micrometer to bring the 0 line of the stage scale in exact alignment with