Shutterstock.com

1 Laboratory

Basic Laboratory Skills

LEARNING OBJECTIVES

Students will….

- acknowledge understanding of laboratory policies and procedures by reading the lab manual, following the instructor's directions, and signing off on the "Safety Statement."
- manipulate the main components of a compound light microscope as they observe a prepared slide and demonstrate proper microscope use to the instructor.
- determine total magnification, field of view, and cell size by using a prepared grid paper and recording calculations.
- practice basic microscopy and observation skills by making slides of live specimens; labeling total magnification, field of view, and cell size; and listing basic characteristics of the specimens that will be reviewed by the instructor and peers.
- practice using specimen information and microscope labels derived by peers to identify unknown specimens.

INTRODUCTION

In this laboratory session you will become familiar with the safety guidelines that should be followed throughout the semester, review general policies and procedures for the course, and become familiar with basic laboratory skills. Specific safety concerns will be addressed in each individual session.

Read through the policies and safety guidelines in the "Getting Oriented" portion of this laboratory manual. You will be addressing these topics in more detail with the help of your laboratory instructor.

Activity One: Basic Microscopy

BACKGROUND

Your instructor will demonstrate the major components of the compound light microscope. The information contained in the demonstration is summarized for you in Appendix A. You can always turn to this appendix to learn about the main components of a microscope, different types of microscopes, the care and handling of a microscope, and other topics in basic microscopy.

▶ PROCEDURE

Using the Compound Light Microscope

1. On your compound microscope, check to see that the lowest power objective lens is directly above the center of the hole in the stage and as far as it will travel away from the stage. Failure to do this may result in smashing the objective into the slide as you try to focus on a specimen.
2. Clean the objective and ocular lenses with a clean piece of lens paper. **Never use anything else to clean the lenses.**
3. Turn on the illuminator to the halfway point and rotate the iris diaphragm to its mid-setting aperture (opening).
4. Obtain a prepared slide from the side counter and place the slide on the stage to be examined. Make sure that the "specimen" is on the top surface of the slide. Place the slide in the slide clamp on the stage.
5. Make sure that the 4× objective lens is in place over your slide. With the coarse focus knob, carefully adjust the distance between the slide and the 4× objective lens to about one inch.
6. Look through both oculars and adjust them until you can comfortably see one image with both eyes open. You may have to move the oculars closer together or push them apart.
7. Once the oculars are properly adjusted, use the coarse focus knob to slowly increase the distance between the lens and the specimen until you get an image. Using the mechanical stage controls, move your slide until the image is in the center of your field of view.
8. Check to see which ocular has a focus ring. Look through only the ocular **without** the focus ring first and bring the image into focus using the fine focus knob. Next, look through the ocular with the focus ring and bring the image into focus using the focus ring. Then look at the image using both eyes.
9. Use the fine focus knob as necessary to improve the focus. You may also need to adjust the amount of light passing through the specimen by rotating the iris diaphragm.
10. To obtain greater magnification, rotate the next higher power objective lens (10×) into position over the specimen. Be sure that the objective lens does not strike the slide when you move it into place. **Use only the fine focus while studying a**

specimen under 10× and 40× higher powers to avoid complete loss of focus and possible contact between the objective lenses and the slide. For the greatest magnification that you will use, rotate the nosepiece to the next position (40× objective lens). Most microscopes are now designed so that the specimen remains in approximate focus without adjustment when switching between objective lenses. These lenses are termed **parfocal**.

11. To observe a different slide, rotate the lowest power objective back into position above the hole in the stage and repeat steps 2–7 to focus on a new specimen.
12. Always store the microscope by returning the lowest power objective to center stage and removing any slides.

Activity Two: Determining Total Magnification

The total magnification is the magnification of the ocular lens multiplied by the magnification of the objective lens, such that a 10× objective lens used with a 10× ocular lens gives a total magnification of 100×. What is the total magnification if you are examining a slide using a 4×, a 40×, and a 100× objective lens? Complete Table 1-1 and check all of your answers with the laboratory instructor before proceeding.

Table 1-1. Total magnification for each objective

OBJECTIVE LENS	OCULAR LENS	TOTAL MAGNIFICATION
4×	10×	
10×	10×	
40×	10×	
100×	10×	

Activity Three: Determining Field of View and Cell Size

Basic to any microscopic investigation is developing an appreciation for the size of the organism being examined. To determine the size of an organism, you have to determine total magnification and field of view. The field of view is what is seen through the eyepiece of the microscope.

▶ PROCEDURE

Field of View

1. To determine the field of view, place a piece of transparent grid paper (1 mm × 1 mm) under a microscope at the lowest total magnification (40×). How many grid lines can you observe across the center of the field of view at 40×? This is your field of view in mm.
2. Record it in Table 1-2.
3. The field of view, which is the area you can see through the microscope, is a constant size at a set magnification for the light microscope. In Figure 1-1, the field of view is 2.6 mm at 40×. This is determined by counting the number of squares across the diameter.

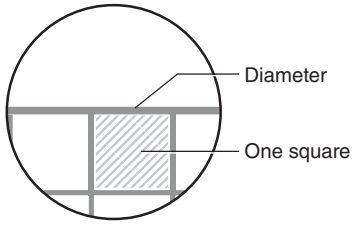


Figure 1-1. Sample Field of View (the white circle) while Examining a Grid

Field of View at Higher Magnifications

1. Because the divisions on the ruler are too thick or too far apart to focus at high magnifications, you will have to estimate the field of view at higher magnifications using the field of view you obtained above. For example, in order to compare the field of view between 40× and 100×, set up the following ratio:

   40/100 = 0.4

   Therefore, at 100×, you should be able to see only 0.4 of what you saw at 40×.

2. You will then multiply the field of view at 40× by the factor you just calculated. In the example in Figure 1-1, the field of view at 40× was 2.6 mm. Simply multiply 2.6 by 0.4 to get the approximate width of the field of view at 100×.

   2.6 mm × 0.4 = 1.04 mm (approximate field of view at 100×)

   Therefore, at 100×, your field of view is 1.04 mm.

3. Using your own measurement of the field of view at 40×, determine the field of view at the magnifications listed in Table 1-2.

Table 1-2. Field of view for each objective on the microscope

TOTAL MAGNIFICATION	40/TOTAL MAGNIFICATION	FIELD OF VIEW (mm)
40×	1	
100×		
400×		
1000×		

▶ PROCEDURE

Cell Sizing

1. To determine cell size, you can count the number of cells that fit along the diameter of the field of view and then divide the field of view by the number of cells.
2. For example, in Figure 1-2, the field of view when using the 10× objective is 2 mm. If eight plant cells extend across the diameter of the field of view, then each cell is 2/8 or 0.25 mm long. Remember that the field of view changes depending on the power of the objective.
3. You will need to determine the size of cells in the next activity. See your laboratory instructor for more information.

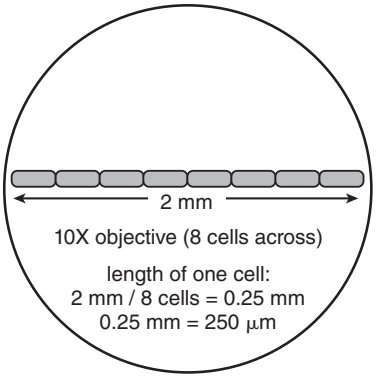


Figure 1-2. Cell Sizing

Activity Four: Examining Live Specimens

BACKGROUND

All science begins with **observations.** For example, suppose a scientist discovers that trees near the smokestack of a factory are dying. This is an example of a simple observation. The scientist then decides she wants to determine if other organisms in the area are also dying. She sets out traps for small mammals. She puts samples of pond water on glass slides and examines them under a microscope to see if they contain protists. She uses a pH meter to determine if increasing acidity is killing invertebrates that live in the soil. She employs a gas analyzer to calculate the percentage of toxic sulfur dioxide in the air around the factory. All of these activities are observations. The traps, microscope, pH meter, and gas analyzer are tools that aid in her investigation.

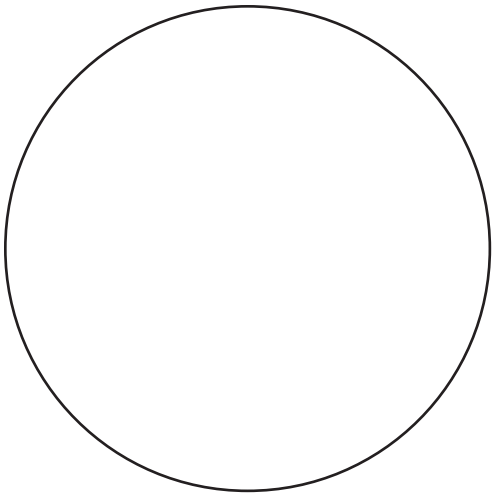
This implies that the foundation of all science is careful observation. To illustrate this, let us retell the following story (Beveridge 2017). There was a veteran and distinguished doctor who, while on rounds, was lecturing interns about the importance of careful observation. As he escorted his covey of eager students down the halls, he remarked on the significance of this or that symptom. The interns expected him to pick up a urine sample to check it for cloudiness, etc. They did not expect him to place a finger in the sample and then in his mouth. "One of the symptoms of

diabetes mellitus, sweet-tasting urine," he remarked as he passed the specimen to the nearest intern. "Check it out." After each of the interns repeated his gesture, trying diligently not to show the slightest bit of hesitation and discomfort at licking a finger dipped in urine, the good doctor launched into another sermon on observation. He ended by pointing out that if they had practiced good observational skills, they would have noted that he stuck his second finger in the urine and his third in his mouth.

We want your observational skills to be better than those of the interns in the story. To start refining these skills, you will have to observe an unknown organism—Organism X, a microscopic organism found on Planet 181.

▶ PROCEDURE

1. You are part of a remote team (your colleagues in your student group) exploring a new habitat, and your task is to describe a type of organism you have found in the area—Organism X.
2. Prepare a wet mount of your specimen:
    a. Obtain a clean glass slide and take it to the side counter near the culture you plan to examine. Place your slide on a flat surface.
    b. Using the dropper labeled for your specimen, pull a small amount of liquid from the culture jar. (Ask your laboratory instructor about the best way to do this to assure that you actually have live material in your dropper.) Place a small drop on the slide. Bring the slide back to your lab table. Obtain a new coverslip and place one edge of your coverslip on one side of your specimen and, with your finger, hold the other edge of the coverslip at an angle over the specimen.
    c. Gently lower the coverslip onto the specimen. Do this slowly and carefully to avoid trapping air bubbles. Wipe up any excess water with a Kimwipe tissue.
3. Draw your specimen in the space provided on the next page. Make sure you include the total magnification, field of view, and cell size in your drawing. Write your observations about distinguishing characteristics.
4. Write a useful and detailed description of your organism so that future scientists (colleagues from other student groups) visiting the area could use it to identify the same organism. Your peers will be using your descriptions. Write the information to be included in Table 1-3.
5. Exchange your description with someone at another table. **NOTE:** Only exchange your written description; no pictures.
6. Using the description you receive from the other person, try to identify their Organism X. If you cannot, what information would you need to be able to do so? Give feedback to the colleague who provided you with a description. Your lab instructor will also help you identify the organism.
7. Once you receive feedback from the person who examined your Organism X, edit your description.

Picture of Organism X:	
Total Magnification:	
Field of View:	
Cell Size:	
Name:	

Your Own Observations:

Table 1-3. Description of "Organism X" to share with other groups

REFERENCE

Beveridge, W. I. B., 2017. The Art of Scientific Investigation. Edizioni Savine.

[Questions]

Name Lab/Section

Partner's Name (if applicable) Date (of Lab Meeting)

1. What must you know in order to calculate total magnification when using a microscope? Give an example of how you would do this.

2. As you increase magnification, what happens to the field of view? Draw a sketch of an organism you saw under your microscope at 40× and how it would look at 100× or 400× (whichever was the most appropriate final magnification).

3. When reading the description of Organism X from another group, what information was most useful in helping you determine the identity of the unknown? What information was not as useful?

12