MODULE 4

Compound Microscope for the Study of Microbes

PREREQUISITE SKILL

MATERIALS

- compound light microscope with oil-immersion objective
- light source
- clear plastic millimeter ruler
- microscope slides (2)
- concentrated salt solution
- prepared slides of stained blood smear
- stained smear of yeast, *Bacillus* sp. (any species of the genus *Bacillus*), *Escherichia coli,* and a yeast–*E. coli* mixture
- prepared slide of mixed bacteria types

OVERALL OBJECTIVE

Demonstrate your ability to use a compound light microscope at all powers of magnification.

Specific Objectives

1. Draw various bacteria using the 10×, 40×, and 100× objectives of the microscope.
2. Define the terms magnification at eyepoint, object, and parfocal.
3. Name the optical parts of the microscope and their functions.
4. Name the movable parts of the microscope that are not part of the optical system and describe their functions.
5. Name the two parts of a microscope that are most critical in controlling light.
6. Label all the parts of your microscope that are described in the module.
7. Relate the magnification of the various objectives to the approximate size of the field.
8. Describe how to manipulate the diaphragm and condenser to improve light and define the object.
9. Explain how to take care of a microscope.
10. Describe how a microscope should be carried.
11. Explain how the different powers of your objectives are identified on the microscope.
12. Calculate the approximate size of a yeast cell.

DISCUSSION

Using a microscope correctly is invaluable in studying microbes because of the size of the microorganisms. You can become an expert microscopist only with practice. Module 4, "Compound Microscope for the Study of Microbes," will show you how to use a microscope.

As a beginning microbiologist, the amount of skill you develop in using the microscope can determine whether you find the course interesting or boring. How the microscope functions is not as important as what you are supposed to see through it. If you wish to learn the mechanism of the microscope, refer to any microbiology text. They contain detailed explanations of resolving power, numerical aperture, real image, virtual image, and refractive index. If you plan to major in or go onto advanced work in microbiology, you will need to learn these functions of the microscope. It is the purpose of this module, however, to allow you to enjoy microbiology by teaching you to use your microscope to its optimum.

The microscope illustrated is an Olympus CHS/CHT series binocular microscope, in which focusing is done by moving the stage. Other makes are similar. Study Figures 4-1 and 4-2. (Figures 4-3 and 4-4 show the Reichert-Jung [AO Spencer] series 150 microscope, in which focusing is done by moving the nosepiece. Familiarize yourself with all the labeled parts and explain their functions. (See Table 4-1.) Study the figures and table. (If your microscope is monocular, all parts are the same except the number of oculars and the interpupillary distance scale.)

Each of the objectives on a microscope bears a magnification number: 10×, 40×, or 100×. The number indicates how many times the object is magnified, or enlarged, by that objective. The 10× objective is the low-power objective, magnifying the object 10 times its actual size. The 40× objective (high power) magnifies it 40 times its actual size, and the 100× objective (oil immersion)

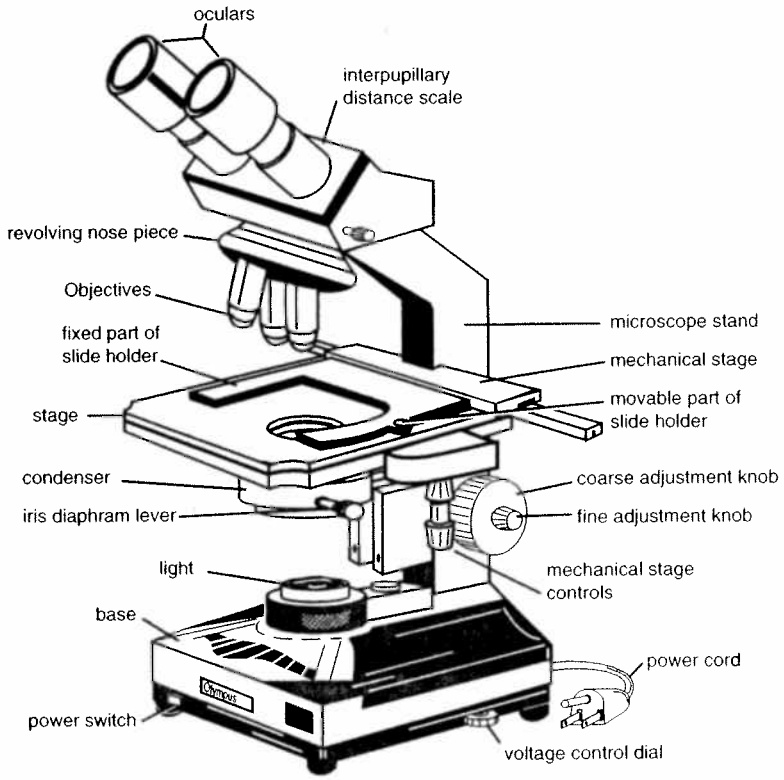

FIGURE 4-1
Olympus CHS/CHT binocular microscope (front view).

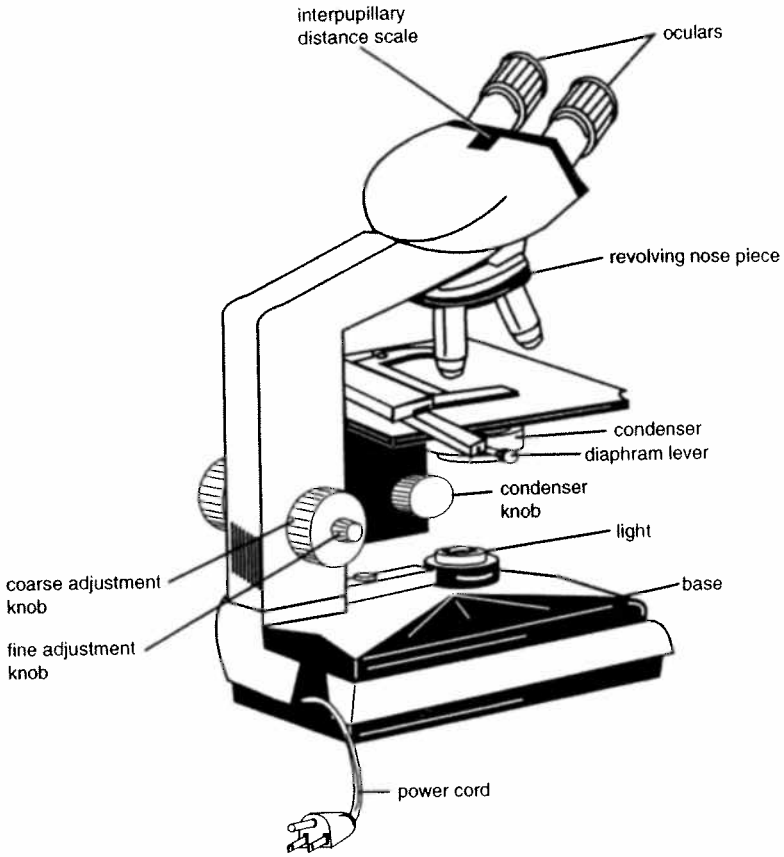

FIGURE 4-2
Olympus CHS/CHT binocular microscope (rear view).

magnifies it 100 times the actual size. Some microscopes are equipped with objectives that vary from the usual 10×, 40×, and 100× combination. The most common variation is a 10×, 43×, and 97× combination. Some microscopes are equipped with a 4× (or 3.5×) scanning lens in addition to the other three objectives.

Objectives are often marked with incised bands around the lower part to allow you to distinguish one from another without seeking the magnification number. These bands are especially important when using the high-power and oil-immersion objectives, which are nearly the same length. The high-power objective (40×) is used dry, and the outer lens can be damaged if immersed in oil. The 10× usually has one band, the 40× two bands, and the oil-immersion objective (100×) three bands. The bands may be colored for still easier recognition, in which case the oil-immersion objective is usually banded in red.

The ocular, or eyepiece, also magnifies, usually 10 times, which means that what you see at eyepoint is magnified 10 times more than the magnification marked on the objective you are using.

The objectives on your microscope, and on most microscopes, are *parfocal*. That means they are mounted so that when an object is in sharp focus with one objective, it will be in approximate focus with the other objectives when they are rotated into working position. If an object is in sharp focus on low power, you can rotate the high-power objective (40×) and achieve sharp focus with only a slight turn of the fine adjustment knob.

Despite the fact that the objectives are parfocal, the novice microscopist frequently "loses" the object when switching to a higher-power objective because each increase in magnification *decreases* the microscopic field by about half. The illuminated field *appears* to be the same size at all powers, but the area being

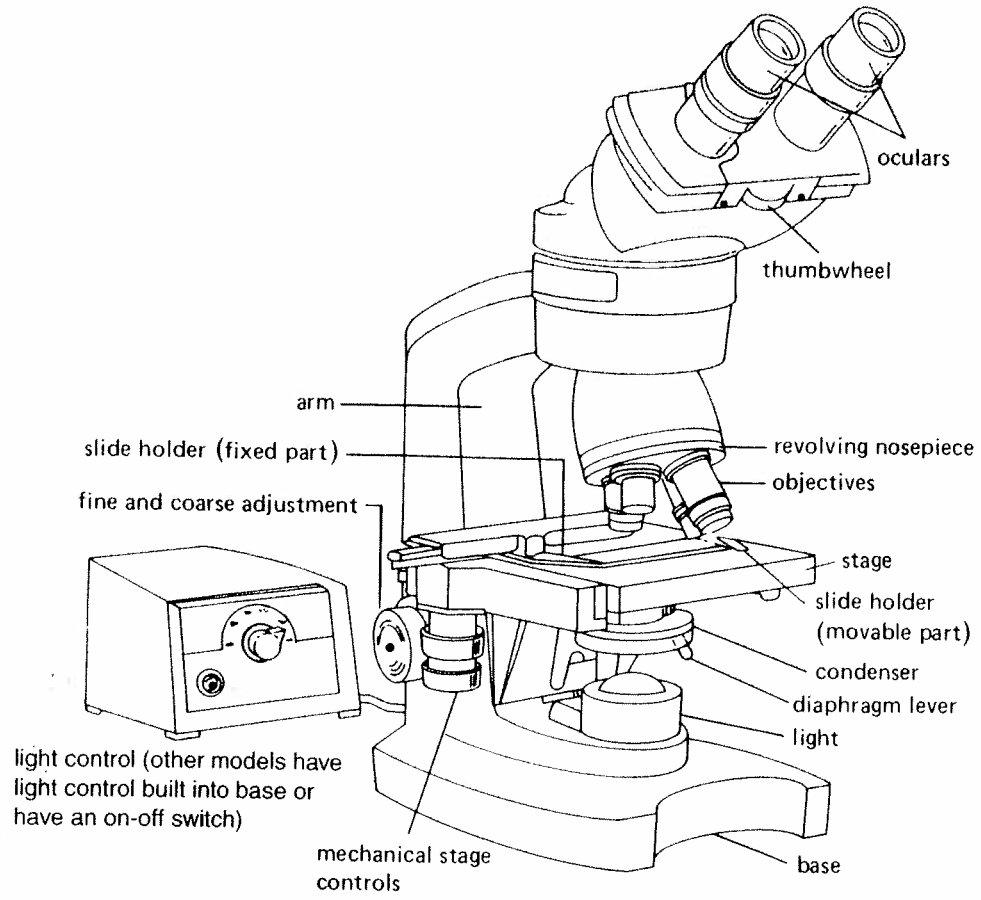

FIGURE 4-3
Reichert-Jung (AO Spencer) series 150 binocular microscope (right front view).

magnified shrinks each time you switch to an objective that magnifies at a higher power. Field diameter is inversely related to power of magnification. That is, when the magnification doubles, the field diameter halves. If the object is not in the center of the field when you switch to a higher power, you can easily lose it from the resulting diminished field. Center the object before you switch to a higher power.

As magnification increases, more light must enter the optical system. Activity 1 will help you master light control, which is critical. Repeat Activity 1 until you master it.

Care of Your Microscope

Read and memorize the following instructions and precautions and you will become a skilled microscopist sooner.

1. Use both hands to carry the microscope. Grasp the stand firmly with one hand and lift it carefully. Place the other hand under the base as you carry it. Keep the microscope vertical because if tilted the oculars could fall out.
2. Clean the optical system (ocular lens, objectives, and condenser lens) before and after each use. This is especially necessary if you share the microscope with a student in another lab section. Use optical lens tissue *only* to clean the optical system. To remove oil or dust from other portions, use a soft cloth or facial-type tissue. Keep the microscope immaculate!
3. Never remove a part of the microscope without consulting your instructor.

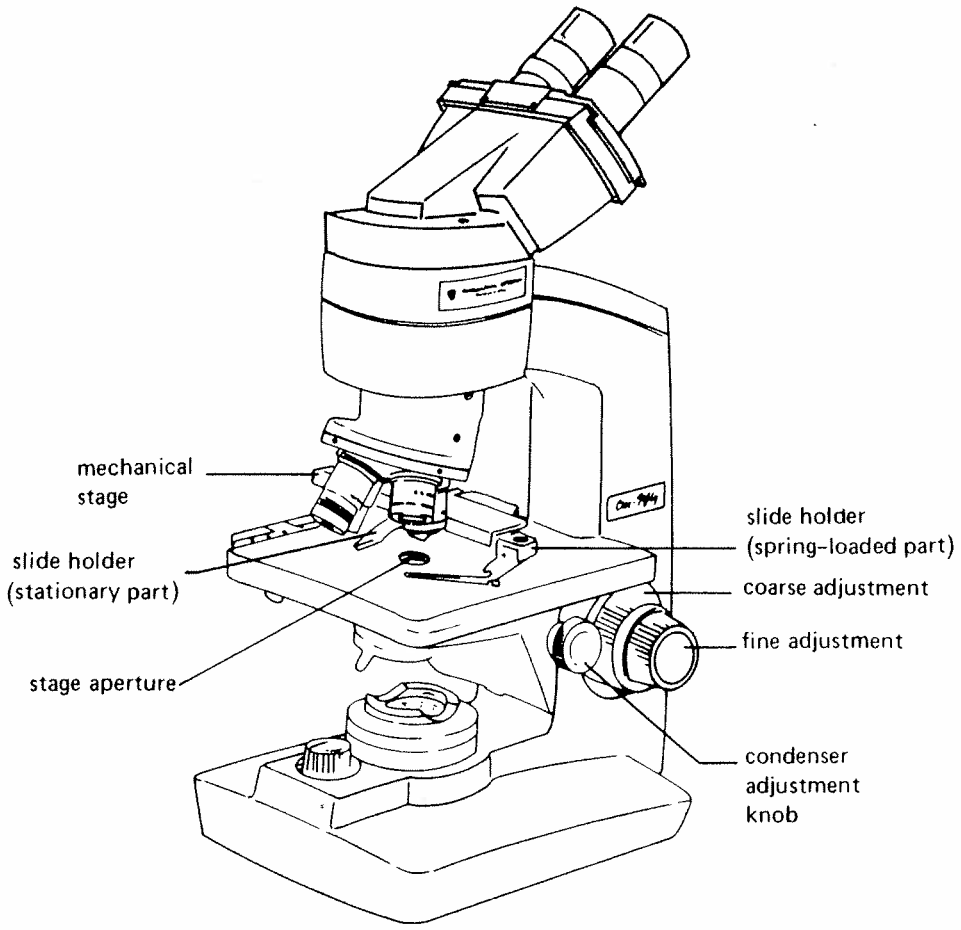

FIGURE 4-4
Reichert-Jung (AO Spencer) series 150 binocular microscope (left front view).

4. When you finish using the microscope and have cleaned it, if the microscope does not have an autofocus stop, place the low-power objective into working position. It is shorter than the other two objectives and less likely to be damaged if it strikes the mechanical stage. Replace the dust cover before you return the microscope to the storage cabinet.
5. If the microscope does not have an autofocus stop, never focus downward while looking through the eyepiece. To prevent breaking slides and damaging the objective, turn the objective to its lowest point while watching from the side before you look through the eyepiece and focus. Do not touch the lenses of the eyepieces because oils in the skin can mar the polished glass surface of the lens.

ACTIVITIES

Activity 1: Practice for Light Control

1. Turn on the light source to maximum intensity.
2. While looking through the oculars, adjust eyespan by gently pushing the oculars closer together or farther apart (use interpupillary distance scale) until you see a single illuminated microscopic field.
3. While looking through the oculars, open and close the iris diaphragm.

TABLE 4-1 The Binocular Microscope

Part	Function
Oculars (eyepieces)	A series of lenses that usually magnify 10 times.
Interpupillary distance scale	Adjusts to your eyespan.
Revolving nosepiece	Rotates for changing from one objective to another.
Objectives	Usually three magnifications (if no scanning lens is present): 10×, low power; 45×, high dry power; and 100×, oil immersion. Powers of the objectives are distinguished by colored bands.
Slide holder	Spring-loaded portion allows for placing the microscope slide in the mechanical stage, which holds it tightly.
Stage	Rises and lowers in focusing.
Diaphragm lever	Opens and closes the diaphragm to control the amount of light that strikes the object.
Condenser	Condenses light waves into a pencil-shaped cone, preventing light from escaping. Controls the intensity of light when raised and lowered.
Condenser adjustment knob	Raises and lowers the condenser.
Mechanical stage	Allows the slide to be moved on the stage.
Mechanical stage controls	Move the slide on two horizontal planes, that is, back and forth and side to side.
Base	Supports the entire microscope.
Power switch	Turns light on and off.
Voltage control	Controls the intensity of light.
Microscope stand	Supports upper half of the microscope.
Coarse adjustment	Moves the stage up and down quickly for approximate focusing.
Fine adjustment	Moves the stage up and down slowly for definitive focusing

- Determine the effect the diaphragm opening has on the brightness of the microscopic field.
- Open and close the diaphragm several times, making a mental note of optimum brightness. Optimum brightness does not necessarily mean maximum brightness.

4. Using the condenser knob, raise and lower the condenser while looking through the oculars.
   - Note what effect the position of the condenser has on the intensity of light. Do this several times, adjusting the condenser to optimum illumination. Do not hesitate to open and close the diaphragm or raise and lower the condenser.
   - Once the condenser is adjusted to optimum light intensity, do not change the setting. The amount of light must now be controlled with the iris diaphragm.

Repeat this practice activity several times. Light control is crucial to good microscopy.

Activity 2: Diameter of the Field

1. Place a flat, clear plastic millimeter ruler on the stage and focus the millimeter marks with your 10× objective.

TABLE 4-2 Magnification at Eyepoint

Ocular		Objective	Magnification at eyepoint
10	×	10×	100×
10	×	40× (43×)	400×(430×)
10	×	100× (43×)	1000×(970×)

2. Position the scale so that one of the unit marks is at the edge of the field and the edge of the ruler runs across the center of the field.
3. Estimate the diameter of the field in millimeters. Record your observation on the worksheet.
4. Convert this figure to micrometers (μm), also called microns, by multiplying by 1000. Record your calculation on the worksheet.
   - The micrometer (micron) is the preferred unit of measure for very small microscopic objects. For example, a red blood cell is about 7.5 μm in diameter.
5. The field of view on high dry power and oil immersion is too small to measure with a ruler. You can, however, calculate the size of the fields using the 10x objective measurement as a basis.
   - High dry power is 4.0 times the magnification of low power. (See Table 4-2.)
   - Because magnification and size of field are inversely related, the field diameter of high dry power must be 4.0 times less than the field diameter of low power.
6. Calculate the size of the oil immersion field.
7. Record the figures for the diameter of the fields on the worksheet.
8. You can determine the approximate size of an object in the microscopic field by estimating the number of objects that would fit across the diameter of the field of view. Then divide the diameter of the field by that number.
   - You estimate that an animal cell is about $^1/_5$ the diameter of the field. That is, about 5 of these cells would fit across the field diameter of the low-power field (10×). The size of the cell is approximately:

Activity 3: Low-Power Observation of Salt Crystals

1. Place a small drop of concentrated salt solution on a microscope slide, spread it out, and allow it to dry.
2. Place the slide in the slide holder as shown in Figure 4-5.
   - Be sure your slide is held against the arm of the mechanical stage.
3. Position the crystals under the objective by using the mechanical stage controls.
4. Using the coarse adjustment, move the 10× objective as close to the slide as possible, that is, until you reach the autostop. Do not force the objective further.
5. Looking through the ocular, turn the coarse adjustment back slowly, moving the objective away from the slide until the salt crystals come into focus.

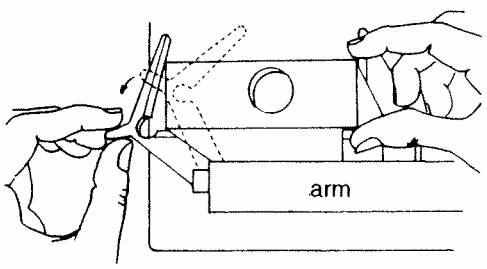

FIGURE 4-5
A slide is placed in the slide holder. Though there are variations of the slide clip, all work on the same principle: spring-loaded clamps place tension on the slide, keeping it in place as it moves with the mechanical stage. (Be sure your slide is held against the arm of the mechanical stage.)

6. Adjust the optical system as you did in Activity 1 until you obtain the best definition of salt crystals.
   - Adjust and readjust the iris diaphragm and condenser.
   - It will probably be necessary to reduce the light intensity. Experiment with the iris diaphragm and condenser until you find the optimum position of each that allows you to see the most definition of the salt crystals. Do not be satisfied with just any image; get the best image you can.

Make a composite drawing of a few salt crystals on the worksheet. Using the mechanical stage, select one crystal from several different fields.

Activity 4: High-Power Observation of a Stained Blood Smear

1. Place a prepared slide of a stained blood smear in the slide holder.
   - Be sure the smear side faces upward.
2. Position the smear under the objective by using the mechanical stage.
3. With the 10× objective in place, adjust the iris diaphragm and condenser for optimum light.
   - Repeat Activity 2 until the blood cells are in sharp focus.
4. Rotate the high-power objective (40×) into working position.
5. Using the fine adjustment, adjust to the sharpest possible focus.

Sketch representative blood cells at 400× on your worksheet. Do not ponder the different types of cells. In the next activity you will study cell types.

Activity 5: Demonstration of Parfocality Using Oil-Immersion Objective

1. With the stained blood smear from Activity 3 still in focus and the 40× objective still in place, adjust for maximum light.
2. Rotate the revolving nosepiece until the space between the 40× and the 100× objective is directly over the center of the smear. No objective is in working position yet.
3. Place a large drop of immersion oil on the smear, being careful that none gets on the microscope stage.
4. Rotate the nosepiece until the 100× objective clicks into working position.
   - The tip of the objective should be immersed in the oil but not quite touching the slide. If the objective is not submerged in the oil, you will not obtain sharp definition of the object.
5. Look through the oculars and focus with the fine adjustment.

Draw several blood cells on your worksheet as they appear at 1000×. See Figure 4-6, charts, and color plates for blood cell types. Include erythrocytes and leukocytes. Calculate the approximate size of a leukocyte.

Repeat this activity using a stained smear of yeast and of bacteria (*Bacillus* sp.) as shown in Figure 4-6.

After you have become proficient in employing parfocality to use the oil-immersion objective, and after you are satisfied that you are getting the maximum amount of definition by using the optimum amount of light, proceed to Activity 6.

Activity 6: Direct Use of the Oil-Immersion Objective

The more expert you are as a microscopist, the more you will use only the oil-immersion objective to study bacterial structures. Modern microscopes have an autofocus stop, so after applying oil to the slide, you can lower the coarse adjustment gently to its positive stop without danger of breaking the slide. The object will come into focus with a slight adjustment of the fine adjustment knob.

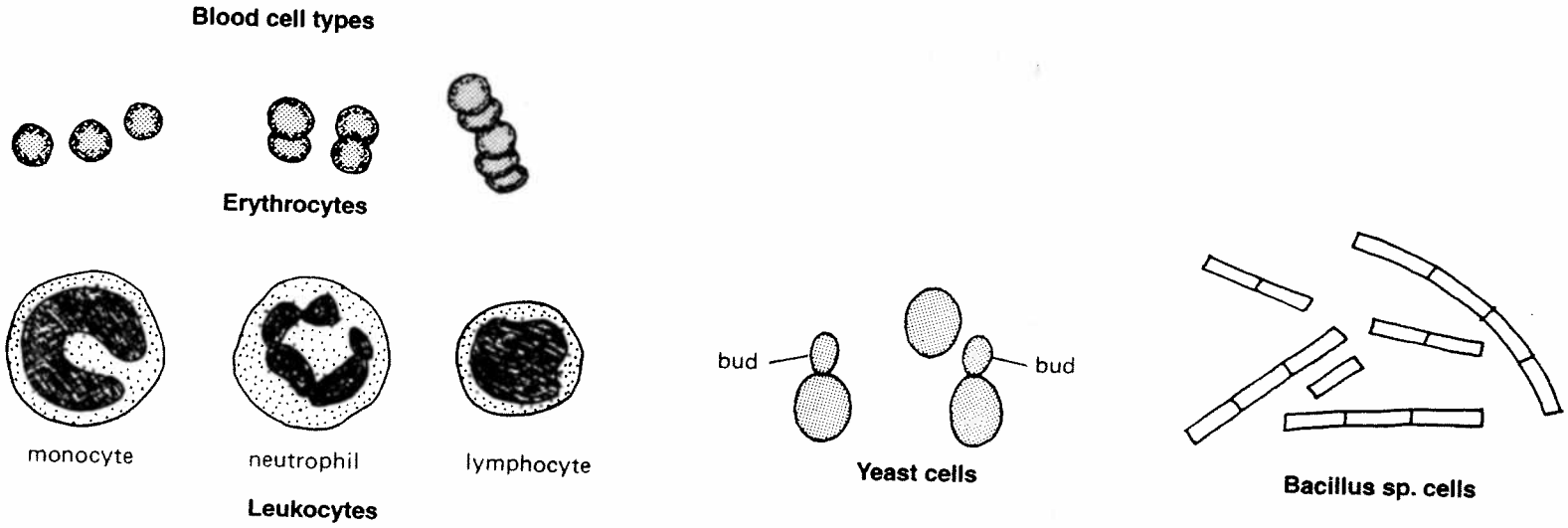

FIGURE 4-6
Blood cell types, yeast cells, and bacterial cells as viewed with an oil-immersion objective (not sized to scale). Use a photographic plate for accuracy. See color insert.

1. Place a simple stain of yeast cells in the mechanical stage.
2. Put a large drop of oil on the slide as described in Activity 5.
3. Using the coarse adjustment, immerse the 100× objective into the oil until the stop is reached.
4. Do not force the coarse adjustment knob downward.
5. Looking through the oculars, slowly turn the coarse adjustment backward; that is, move the oil-immersion objective slowly away from the object until it comes into partial focus.
6. When you can see the object vaguely, use the fine adjustment for definitive focus.

Precaution: The fine adjustment should be forward or in the middle of its track before you begin this activity. It should not be turned toward you, where there is no more focus adjustment.

Adjust the light for optimum definition. Draw several yeast cells on the worksheet.

Repeat the procedure using stained smears of *Bacillus* sp., *Escherichia coli*, and a yeast–*E. coli* mixture. Don't hesitate to reread the instructions. If you take precautions and time now, you will become expert sooner. Time, practice, and mastery of the microscope will make microbiology more rewarding.

Draw numerous cells from the *Bacillus* sp., *E. coli*, and the mixed slide on your worksheet. Calculate the approximate sizes of a yeast cell and an *E. coli* cell.

When you are ready to make your drawings of *E. coli*, ask your instructor to look through your microscope to check your illumination. It is good to know if the organisms you are observing have the maximum visibility.

Activity 7: Continued Practice Using the Oil-Immersion Objective

Examine a prepared smear of mixed bacterial types or other available smears with the oil-immersion objective. This activity repeats Activity 6 and offers more practice in finding the object with the oil-immersion objective.

Permanent, commercially prepared slides have cover slips over the smear. Place the immersion oil directly on the cover slip, and immerse the oil-immersion objective carefully.

Draw several representatives of each bacterial cell type on the worksheet. Figures 4-6 and 5-1 may help you identify cell types.

When you think you are well acquainted with the microscope and its parts, take the post test. If you are not satisfied with your results, review this module before going on. Your success in this course depends on your being honest with yourself.

Phonetic Pronunciation

Bacillus = buh-sill´-us

Escherichia coli = esh-ur-eek´-ee-uh koe´-lee

POST TEST

Part I: True or False

____ 1. When using the oil-immersion objective, the image you perceive at eyepoint is 97 to 100 times larger than the object.

____ 2. If two objectives are parfocal, one will be in approximate focus when the other is in sharp focus.

____ 3. The two functions of the optical parts of a microscope are to project light waves onto the object and to form a magnified image of the object.

____ 4. The iris diaphragm, condenser, and ocular are the three parts of the microscope most critical in controlling light.

____ 5. The size of the microscopic field remains constant regardless of the magnification of the objective you are using.

____ 6. Facial-type tissue can be used to clean the optical parts of a microscope.

____ 7. A microscope should be cleaned thoroughly with the low-power objective rotated into working position and the dust cover replaced before it is stored.

____ 8. Magnification at eyepoint equals magnification of the objective multiplied by magnification of the ocular.

____ 9. Once an image is achieved, the optical parts that control light striking the object should be moved as little as possible.

Part II

List the four major points to remember when carrying a microscope.

1. _____

2. _____

3. _____

4. _____

Part III

Match each part of the microscope with its function.

____ 1. Ocular

____ 2. Interpupillary distance scale

____ 3. Revolving nosepiece

____ 4. Objectives

____ 5. Slide holder

____ 6. Stage aperture

____ 7. Stage

____ 8. Diaphragm lever

____ 9. Condenser

____ 10. Condenser adjustment knob

____ 11. Mechanical stage

____ 12. Mechanical stage controls

____ 13. Base

____ 14. Microscope stand

____ 15. Coarse adjustment

____ 16. Fine adjustment

a. Opens and closes diaphragm to control amount of light striking object.

b. A series of lenses that usually magnify 10 times.

c. Condenses light waves into pencil-shaped cone, thereby preventing escape of light waves; also controls amount of light striking object.

d. Is raised and lowered in focusing some microscopes.

e. Usually has three magnifications.

f. Raises and lowers condenser.

g. Supports upper portion of microscope.

h. Rotates to change from one objective to another.

i. Adjusts for your eyespan.

j. Moves stage up and down quickly for approximate focusing.

k. Allows the slide to be moved.

l. Hole in stage to allow light waves to strike object.

m. Supports entire microscope.

n. Spring-loaded portion allows for placement of the slide in the mechanical stage, where it is held tightly.

o. Moves stage up and down slowly for definitive focusing.

p. Moves the slide on two horizontal planes.

Part IV

List the three parts of the binocular microscope that are critical in light control and resulting good definition.

1. _____
2. _____
3. _____

Part V

Describe the markings on the following objectives in the microscope you use:

1. Scanning_____
2. Low power_____
3. High power_____
4. Oil immersion_____

Name_____

Lab Section_____

MODULE 4: COMPOUND MICROSCOPE FOR THE STUDY OF MICROBES

Activity 2: Diameter of Field

Diameter of 4× field (scanning) [40× at eyepoint]: measured

_____ mm = _____ µm

Diameter of 10× field (low power) [100× at eyepoint]: measured

_____ mm = _____ µm

Diameter of 40× field (high power) [400× at eyepoint]: calculated

Calculations:

_____ mm = _____ µm

Diameter of 100× field (oil immersion) [1000× at eyepoint]: calculated

Calculations:

_____ mm = _____ µm

Sodium Chloride Crystals

Stained Blood Smear

Direct Use of Oil-Immersion Objective

Continued Practice Using Oil-Immersion Objective

Rank the cells in order of size from small to large when observed with the oil-immersion objective.

yeast	Smallest	_____
Bacillus sp.		_____
E. coli		_____
leukocyte		_____
erythrocyte	Largest	_____