(Note: Bibulous paper is specially prepared blotting paper with little or no extraneous paper fibers. This eliminates the sometimes annoying and confusing incidence of focusing on paper fibers under the microscope.) Figure 1.4 shows the slide being blotted dry.

6. Pour the residual stain in the staining tray into an appropriate discard container if directed to do so.

THE MICROSCOPE

A major component of this part of the course is staining and observing various types of microbes such as bacteria, fungi, and protozoans. (Viruses are too small to be seen with a conventional microscope.) Use of the microscope is an integral part of this study. These organisms, especially bacteria, are significantly smaller than any human or mammalian cells you may have seen in any Anatomy and Physiology or Biology course. Therefore, it is important that this device be used properly so that you can see the fine shapes, sizes, and structures of such small organisms. See Figure 1.5 for a chart showing the relative sizes of microscopic structures.

The type of microscope used in most courses is a bright field, binocular, compound microscope. It is a bright field because it projects bright light through the image on the slide; it is binocular because you can use both eyes to view the object; and it is compound because it uses a series of lenses to achieve magnifications of up to 1000 times. The following is a basic review and operating guide for using your microscope. Gaining familiarity with the microscope components and procedures for its use will certainly enhance your use of this instrument.

Microscope Components (Plate 1)

(See Figs. 1.6 and 1.7 which show different, labeled views of a compound microscope.)

Ocular or Eyepiece. The typical ocular has a 10X magnification; that is, it will magnify any object 10 times its size. If you are using a binocular microscope, you must make certain adjustments so that you can use both eyes to view the object on the slide. The oculars must be adjusted to account for the distance between the eyes as well as for differences in focusing ability between the right and left eye. Notice that the oculars can be moved closer and further away from each other to adjust for the distance between the eyes, and they can also be focused independently of each other to adjust for the different focusing ability of each eye. Most binocular microscopes have one fixed focus ocular and one ocular that can be adjusted. (Some binocular microscopes allow both oculars to be adjusted.)

With the adjustable lens set at the neutral ("0") position, focus the microscope at a higher magnification using only the fixed focus lens. For example, if the fixed focus lens is the right lens, look through this lens with your right eye and adjust the microscope so that the target object is in focus. Once in focus, look through the adjustable ocular and determine whether the target object is still in focus. If in focus, both eyes are able to focus at the same point in space and no further modifications are necessary. If the object looks out of focus with the adjustable lens set at "0," turn this lens clock-
wise or counterclockwise until the object is in focus. If you are near-sighted or far-sighted in one eye compared to the other eye, this procedure will adjust the microscope for your eyes and no eyeglasses will be needed. If you have astigmatism, however, you will still have to use eyeglasses if you want to use both oculars.

**Objectives.** Three objectives are typically used in this course. The 10X objective or low-power lens, which will give a total magnification of 100X when used with the ocular (10 X 10), is used to initially focus the object on the slide. The 40X or high-dry objective (total magnification of 400X) will enlarge the object 4 times more than the 10X lens. It can also be used to select an interesting field of vision, or view, before changing to the 100X or oil immersion objective. Finally, the oil immersion objective (1000X total magnification) is used to view all the slides prepared in this class. Most microbes are so small that even at 1000 magnifications they will be just visible. Notice that these lenses can easily be utilized by rotating the base or nosepiece where they attach to the body of the microscope.

**Mechanical Stage.** The mechanical stage is where the slide is placed for viewing. Notice the stage clip that is used to hold the slide in place. Most microscopes have stages and stage clips that can be manipulated by turning two knobs located below the stage.
Coarse and Fine Adjustments. These knobs are usually located on both sides of the body at or below the level of the stage. The coarse adjustment knob is the larger of the two and is usually located closer to the body of the microscope. By turning it with the low power (10X) in place, you should readily see the stage move up and down unless the stage is at the highest position. The only time the coarse adjustment knob is used is when the low power lens is in place. If used with the higher power lenses, damage to the slide and the lens itself may occur. Most microscopes have a safety stop built into the coarse adjustment knob that prevents it from being raised too high. Once you reach this level, do not continue to turn the knob, for it may damage the microscope. Even with a safety stop, always view this adjustment of the lens and stage from the side to ensure that you do not damage any microscope components.

The fine adjustment knob is the smaller knob that is usually attached to the side of the coarse adjustment knob. By turning this knob, the stage will also move up and down but in much smaller increments. The movement is so minuscule that few students can see the stage move at all. One of these fine adjustment knobs may have a scale attached to it, which is useful to measure the thickness of cells under the microscope. Each notch on this scale usually measures an increment of only 2 micrometers (µm), about one-fourth the diameter of a human red blood cell. This knob will be the only one used to focus the microscope when using the higher magnifications.

Light. Most microscopes use the following components to adjust the light for optimum viewing.

Illuminator rheostat—Most microscopes make use of a rheostat to adjust the amount of electricity through the light bulb located beneath the stage. As the amount of electrical current increases, so does the illumination. Depending on the type of microscope used, your rheostat may be part of an on-off switch or it may operate separately. Follow your instructor’s directions in operating the rheostat.

Note: To prevent premature lamp burnout, the rheostat must be turned to its lowest position before turning the microscope on and off.

Condenser—The light is focused through the slide with this lens. Its ideal position is just below its highest position beneath the stage. To adjust this lens, locate the condenser focus knob under the stage and move the lens as directed, usually to its highest point.

Iris diaphragm—Once the rheostat and condensing lens are set, light passing through the slide can be regulated by simply adjusting the iris diaphragm located on the condenser itself. An adjustment knob or a lever is used to readily control the passage of light through the condensing lens. When using the microscope under low power (10X objective), adjust the opening of the diaphragm so that a minimum amount of light passes through the slide. As the magnification of the microscope is increased, you will have to increase the light transmitted through the slide by increasing the size of the opening of the iris diaphragm.

One of the most common problems students encounter with their microscopes is improper illumination. If you are having trouble seeing the object, the light adjustment should be one of the first things you should check.

PROCEDURE FOR USING THE MICROSCOPE

Step 1. Clean the lenses using lens tissue only. Start with the oculars and then the objectives. Clean the oil immersion objective last, so that any residual oil left from previous classes will not smear the oculars or other objectives.

Step 2. Set the microscope up as follows:
Have the 10X or low power objective in place.
Set the stage as high as it will go or lower the nosepiece to minimum working distance.
Set the rheostat, condensing lens, and iris diaphragm as directed.
Have the cheek cell slide, previously stained, or a prepared slide centered on the stage. (The light coming from the condensing lens acts as a spotlight to easily target the slide.)

Step 3. Look through the ocular and lower the stage. If you are looking at your own cheek smear, epithelial cells will eventually come into focus. If you are looking at a prepared slide, you will see extremely small structures. It may be advisable to focus in on the edge of the coverslip or a mark on the slide for the initial focusing. Once in focus, adjust the light and maneuver the slide so that the cells are in the center of the field of vision. (The circle of light you see through the ocular is the field of vision.)

Step 4. Without moving the stage, rotate the nosepiece until the high-dry (40X) objective is in place. Notice that this objective just barely misses the slide as it is rotated into place. This is why you must use only the fine focus with the higher magnifications! USE OF THE COARSE ADJUSTMENT MAY DAMAGE THE SLIDE AND THE LENS! You should notice that you can see the object, but it may be slightly out
of focus. These microscopes are designed to be parfocal; that is, the lenses have been adjusted to focus at the same point in space. This means that if you get the object in focus under low power, it will be in focus (or nearly so) under the other magnifications. Once you use the fine adjustment to focus, you may have to adjust the light. The cells seen are now 4 times larger (400X versus 100X), and the field of vision is now 4 times smaller. Because of this smaller field of vision, a cell on the periphery of the field at 100X magnification will not be seen at 400X magnification.

**Step 5.** Rotate the nosepiece once again until the oil immersion (100X) is about to lock into place. Place 1 to 2 drops of immersion oil on the slide in the location where the lens will rest, and then complete the rotation and lock the objective in place. The light from the condensing lens will guide you to the exact location for placement of the oil. The objective should now be touching the oil. Under this magnification, the oil increases the resolution of the microscope. That is, it will give a sharp, clear image. Focus with the fine focus knob and adjust the light using the iris diaphragm.

⚠️ **REMEMBER NEVER USE THE COARSE ADJUSTMENT WITH THE HIGH-DRY OR OIL IMMERSION OBJECTIVES.**

**Troubleshooting**

If you are having trouble getting the object in focus with the microscope under higher magnifications, consider the following:

1. **Is the light adjusted properly?** If not, review the steps in adjusting the light.
2. **Is the slide upside down?** If it is, you will get the object in focus under low power but not with the other objectives. *Hint:* Mark a part of the slide with a marking pen or pencil for a reference point. Prepared slides will have a label and coverslip, so this will not be a problem whenever these are used.
3. **Was the object in focus under low power?** Remember that these objectives are parfocal. If it is out of focus in low power, it will be out of focus with the others. If you are having trouble focusing the object under low power, focus on a mark placed near the smear or on the edge of the coverslip.
4. **Is the oil touching the lens?** You will not get a high-resolution image with the oil immersion objective unless it is in contact with the oil.
5. **Is the lens dirty?** Use lens tissue to clean the lenses.

**EUKARYOTIC VERSUS PROKARYOTIC CELLS (PLATE 2)**

One of the many methods of classifying organisms is to divide them into two major groups based on cellular structure. **Eukaryotic cells** (eu = true or real, kary = nuclear or chromosomal material), exemplified by human cells, have characteristics that include a membrane-covered nucleus with paired chromosomes and tend to be relatively large. **Prokaryotic cells** (pro = before) have no nucleus, only one chromosome, and they are small compared to eukaryotic cells. Prokaryotic cells are bacteria. Human epithelial cells tend to be 30 to 40 μm in diameter, whereas most prokaryotic cells are only 1 to 2 μm wide. The most typical prokaryotic cell found on the surface of human cheek cells is a paired circular-shaped bacterium called a diplococcus *(dipto = paired, coccus = berry or round)*. Even with 1000X magnification, the diplococci *(cocci = plural of diplococcus)* will just barely be visible. Figure 1.8 shows epithelial and diplococci together. The diplococci that will be observed under the microscope are most likely streptococci or chains of cocci when grown in the laboratory. In the mouth, they tend to be found as pairs.

Adjust the focus and light so that the bacterial cells are as clear as possible. Notice that if you rotate the fine adjustment knob so that the focus changes only 2 μm, these cocci will no longer be in focus. If you change the amount of light going through the slide by adjusting the iris diaphragm, notice that the cocci will be much more difficult to see. This is why you must be very precise in your operation of the microscope.

While observing these diplococci, see if you can also see three slightly different shapes of these cells. All three shapes are usually found in the mouth. If you have a good smear and a good stain, and if you focus the microscope properly, you will have a good chance of observing all three on your slide. (One type will look like perfectly round pairs of cells. Another type will look like two elongated letter D's back to back, while the third pair will look like two kidney beans facing each other.)

![Human epithelial cell (Eukaryotic)](image1)

![Cell nucleus](image2)

![Diplococcus (Prokaryotic)](image3)

**FIG. 1.8.** Human epithelial cells and diplococci.
LABORATORY CLEANUP

An important part of this course is leaving your equipment and work area in proper condition for the next person to use.

Microscope Cleanup
1. Remove the slide.
2. Adjust the rheostat to dim the illuminator; then turn off the microscope lamp. As previously stated, this procedure increases the life of the bulb.
3. Clean the lenses of the microscope with lens tissue only in the same manner as before. Make sure the low-power (10X) objective is pointing downward. Wipe all the oil off of the 100X objective.
4. Clean the stage if necessary.
5. Wrap the power cord around the microscope.
6. Cover the microscope if a cover is available, and store the microscope in its assigned place.

Discards
Discard used and broken slides in the designated sharps container or in a container of disinfectant solution such as 10% bleach.

General Cleanup
1. Wipe your work area down with disinfectant solution.
2. Return the stains, immersion oil bottles, staining trays, prepared slides, and all other equipment and materials to their proper locations.

Speaking of Safety
The goal of every microbiology instructor is to create a safety consciousness in students that will continue to affect them in other laboratory courses, at home, and in the workplace years after the college laboratory experience is over.

A laboratory accident may seem an unlikely event to many students, yet year after year hundreds of undergraduate laboratory accidents occur nationwide. Every precaution must be taken to assure a safe, enjoyable laboratory program.

⚠️ REMEMBER THESE SAFETY RULES
1. No food or beverage is to be taken into a laboratory where accidental hand-to-mouth contamination or ingestion can occur. This is one of the most common ways in which dangerous microbes can enter the body.
2. Never place personal items such as backpacks or clothing on your laboratory table. Not only will they very likely get stained and dirty, but they also may become contaminated by microbes.
3. Wear appropriate dress: Use a lab coat, tie back long hair or use a hairnet, and put on protective gloves and eyewear when needed. Microbiological stains do one thing very well—they stain. Do not wear open shoes or apply makeup in the lab. It is better to get these stains on a lab coat than on your personal clothing. Long hair may get scorched in the Bunsen burner or pick up unwanted stains.
4. Know where the first aid kits, safety equipment, and exits are located in your laboratory. It is too late to study a floor plan when the lab is filled with fumes.
5. Follow your instructor’s explicit instructions in the event that the laboratory must be evacuated. After evacuation, stay with your instructor. Never leave until you are permitted to do so.
6. Never put anything in your mouth while in the laboratory. No solutions should be pipetted by mouth. Again, this is one of the most common ways in which microbes can enter the body.
7. Never use a substance or chemical that is missing a label.
8. Always use the fume hood when instructed to do so. A chemical does not have to have an obnoxious odor to be toxic.
9. Notify the instructor in the event of a chemical spill or accident. Certain spilled chemicals will rapidly fill the lab with fumes.
10. If at all possible, do not share Bunsen burners; this can lead to singed fingers.
11. Follow laboratory housekeeping rules such as washing down your tables with disinfectant before and after use. Allow the disinfectant to air dry. For maximum effectiveness, do not towel dry the table. This will make for a clean and safe working environment.
12. Always wash your hands before leaving the laboratory. (Are you detecting a pattern here?)
13. Replace your lab stool or chair under the table before you leave, and store your lab coat properly or place it in a sealed plastic bag before you leave the lab. Make sure all materials have been returned to their proper location.
15. Make sure all gas jets are shut. If you smell gas, notify your instructor.