Microscopy

When you have completed this exercise, you will be able to:

1. Identify the parts of a microscope.
2. Match the parts of a microscope with their functions and uses.
3. Calculate the total magnification achieved when different objective and ocular lenses are used.
4. Demonstrate and explain proper procedures for microscope use.
5. Explain the meaning of “parfocal.”
6. Describe and draw types of microorganisms observed in pond water, a hay infusion culture, and a pure culture of *Amoeba proteus*.
7. Compare the biological diversity of natural and altered environments.
8. Predict differences to be expected when comparing natural and altered environments.

The microscope is a very important tool in environmental science and all of biology. It makes microorganisms visible, opening up a vast new world to the student. In this class, you will have the opportunity to observe microorganisms from water and soil samples. The diversity of microorganisms present in these samples amazes many students.

Microscopes are expensive tools that should last for many years with proper care and maintenance. Everyone who uses a microscope helps determine how long it will be of service. Be sure that you use your microscope properly so that it may be used by many future students.

Parts of the Microscope

Three parts comprise the physical backbone of the microscope: the base, the arm, and the stage. The microscope sits upon its base; the arm connects the base to the stage and to other parts of the microscope. To safely carry the microscope, hold one hand at the arm and one beneath the base. Be sure that all cords are tucked in so that you do not trip. The stage is the site of the action; the microscope slide is placed on it to be observed.

Note whether your microscope uses stage clips to hold the slide in place or is equipped with a mechanical stage. With stage clips, the microscope slide is slid beneath the clips so that the slide is held in place. With this type of stage, you must move the slide around with your hands. This can be tricky, especially at high magnifications; therefore, a mechanical stage is preferable. With a mechanical stage, the slide is placed on the stage and slid into place as the clamps are held open and then released to hold the slide in place. Two knobs beneath the stage move the slide around the stage. One knob moves the slide horizontally; the other moves it vertically. It is much easier to control small movements using the mechanical stage.

Light comes into the microscope from a light source. In modern microscopes, the light is part of the microscope and is fixed in the base. Filters may be set on top of the light to change its color or other attributes. Some microscopes have controls to adjust the brightness of the light. These work like the dimmer switches you may have in your home. If you do not find this feature on your microscope, the diaphragm located above the light can provide a similar effect. The diaphragm opens and closes the aperture through which the light shines by producing a larger or smaller hole as the diaphragm control lever is moved from side to side. The smaller hole lets through very little light and allows you to observe slight shadows around rather clear specimens. This can make these specimens visible without staining.

Microscopes magnify the images of specimens by using lenses. Three types of lenses are found in most compound microscopes: the condenser lens, the objective lens, and the ocular lens. The condenser lens is found beneath the stage aperture where it focuses the light on the specimen. Some condensers are fixed and cannot be adjusted. Others, with adjustable condensers, allow the user to focus the light away from the specimen. This, like the use of low light, provides contrast to rather clear specimens, making them more visible. After coming through the condenser, the light strikes the specimen. An image is projected up to the lens above the slide. This lens is the objective lens. Note that your microscope has from two to four objective lenses, all mounted on a revolving nosepiece that brings each lens into viewing position in turn. These objective lenses differ in magnifying power, the number of times the image is magnified by the
lens (for example, 10×). The magnifying power is labeled on each lens, and the colors for the labels are standardized as well:

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Magnifying Power</th>
<th>Label</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning lens</td>
<td>4×</td>
<td></td>
<td>Black</td>
</tr>
<tr>
<td>Low power</td>
<td>10×</td>
<td></td>
<td>Green</td>
</tr>
<tr>
<td>High (“dry”) power</td>
<td>43×</td>
<td></td>
<td>Yellow</td>
</tr>
<tr>
<td>High-power oil immersion</td>
<td>100×</td>
<td></td>
<td>Red</td>
</tr>
</tbody>
</table>

The third type of lens found in a compound microscope is the ocular lens, or eyepiece. This lens receives the magnified image produced by the objective lens in viewing position. Often, unseen mirrors reflect the image to the ocular lens. The ocular lens magnifies the image again, usually tenfold. The total magnification of the image is the product of the magnification of the objective lens times the magnification of the ocular lens. The compound microscope is so named because of the “compounded” magnification of the lenses it uses.

Your microscope may have one or two ocular lenses. With one ocular lens, your microscope is called monocular. To look into it, you use your dominant eye. Keep your other eye open and ignore what it is seeing. This is not as difficult as it sounds, but it does take practice. If your microscope has two ocular lenses, it is binocular. The image observed is not three-dimensional, but both eyes receive the image. Less eyestrain occurs when the binocular microscope is used, but the eyepieces must be adjusted for each individual user. This may be why monocular microscopes are most often found in classrooms.

The image is focused by using the coarse and fine focus knobs. These controls move either the stage or the objective’s nosepiece up and down to bring the image into focus. The coarse focus adjustment is done first, and the fine focus adjustment merely perfects the focus by making only small adjustments.

**Viewing Tips**

Another feature of modern microscopes is that the lenses are mounted to be parfocal. That is, when you have achieved a coarse focus with the lowest power objective lens in place, you can switch to the next higher magnification lens and still maintain the coarse focus. This leaves a space above the slide and below the lens, the “working distance.” Note that the working distance declines as the magnifying power increases. With the highest power objective in place, there is little room for movement of slides, so be careful to never allow the lens to touch the slide. It is best to always return the lowest power objective to the viewing position before removing or adding a slide. This allows you to manipulate the slides with the greatest working distance.

When you look through the microscope, you will observe a circle of light and, if a slide is in place, your specimen. This area is known as your microscopic field. Note that as you increase the magnification, the area of the specimen viewed in the microscopic field is smaller. This is logical because when the magnification increases, you can only observe a smaller portion of your slide.

**By the way . . .**

The primary reason a student is unable to see a specimen clearly is a dirty lens. If your view is fuzzy, clean the lenses with special lint-free lens tissue or lens paper. Do not use a substitute or your lens will be permanently scratched and ruined. Quite often, simple cleaning can effect a remarkable improvement in the quality of the image observed. If simple cleaning using a lens tissue does not improve the image, tell your instructor. There are special cleaning fluids that may be helpful, or perhaps a more serious problem is present.

Microscope lenses differ in resolving power, the ability of a lens to separate two small, nearby objects into two distinct images. A high-quality lens will have greater resolving power than a lesser quality lens. Most student microscopes will resolve two small objects 0.5 μm apart. If the objects are closer together, they will appear as one. Higher quality microscopes can resolve even smaller distances.

The oil immersion lens provides the highest magnification (and the highest resolution as well). A drop of special, optically pure immersion oil is placed on the slide before viewing, and the oil immersion objective is turned into place and immersed in the oil to view the slide. The use of oil decreases image distortion. You will probably not use this type of lens in this class, but if you do, be sure to remove all the oil when you are done. Residual oil left on a lens can seep into the housing of the lens and ruin it.

Quality lenses are very expensive, and the replacement cost for one lens may exceed the cost of an entire new, lesser quality microscope! The utmost care must be used to ensure that lenses are not damaged.

**Stereomicroscopes**

Stereomicroscopes, also called dissecting microscopes, have two ocular lenses and two objective lenses. This allows stereoscopic vision so that when the stereoscope is used, a three-dimensional image is seen. Thicker specimens, such as live, whole small organisms, may be observed by using the stereomicroscope.

Modifications of the procedures used for the light microscope apply when using the stereomicroscope. The specimen is placed in the viewing area. The eyepieces are adjusted to the user’s eye width. Finally, the light is adjusted to best illuminate the specimen. Some stereomicroscopes provide “zoom” features that allow you to adjust the magnification to best display your image.

**Activity 1: Using the Light Microscope**

**Materials**

- Compound microscopes
- Prepared letter “e” slides
Procedure

1. Carry the microscope upright with both hands, holding onto the base and the arm.
2. Plug in the microscope, being sure the cord is placed safely.
3. Turn on the light.
4. Place a letter “e” slide on the stage and slide it into place. Use your stage clips or mechanical stage to hold the slide in place, with the letter “e” centered over the circle of light above the condenser.
5. Look through the ocular lens.
6. With the lowest magnification objective lens in place above your slide, use the coarse focus knob to bring the letter “e” into focus; adjust the focus further by using the fine focus knob. If your microscope is binocular, use the following steps to adjust the two lenses to your eyes:
   a. Pull or push the lenses apart or together to fit the width of your eye spacing.
   b. Determine which of the ocular lenses has an adjustable focus. Look only through the unadjustable one and focus carefully using the fine focus adjustment.
   c. Now adjust the focus on the adjustable ocular lens so that both of your eyes see a clear image. Your microscope is now adjusted to your eyes. If you share a microscope with a partner, you may need to make these adjustments often.
7. Re-center the letter “e” in your field. Draw what you see in the circle below.

8. Note the orientation of the letter as it appears with your naked eye. Is it the same as through your microscope? Why do you think it appears this way? Hint: What can be found between the objective and ocular lenses?
9. To increase the magnification of the image, rotate the next higher power objective lens into place. If the image of the letter “e” disappears, switch back to the lower power lens and re-center the image. Then try switching to the higher powered objective again. Once you see the letter “e” or a portion of it, draw what you see in the circle below. Why do you think the image has changed more than just getting bigger?

10. To increase the magnification further, re-center the image, switch objective lenses, and refocus with the fine focus knob. Add a drop of oil before switching to the high-power oil immersion lens. Note: If a slide, coverslip, or specimen is too thick for the oil immersion lens to be turned into place, do not try to force it. You will only succeed in breaking the slide and probably scratching either or both the oil immersion objective and condenser lenses.
11. When you are finished observing a slide, switch to the lowest magnification objective lens. Use the coarse focus knob to raise the objectives (or lower the stage, depending on your microscope). Remove the slide, wipe off any immersion oil (if used), and return the slide to its proper storage place.
12. When you are done using the microscope, clean the lenses with lens tissue if necessary. Then securely wrap the cord around the base. Do not wrap it too tightly because this may damage the cord and cause an electrical short to develop. Using both hands, carry the microscope to the storage cabinet and put it in its proper place.

Activity 2: Preparing Wet Mounts and Observing Specimens

Ecological dogma states that undisturbed natural environments have greater biological diversity (more different species of organisms in a given space or volume) than disturbed environments that have been altered by humans. Keep this dogma in mind as you observe these samples. You will observe three samples: pond water, a hay infusion culture, and a pure culture of Amoeba proteus. You will prepare a wet mount of each, draw pictures of what you observe, and critically compare the three specimens.

The pond water is the most natural of the three samples. The hay infusion culture was prepared by adding water to hay and allowing it to sit (“culture”) for a few days. This specimen can be more or less “natural,” depending upon the source and type of hay. (Ask your instructor how this particular culture was prepared.) The pure culture of A. proteus is the most unnatural sample of the three. It was prepared in the laboratory of a biological supply company. We call it a pure culture because it contains only one species of organism. Special techniques are required to maintain it as a pure culture because in the real world there are always organisms ready and able to contaminate the culture. In fact, it is probable that your class will contaminate the A. proteus pure culture by the end of this lab by introducing organisms from students’ hands or from the other cultures. If another class used the culture before yours, perhaps it already contains more than one species.

Materials

Microscope slides
Coverslips
Eyedroppers or Pasteur pipettes
Pond water
Hay infusion culture
Pure culture of Amoeba proteus
**Procedure**

1. Obtain a clean microscope slide, a coverslip, and an eyedropper from the supply area.
2. Obtain a drop of pond water from the bottom of the vessel. (It is best to collect the sample from the bottom of the container because you will find the most organisms there. Since aquatic microorganisms are affected by gravity, many are found at the bottom.)
3. Place the drop on the center of a microscope slide (Figure 2.1a). Sometimes it is useful to add a drop of stain to make the microorganisms more visible.
4. Place a coverslip with one edge at one side of the drop. Draw the touching edge toward the drop until it spreads out by capillary action as shown in Figure 2.1b.
5. Slowly release the coverslip as you place it the rest of the way down onto the slide (Figure 2.1c). Try not to produce bubbles; however, note that bubbles are round and refractive when you look at them with your naked eye. They are similarly round and refractive when viewed through the microscope. Organisms are not as perfectly round, do not have refractive edges, and are not perfectly clear in their interior. If you observe round clear objects with refractive edges through the microscope, you are observing air bubbles in your wet mount; these are considered artifacts.
6. Draw the microorganisms you observe in the pond water and record your observations about this sample in Table 2.1 in your lab report. Record the total number of different microorganisms observed. Notice the relative abundance of microorganisms in your specimen and estimate the number of microorganisms seen per low-power field.
7. Make a wet mount of hay infusion water, collecting your sample from the bottom of the container. Draw the microorganisms you observe and record your observations about this sample in Table 2.1 in your lab report. Record the total number of different microorganisms observed. Notice the relative abundance of microorganisms in your specimen and estimate the number of microorganisms seen per low-power field.
8. Make a wet mount of the *Amoeba proteus* pure culture, collecting your sample from the bottom of the container. Draw the microorganism(s) you observe and record your observations about this sample in Table 2.1 in your lab report. Record the total number of different microorganisms observed. Notice the relative abundance of microorganisms in your specimen and estimate the number of microorganisms seen per low-power field.

**Lab Report**

Your lab report may be written in standard lab report format (see Appendix A). Alternatively, your instructor may require that you use the lab report form on the following pages.
Lab Report 2  Microscopy

1. Label the parts of the microscope shown in Figure 2.2 by placing the appropriate letters in the spaces below:

   _____arm   _____condenser   _____mechanical stage knobs
   _____base   _____diaphragm control lever   _____nosepiece
   _____binocular lenses   _____fine focus knob   _____objective lenses
   _____coarse focus knob   _____light source   _____stage

Figure 2.2  A compound microscope.
2. If your microscope’s ocular lens has a magnification of 10× and your microscope’s objective lens has a magnification of 43×, what is the total magnification?

3. How should you carry the microscope? Why?

4. What should you use to clean the microscope lenses? Why are other materials not used?

5. Which objective lens should be in place when you begin and finish using the microscope?

6. Describe and draw the microorganisms you observed in each sample in the spaces provided in Table 2.1.

7. Compare the biological diversity of the three samples. Which one would you expect to be the most diverse? Which one was?

8. Predict the differences in organism diversity that you would expect to find when comparing the microorganisms in soil from an agricultural field (e.g., a cornfield) to soil from a natural environment. Use the results of this exercise as your guide. Explain your reasoning.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Drawings of Microorganisms Observed</th>
</tr>
</thead>
</table>
| Pond water           | Total number of different microorganisms observed ____  
                      | Estimate of microorganisms per low-power field ____    |
| Hay infusion         | Total number of different microorganisms observed ____  
                      | Estimate of microorganisms per low-power field ____    |
| *Amoeba proteus* pure culture | Total number of different microorganisms observed ____  
                          | Estimate of microorganisms per low-power field ____    |