Honors Biology
Introduction to Microscopy 2020

Name: _____
Date _____ Block _____

Viewing the Hidden World – Part 1

An introduction to microscopes and their proper use

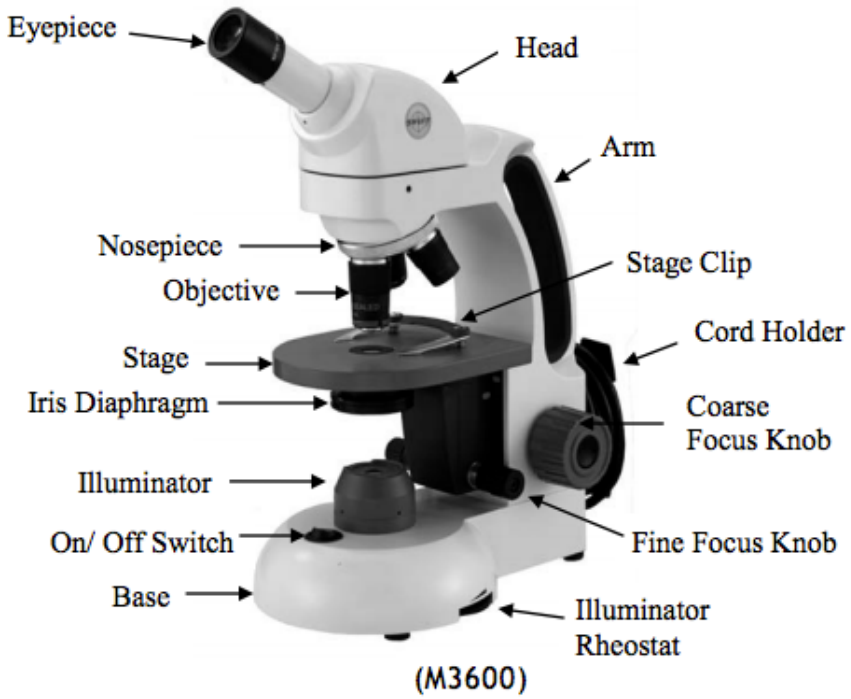
You are responsible for ALL of the information contained in this lab. Be sure to READ EVERYTHING carefully!

Introduction: The microscope is one of the most important tools used by biologists. Unaided, the human eye cannot distinguish objects much smaller than 0.1 mm. The microscope *magnifies* objects, or makes them look larger. Microscopes also increase the *resolution* of an image. Resolution is the ability to distinguish between two points that are close together. The greater the resolution, the sharper the image. Together, magnification and resolution enable the microscope to show details that are too small to be seen by the unaided eye.

The invention of the *compound microscope* was an important advancement. Earlier microscopes used only a single lens and had to be held very close to the eye. The compound microscope, in contrast, has two lenses. Together, they magnify objects more than a single-lens microscope but need not be held so close to the eye.

Prelab Activities:

A. Review the following diagram, becoming familiar with the location and function of each of the labeled parts. Identify each of the labeled parts on your actual microscope. ***With a partner, quiz each other on the parts of the scope.*** Note the differences between the diagram below and your own microscope.



Please record 2 or more differences between the diagram of the microscope above and your microscope in the space below:

B. As shown in the diagram, the lens system includes the *objective lenses*, which are in the nosepiece of the microscope, and the *ocular lens*, or eyepiece. Stamped on each lens is its magnification power. For example, a lens marked 4X is a four-power lens and makes objects seen through it look four times their actual size. If no number is stamped on the eyepiece, assume it is 10X. Compound microscopes generally have between two and four objective lenses of differing power. The *total magnification* of a microscope at a given setting is found by multiplying the objective lens magnification by the eyepiece magnification. For example, a microscope set with a 40X objective and a 10X eyepiece magnifies an object 400X.

Find the total magnification of your microscope under low, medium and high power by filling in the chart below.

	Magnification of Eyepiece	Magnification of Objective	Total Magnification
Low Power			
Medium Power			
High Power			

The lighting system of the microscope is under the stage. It sends light through the specimen and up through the lenses to the eye, and consists of either a mirror or a light source. The *diaphragm*, found under the stage, is an adjustable opening to control the amount of light passing through the specimen. Some specimens are best viewed in dim light, others in bright light.

The focusing system of a microscope changes the distance between the specimen and the objective lens in order to bring the specimen into sharp focus. The focus is controlled by two knobs: a *coarse adjustment* knob and a *fine adjustment* knob.

<u>Functions of the Parts of the Microscope</u>:

Briefly describe the function of the following microscope parts:

1. Eyepiece: _____
2. Objectives: _____
3. Light source/Mirror: _____
4. Stage Clips: _____
5. Diaphragm: _____
6. Coarse adjustment: _____
7. Fine adjustment: _____
8. Knobs under stage (stage controls): _____

✶✶ Rules for Microscope Use ✶✶

1. Carry the microscope with two hands, one on the base and one on the arm.
2. Set the microscope at least 10 cm from the edge of the table or lab bench.
3. Use only lens paper to clean lenses and other glass surfaces on the microscope.
4. Some scopes use mirror as a light source. NEVER USE DIRECT SUNLIGHT as a light source.
5. Always place a specimen on a slide and use a coverslip. Do not place anything directly on the stage. (Exceptions on dissecting scopes)
6. Attempt to look at the object being viewed by keeping both eyes open. The eye not being used will naturally drop out of focus.
7. To focus on a specimen, ***always begin by using the low-power objective*** and the coarse adjustment knob. The fine adjustment knob should then be used to refine the sharpness of the image.
8. When changing from low to high power, make sure the specimen is in the center of the field of view. Now turn the revolving nosepiece to bring the high power objective into place. You will hear a distinct "click" when it snaps into place. **Use the fine adjustment knob to focus. NEVER TURN THE COARSE ADJUSTMENT KNOB WHILE USING THE HIGH POWER OBJECTIVE!** Doing so may break the slide and damage the lens.
9. When cleaning up, make sure your microscope stage is clean and dry. Switch to the low power objective, wrap the power cord neatly around the base, replace the dust cover if available and store the microscope *neatly* in the closet.

Procedure:

A. Setting up the Microscope

Make sure the low power objective (4x) is "clicked" into place. If the objective is out of line with the arm, no light will be able to get through. Adjust the light so it is reflected upward through the opening in the stage. Locate the diaphragm directly under the stage. Change the setting of the diaphragm while looking through the eyepiece.

What occurs when you change the setting of the diaphragm?

_____

_____

B. Preparing a Wet-mount Slide

Specimens that are viewed under the microscope are mounted on one of two types of glass slides. *Prepared slides* are specially treated for repeated and indefinite use. Schools often purchase prepared slides from biological supply companies for students to view. *Wet-mount slides* are for temporary use. Your next task is to make a wet-mount slide.

1. Review the "Rules for Microscope Use" found above and follow them at all times!
2. Rotate the revolving nosepiece so that the low power objective (4x) is snapped into place. Look through the eyepiece. Adjust the light and the diaphragm until the maximum amount of light is passing through the stage opening.
3. With your scissors, cut out the letter "d" from the newsprint (use the *smallest* 'd').
4. Place it on the glass slide *right-side up* so as to look like (d). THIS IS A VERY IMPORTANT STEP!
5. Cover it with a clean cover slip. See **Figure A** below. Notice the 'd' is in the proper reading position.

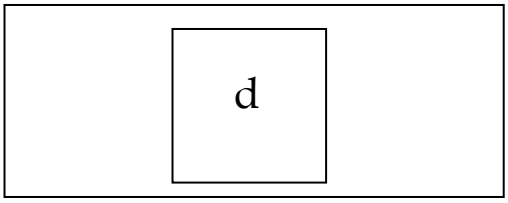


Figure A

6. Using your eyedropper, place a drop of water on the edge of the cover slip where it touches the glass slide. The water should be sucked under the slide if done properly. You may need to put a drop of water on the "d" before placing the coverslip on top. This will work too, but the "d" may change its orientation. *It is important that the "d" be right-side up when viewing under the microscope.*
7. Place the slide on the stage, making sure the "d" is facing the normal reading position (*See Figure A*). Using the course adjustment and low power, focus the image until the "d" can be seen clearly. Draw what you see in the space below (Figure 1-a).

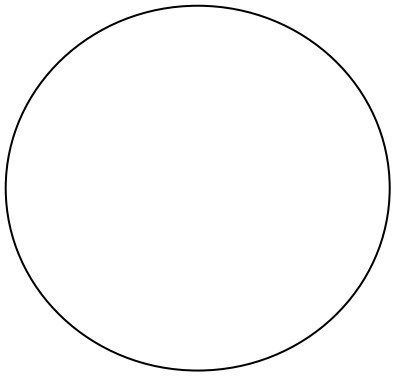


Figure 1-a
Magnification _____

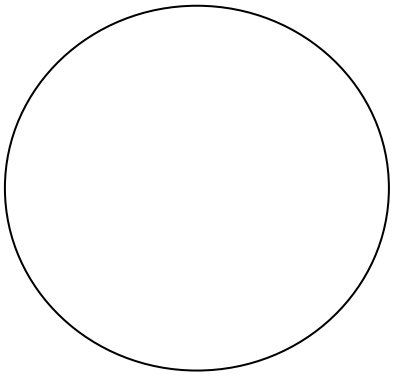


Figure 1-b
Magnification _____

8. Compare orientation of the letter "d" as it looks to the naked eye and as it looks through the microscope.

_____

_____

9. Look through the eyepiece at the letter and using the stage controls, move the slide slightly to the left, to the right, away from you, and toward you. What do you notice about the direction the image moves as compared to the direction you actually move the slide?

_____

_____

10. Again ***center*** the letter "d" in the field of view. Make sure the "d" is in focus. To observe the specimen under high-power magnification, turn the nosepiece until the high-power objective "clicks" into place**. Use only the fine adjustment knob to bring the specimen into focus.** Sketch what you see in the circle in Figure 1-b. Record the magnification. How does the ink that was used to print the letter differ in appearance when you see it with the unaided eye compared with the way it appears under the microscope?

_____

_____

11. Prepare a wet-mount slide of two human hairs. Use one of your own and "borrow" one from a classmate whose hair is a different color. Cross the hairs to make an "X." Use low power to focus on the center of the "X" and then switch to high power.

    Can you focus sharply on both hairs at the same time? _____
    Slowly change the fine adjustment to focus on one hair at a time.

Sketch what you see under high power in the circle in Figure 2. Record the magnification.

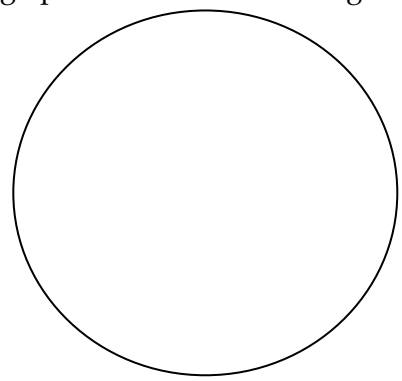


Figure 2
Magnification _____

Questions: (*Answer in complete sentences where appropriate*)

1. Why is only the fine adjustment used under high power?

2. Using the idea of depth of field, how can you tell which hair was above the other?

3. Imagine you carefully prepare a wet mount of living cells. You put the slide on the stage and look through the eyepiece. You see no light at all; the entire field of view is dark. List three things you could check to ensure that enough light was passing through the body tube?

4. Explain why a specimen to be viewed under the microscope must be thin.

5. Suppose you were observing a living organism under the microscope and noticed that it moved toward the bottom of the slide and then it moved to the left. What does this tell you about the actual movement of the organism?

6. Microscope A has a 20x eyepiece and a 30x objective; microscope B has a 5x eyepiece and a 40x objective. Which microscope has the greatest magnification? _____

7. What is the correct way to carry the microscope? _____
8. Why should you never use the coarse adjustment when viewing under high power?

Viewing the Hidden World-Part 2

Investigating the Field of View

The *field of view* is the circular area seen by looking through the eyepiece. The diameter of the field of view can be used to estimate the actual size of a specimen. In part II of the microscope lab you will measure your field of view and use that measurement to estimate the size of a unicellular organism.

Procedure:

1. Record magnification of the microscope you will be using below:
   Low: _____ Med: _____ High: _____

2. Examine the markings on a metric ruler. Decide which marks indicate millimeter lengths. To measure the field of view, place the ruler on the stage so that it covers half of the stage opening as in Figure 1.

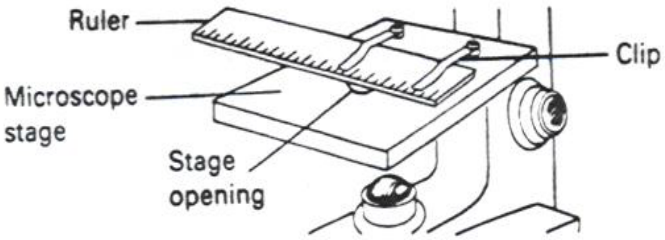


Figure 1

3. Observe the ruler under low power and focus on the millimeter marks. (See Figure 2) Move the ruler slowly until one of the millimeter marks is all the way to the edge of the field of view. Estimate the diameter of the field of view under low power to the nearest tenth of a millimeter and record in the chart below. Note that 1 millimeter is the distance from the middle of one mark to the middle of the next mark.

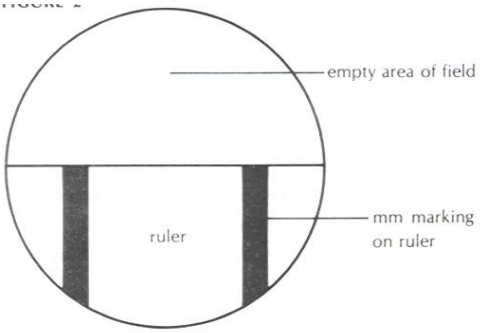


Figure 2

4. Since microscopic dimensions are very small, it is useful to measure them in microns (μm) rather than in millimeters. One millimeter equals one thousand microns. (Microns are also called micrometers). Convert each diameter measurement into microns and record in the data table below.

Data Table 1: Dimensions of the Field of View- Low Power

	Diameter (mm)	Diameter (μm)
Low Power		

Dimensions of the Field of View- Medium & High Powers

The technique used to find the field of view for low power cannot be implemented to find the high power field of view diameter. This diameter may be calculated by dividing the low power diameter measured above *by the factor of difference* in magnification between the medium/high and low powers (*Factor of Difference:* High or medium power magnification divided by low power magnification). Use these values to calculate the diameter of the Medium and High power diameter. ***Show all work below and use units***.

Record your diameter values of the field of view on the class data table on the white board. Once the entire class has recorded their data, find the average diameter of the field of view for each magnification and record in the table below. This will be the 'official' diameter of our classroom microscopes going forward.

Data Table 2: Class Averages for Diameter of Field of View

Magnification	Average Diameter (μm)
Low Power	
Medium Power	
High Power	

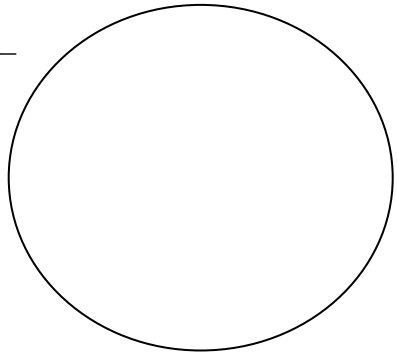
Which has the larger field of view, low or high power? _____

Observing a Prepared Slide

1. Examine a prepared slide of your choice under low and then high power. Sketch what you see under high power. Label the magnification and sample.

Sample: _____

Magnification _____



2. Based on your determined dimensions of the high power field of view, estimate the length of one of the sample cells in microns. _____
(Hint: Begin by estimating how many cells could line up across the diameter of the field of view.)
Show calculations below.

Questions & Analysis: *Answer in complete sentences where appropriate.*

1. Why is the diameter of the field of view under low power different from the diameter under high power? _____

2. Should the length of your specimen change when you switch from low to high power? *Explain your answer.* _____

3. Find the diameter of the high power field of view with a low power objective marked 10x, a high power objective marked 40x, and the low power field of view diameter is measured to be 1800 microns. (*Show work*)

4. When searching a slide for a small object under the microscope, with which objective is it best to begin? Why? *Explain as it connects to the difference in the size of the field of view between low and high power.* _____

5. Why can't you use the technique used for finding the diameter of the low power field of view for the high power field of view diameter? _____