LEARNING OBJECTIVES

In this lab, students will:

- Learn to recognize and use the parts of a compound microscope.
- Determine magnification power.
- Focus a microscope.
- Make a wet mount.
- Recognize the difference between a compound microscope and a stereomicroscope.

INTRODUCTION

The basic tool of most biologists is a microscope. Since the human eye is unable to distinguish objects that are smaller than 0.1mm, biology laboratories use microscopes to observe the basic structure of cells and their components. A microscope can provide both magnification of an object and resolution, that is the ability to distinguish between two points in a specimen.

The father of microbiology is Anton van Leeuwenhoek (1632–1723). He built and used a single-lens microscope. Although the magnifying ability of glass had been observed since Roman times, Leeuwenhoek perfected the grinding of lenses and then used these lenses to make some important microscopic discoveries. The first microorganisms seen were bacteria, yeasts, and protists.

Leeuwenhoek constructed only single-lens microscopes. To increase magnification, the lens’ diameter was decreased, which made it difficult to see through because they were so small. A two-lens system was created in which the second lens magnified the image of the first lens. This construction led to the compound microscope, a microscope that uses a two-lens system. Today, biologists use many types of microscopes. Most of them have the same basic parts and functions. In today’s lab you will learn the parts of a compound microscope, its magnification and how to focus on a specimen mounted on a slide.
THE BASIC PARTS AND FUNCTIONS OF THE COMPOUND MICROSCOPE

As you examine the compound microscope, review Table 1, where each part is listed and described. In the last column of the table, indicate which number on the microscope diagram (Figure 1) corresponds to the location of that particular part.

**Table 1**: The parts and functions of the compound microscope.

<table>
<thead>
<tr>
<th>Part</th>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular Lenses</td>
<td>Houses first lens system. Eye looks through this part. May be one or two, also called eyepiece.</td>
<td></td>
</tr>
<tr>
<td>Body Tube</td>
<td>Holds ocular on one end and nosepiece at the other.</td>
<td></td>
</tr>
<tr>
<td>Arm</td>
<td>Provides support, and serves as a handle for carrying.</td>
<td></td>
</tr>
<tr>
<td>Nosepiece</td>
<td>Holds the objectives and is able to rotate.</td>
<td></td>
</tr>
<tr>
<td>Objectives Lenses</td>
<td>Series of tubes that house the second lens system: scanning, low power, high power, and oil.</td>
<td></td>
</tr>
<tr>
<td>Coarse Adjustment Knob</td>
<td>Larger knob used to make large adjustments. Should only be used with the scanning objective.</td>
<td></td>
</tr>
<tr>
<td>Fine Adjustment Knob</td>
<td>Smaller knob used to make tiny adjustments.</td>
<td></td>
</tr>
<tr>
<td>Condenser</td>
<td>Found below the stage, and used to focus beam of light from below.</td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td>Aperture that controls the amount of light hitting object.</td>
<td></td>
</tr>
<tr>
<td>Light Source</td>
<td>Lamp that provides light for illumination.</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>The bottom of the microscope.</td>
<td></td>
</tr>
<tr>
<td>Stage/Mechanical Stage</td>
<td>Flat surface that support the slides. Usually has clips to hold the slide in place. Some have adjustment knobs to move slide.</td>
<td></td>
</tr>
</tbody>
</table>
A good source of information about microscopes can be found at the following URL:

http://www.southwestschools.org/jsfaculty/Microscopes/microscopeparts.html

**MAGNIFICATION**

1. Obtain a clean slide and mark a very small “e” with a wax pencil on the center of the slide.

2. Place the slide on the stage; adjust the slide on the stage so that the center of slide with “e” letter is at the center of opening of the stage.

3. Use scanning objective (4X) to observe letter “e”. Position the scanning objective so that it is just above the opening of the stage. Turn on the illuminator.

4. With coarse adjustment knob, turn the body up and down in order to focus the letter. Use fine adjustment knob to fine focus. Repeat this experiment with objectives 10X and 40X. Observe the results and compare to your initial observations.
Observe the letter “e” and record your observations
Please see the questions 1-4 at the end of this chapter in the Lab Report.

**MAGNIFICATION IN THE MICROSCOPE**

1. There are two lens systems in the microscope, one located in the ocular and the other in the objectives. Examine the ocular(s) of the microscope. What is printed on the side or top of the **ocular lens**?

2. Notice the number followed by an “X”; this is the **magnification power** of the ocular. Write the magnification of the ocular in the space provided. This means that the lens in the ocular makes the object appear larger by this factor.

   Magnification of the ocular:

3. Next, examine the objectives (there are 3 or 4). Examine the numbers printed on the side of each **objective lens**. Draw a quick sketch of one of the objectives, indicating the markings on it. (Instructor may have to provide data.)

4. One number indicates the **magnification** of the objective; it is usually a whole number such as 4, 10, or 40. Another number commonly found on the objectives indicates the **aperture** of the objective. This number is usually a small number that often includes a decimal; and it indicates how large a cone of light hits the object being viewed. The larger the numerical aperture, the larger the cone of light that will be focused on the object, and the more resolution you will have.

5. The objectives vary in size, and the smallest one is the **scanning objective**. In Table 2, list the magnification and numerical aperture for each lens, and describe how you would distinguish each objective from the others.
**Table 2**: Information on objective lenses.

<table>
<thead>
<tr>
<th>Objective</th>
<th>Magnification</th>
<th>Numerical Aperture</th>
<th>Distinguishing Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Power</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Power</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(dry)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil Immersion</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL MAGNIFICATION**

1. With two-lens systems, there is a simple method to figure the total magnification when using each objective. The total magnification is equal to the magnification of the ocular multiplied by the magnification of the objective.

2. For each objective, determine the total magnification and write it in Table 2.

**Table 3**: Total magnification for objective lenses.

<table>
<thead>
<tr>
<th>Objective</th>
<th>Total Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FOCUSING THE MICROSCOPE

There are some basic steps to follow when focusing the microscope.

1. Always begin with the objective that has the lowest magnification—the **scanning objective**. Turn the revolving **nosepiece** until this objective is over the **stage**.

2. Use the **coarse adjustment knob** to move the objective so that it is the furthest away from the stage.

3. Place a slide on the stage and secure it with the clips or the spring lever.

4. While looking through the ocular, slowly move the objective closer to the slide using the coarse adjustment knob.

5. The **iris diaphragm lever** can be used to increase or decrease the amount of light coming through the stage opening.

6. Be sure that you have the object clearly focused at this point.

7. Now you are ready to move to the next objective. The term **parfocal** is used to describe a microscope’s ability to remain in focus no matter what objective is used. The key to this parfocal ability is to always begin with the scanning objective.

8. Keep in mind that as you move up in magnification, you will **not** be able to use the coarse adjustment knob. The objectives are too long and will crack the slide. Use only the **FINE ADJUSTMENT KNOB**. When using the high dry or oil immersion objective lens.

9. Rotate the revolving nosepiece to the next objective—**low power**. You should only need to use the fine adjustment knob to sharpen the focus.

10. Now that you have the slide in focus at this power, move the revolving nosepiece to the next objective—**high power**—and repeat the process described in step 9.

11. The **oil immersion lens** can only be used with immersion oil. It is commonly needed to see very small objects such as bacteria. **DO NOT USE THIS OBJECTIVE LENS.**
Your First Observation

1. It is time to practice using the microscope. Carefully cut out a line of words from a piece of newspaper.

2. Place your sample on a microscope slide, moisten it with water, and place a cover slide on top.

3. Write the line of words, as they appear, in the space below.

4. Using the steps for focusing, examine your line of words using the scanning objective. How do they look?

5. Now view the same line of words using the low power objective. How do they look?

6. Finally, examine the words using the high power objective. How do they look?

7. Explain the differences that you have seen using each objective.
FIELD OF VISION

1. The **field of vision** of a microscope is the circle of light that is visible through the lens. How does increasing the magnification affect the field of vision?

2. The diameter of the field of vision is the length of the field from one edge to the other. To determine the diameter, use a slide that has a clear grid attached to it. The grid is composed of 1 millimeter squares. Use the scanning objective to determine the length of the diameter in millimeters. The circle represents the field of vision. See Figure 2.

![Figure 2: Determining field of vision using a micrometer slide with 1mm blocks.](image)
3. Repeat the process using low power.

4. The diameter of the field of vision using the scanning objective is:

5. The diameter of the field of vision using the low power objective is:

6. How do the diameters compare?

Please see the questions 5-8 at the end of this chapter in the Lab Report.

**DEPTH OF FOCUS**

1. Obtain a prepared slide of three or more colored threads mounted together. Using the scanning objective, focus at the point where threads cross.

2. Change to low power. Slowly focus up and down using the fine adjustment knob. How do threads appear as you focus up and down?

3. What color thread is:
   - on top?
   - in the middle?
   - on the bottom?

4. Switch to high power. Again, using the fine adjustment knob, focus up and down. How does the depth of focus at high power compare with the depth of focus at low power?
RESOLUTION

Resolution refers to the ability to distinguish two points as separate points. As you might think, resolution is dependent upon the distance between the two points. When using a microscope or any optical instrument, the closer the points are, the more difficult it becomes to see them as separate points. Numerical aperture is related to resolution, although magnification has very little to do with it. The higher the numerical aperture, the better the resolution will be. An object can be magnified larger and still be blurry, because although magnification has increased the resolution has not improved.

CARE OF MICROSCOPE

The microscope should be carried properly in an upright position. Both the hands should be used to carry the microscope, use one hand to hold the base and other grasping hand to hold the arm.

It is important to clean the objective, and ocular lenses before and after the use. Lens should be cleaned with lens paper only.

Before putting away the microscope, change the magnification to lowest power, and body tube turned down near the stage.
1. What happened to the orientation of the letter?

2. When you move the slide to your right, in which direction does the image move.

![Figure 3: The letter “e” as observed by your eye only.](image)

3. What happened to the size of field?

4. What happened to the brightness of the object?

5. When comparing low power to high power, which one has the largest diameter field of vision?

6. Which power (low or high) will allow you to see more of the object?
7. Which power (low or high) will magnify the object more?

8. While viewing an object under low power you see a small organism at the edge of the field of vision. You move to the high-power lens, but the organism is no longer visible. What has occurred? How do you correct it?

9. What common parts are found in both the compound microscope and stereo microscope?

10. You have placed a letter “e” on a slide. When viewing it with your “eye” only, the letter appears as in Figure 3. How will it appear when you view it using a microscope?

11. Suppose you measure the low-power field of vision diameter with a ruler and it is 2 mm. If high power is 10X more magnification than low power, how big will the diameter of the field of vision be using high power?