CHAPTER

1

Introduction to Botany and Microscopy

LABORATORY ACTIVITIES

Activity 1.1: Parts of the Compound Light Microscope
Activity 1.2: Viewing an Object with the Compound Light Microscope
Activity 1.3: Measuring the Working Distance
Activity 1.4: Measuring the Diameter of the Field of View
Activity 1.5: Measuring the Depth of Field
Activity 1.6: Cleaning the Lenses of the Microscope
Activity 1.7: Storing the Microscope
Activity 1.8: Stereomicroscope

GOALS

Following this exercise, students should be able to:

- Identify the parts of a microscope.
- Understand the differences between the types of microscopes described.
- Use the microscope effectively.

INTRODUCTION

Botany is the scientific study of plants. It can be subdivided and incorporated into many fields: plant anatomy, plant physiology, ecology, cell biology, molecular biology, genetics, and many others. The study of plants can be approached as an experimental science using **controlled experimentation** to test **hypotheses**. A hypothesis is a plausible explanation of natural, observable phenomena that is testable. Human interest in plants began as a practical interest in obtaining more foods, fibers, and other plant-based goods for human use. Over time, curiosity about how plants work developed and gave life to the field of botany.

In many ways, the advancements in botanical science are directly correlated to the available technology. Many tools are used in modern-day botany; however, the microscope has had a large impact on all aspects of biology. Cells were first identified and named through the study of cork. Our understanding of the cell and its components has increased as new technologies were developed.

Early microscopes were simple designs that relied on a single lens to magnify the object. These microscopes provided limited magnification, approximately equal to what one might experience with a hand lens. The **compound light microscope** is one of the most commonly used microscopes. A compound microscope sends a beam of light through a thin section of a specimen and uses multiple lenses to enlarge the image. A series of lenses is beneficial because the second lens **compounds** the magnification of the first. Most compound light microscopes are used to magnify images up to 1,000×. Magnification beyond this limit causes a problem with **resolution**. **Magnification** simply refers to the process of making an object appear larger. Resolution refers to the ability of a lens to distinguish between two closely adjacent points. Beyond a certain point, increased magnification does not result in increased resolution. Therefore, even though the image would increase in size, objects would not become any clearer.

Although widely used, compound light microscopes are limited in their effectiveness. They rely on light passing through a thin section of the specimen. These microscopes are not as useful for observing the surface of intact, multicellular organisms. For those applications a **stereomicroscope**, also called a dissecting microscope, is useful. Typical stereomicroscopes can magnify images from about 5× to 40×. Though the magnification is much lower than that of a compound light microscope, stereomicroscopes are useful in other ways. Stereomicroscopes are almost always **binocular**, having two eyepieces through which one observes the specimen. This enhances the three-dimensional appearance of the specimen. Conversely, compound light microscopes provide a two-dimensional view of an object. Student compound microscopes may be either monocular or binocular.

When higher magnification with high resolution is needed, light microscopes are insufficient. Instead, electron microscopes can be used. Electron microscopes use a beam of electrons to form the image being magnified. Electrons allow for much higher resolution, and electron microscopes provide highly magnified images with exceptional resolution.

A **transmission electron microscope** is analogous to a compound light microscope. It provides detailed images of the internal structure of a specimen. The beam of electrons must pass through the specimen, so each specimen must be carefully prepared and sliced with a diamond knife to generate extremely thin sections.

The **scanning electron microscope** provides detailed images of the surface of a three-dimensional object. Electrons bounce off the surface of the specimen instead of passing through it; therefore, the specimen does not need to be sliced and can be left intact. The primary advantage of a scanning electron microscope is the generation of a highly detailed, clear image of a structure's surface.

Although both types of electron microscopes have provided a wealth of information about cell structure, many scientists still rely on the less-expensive and more-common light microscope. Throughout this semester, you will be using light microscopes extensively to study plant anatomy and physiology. Typically, you will be using compound microscopes; however, stereomicroscopes can be used to observe some of the larger structures. In this lab, we examine both types of light microscopes and their uses.

ACTIVITIES

Name

Date Section

Activity 1.1: Parts of the Compound Light Microscope

Materials:

- Compound light microscope

1. Acquire your assigned compound microscope per your instructor's directions.
2. Refer to Figure 1-1 as you identify all the parts of the microscope described below.

The compound light microscope is a familiar fixture in many biology labs. Many are binocular, containing two **eyepieces**, whereas others are monocular and have only one eyepiece. As you look into the eyepiece, you will see the **ocular lens**. The ocular lens typically has a magnification of 10×. This should be labeled on the outside of the eyepiece. Below the eyepiece you will find the **body**, which is the housing that contains the internal structure of the microscope. It connects the ocular lens to the objective lens and keeps the lenses aligned. The objective lenses are found just above the **stage**, where the specimen is placed. Each microscope has three or four **objective lenses** attached to a revolving nosepiece. The objective lens in use can be changed by twisting the revolving nosepiece until the next objective lens clicks into place. The objective lenses have specific names. The **scanning-power objective** has the lowest magnification, usually 4×. It is also the shortest objective. The **low-power objective** is somewhat longer and has a magnification of 10×. The **high-power objective**, sometimes called high-dry power, has 40× magnification. A fourth objective, if present, is an **oil-immersion objective**; it has a magnification of 100×. This level of magnification is typically not needed to view botanical specimens. You can calculate the **total magnification** of an image as it reaches your eye by multiplying the magnification of the objective lens in use by the magnification of the ocular lens. For example, if you used the scanning lens (4×) to observe a specimen, total magnification is 40× (total magnification = 4× times 10× = 40×).

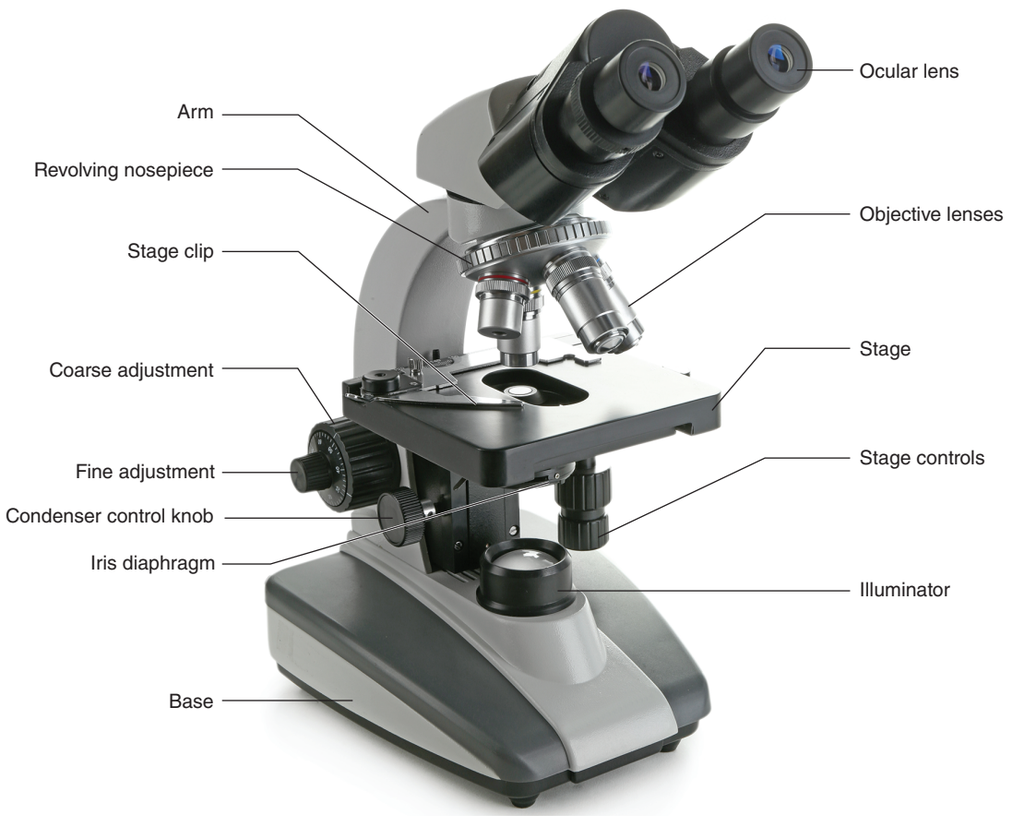

FIGURE 1-1 The parts of a compound light microscope.

The part of the microscope that sits directly on the table top is the base. Within the base is the **illuminator**; in most modern microscopes this is a light bulb. The arm of the microscope extends upward from the base and supports the eyepieces. The stage is controlled by two focus adjustment knobs. Typically, these knobs are stacked on each other and are found on both sides of the arm, near the base. The **coarse adjustment knob** is the larger of the two and moves fairly rapidly in a vertical plane. You use this for focusing on an object with the scanning- or low-power objectives. The smaller of the two knobs is the **fine adjustment knob**. It moves the stage vertically, as well; however, it moves the stage much more slowly. On the upper surface of the stage is a metal stage clip. The **stage clip** is composed of a fixed metal bracket on one side with a moveable lever (clip) on the other side. When used properly it holds the slide in place and makes the mechanical stage more useful. The stage is controlled mechanically by a set of stacked knobs, called the **mechanical stage controls**, found on the lower side of the stage. The upper knob moves the stage forward and backward, whereas the lower knob moves the slide from left to right.

Just beneath the stage is the **condenser**. You can see it through the opening in the center of the stage. The condenser is a lens that focuses light from the illuminator onto the specimen on the slide. It is controlled by a **condenser control knob** found beneath the stage near the arm. In the same area with the condenser is the **iris diaphragm**. The iris diaphragm controls how much light reaches the specimen. Most microscopes have a small lever present on the side of the condenser that controls the diaphragm. Some microscopes have diaphragms that are controlled by turning the condenser housing. Your instructor will demonstrate how to use the diaphragm on your microscope.

When carrying a microscope always use two hands. Place one hand on the base and one on the arm. It is important to be careful because these are expensive pieces of equipment.

3. Provide the total magnification for each lens below (assuming the current ocular lens is in use).

a. Scanning (4×)

_____

b. Low power (10×)

_____

c. High power (40×)

_____

d. Oil immersion (100× if present)

_____

Activity 1.2: Viewing an Object with the Compound Light Microscope

Materials:

- Compound light microscope
- Letter "e" slide

As you view your first slide under the microscope, remember that the lenses in a microscope invert the image as they magnify it. This inversion of the image is due to the nature of light reflection by a lens.

1. Obtain a prepared slide of the letter "e."

2. Observe the letter "e" slide with your unaided eyes. Diagram what you see in the space below, and make note of the orientation of the letter "e."

3. Observe the slide under scanning power (4×). Use the course adjustment knob to bring the specimen into focus. Always begin focusing on an object by using the lowest power (scanning) objective. Diagram what you see using the space below, and make note of the orientation of the letter "e" as you see it through the microscope.

4. Compare the orientation of the letter "e" as seen with the microscope with the orientation seen with the unaided eye. Do they appear the same in orientation?

5. Switch to low power. Do not move the focus knobs or mechanical stage. Simply turn the revolving nosepiece until the low-power objective clicks into place. If it does not click into place, you will not be able to see light through the eyepiece. You do not need to readjust the stage each time you increase magnification; you need only click the next objective into place and focus with the fine adjustment knob. You should be able to bring the specimen into sharp focus with just a few turns of the fine adjustment knob. This action is possible because the microscope is **parfocal.** That is, when an object is centered and in focus with one objective, it remains roughly centered and in focus when the objective is changed.

6. How does the letter "e" appear different under low power as opposed to scanning power?

7. Diagram the letter "e" as seen on low power.

8. Now switch to high power. How does the letter "e" appear now? Diagram the letter "e" as seen under high power.

Activity 1.3: Measuring the Working Distance

Materials:

- Compound light microscope
- Letter "e" slide
- Flexible plastic ruler

Notice, as you moved from relatively low magnification objectives up to the high-power objective, the distance between the objective and the slide decreased. The distance between the objective lens and the surface of the slide is called the **working distance**.

1. Measure the working distance for each lens by holding the ruler vertically alongside the stage; gently bend it to get the most accurate measurements. Carefully measure the distance between the tip of the objective lens and the surface of the slide on the stage.
   Record the working distance (in mm) for each lens (assuming the current ocular lens is in use).
   a. Scanning (4×)

   b. Low power (10×)

   c. High power (40×)

   d. Oil immersion (100× if present)

Activity 1.4: Measuring the Diameter of the Field of View

Materials:

- Compound light microscope
- Thin, flexible, transparent plastic ruler or micrometer

Each objective lens has a different **field of view**. The field of view is the size of the area that can be seen at any one time using a particular lens. You can measure the size of the field of view by placing a thin, transparent ruler across the stage of the microscope within the field of view or by viewing a micrometer if one is available.

1. Place the centimeter side of the ruler across the field of view, bring the marks on the ruler into focus using each objective starting with the scanning-power objective, and count how many tick marks are visible through the microscope. Each tick mark within a centimeter is 1 mm.
   Measure and record the diameter of the field of view (in mm) for each lens (assuming the current ocular lens is in use).
   a. Scanning (4×)

   b. Low power (10×)

c. High power (40×)

d. Oil immersion (100× if present)

Activity 1.5: Measuring the Depth of Field

Materials:

- Compound light microscope
- Crossed-threads slide

The **depth of field** is another important concept in microscopy. Depth of field refers to the thickness of the specimen that is in focus at any point in time.

1. Obtain a prepared slide of crossed threads. Move the slide so that the crossed portions of the threads are in view.
2. Observe the specimen under scanning power (4×). How many threads are in focus?

3. Switch to low power (10×). How many threads are in focus now? Can you tell which is on top of the others?

4. Switch to high power (40×). How many threads are in focus at one time? Which one is on top?

As you moved from the lowest magnification to the highest magnification, you should have noticed that fewer threads were in focus at a given point in time. Objectives that have a large depth of field allow you to see thick objects, or multiple threads, with a high percentage of the specimens in sharp focus at one time. Conversely, objectives with low depth of field only allow you to see small portions of the specimen in focus at one time.

5. Observe any other specimens your instructor has out for display.

COMPARE AND CONTRAST

1. Which objective has the shortest working distance?

2. Which objective has the smallest depth of field?

3. Which objective has the largest field of view?

Activity 1.6: Cleaning the Lenses of the Microscope

Materials:

- Compound light microscope
- Lens paper
- Lens cleaning solution

Look closely at the 100× or highest power objective lens, and you might see the words "oil immersion" imprinted on the side. These types of lenses require the tip of the lens to be submerged in a drop of oil to boost resolving power. Thorough cleaning of the lenses is always necessary following oil immersion. Although oil immersion is not usually necessary in general botany, cleaning of the lenses is also important after viewing wet-mounted slides. Always use only the lens paper and cleaning solution provided by your instructor to clean the lenses of the microscope. Follow the procedure below or watch your lab instructor demonstrate how to properly clean the lenses of the microscope.

1. Obtain lens paper and cleaning solution from your lab instructor.
2. Tear out a narrow strip of lens paper about 1" wide.
3. Lay the strip out before you, and place a drop of lens cleaner near one end of the strip of paper.
4. Holding the strip lightly at both ends, allow the spot of cleaner to come in contact with the tip of the objective lens.
5. Still holding the strip at both ends, gently allow the length of the paper to travel over the surface of the lens leaving a streak of cleaner and/or oil. Do not rub the lens with your finger. Only allow the paper to travel across the surface very lightly until there is no longer a streak of cleaner produced.
6. Repeat with the other lenses.

Activity 1.7: Storing the Microscope

Storage is an important component of caring for a microscope to ensure it is in working order the next time you use it. Follow these steps to properly store your microscope.

1. Turn off your microscope and unplug it.
2. Adjust the stage to its lowest position.
3. Place the scanning objective (lowest power) in the operating position.
4. Coil the cord around the base, making sure it does not come in contact with the substage apparatus.
5. Carefully carry the microscope to the proper storage location.

Activity 1.8: Stereomicroscope

Materials:

- Stereomicroscope
- Letter "e" slide

Stereomicroscopes—also known as dissecting microscopes—are relatively simple in design. They have many of the same parts as a compound microscope. They are typically equipped with binocular eyepieces and objective lenses that are located on a turret. The objective turret twists so that you can change objective lenses. The base has a stage plate and usually has one or two stage clips to anchor the slide. These microscopes can have two illuminators. Most stereomicroscopes have an illuminator just below the objectives that shines light on the surface of a large object from above. Sometimes, one is also present in the base, beneath the stage plate, to illuminate the specimen from below.

1. Pick up your stereomicroscope as directed by your instructor.
2. Identify the parts of the microscope.
3. Obtain a prepared slide of the letter "e." Observe it with your unaided eye. Diagram what you see in the space below.

4. View the letter "e" slide under the dissecting scope using the lowest possible magnification. Diagram what you observe in the space below.

5. How does the letter "e" compare as viewed through the microscope versus how it appears to your unaided eye?

6. Switch to a higher magnification. How does the "e" appear now?

7. Observe any other specimens your instructor has on display.

COMPARE AND CONTRAST

1. How does the letter "e" appear differently when viewed through a stereomicroscope as opposed to a compound microscope?

2. Describe the benefits of both the compound microscope and the stereomicroscope.

CASE STUDY—Adventures in Microscopy

Savannah is enrolled in a Botany course and is using a microscope for the first time. She needs to view some specialized root structures on a slide purchased from a biological supply company. These purchased slides are special because they are differentially stained, which makes them quite valuable and expensive. She sets up the microscope on her desk, places the slide in the clips on the stage, and looks through the eyepiece. Then, she decides that because the cells are very small, she will need to start out with a higher magnification than the scanning power, so she clicks the 100× magnification lens into place. While looking through the eyepiece, she tries to bring the object into focus by drawing the stage toward the objective lens with the course adjustment knob. She does not see the image come into focus, so she continues to coarse adjust until she is startled to hear the distinctive *CRACK* sound of glass breaking. The rest of the class hears the noise, and Savannah sees her teacher's head

pop up to investigate the situation. She is upset when she looks down at the stage and sees that the expensive slide is broken into pieces. Her teacher comes over and explains what she did wrong and how to prevent this situation from repeating itself.

1. What did Savannah do wrong? What part or parts of proper microscope procedure did she not follow?

2. How exactly can she make sure not to break any more slides?

3. How should the broken slide be properly disposed of in the lab?

STUDY GUIDE

- Be able to define the terms in bold.
- Be able to describe the correct operation of the compound light microscope.
- Be able to differentiate between the lenses on the compound light microscope and their characteristics.
- Understand the relationship between magnification and diameter of field, working distance, and depth of field.

Conclusions

1. How do you calculate the total magnification provided by a compound light microscope?

2. What total magnification is provided by the scanning, low-power, and high-power objectives?

3. What is the field of view? How does it change with increasing magnification?

4. What is the depth of field? How does it change with increasing magnification?

5. What is the working distance of an objective? How does it change with increasing magnification?

6. What is the advantage of a parfocal microscope?

7. What is resolution? How does it affect the maximum available magnification of a microscope?

8. Describe the difference in how specimens appear with a stereomicroscope versus a compound microscope.

9. If you wanted to see a highly detailed image of the surface of a pollen grain, which type of microscope would be optimal? Why?

10. You are observing a slide of onion epidermal peel using the low-power objective on your lab microscope. What is the total magnification of the epidermal peel as you see it?

11. Describe three differences between your lab's compound microscopes and stereomicroscopes.

12. On Figure 1-2, label the diagram of a microscope.

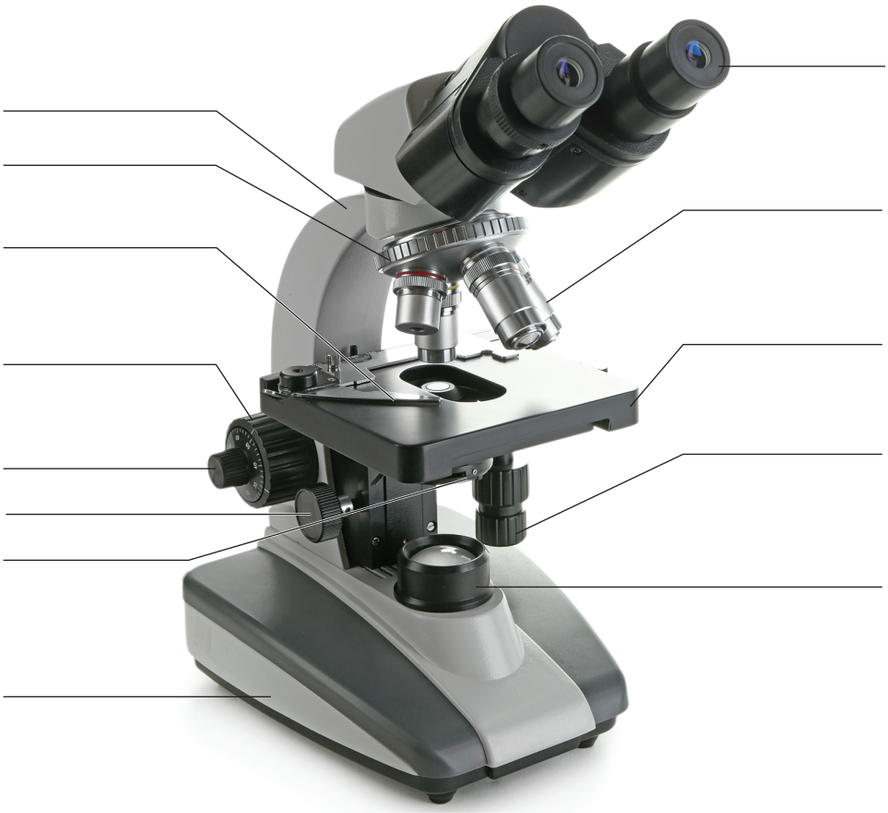

FIGURE 1-2 The compound light microscope.
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