

## LESSON

1-10

# The Microscope

## LESSON OBJECTIVES

After studying this lesson, the student will:

- Locate and name the parts of a light microscope.
- Explain the function of each part of the light microscope.
- Explain the use of coarse and fine adjustments.
- Use the low-power objective to view a specimen.
- Use the high-power objective to view a specimen.
- Use the oil-immersion objective to view a specimen.
- Adjust the condenser and iris diaphragm.
- Use Köhler illumination to align the microscope light path.
- Explain how to perform interpupillary distance and diopter adjustments.
- Explain when Standard Precautions and personal protective equipment should be used while using the microscope.
- Explain how light microscopes differ from electron microscopes.
- Explain how the proper care and storage of the microscope can affect the quality of results.
- Define the glossary terms.

## GLOSSARY

**binocular** / having two oculars or eyepieces

**coarse adjustment** / control that adjusts position of microscope objectives and is used to initially bring objects into focus

**condenser** / apparatus located below the microscope stage that directs light into the objective

**electron microscope** / a microscope that uses an electron beam to create images from a specimen and that is capable of much greater magnification and resolving power than a light microscope

**eyepiece** / ocular

**field diaphragm** / adjustable aperture attached to microscope base

**fine adjustment** / control that adjusts position of microscope objectives and is used to sharpen focus

**iris diaphragm** / device that regulates the amount of light striking the specimen being viewed through the microscope

**Köhler illumination** / alignment of illuminating light for microscopy; double diaphragm illumination

**lens** / a curved transparent material that spreads or focuses light

**lens paper** / a special nonabrasive material used to clean optical lenses

**microscope arm** / the portion of the microscope that connects the lenses to the base

**microscope base** / the portion of the microscope that rests on the table and supports the microscope

**monocular** / having one ocular or eyepiece

**nosepiece** / revolving unit to which microscope objectives are attached

**objective** / magnifying lens closest to the object being viewed with a microscope

**ocular** / eyepiece of the microscope that contains a magnifying lens

**parfocal** / having objectives that can be interchanged without varying the instrument's focus

**resolving power** / the ability of a microscope to produce separate images of two closely spaced objects

**stage** / platform that holds the object to be viewed microscopically

**working distance** / distance between the microscope objective and the microscope slide when the object is in sharp focus

## INTRODUCTION

Microscopes are used in many clinical laboratory departments to evaluate stained blood smears and tissue sections, perform cell counts, examine urine sediment, observe cellular reactions, and interpret smears containing microorganisms. The microscopist must be skilled in microscope use if maximum information is to be gained from prepared slides.

This lesson is an introduction to microscopy and includes descriptions of several types of microscopes used in medicine and research. The proper use of the bright-field clinical microscope is described in detail. Because the microscope is a delicate, expensive instrument, special care must be taken in its use, cleaning, and storage.

## TYPES OF MICROSCOPES

Microscopes come in various sizes, prices, designs, and capabilities. Microscopes can be divided into two categories based on the type of illumination used. Clinical microscopes are *light microscopes*, meaning that the specimen is illuminated using a light source such as a tungsten, halogen, or mercury lamp. Tungsten and halogen lamps emit a full spectrum of light (white light); mercury lamps emit light in the ultraviolet range. The other major category of microscopes is the *electron microscopes*. These are named because specimens are visualized by focusing an electron beam on the specimen rather than light waves.

### Light Microscopes

Modern light microscopes are also called *compound* light microscopes because they have two lens systems, one system in the oculars and one system in the objectives. Common types of light microscopes in the clinical laboratory are the bright-field microscope, phase-contrast microscope, and epi-fluorescence microscope.

### Bright-field Microscope

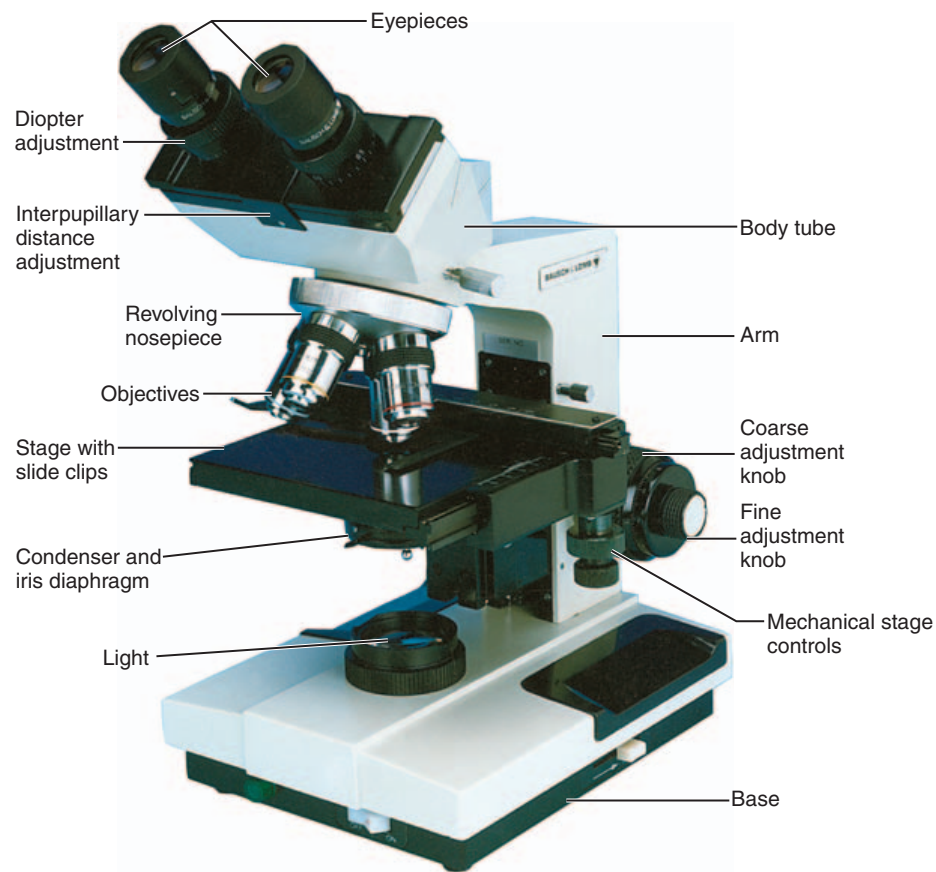
The bright-field microscope is the workhorse of the clinical laboratory (Figure 1-54). The microscope is named bright-field because the object being viewed is seen against a *bright field* of view. All routine clinical laboratory tests requiring a microscope can be performed using the bright-field microscope. Bright-field microscopes are especially well suited for viewing stained specimens, such as stained blood smears. Figure 1-55A shows an image as seen with a bright-field microscope.

### Phase-Contrast Microscope

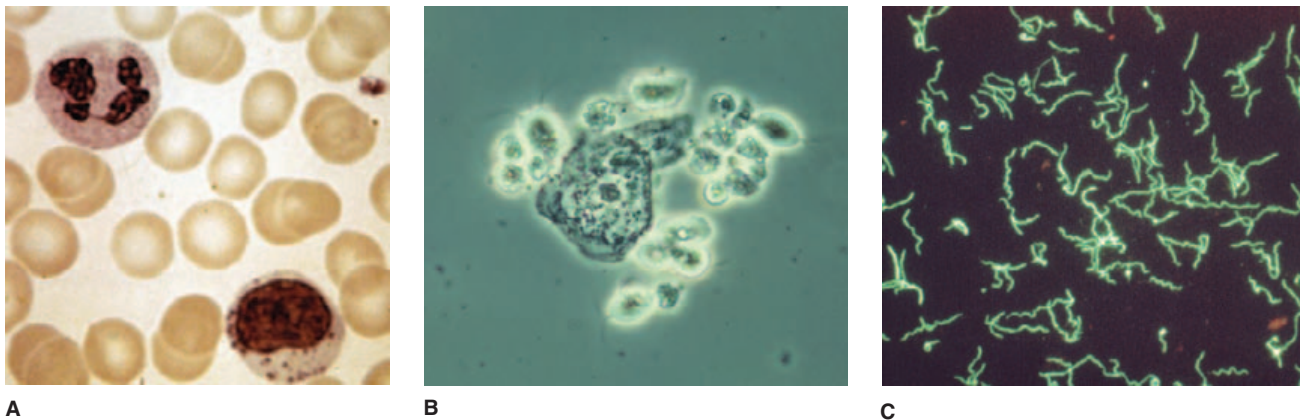
The phase-contrast microscope provides an improved way of viewing unstained cells, which are nearly transparent. By installing special objectives and a phase condenser, bright-field microscopes can be equipped for phase contrast. Phase contrast is useful for viewing specimens such as urine sediments and for performing platelet counts using the hemacytometer. With phase-contrast microscopy, the background (field) appears grey and the specimen is bright. Figure 1-55B shows an image viewed with phase-contrast microscopy.

### Epi-fluorescence Microscope

Epi-fluorescence microscopes use ultraviolet light to illuminate the specimen. The epi-fluorescence microscope enables objects that have been stained with fluorescent dyes to be observed. When these dyes are combined with antibodies, it is possible to identify specific areas of reaction within a cell or on a cell surface. The epi-fluorescence microscope can be used to identify microorganisms such as mycobacteria, and to detect the presence of antibodies in certain diseases such as syphilis and lupus erythematosus. Figure 1-55C shows *Borrelia burgdorferi*, the cause of Lyme disease. The bacteria are stained with a fluorescently labeled antibody and viewed with epi-fluorescence microscopy.



**FIGURE 1-54** Binocular bright-field microscope with parts labeled



**FIGURE 1-55** Microscopy images: (A) stained cells viewed with a bright-field microscope; (B) phase-contrast image; (C) *Borrelia burgdorferi* stained with fluorescent antibody and viewed with epi-fluorescence microscopy (Photo A courtesy of Abbott Laboratories, Abbot Park, IL; photo B courtesy of CDC, Atlanta, GA)

## Electron Microscopes

**Electron microscopes** provide much greater magnification and resolving power than light microscopes. The image from an electron microscope is created by exposing specimens to an electron beam, rather than illuminating them with a light source. Electron microscopes have been used in medical research for several years, but have been limited for the most part to pathology and virology. However, with new knowledge and techniques, their use in clinical medicine is increasing.

With the electron microscope, objects as small as  $0.001 \mu\text{m}$  (too small to be seen with light microscopes) can be viewed. The two types of electron microscopes are the *transmission electron microscope* (TEM) and the *scanning electron microscope* (SEM), shown in Figures 1-56 and 1-57.

Objects are visualized in the TEM by passing an electron beam through the specimen. Minute details inside a cell, such as nuclear structure, can be seen (Figure 1-58A). The image is displayed on a phosphorescent screen and viewed through protective glass or projected onto a monitor (Figure 1-58A). In the



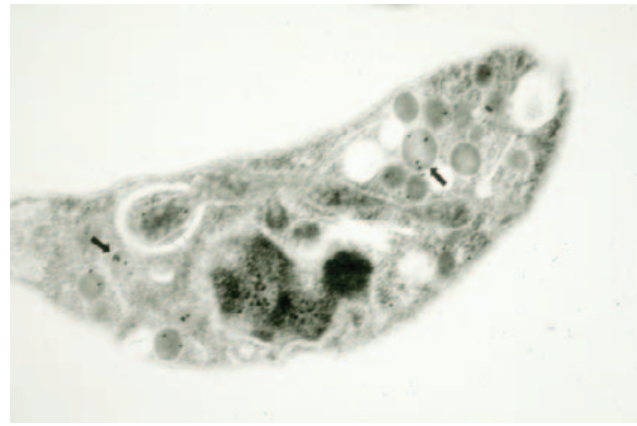
FIGURE 1-56 Transmission electron microscope



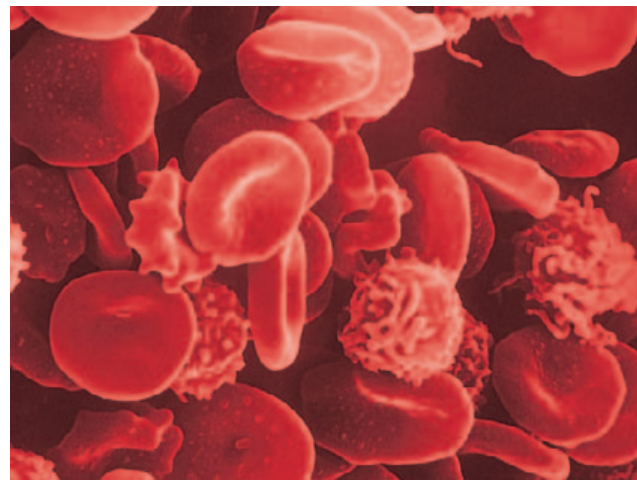
FIGURE 1-57 Scanning electron microscope

SEM, the electron beam is scanned over the surface of a metal-coated specimen, causing electrons to bounce off the specimen. These deflected electrons are measured with a detector and converted into a three-dimensional image similar to an image on a TV screen. Figure 1-58B is a scanning electron microscope image of the surface of blood cells.

Electron microscopes are very expensive and require lengthy specimen preparation and special expertise to operate.



A



B

FIGURE 1-58 Electron microscopy images: (A) cell viewed with transmission electron microscope (*Courtesy of C.A. Sundermann, Auburn University, AL*); (B) blood cells as seen with the scanning electron microscope (*Courtesy Philips Electronics Instruments Co.*)

Reference laboratories, medical schools, and teaching hospitals are the most likely clinical locations for electron microscopes.

## PARTS OF THE MICROSCOPE

Microscope design can differ slightly from one model to another. However, some parts are common to all microscopes. The microscope shown in Figure 1-54 has the parts labeled.

### Oculars

A microscope can be **monocular** or **binocular**. Monocular microscopes have only one **ocular**, or **eyepiece**. Because of this, most people find it difficult to use them without eyestrain. Binocular microscopes have two eyepieces to allow viewing with both eyes, resulting in less eyestrain (Figure 1-54).

The oculars, or eyepieces, located at the top of the microscope, are attached to a barrel or tube connected to the **micro-**

**scope arm.** Each ocular, through which the object is viewed, contains a magnifying **lens**. The usual magnification is 10 times ( $10\times$ ), but oculars are also available in  $15\times$  and  $20\times$ .

## Objective Lenses

The underside of the microscope arm contains a revolving **nosepiece** to which the **objectives** are attached. Most microscopes have at least three objectives or magnifying lenses: the low-power objective, which magnifies  $\times 10$  or  $\times 20$ ; the high-power objective, which magnifies  $\times 40$ ,  $43$ , or  $45$ ; and the oil-immersion objective, which magnifies  $\times 95$ ,  $97$ , or  $100$ . Each objective is marked with color-coded bands and the power of magnification (Figure 1-54).

To determine the degree of magnification, the magnification listed on the ocular (usually  $10\times$ ) is multiplied by the magnification listed on the objective being used (Table 1-33). For example, an object viewed with a  $10\times$  ocular and high-power ( $43\times$ ) objective would be magnified 430 times ( $430\times$ ). An object viewed with a  $10\times$  ocular and the oil-immersion objective ( $97\times$ ) would be magnified 970 times.

There is a limit to the degree of magnification that can be obtained with a light microscope and still yield a clear image. The ability of a microscope to produce separate images of closely spaced details in the object being viewed is called its **resolving power**. The resolving power is determined by the quality of the objective lenses.

## Light Source, Condenser, and Diaphragm

The microscope arm connects the objectives and eyepiece(s) to the **microscope base**, which supports the microscope. The base also contains the light, which illuminates the object viewed. Located above the light is the moveable condenser and iris diaphragm (Figure 1-54). The **condenser** focuses or directs the available light into the objective as it is raised or lowered and enhances specimen contrast (Figure 1-59). The **iris diaphragm**, located in the condenser unit, regulates the amount of light that strikes the object being viewed (much like the shutter of a camera). The iris diaphragm can be adjusted by a movable lever. Microscopes can also have an adjustable **field diaphragm**, located just over the light source. The field diaphragm is used

to help align or focus the light in a procedure called **Köhler illumination**.

## Coarse and Fine Focus Adjustments

The two focusing knobs are usually located on the sides of the microscope base. The **coarse adjustment** is used to focus with the low-power objective only. The **fine adjustment** is used to give a sharper image after the object is brought into view with the coarse adjustment (Figure 1-54).

The **working distance** is the distance between the objective and the specimen slide when the object is in sharp focus. The higher the magnification of the objective, the shorter the working distance will be. The coarse adjustment should *not* be used when using the higher magnifications to prevent the objective from accidentally striking the slide and becoming damaged.

## Stage

The **stage** of the microscope is supported by the arm and is located between the nosepiece and the light source. The stage serves as the support for the object being viewed and has a stage clip to keep slides stationary. The stage, called a mechanical stage, can be moved by using knobs located just below the stage. These move the stage in a horizontal plane left and right or backward and forward (Figure 1-54).

## HOW THE IMAGE IS PRODUCED

To see an image through the microscope oculars, the image must be magnified, focused, and directed into the oculars (Figure 1-59). The light in the microscope base is directed upward through the condenser, which focuses the light beam on the specimen. When light strikes the specimen, some is absorbed, some is deflected, and some is transmitted (passes through the specimen). The transmitted light enters the lens of the objective which magnifies the image. The light then strikes a prism or mirror located between the objective and ocular. This prism deflects or bends the light (image) to direct it into the oculars. The oculars contain magnifying lenses that enlarge the image again and allow the eye to focus on the image. The combination of these two magnifying lens systems is the basis for the compound microscope.

TABLE 1-33. Calculation of total magnification in a compound microscope

OBJECTIVE LENS	MAGNIFICATION STRENGTH	$\times$	OCULAR STRENGTH	=	TOTAL MAGNIFICATION
Low power	$10\times$	$\times$	$10\times$	=	$100\times$
High power	$40\times$	$\times$	$10\times$	=	$400\times$
Oil immersion	$100\times$	$\times$	$10\times$	=	$1000\times$

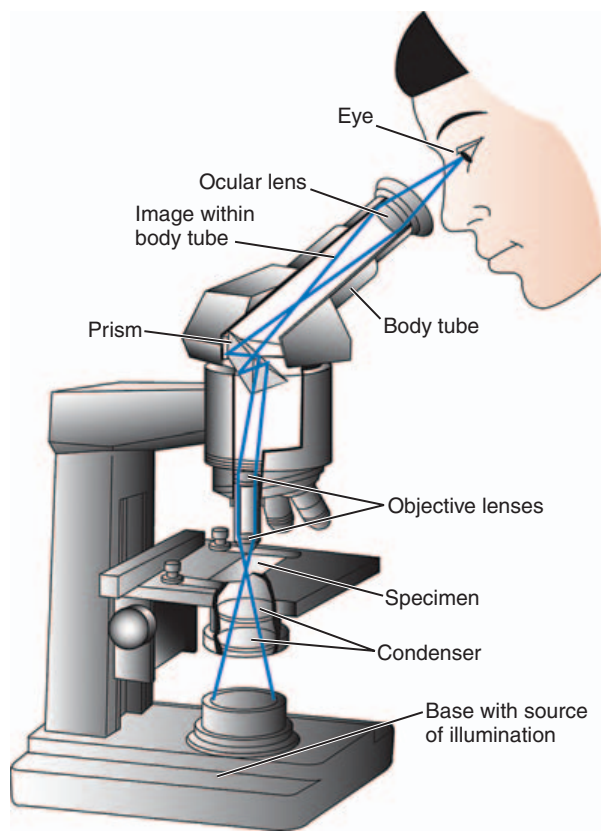


FIGURE 1-59 Light path in a compound microscope

## USING THE MICROSCOPE

Much practice is required to become competent and adept at clinical microscopy. Microscopists must be proficient in making all of the adjustments required to achieve an optimal image of various types of biological specimens. They must also use proper safety measures and cleaning and maintenance techniques to keep the instrument operating at maximum capacity. The microscope should be placed on a sturdy table at a comfortable height for the technician. When not in use, the microscope should be left with the low-power objective in position.

### Microscope Safety



Appropriate safety rules for electrical equipment must be followed when using the microscope. Electrical cords must not be frayed and should be plugged into a grounded receptacle. Cords must be kept away from liquids. The microscope should be unplugged before attempting to do any maintenance, repairs, or bulb replacement.

Glass slides should be handled carefully to avoid the chance of chipping or breaking. If an unfixed or fluid biological specimen (such as urine sediment) is to be examined, Standard Precautions must be observed and appropriate personal protective equipment (PPE) must be worn. The microscope stage must be disinfected after examining such a specimen.

## Quality Assessment



The microscopist should follow good microscopy practice in the care and use of the microscope to avoid damage to the instrument. Patient results should only be reported by experienced technicians who have demonstrated the required level of competency.

### Care and Cleaning of Lenses

The amount and quality of information that can be gained from microscopic examination of a specimen is dependent on the condition of the objective lenses. Oculars and objective lenses should be cleaned before and after each use with **lens paper**. Materials such as laboratory tissue, cotton balls, paper towels, or gauze squares must not be used because they can scratch the lenses. Lenses that become damaged or cloudy should be replaced since important information can be missed when viewing a specimen through a cloudy lens.

### Immersion Oil

Only immersion oil manufactured for microscopy should be used with the oil-immersion objective. Immersion oil should never be allowed to touch the other (low- or high-power) objectives. After the oil-immersion objective is used, the objective lenses and condenser should be cleaned to remove any residual oil. It is especially important that lenses never be left with oil on them, as oil will soften the cement that holds the lens in place in the objective. Lens cleaner, similar to a glass-cleaning solution, can be used with lens paper to remove oil from objectives.

## Focusing with the Low-Power Objective

The low-power objective is used to initially locate objects and to view large objects. The coarse adjustment focus knob is used to bring the objective and the slide as close together as possible. Then, while looking through the ocular, the coarse adjustment is used to move the objective and slide apart until the object on the slide comes into focus. (In some microscopes, the coarse adjustment raises and lowers the objectives. In other microscopes, the stage is raised and lowered when the coarse adjustment knob is turned.) The fine focus adjustment knob can then be used to bring the image into sharp focus. When viewing objects using the low-power objective, the light intensity may need to be decreased.

## Adjusting Oculars on Binocular Microscopes

Once the object is in view, the oculars should be adjusted to accommodate each microscopist's eyes. Two adjustments are made, the *interpupillary distance adjustment* and the *dioptric adjustment*.

The interpupillary distance is adjusted by sliding the oculars either closer together or further apart until only one image is

seen when looking through both oculars. This adjustment is like the one you would make when adjusting binoculars to your eyes.

Once the interpupillary distance is correct, then the dioptic adjustment should be made. This adjustment compensates for the microscopist's vision. The microscopist should look through the right ocular with the right eye (left eye closed) and use the coarse adjustment to bring the object into sharp focus. Then the microscopist should look through the left ocular with the left eye (right eye closed) and use the knurled collar (Figure 1-54) on the ocular (*not* the coarse focus adjustment) to bring the specimen into focus.

## Alignment of Illumination

The path of light through the microscope must be properly aligned to have good resolution. Incorrect alignment causes poor resolution, artifacts, and unevenly lit field of view. The illumination alignment of many student microscopes is pre-set during manufacture and realignment is done periodically by a professional microscope repair and service company. In these cases the microscopist does not perform the alignment procedure; it has been done for them.

Clinical microscopes that have an adjustable field diaphragm built into the light source allow the microscopist to manually align the illumination. The technique of aligning or focusing the light path using the field diaphragm is called Köhler illumination, and must be performed each time the microscope is used before viewing specimens with the microscope.

A specimen slide is placed on the microscope stage (specimen-side up) and positioned so that the specimen is directly beneath the low power objective lens and the microscope light is turned on. The specimen is brought into focus. The field diaphragm is closed as much as possible and the edges of the diaphragm are brought into focus by raising or lowering the condenser. Both the specimen and diaphragm edge should be in focus and the diaphragm outline should be centered in the field of view (Figure 1-60, far left and second from left). The field diaphragm image is centered using the condenser centering knobs. The field diaphragm is then opened so that the edges lie at the edge of the field of view (Figure 1-60, far right). The condenser iris diaphragm is adjusted to produce the desired contrast. The light intensity is adjusted using the voltage control (rheostat). The microscope is now correctly adjusted and ready for viewing specimens.

## Using the High-Power Objective

The high-power ( $40\times$ ) objective is used when greater magnification is needed, such as for cell counts and viewing urine



**FIGURE 1-60** Köhler illumination. Far left, closed, off-center field diaphragm; second from left, closed, centered field diaphragm; far right, open, centered field diaphragm

sediments. After initial focusing with the low-power objective and illumination alignment (if applicable), the high-power objective is carefully rotated into position. The fine adjustment is used to bring the object into sharp focus. Most microscope objectives are **parfocal** and therefore require only slight changes in the fine adjustment when rotating between objectives.

*Because the working distance between the slide and the high-power objective is so small, only the fine adjustment should be used when the high-power objective is in place. This avoids the possibility of the objective striking the slide and possibly damaging the objective or the slide.*

To view unstained specimens using the high-power objective, light intensity and iris diaphragm should be adjusted to provide proper lighting and contrast. When viewing most stained preparations with the high-power objective, the condenser should be raised, the diaphragm opened, and light intensity increased.

## Using the Oil-Immersion Objective

The oil-immersion objective is used to view stained blood cells, tissue sections, and stained slides containing microorganisms. This objective gives the highest magnification of the bright-field objectives. After initial focusing with the low-power objective, the objective is slightly rotated to the side. A drop of immersion oil is placed on the slide directly over the condenser. The oil immersion objective is then carefully rotated into the drop of oil, taking care that no other objectives contact the oil and that the oil immersion objective does not strike the slide (Figure 1-61A). The object is then brought into sharp focus using only the fine adjustment. *The coarse adjustment should never be used when the oil immersion objective is in position.* When viewing specimens with the oil-immersion objective, the condenser should be raised to its highest position (almost touching the bottom of the slide). The iris diaphragm should be open and maximum light should be used.

After completing examination of the slide, the low-power objective is rotated into position and the slide is removed from the stage. All oil must be cleaned from the oil-immersion objective with lens paper. The stage and condenser should be cleaned if necessary.

## Transporting and Storing the Microscope

Microscopes should be left in a permanent position on a sturdy table where they cannot be jarred. However, if a microscope must be moved, it should be held securely, with one hand supporting the base and the other holding the arm (Figure 1-61B). The microscope should be placed gently to avoid jarring.

## Microscope Storage

When the microscope is not being used, it should be left with the low-power objective in position and the nosepiece in the lowest position. The stage should be centered so that it does not project from either side of the microscope. The microscope should be stored under a dust-proof cover.





A

**FIGURE 1-61** Proper use and care of the microscope:  
 (A) observe the slide and objectives when changing  
 from high-power to oil-immersion objective;  
 (B) proper way to carry a microscope



B

### SAFETY Reminders

- Use Standard Precautions when examining unfixed biological materials microscopically. 
- Clean the microscope stage with a surface disinfectant after examining fluid samples such as urine sediment. 
- Unplug the microscope before attempting to replace the bulb or perform any electrical repair.

### PROCEDURAL Reminders

- Clean all oculars and objectives with lens paper before and after each use. *QA*
- Use the coarse adjustment with the low-power objective only.
- Use immersion oil with the oil-immersion objective only.
- Adjust interpupillary distance, diopter, and align illumination before using.
- Store the covered microscope in a protected area.
- Avoid jarring or bumping the microscope.
- Transport the microscope with one hand under the base and the other hand gripping the arm.

## CRITICAL THINKING 1

Sheila was performing urine microscopic examinations when the light went out on her microscope. She removed the microscope slide, turned the microscope over, and opened up the light compartment. She removed the light bulb with difficulty, saw that the filament was broken, and replaced the bulb. As soon as the bulb was fitted into the holder, the microscope light came on. Comment on Sheila's microscope repair technique.

## CRITICAL THINKING 2

Roberta needed to use a microscope to examine a blood smear. She cleaned the oculars and the 10 $\times$ , 40 $\times$  and 100 $\times$  oil-immersion objectives with lens paper, using a clean section of lens paper for each objective. The lens paper used to clean the oil-immersion objective revealed oil on the objective. Roberta mentioned this to Jack, a technician who worked regularly with that microscope, and he replied that it was only necessary to remove oil from the objective once or twice a shift since the objective might be used as many as 10 to 12 times during the day. Is Jack correct? Explain.

## SUMMARY

The microscope is a valuable instrument used in many departments in the clinical laboratory. A large clinical laboratory will usually have several bright-field microscopes and also may have phase-contrast and epi-fluorescence microscopes. Electron microscopes are used primarily in research.

Urine sediments, stained blood smears, and bacterial stains are evaluated using the microscope. Specialized tests such as fluorescent antibody techniques also can require microscope use. Microscopists must become skilled in the proper operation of the microscope. Because microscopes are expensive, precision instruments, the technician must use the microscope with care, maintain the microscope in good working order, and be sure that it is cleaned and stored properly after each use. Much practice is required to become a competent microscopist.

## REVIEW QUESTIONS

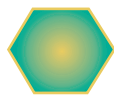
1. Explain the functions of the iris diaphragm and condenser.
2. Name the three objectives commonly used on a clinical microscope.
3. Explain the uses of the coarse and fine adjustments.
4. What is the proper method of cleaning a microscope after use?
5. How should a microscope be stored when not in use?
6. When is the oil-immersion objective used?
7. When is immersion oil used on a slide?
8. Explain how to adjust the interpupillary distance on a binocular microscope.
9. What is the purpose of making a dioptic adjustment? Explain how the adjustment is made.
10. How is total magnification calculated in the compound microscope?
11. How do electron microscopes differ from light microscopes?
12. When must Standard Precautions be used with the microscope?
13. What is Köhler illumination? How is it performed?
14. Define binocular, coarse adjustment, condenser, electron microscope, eyepiece, field diaphragm, fine adjustment, iris diaphragm, Köhler illumination, lens, lens paper, microscope arm, microscope base, monocular, nosepiece, objective, ocular, parfocal, resolving power, stage, and working distance.

## STUDENT ACTIVITIES

1. Complete the written examination for this lesson.
2. Obtain a microscope from the instructor. Locate and identify the following parts: oculars, condenser, condenser adjustment knob, condenser centering knobs, field diaphragm, iris diaphragm, light, light intensity control, nosepiece, arm, base, stage, stage controls, coarse and fine adjustment knobs, and diopter adjustment ring. Note which (if any) parts are not found on your microscope.
3. Obtain a stained slide from your instructor. View the specimen with the low-power, high-power, and oil-immersion objectives. Draw the structure(s) you see when using each objective. Observe the specimen when the condenser is raised and when it is lowered, and with the iris diaphragm wide open and closed. Discuss the differences in the image you see in each of these conditions. What adjustments provide the most information from your specimen?
4. If you live near a university or a large research laboratory, find out if they have an electron microscope. If so, try to arrange a visit to the facility.
5. Practice using a microscope following the procedure outlined in the Student Performance Guide.

## WEB ACTIVITIES

1. Find images taken with transmission and scanning electron microscopes using the Internet.
2. Use the Internet to find information about various types of light microscopes. Try to find examples of phase-contrast images and fluorescent images. Compare these to the way stained images look with the bright-field microscope in your laboratory.



# Student Performance Guide

## LESSON 1-10 The Microscope

Name \_\_\_\_\_ Date \_\_\_\_\_

### INSTRUCTIONS

1. Practice using the microscope following the step-by-step procedure.
2. Demonstrate the proper use of the microscope satisfactorily for the instructor using the Student Performance Guide. Your instructor will determine the level of competency you must achieve to obtain a satisfactory (S) grade.

**NOTE:** Procedure will vary slightly according to microscope design. Consult operating

procedure in microscope manual for specific instructions.

### MATERIALS AND EQUIPMENT

- antiseptic
- microscope
- lens paper
- lens cleaner
- prepared slides (commercially available)
- immersion oil
- surface disinfectant

### PROCEDURE



Record in the comment section any problems encountered while practicing the procedure (or have a fellow student or the instructor evaluate your performance).

S = Satisfactory  
U = Unsatisfactory

You must:	S	U	Comments
1. Wash hands			
2. Assemble equipment and materials			
3. Clean the oculars and objectives with lens paper			
4. Use the coarse adjustment to raise the nosepiece unit			
5. Raise the condenser as far as possible by turning the condenser knob			
6. Rotate the low-power (10×) objective into position, so it is directly over the opening in the stage			
7. Turn on the microscope light			
8. Open the iris diaphragm until maximum light comes up through the condenser			
9. Place slide on stage (specimen side up) and secure with clips. Position the condenser so it is almost touching the bottom of the slide			

You must:	S	U	Comments
10. Locate the coarse adjustment			
11. Look directly at the stage and low-power (10×) objective and turn the coarse adjustment until the objective is as close to the slide as it will go. Stop turning when the objective no longer moves <b>NOTE:</b> Do not move any objective toward a slide while looking through the oculars			
12. Look into the ocular(s) and slowly turn the coarse adjustment in the opposite direction (from step 11) to raise the objective (or lower the stage) until the object on the slide comes into focus			
13. Locate the fine adjustment and use it to sharpen the focus of the image			
14. Adjust the oculars for your eyes (steps 14a–14b) <ol style="list-style-type: none"> <li>a. Adjust interpupillary distance by adjusting distance between oculars so one image is seen (as when using binoculars)</li> <li>b. Make dioptic adjustment following steps 14b1–14b3               <ol style="list-style-type: none"> <li>1) Use coarse and fine adjustments to bring object into focus while looking through the right ocular with right eye (and with left eye closed)</li> <li>2) Close the right eye, look into the left ocular with left eye, and <i>use the knurled collar on the left ocular</i> to bring the object into sharp focus. (Do not turn coarse or fine adjustment at this time.)</li> <li>3) Look into oculars with both eyes to observe that object is in clear focus. If not, repeat the procedure</li> </ol> </li> </ol>			
15. If field diaphragm is present, perform Köhler illumination, following steps 15a–15g. If field diaphragm is not present, go to step 16 <ol style="list-style-type: none"> <li>a. Stop down (close) the field diaphragm located on the microscope base</li> <li>b. Bring the edge of the diaphragm into focus using the condenser adjustment knob (which raises and lowers the condenser)</li> <li>c. Confirm that both the specimen and the diaphragm edge are in focus</li> <li>d. Center the image of the field diaphragm using the condenser-centering knobs</li> <li>e. Open the centered and focused field diaphragm so the edges lie just beyond the field of view</li> <li>f. Adjust the condenser iris diaphragm to increase or decrease image contrast; the proper position depends on the specimen</li> <li>g. Adjust light intensity by adjusting the voltage to the light source with the power supply rheostat</li> </ol>			

You must:	S	U	Comments
16. Scan the slide using the stage controls to move the slide in a left and right or backward and forward pattern while looking through the oculars			
17. Rotate the high-power (40×) objective into position while observing the objective and the slide to see that the objective does not strike the slide			
18. Look through the oculars to view the object on the slide; it should almost be in focus			
19. Locate the fine adjustment			
20. Look through the oculars and turn the fine adjustment until the object is in focus. Do not use the coarse adjustment.			
21. Check alignment of Köhler illumination. Repeat steps 15a–15g if necessary			
22. Scan the slide as in step 16, using the fine adjustment if necessary to keep the object in focus			
23. Rotate the oil-immersion objective slightly to the side			
24. Place one drop of immersion oil on the portion of the slide directly over the condenser			
25. Rotate the oil-immersion objective into position, being careful not to rotate the high-power (40×) objective through the oil. Look to see that the oil-immersion objective is touching the drop of oil			
26. Look through the oculars and slowly turn the fine adjustment until the image is clear. Use only the fine adjustment to focus the oil-immersion objective. Scan the slide as in step 16			
27. Rotate the low-power (10×) objective into position; do not allow high-power (40×) objective to touch oil			
28. Remove the slide from the microscope stage and gently blot the oil from the slide with lens paper			
29. Clean the oculars, low-power (10×) objective, and high-power (40×) objective with clean lens paper			
30. Clean the oil-immersion objective with lens paper to remove all oil			
31. Clean all oil from the microscope stage and condenser			
32. Turn off the microscope light and unplug the microscope			
33. Position the nosepiece in the lowest position using the coarse adjustment			

You must:	S	U	Comments
34. Center the stage so it does not project from either side of the microscope			
35. Cover the microscope and return it to storage			
36. Clean work area; return slides to storage			
37. Wash hands			
<b>Evaluator Comments:</b>			
Evaluator _____ Date _____			