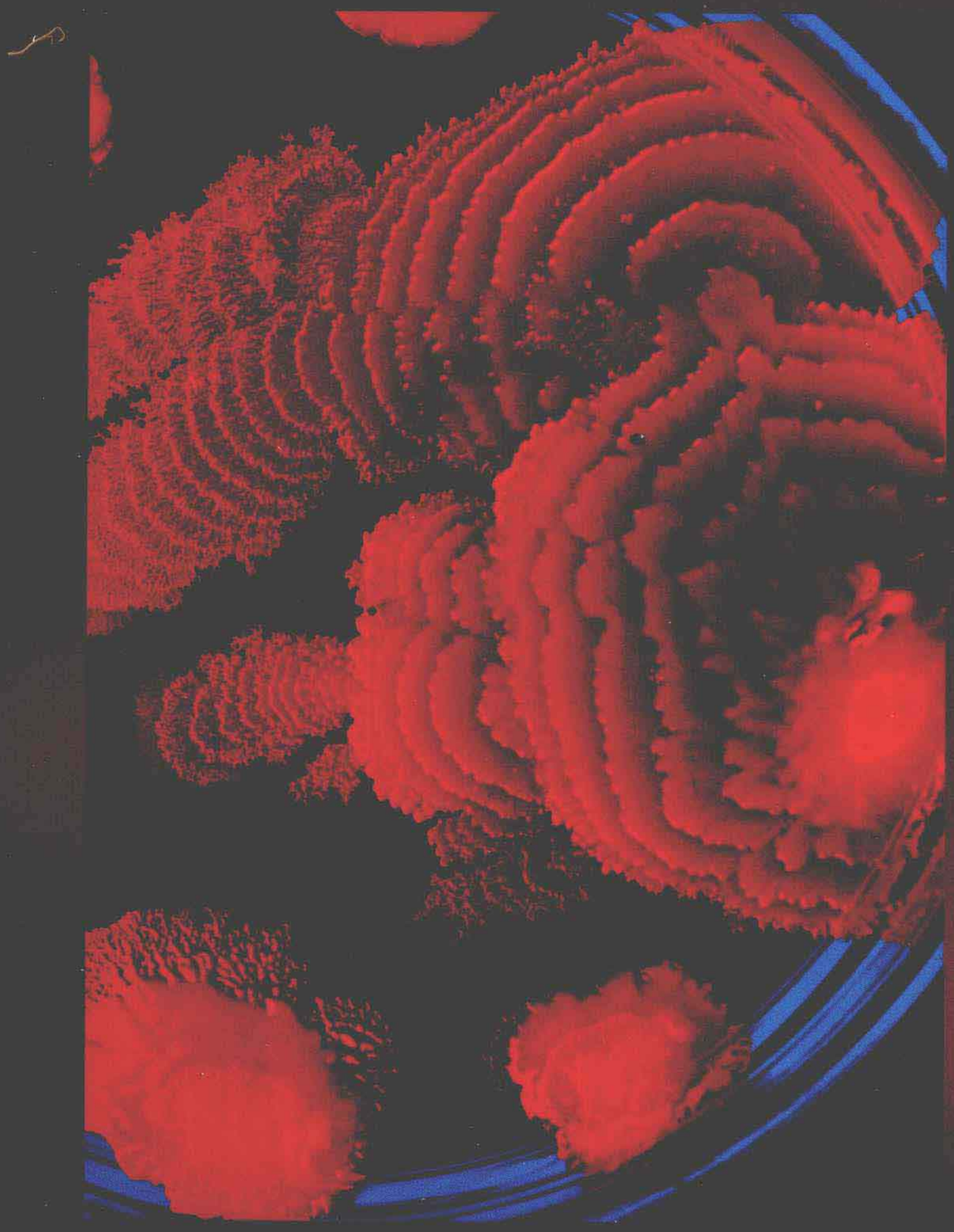
Eighth Edition

Microbiological Applications

Laboratory Manual in General Microbiology



Benson

SHORT VERSION

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Laboratory Manual in General Microbiology

Eighth Edition

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McGraw-Hill Higher Education
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MICROBIOLOGICAL APPLICATIONS: LABORATORY MANUAL IN GENERAL MICROBIOLOGY/SHORT VERSION, EIGHTH EDITION

Published by McGraw-Hill, a business unit of The McGraw-Hill Companies, Inc., 1221 Avenue of the Americas, New York, NY 10020.

This book is printed on recycled, acid-free paper containing 10% postconsumer waste.

4 5 6 7 8 9 0 QPD/QPD 0 9 8 7 6 5 4 3

ISBN 0–07–231889–9

Publisher: *James M. Smith*
Senior developmental editor: *Jean Sims Fornango*
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Marketing coordinator: *Tami Petsche*
Project manager: *Joyce Watters*
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Coordinator of freelance design: *Michelle M. Meerdink*
Freelance cover designer: *Lisa Gravunder*
Cover image: Colony of *Proteus mirabilis* by James A. Shapiro, University of Chicago
Senior supplement producer: *Stacy A. Patch*
Compositor: *Carlisle Communications, Ltd.*
Typeface: *11/12 Times Roman*
Printer: *Quebecor World Dubuque, IA*

www.mhhe.com

Preface

Although the Complete Version of this book has been expanded and changed in several respects, this shorter version has picked up only one new exercise and remains less altered. Most of the changes in this version pertain to rewriting of some experiments and improving illustrations where necessary.

The only new exercise in this version is Exercise 7: Aseptic Technique. This exercise has been requested by some users of the book who feel that more emphasis was needed on the handling of bacterial cultures. The philosophy of previous editions was to incorporate in each exercise those aseptic techniques that were required to perform the specific experiment at hand. Some users of this book may see this as unnecessary redundancy; others will feel that the topic of aseptic technique cannot be over-emphasized.

To further augment laboratory efficiency and safety, two other changes have been incorporated in this edition: (1) a three page set of instructions entitled *Laboratory Protocol* has been inserted following this Preface, and (2) highlighted cautionary boxes have been incorporated into every exercise where applicable.

In the *Laboratory Protocol* many of the "Rules of the Lab" from the previous *Introduction* are included, as well as new terminology, aseptic techniques, and the handling of accidental spills. Although most of the cautionary statements highlighted in the caution boxes existed in the previous edition, placing them in special boxes makes these warnings more visible, thus contributing to greater laboratory safety.

Although several exercises have been slightly reformatted, the only one to have been altered considerably is Exercise 40 that pertains to oxidative and fermentative tests employed in the identification of unknown bacteria. More clarity has been introduced here to help students to understand the complex set of tests that are performed in this exercise.

In addition to the above changes there has been considerable upgrading of graphics throughout the book. Approximately thirty illustrations have been replaced. Several critical color photographs pertaining to molds and physiological tests were also replaced to bring about more faithful color representation.

I am greatly indebted to my editors, Jean Fornango and Jim Smith, who made the necessary contacts for critical reviews. As a result of their efforts the following individuals have provided me with excellent suggestions for improvement of this manual: Barbara Collins at California Lutheran University, Thousand Oaks, CA; Alfred Brown of Auburn University, Auburn, AL; Lester A. Scharlin at El Camino College, Torrance, CA; and Hershell Hanks at Collin County Community College, Plano, TX.

Laboratory Protocol

Welcome to the exciting field of microbiology! The intent of this laboratory manual is to provide you with basic skills and tools that will enable you to explore a vast microbial world. Its scope is incredibly broad in that it includes a multitude of viruses, bacteria, protozoans, yeasts, and molds. Both beneficial and harmful ones will be studied. Although an in-depth study of any single one of these groups could constitute a full course by itself, we will be able to barely get acquainted with them.

To embark on this study it will be necessary for you to learn how to handle cultures in such a way that they are not contaminated or inadvertently dispersed throughout the classroom. This involves learning aseptic techniques and practicing preventive safety measures. The procedures outlined here address these two aspects. **It is of paramount importance that you know all the regulations that are laid down here as Laboratory Protocol.**

Scheduling During the first week of this course your instructor will provide you with a schedule of laboratory exercises arranged in the order of their performance. Before attending laboratory each day, check the schedule to see what experiment or experiments will be performed and prepare yourself so that you understand what will be done.

Each laboratory session will begin with a short discussion to brief you on the availability of materials and procedures. *Since the preliminary instructions start promptly at the beginning of the period, it is* ***extremely important that you are not late to class.***

Personal Items When you first enter the lab, place all personal items such as jackets, bags, and books in some out of the way place for storage. Don't stack them on your desktop. Desk space is minimal and must be reserved for essential equipment and your laboratory manual. The storage place may be a drawer, locker, coatrack, or perimeter counter. Your instructor will indicate where they should be placed.

Attire A lab coat or apron must be worn at all times in the laboratory. It will protect your clothing from accidental contamination and stains in the lab. When leaving the laboratory, remove the coat or apron. In addition, long hair must be secured in a ponytail to prevent injury from Bunsen burners and contamination of culture material.

Terminology

Various terms such as sterilization, disinfection, germicides, sepsis, and aseptic techniques will be used here. To be sure that you understand exactly what they mean, the following definitions are provided.

Sterilization is a process in which all living microorganisms, including viruses, are destroyed. The organisms may be killed with steam, dry heat, or incineration. If we say an article is **sterile,** we understand that it is completely free of all living microorganisms. Generally speaking, when we refer to sterilization as it pertains here to laboratory safety, we think, primarily, in terms of steam sterilization with the autoclave. The ultimate method of sterilization is to burn up the infectious agents or ***incinerate*** them. All biological wastes must ultimately be incinerated for disposal.

Disinfection is a process in which vegetative, nonsporing microorganisms are destroyed. Agents that cause disinfection are called **disinfectants** or **germicides.** Such agents are used only on inanimate objects because they are toxic to human and animal tissues.

Sepsis is defined as the growth (multiplication) of microorganisms in tissues of the body. The term **asepsis** refers to any procedure that prevents the entrance of infectious agents into sterile tissues, thus preventing infection. **Aseptic techniques** refer to those practices that are used by microbiologists to exclude all organisms from contaminating media or contacting living tissues. **Antiseptics** are chemical agents (often dilute disinfectants) that can be safely applied externally to human tissues to destroy or inhibit vegetative bacteria.

Aseptic Techniques

When you start handling bacterial cultures as in Exercises 9 and 10, you will learn the specifics of aseptic techniques. Some of the basic things you will do are as follows:

Hand Washing Before you start working in the lab, wash your hands with a liquid detergent and dry them with paper toweling. At the end of the period, before leaving the laboratory, wash them again.

Tabletop Disinfection The first chore of the day will be to sponge down your desktop with a disinfectant. This process removes any dust that may be present and minimizes the chances of bacterial contamination of cultures that you are about to handle.

Your instructor will indicate where the bottles of disinfectant and sponges are located. At the end of the period before leaving the laboratory, perform the same procedure to protect students that may occupy your desk in the next class.

Bunsen Burner Usage When using a Bunsen burner to flame loops, needles, and test-tubes, follow the procedures outlined in Exercise 7. Inoculating loops and needles should be heated until they are red-hot. Before they are introduced into cultures, they must be allowed to cool down sufficiently to prevent killing organisms that are to be transferred.

If your burner has a pilot on it and you plan to use the burner only intermittently, use it. If your burner lacks a pilot, turn off the burner when it is not being used. Excessive unnecessary use of Bunsen burners in a small laboratory can actually raise the temperature of the room. More important is the fact that unattended burner flames are a constant hazard to hair, clothing, and skin.

The proper handling of test-tubes, while transferring bacteria from one tube to another, requires a certain amount of skill. Test-tube caps must never be placed down on the desktop while you are making inoculations. Techniques that enable you to make transfers properly must be mastered. Exercise 7 pertains to these skills.

Pipetting Transferring solutions or cultures by pipette must always be performed with a mechanical suction device. Under no circumstances is pipetting by mouth allowed in this laboratory.

Disposal of Cultures and Broken Glass The following rules apply to culture and broken glass disposal:

1. Petri dishes must be placed in a plastic bag to be autoclaved.
2. Unneeded test-tube cultures must be placed in a wire basket to be autoclaved.
3. Used pipettes must be placed in a plastic bag for autoclaving.
4. Broken glass should be swept up into a dustpan and placed in a container reserved for broken glass. Don't try to pick up the glass fragments with your fingers.
5. Contaminated material must never be placed in a wastebasket.

Accidental Spills

All accidental spills, whether chemical or biological, must be reported immediately to your instructor. Although the majority of microorganisms used in this laboratory are nonpathogens, some pathogens will be encountered. It is for this reason that we must treat all accidental biological spills as if pathogens were involved.

Chemical spills are just as important to report because some agents used in this laboratory may be carcinogenic; others are poisonous; and some can cause dermal damage such as blistering and depigmentation.

Decontamination Procedure Once your instructor is notified of an accidental spill; the following steps will take place:

1. Any clothing that is contaminated should be placed in an autoclavable plastic bag and autoclaved.
2. Paper towels, soaked in a suitable germicide such as 5% bleach, are placed over the spill.
3. Additional germicide should be poured around the edges of the spill to prevent further aerosolization.
4. After approximately 20 minutes, the paper towels should be scraped up off the floor with an autoclavable squeegee into an autoclavable dustpan.
5. The contents of the dustpan are transferred to an autoclavable plastic bag, which may itself be placed in a stainless steel bucket or pan for transport to an autoclave.
6. All materials, including the squeegee and dustpan, are autoclaved.

Additional Important Regulations

Here are a few additional laboratory rules:

1. Don't remove cultures, reagents, or other materials from the laboratory unless you have been granted specific permission.
2. Don't smoke or eat food in the laboratory.
3. Make it a habit to keep your hands away from your mouth. Obviously, labels are never moistened with the tongue; use tap water or self-adhesive labels instead.
4. Always clean up after yourself. Gram-stained slides that have no further use to you should be washed and dried and returned to a slide box. Coverslips should be cleaned, and dried, and returned. Staining trays should be rinsed out and returned to their storage place.
5. Return all bulk reagent bottles to places of storage.
6. Return inoculating loops and needles to your storage container. Be sure that they are not upside down.

7. If you have borrowed something from someone, return it.
8. Do not leave any items on your desk at the end of the period.
9. Do not disturb another class at any time. Wait until the class is dismissed.
10. Treat all instruments, especially microscopes, with extreme care. If you don't understand how a piece of equipment functions, ask your instructor.
11. Work cooperatively with other students in group-assigned experiments, but do your own analyses of experimental results.

Contents

PART 1 Microscopy

Microscopes of various types are available to the microbiologist. Each type has its specific applications and limitations. No instrument functions best in all applications. The principal types are the brightfield, phase contrast, fluorescence, and electron microscopes.

Since the brightfield and phase-contrast microscopes will be most widely used in this course, two exercises (1 and 3) relate to these instruments. Exercise 2, which pertains to darkfield microscopy, will also be studied. Its applications are more limited. Understanding the basic principles and applications of these three types of microscopy is a fundamental goal in this course.

Exercise 4 pertains to the use of an ocular micrometer in determining the sizes of microscopic organisms. The technique outlined here applies, primarily, to brightfield and phase-contrast microscopes.

Before using any of these microscopes in the laboratory it is best to read over the exercises and answer the questions on the Laboratory Report before coming to the laboratory. Your instructor may even want you to hand in the Laboratory Report at the beginning of the period when an instrument is first used.

Brightfield Microscopy

1

A microscope that allows light rays to pass directly through to the eye without being deflected by an intervening opaque plate in the condenser is called a ***brightfield microscope.*** This is the conventional type of instrument encountered by students in beginning courses in biology; it is also the first type to be used in this laboratory.

All brightfield microscopes have certain things in common, yet they differ somewhat in mechanical operation. An attempt will be made in this exercise to point out the similarities and differences of various makes so that you will know how to use the instrument that is available to you. Before attending the first laboratory session in which the microscope will be used, read over this exercise and answer all the questions on the Laboratory Report. Your instructor may require that the Laboratory Report be handed in prior to doing any laboratory work.

Care of the Instrument

Microscopes represent considerable investment and can be damaged rather easily if certain precautions are not observed. The following suggestions cover most hazards.

Transport When carrying your microscope from one part of the room to another, use both hands when holding the instrument, as illustrated in figure 1.1. If it is carried with only one hand and allowed to dangle at your side, there is always the danger of collision with furniture or some other object. And, incidentally, *under no circumstances should one attempt to carry two microscopes at one time.*

Clutter Keep your workstation uncluttered while doing microscopy. Keep unnecessary books, lunches, and other unneeded objects away from your work area. A clear work area promotes efficiency and results in fewer accidents.

Electric Cord Microscopes have been known to tumble off of tabletops when students have entangled a foot in a dangling electric cord. Don't let the light cord on your microscope dangle in such a way as to hazard foot entanglement.

Lens Care At the beginning of each laboratory period check the lenses to make sure they are clean. At the end of each lab session be sure to wipe any immersion oil off the immersion lens if it has been used. More specifics about lens care are provided on page 6.

Dust Protection In most laboratories dustcovers are used to protect the instruments during storage. If one is available, place it over the microscope at the end of the period.

Components

Before we discuss the procedures for using a microscope, let's identify the principal parts of the instrument as illustrated in figure 1.2.

Framework All microscopes have a basic frame structure, which includes the **arm** and **base.** To this framework all other parts are attached. On many of the older microscopes the base is not rigidly attached to the arm as is the case in figure 1.2; instead, a pivot point is present that enables one to tilt the arm backward to adjust the eyepoint height.

Stage The horizontal platform that supports the microscope slide is called the *stage.* Note that it has a clamping device, the **mechanical stage,** which is used for holding and moving the slide around on the stage.

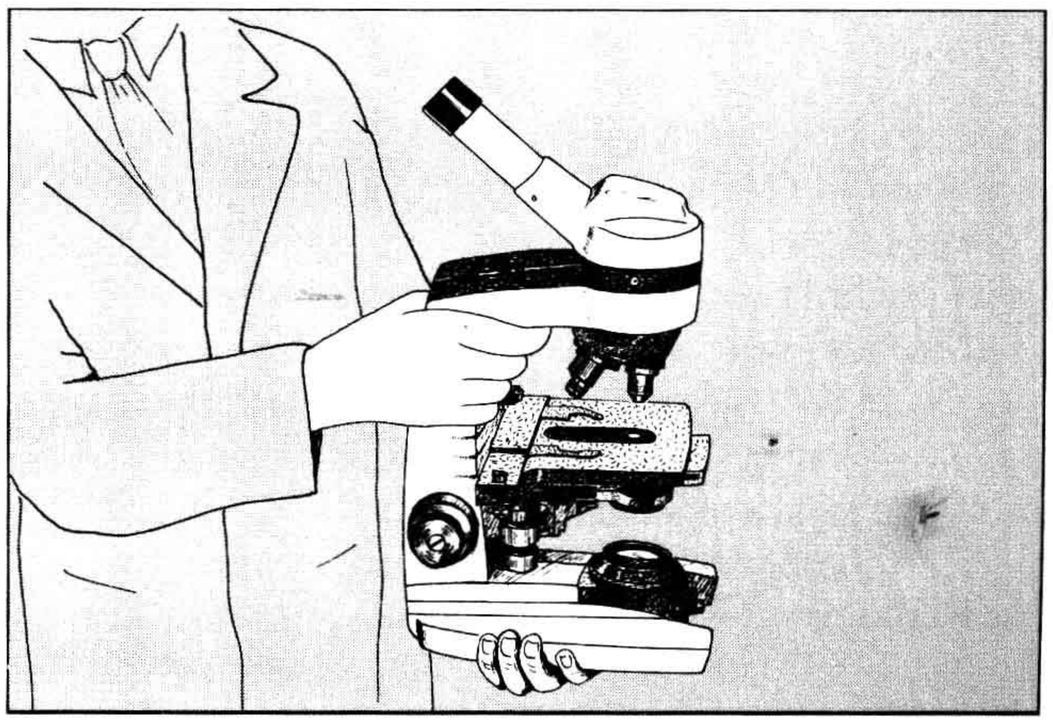

Figure 1.1 **The microscope should be held firmly with both hands while carrying it.**

Note, also, the location of the **mechanical stage control** in figure 1.2.

Light Source In the base of most microscopes is positioned some kind of light source. Ideally, the lamp should have a **voltage control** to vary the intensity of light. The microscope in figure 1.2 has a knurled wheel on the right side of its base to regulate the voltage supplied to the light bulb. The microscope base in figure 1.4 has a knob (the left one) that controls voltage.

Most microscopes have some provision for reducing light intensity with a **neutral density filter.** Such a filter is often needed to reduce the intensity of light below the lower limit allowed by the voltage control. On microscopes such as the Olympus CH-2, one can simply place a neutral density filter over the light source in the base. On some microscopes a filter is built into the base.

Lens Systems All microscopes have three lens systems: the oculars, the objectives, and the condenser.

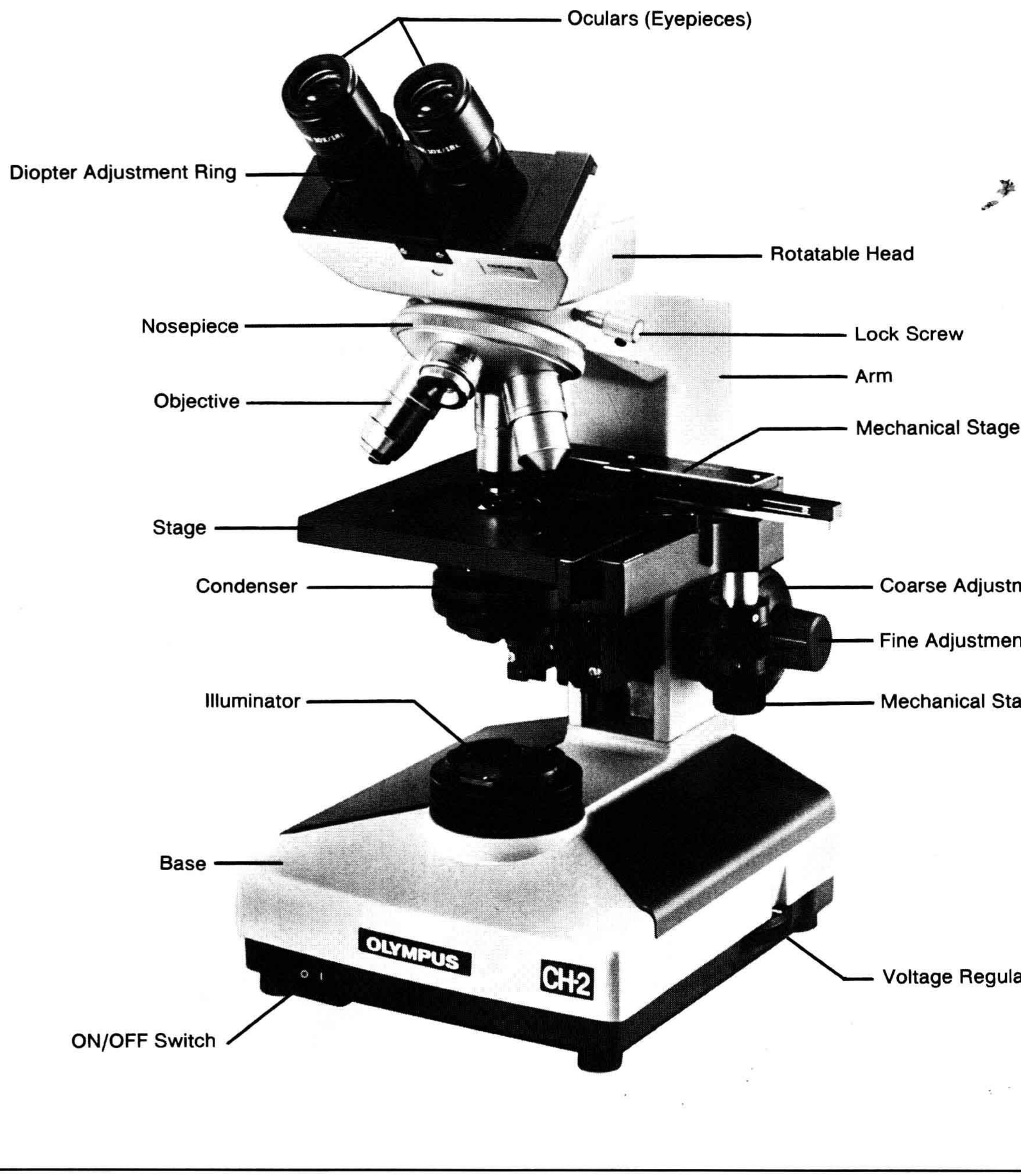

Figure 1.2 **The compound microscope** Courtesy of the Olympus Corporation, Lake Success, N.Y.

Figure 1.3 illustrates the light path through these three systems.

The **ocular,** or eyepiece, is a complex piece, located at the top of the instrument, that consists of two or more internal lenses and usually has a magnification of 10×. Although the microscope in figure 1.2 has two oculars (binocular), a microscope often has only one.

Three or more **objectives** are usually present. Note that they are attached to a rotatable **nosepiece,** which makes it possible to move them into position over a slide. Objectives on most laboratory microscopes have magnifications of 10×, 45×, and 100×, designated as **low power, high-dry,** and **oil immersion,** respectively. Some microscopes will have a fourth objective for rapid scanning of microscopic fields that is only 4×.

The third lens system is the **condenser,** which is located under the stage. It collects and directs the light from the lamp to the slide being studied. The condenser can be moved up and down by a knob under the stage. A **diaphragm** within the condenser regulates the amount of light that reaches the slide. Microscopes that lack a voltage control on the light source rely entirely on the diaphragm for controlling light intensity. On the Olympus microscope in figure 1.2 the diaphragm is controlled by turning a knurled ring. On some microscopes a diaphragm lever is present. Figure 1.3 illustrates the location of the condenser and diaphragm.

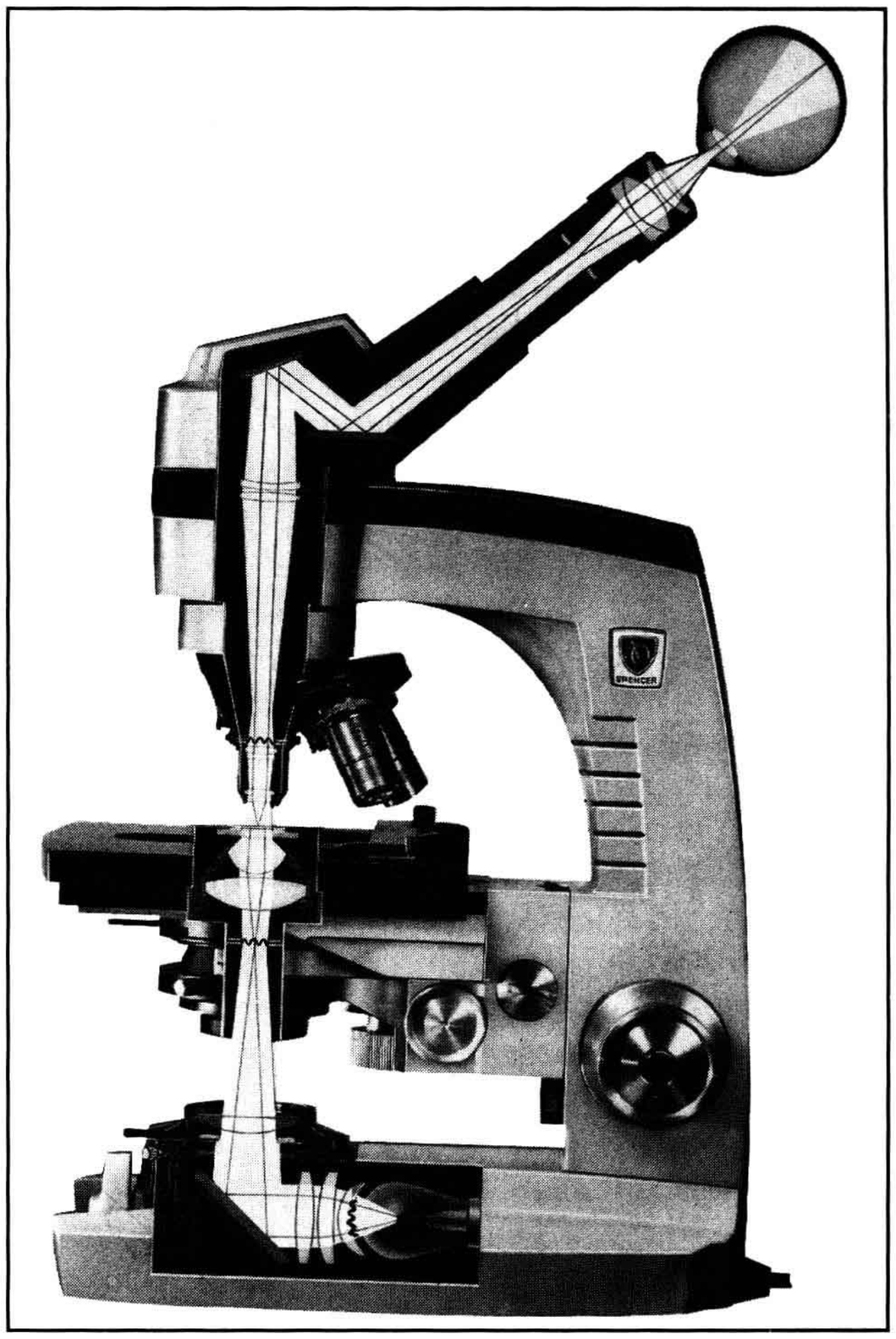

Figure 1.3 The light pathway of a microscope

Focusing Knobs The concentrically arranged **coarse adjustment** and **fine adjustment knobs** on the side of the microscope are used for bringing objects into focus when studying an object on a slide. On some microscopes these knobs are not positioned concentrically as shown here.

Ocular Adjustments On binocular microscopes one must be able to change the distance between the oculars and to make diopter changes for eye differences. On most microscopes the interocular distance is changed by simply pulling apart or pushing together the oculars.

To make diopter adjustments, one focuses first with the right eye only. Without touching the focusing knobs, diopter adjustments are then made on the left eye by turning the knurled **diopter adjustment ring** (figure 1.2) on the left ocular until a sharp image is seen. One should now be able to see sharp images with both eyes.

Resolution

The resolution limit, or **resolving power,** of a microscope lens system is a function of its numerical aperture, the wavelength of light, and the design of the condenser. The optimum resolution of the best microscopes with oil immersion lenses is around 0.2 μm. This means that two small objects that are 0.2 μm apart will be seen as separate entities; objects closer than that will be seen as a single object.

To get the maximum amount of resolution from a lens system, the following factors must be taken into consideration:

- A **blue filter** should be in place over the light source because the short wavelength of blue light provides maximum resolution.
- The **condenser** should be kept at its highest position where it allows a maximum amount of light to enter the objective.
- The **diaphragm** should not be stopped down too much. Although stopping down improves contrast, it reduces the numerical aperture.
- **Immersion oil** should be used between the slide and the 100× objective.

Of significance is the fact that as magnification is increased, the resolution must also increase. Simply

increasing magnification by using a 20× ocular won't increase the resolution.

Lens Care

Keeping the lenses of your microscope clean is a constant concern. Unless all lenses are kept free of dust, oil, and other contaminants, they are unable to achieve the degree of resolution that is intended. Consider the following suggestions for cleaning the various lens components:

Cleaning Tissues Only lint-free, optically safe tissues should be used to clean lenses. Tissues free of abrasive grit fall in this category. Booklets of lens tissue are most widely used for this purpose. Although several types of boxed tissues are also safe, *use only the type of tissue that is recommended by your instructor.*

Solvents Various liquids can be used for cleaning microscope lenses. Green soap with warm water works very well. Xylene is universally acceptable. Alcohol and acetone are also recommended, but often with some reservations. Acetone is a powerful solvent that could possibly dissolve the lens mounting cement in some objective lenses if it were used too liberally. When it is used it should be used sparingly. Your instructor will inform you as to what solvents can be used on the lenses of your microscope.

Oculars The best way to determine if your eyepiece is clean is to rotate it between the thumb and forefinger as you look through the microscope. A rotating pattern will be evidence of dirt.

If cleaning the top lens of the ocular with lens tissue fails to remove the debris, one should try cleaning the lower lens with lens tissue and blowing off any excess lint with an air syringe, or gas cannister. *Whenever the ocular is removed from the microscope, it is imperative that a piece of lens tissue be placed over the open end of the microscope as illustrated in figure 1.5.*

Objectives Objective lenses often become soiled by materials from slides or fingers. A piece of lens tissue moistened with green soap and water, or one of the acceptable solvents mentioned above, will usually remove whatever is on the lens. Sometimes a cotton swab with a solvent will work better than lens tissue. At any time that the image on the slide is unclear or cloudy, assume at once that the objective you are using is soiled.

Condenser Dust often accumulates on the top surface of the condenser; thus, wiping it off occasionally with lens tissue is desirable.

Procedures

If your microscope has three objectives you have three magnification options: (1) low-power, or 100× total magnification, (2) high-dry magnification, which is 450× total with a 45× objective, and (3) 1000× total magnification with a 100× oil immersion objective. Note that the total magnification seen through an objective is calculated by simply multiplying the power of the ocular by the power of the objective.

Whether you use the low-power objective or the oil immersion objective will depend on how much magnification is necessary. Generally speaking, however, it is best to start with the low-power objective

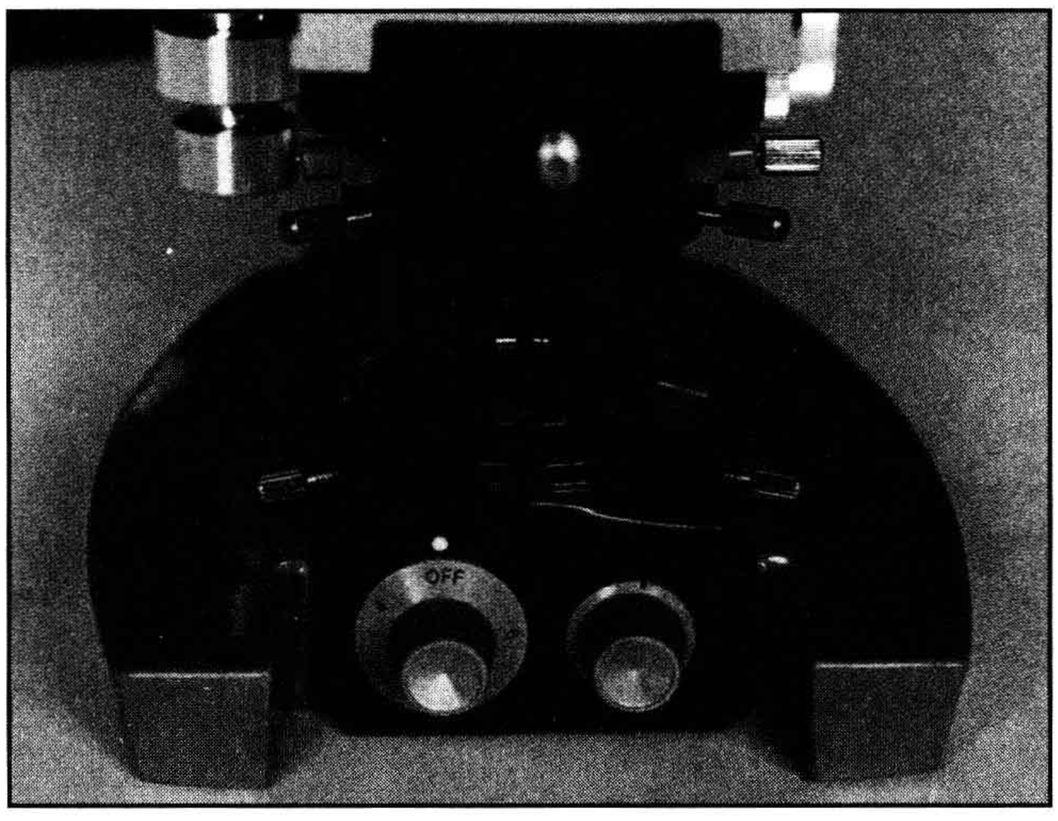

Figure 1.4 On this microscope, the left knob controls voltage. The other knob is used for moving a neutral density filter into position.

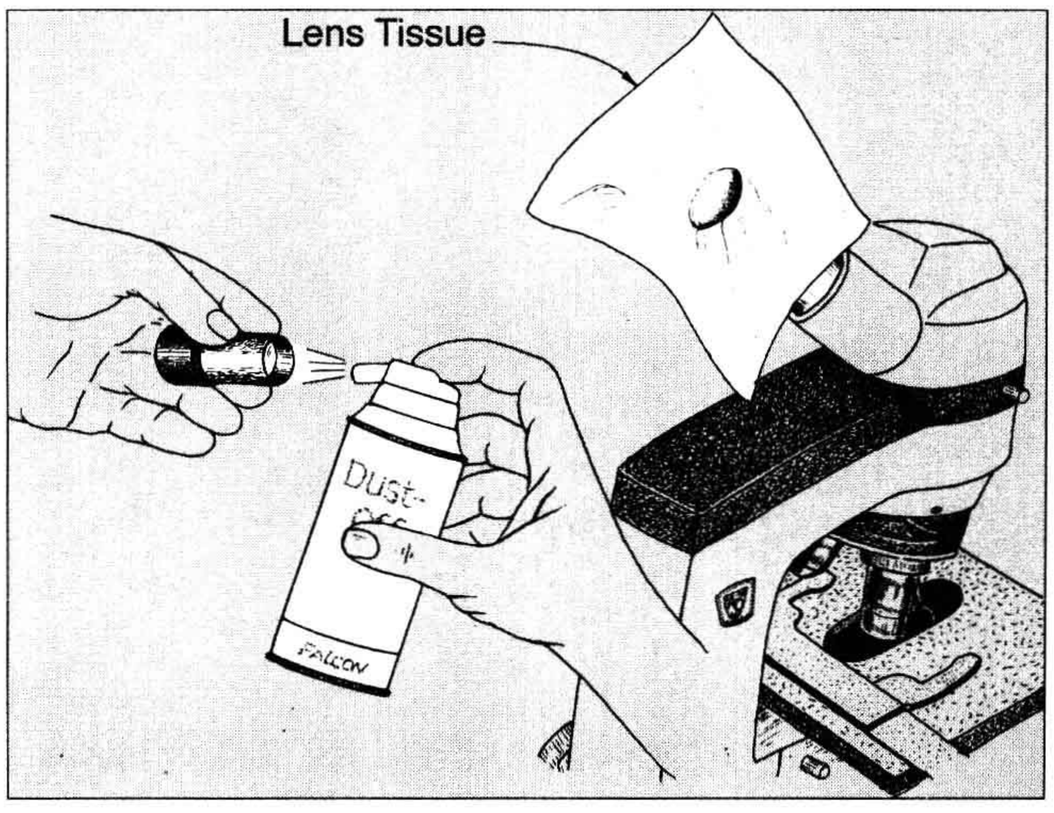

Figure 1.5 When oculars are removed for cleaning, cover the ocular opening with lens tissue. A blast from an air syringe or gas cannister removes dust and lint.

and progress to the higher magnifications as your study progresses. Consider the following suggestions for setting up your microscope and making microscopic observations.

Viewing Setup If your microscope has a rotatable head, such as the ones being used by the two students in figure 1.6, there are two ways that you can use the instrument. Note that the student on the left has the arm of the microscope *near* him, and the other student has the arm *away from* her. With this type of microscope, the student on the right has the advantage in that the stage is easier to observe. Note, also that when focusing the instrument she is able to rest her arm on the table. The manufacturer of this type of microscope intended that the instrument be used in the way demonstrated by the young lady. If the microscope head is not rotatable, it will be necessary to use the other position.

Low-Power Examination The main reason for starting with the low-power objective is to enable you to explore the slide to look for the object you are planning to study. Once you have found what you are looking for, you can proceed to higher magnifications. Use the following steps when exploring a slide with the low-power objective:

1. Position the slide on the stage with the material to be studied on the *upper* surface of the slide. Figure 1.7 illustrates how the slide must be held in place by the mechanical stage retainer lever.
2. Turn on the light source, using a *minimum* amount of voltage. If necessary, reposition the slide so that the stained material on the slide is in the *exact center* of the light source.
3. Check the condenser to see that it has been raised to its highest point.
4. If the low-power objective is not directly over the center of the stage, rotate it into position. Be sure that as you rotate the objective into position it clicks into its locked position.
5. Turn the coarse adjustment knob to lower the objective until it stops. A built-in stop will prevent the objective from touching the slide.
6. While looking down through the ocular (or oculars), bring the object into focus by turning the fine adjustment focusing knob. Don't readjust the coarse adjustment knob. If you are using a binocular microscope it will also be necessary to adjust the interocular distance and diopter adjustment to match your eyes.
7. Manipulate the diaphragm lever to reduce or increase the light intensity to produce the clearest, sharpest image. Note that as you close down the diaphragm to reduce the light intensity, the contrast improves and the depth of field increases. Stopping down the diaphragm when using the low-power objective does not decrease resolution.
8. Once an image is visible, move the slide about to search out what you are looking for. The slide is moved by turning the knobs that move the mechanical stage.
9. Check the cleanliness of the ocular, using the procedure outlined earlier.
10. Once you have identified the structures to be studied and wish to increase the magnification, you may proceed to either high-dry or oil immersion magnification. However, before changing objectives, *be sure to center the object you wish to observe.*

High-Dry Examination To proceed from low-power to high-dry magnification, all that is necessary is to rotate the high-dry objective into position and open up the diaphragm somewhat. It may be necessary to make a minor adjustment with the fine adjustment knob to sharpen up the image, but *the coarse adjustment knob should not be touched.*

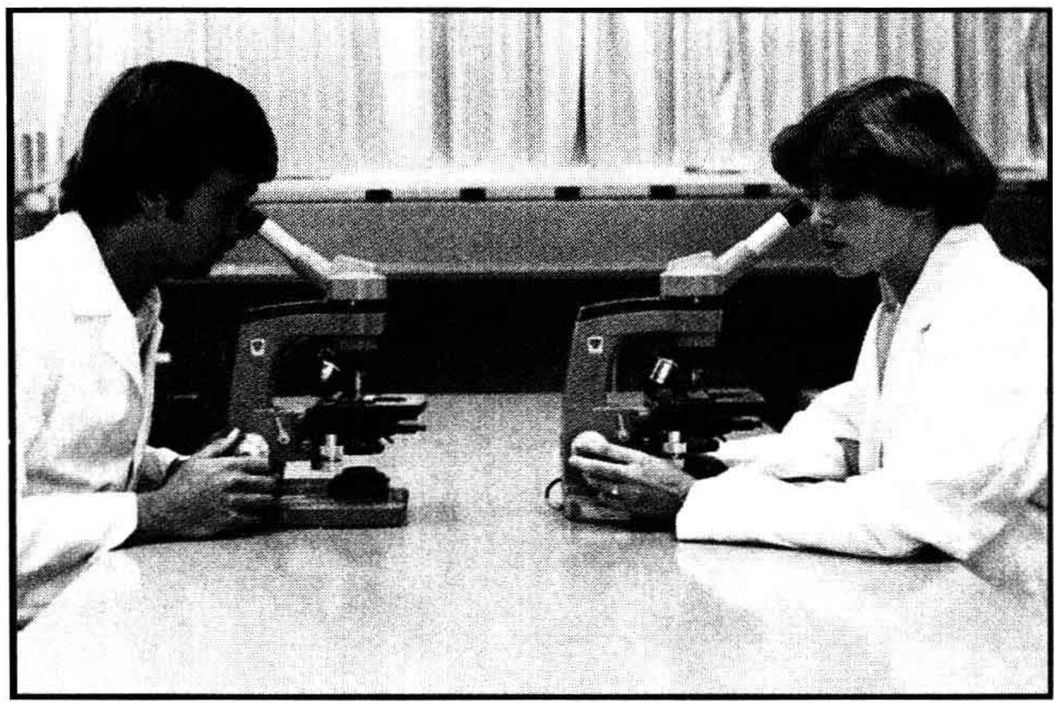

Figure 1.6 The microscope position on the right has the advantage of stage accessibility.

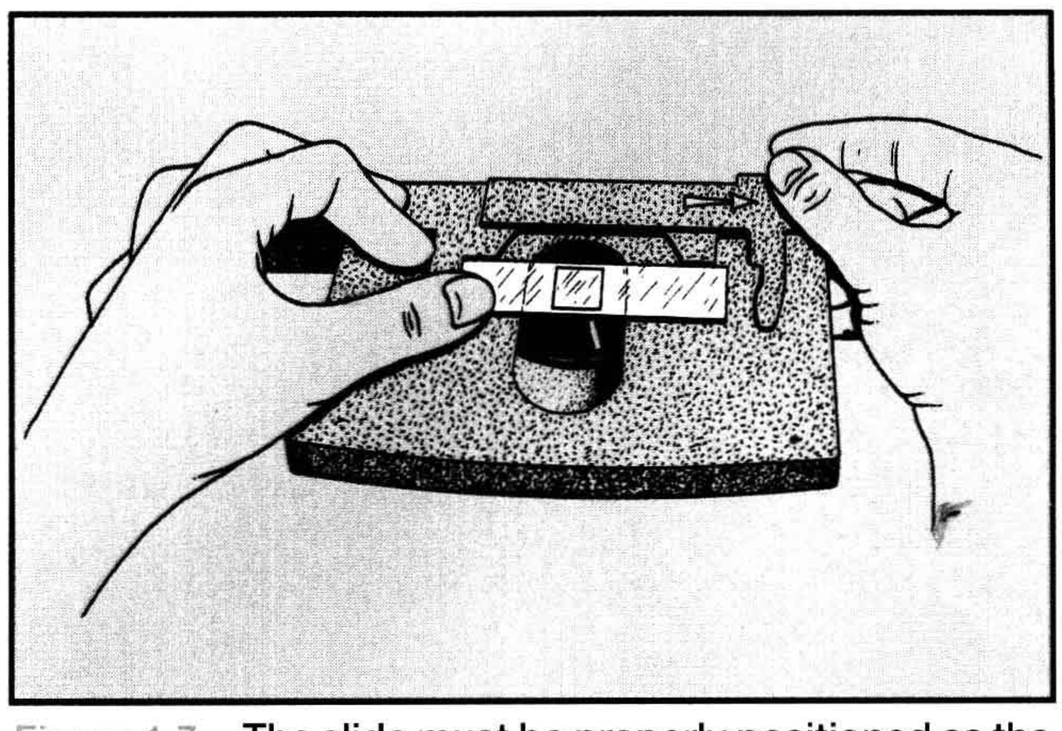

Figure 1.7 The slide must be properly positioned as the retainer lever is moved to the right.

If a microscope is of good quality, only minor focusing adjustments are needed when changing from low power to high-dry because all the objectives will be **parfocalized.** Nonparfocalized microscopes do require considerable refocusing when changing objectives.

High-dry objectives should be used only on slides that have cover glasses; without them, images are usually unclear. When increasing the lighting, be sure to open up the diaphragm first instead of increasing the voltage on your lamp; reason: *lamp life is greatly extended when used at low voltage.* If the field is not bright enough after opening the diaphragm, feel free to increase the voltage. A final point: Keep the condenser at its highest point.

Oil Immersion Techniques The oil immersion lens derives its name from the fact that a special mineral oil is interposed between the lens and the microscope slide. The oil is used because it has the same refractive index as glass, which prevents the loss of light due to the bending of light rays as they pass through air. The use of oil in this way enhances the resolving power of the microscope. Figure 1.8 reveals this phenomenon.

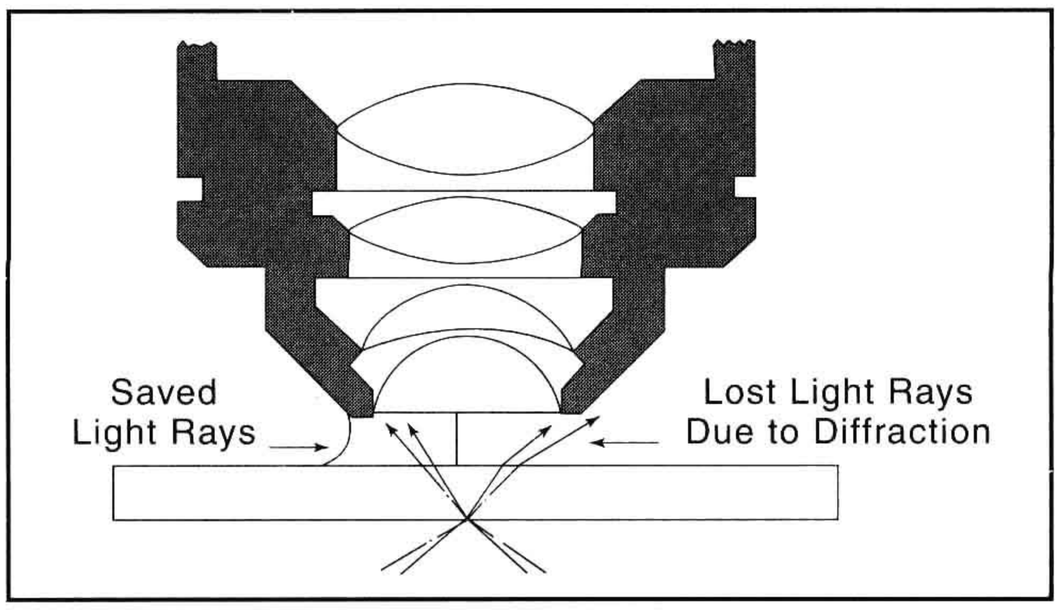

Figure 1.8 **Immersion oil, having the same refractive index as glass, prevents light loss due to diffraction.**

With parfocalized objectives one can go to oil immersion from either low power or high-dry. On some microscopes, however, going from low power to high power and then to oil immersion is better. Once the microscope has been brought into focus at one magnification, the oil immersion lens can be rotated into position without fear of striking the slide.

Before rotating the oil immersion lens into position, however, a drop of immersion oil must be placed on the slide. An oil immersion lens should never be used without oil. Incidentally, if the oil appears cloudy it should be discarded.

When using the oil immersion lens it is best to open the diaphragm as much as possible. Stopping down the diaphragm tends to limit the resolving power of the optics. In addition, the condenser must be kept at its highest point. If different colored filters are available for the lamp housing, it is best to use blue or greenish filters to enhance the resolving power.

Since the oil immersion lens will be used extensively in all bacteriological studies, it is of paramount importance that you learn how to use this lens properly. Using this lens takes a little practice due to the difficulties usually encountered in manipulating the lighting. A final comment of importance: At the end of the laboratory period remove all immersion oil from the lens tip with lens tissue.

Putting It Away

When you take a microscope from the cabinet at the beginning of the period, you expect it to be clean and in proper working condition. The next person to use the instrument after you have used it will expect the same consideration. A few moments of care at the end of the period will ensure these conditions. Check over this list of items at the end of each period before you return the microscope to the cabinet.

1. Remove the slide from the stage.
2. If immersion oil has been used, wipe it off the lens and stage with lens tissue. (Do not wipe oil off slides you wish to keep. Simply put them into a slide box and let the oil drain off.)
3. Rotate the low-power objective into position.
4. If the microscope has been inclined, return it to an erect position.
5. If the microscope has a built-in movable lamp, raise the lamp to its highest position.
6. If the microscope has a long attached electric cord, wrap it around the base.
7. Adjust the mechanical stage so that it does not project too far on either side.
8. Replace the dustcover.
9. If the microscope has a separate transformer, return it to its designated place.
10. Return the microscope to its correct place in the cabinet.

Laboratory Report

Before the microscope is to be used in the laboratory, answer all the questions on Laboratory Report 1,2 that pertain to brightfield microscopy. Preparation on your part prior to going to the laboratory will greatly facilitate your understanding. Your instructor may wish to collect this report at the *beginning of the period* on the first day that the microscope is to be used in class.