

MICROSCOPE LAB

PART I: COMPOUND MICROSCOPE

OBJECTIVES: After completing this exercise you should be able to:

- ▶ Demonstrate proper care and use of a compound microscope.
- ▶ Identify the parts of the compound light microscope and describe the function of each part.
- ▶ Compare magnification, resolving power, and contrast.
- ▶ Demonstrate proper technique of preparing a wet mount slide.
- ▶ Demonstrate inversion and depth of field.
- ▶ Use the compound microscope as an instrument of measurement.

INTRODUCTION:

The unaided human eye can detect objects as small as 0.1 mm in diameter. Most cells are between 0.01 mm and 0.1 mm in diameter and cannot be seen without a microscope. A microscope contains one or more lenses and is used to view detail that cannot be seen with the unaided eye. The light microscope, by virtue of its lens system, extends our vision a thousand times so that object as small as 0.1 micrometer (μm) in diameter can be seen. The electron microscope further extends our viewing capability down to 1 nanometer (nm). At this magnification it is possible to see a virus and the outline of individual protein or nucleic acid molecules. A lens functions by *refracting* (bending) light rays coming from an object and focusing them to form an image of that object. Refraction of light is due to the angle at which it passes from one transparent medium to another (for example, air to glass) and the difference in density between the media. A magnifying glass is a simple light microscope. The microscope consists of a set of lenses that focus an enlarged image of an object on the retina of the eye. The greater the area of the retina covered by the image of a specimen, the greater its magnification.

A: PURPOSE OF THE MICROSCOPE

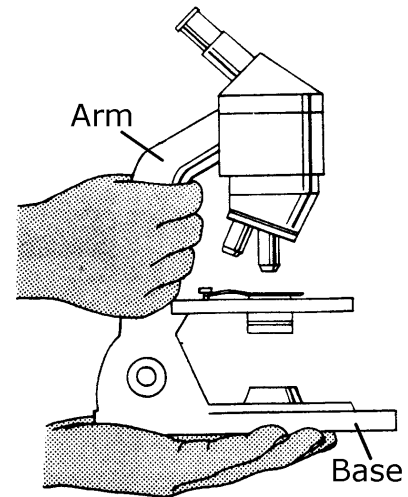
The microscope is useful in making observations and collecting data in scientific experiments. Microscopy involves three basic concepts:

Magnification: The degree to which the image of a specimen is enlarged.

Resolving power: How well specimen detail is preserved during the magnifying process.

Contrast: The ability to see specimen detail against its background. Stains and dyes are added to sections of biological specimens to increase contrast.

The microscope is an expensive precision instrument. When removing the microscope from the storage area, always grasp it with both hands. Place one hand around the arm and the other hand firmly under the base. Hold it close to your body for stability. Once you reach your work area, set the microscope down gently on the table with the arm toward you.



1. Get a microscope from the microscope cabinet and bring it back to your desk.

B: THE COMPOUND MICROSCOPE

This section covers the parts of a compound microscope. Make sure you can identify each of the parts listed in this section on your microscope.

1. Support Structures

- Arm:** Supports the body tube and the stage of the microscope
Stage: Platform where the slide is placed for viewing
Stage clip: Holds the slide firmly in place on the stage
Stage opening: The hole in the stage that allows light to pass from the lamp, through the specimen, and into the body tube.
Base: Lowermost part of the microscope; provides a firm and steady support
Body tube: Holds the eyepiece lens and objective lens at the correct distance for magnification.

Rotate the coarse focus knob. Does the stage or body tube move?

2. Lighting

- Lamp:** Provides the light needed to view the specimen.
Diaphragm: A disk located directly below the stage of the microscope; regulates the amount of light passing through the stage opening.
Condenser: Focuses the beam of light; located below the stage

Does your microscope have a condenser? _____

Describe the diaphragm on your microscope. _____

What diaphragm setting (number) allows the most light to pass through the specimen?

What diaphragm setting (number) allows the least amount of light to pass through the specimen?

3. Focus

Coarse focus knob: Larger knob used to elevate or lower the body tube or stage a large distance with each turn.

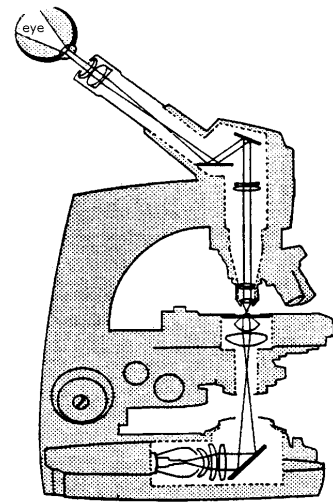
Fine focus knob: Smaller knob used to elevate or lower the body tube or stage a small distance with each turn; used to make fine adjustments when focusing on a specimen.

Where are the coarse focus knobs located on your microscope?

Where are the fine focus knobs located on your microscope?

4. Optics

The compound microscope consists of a set of lenses that gather light transmitted through a specimen and focus this light on the retina of the eye. The diagram below shows the path of light as it passes from the lamp, through the microscope, and into the eye.



The compound microscope has at least two lens systems: an **eyepiece** that you look into and an **objective** that magnifies the specimen.

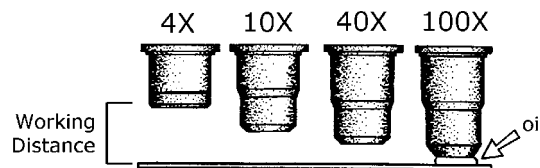
Eyepiece lens: Located in the upper end of the body tube and focuses light on the retina of the eye. The power of the eyepiece is usually 10X.

How many eyepieces does your microscope have? _____

Is it monocular or binocular? _____

Objective lenses: Attached to the revolving nosepiece. The number and magnification of the objective lenses will vary with the type of microscope. The objective lenses are housed in one end of several steel tubes that are threaded into the **revolving nosepiece**. The desired objective lens is placed in position by rotating the nosepiece until it clicks into place. The microscopes used in this class have either three or four objective lenses. The objectives include the scanning lens (4X), lower power lens (10X), high power lens (40X), and the oil immersion lens (100X) in some microscopes.

The drawing below shows the distance between the objective lens and the slide. This distance decreases with higher magnification; therefore it is important to use care when focusing with higher magnification. Only the fine focus knob should be used. Notice the oil immersion lens requires a drop of oil between the lens and the slide.



The Lens Table below will help you see the relationship between the unaided eye and the magnification made possible by the light microscope. The magnification is marked on the housing of each lens. The power of the microscope is determined by multiplying the power of the eyepiece lens times the power of the objective lens. The objective lenses have a color coded ring around each lens which indicates the magnification of that lens.

- Complete the Table below.

Lens	Lens Magnification	Ring Color	Total Magnification
Eyepiece			
Scanning			
Low Power			
High Power			

C: FOCUSING THE MICROSCOPE

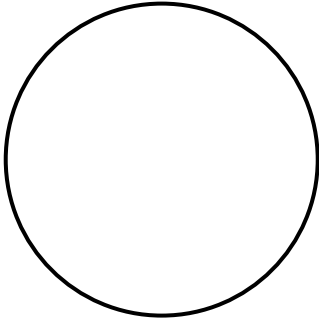
- Obtain a prepared slide from the supply area.
- Make sure the scanning lens or the lower power lens is in place.
- Raise the body tube or lower the stage just enough to allow you to place the slide on the stage without hitting the objective lens.

9. Place the slide on the stage of your microscope and clip it into place. Move the slide so the specimen is over the stage opening.
10. While looking at the microscope from the side, move the body tube all the way down or move the stage all the way up.
11. While looking through the eyepiece, move the body tube up or move the stage down until the specimen comes into focus.
12. Adjust the diaphragm opening until you have the best view of the specimen.
13. With the specimen in focus and positioned in the center of the field of view, rotate the nosepiece lens to the high power objective (40X). **DO NOT move the coarse focus.** Only fine focus should be necessary to bring the specimen into sharp focus. The ability of the microscope to remain in focus when switching from one objective lens to the next highest power is called **parfocal**.
14. Adjust the diaphragm opening until you have the best view of the specimen.
12. Have your partner repeat steps 6 – 12.
13. Return the prepared slide to the supply area.

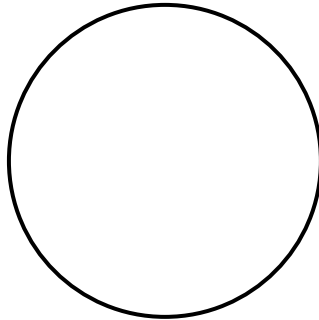
D: SPECIMEN ORIENTATION

14. Use the prepared slide of the letter "e".
15. Place the letter e slide **right** side up on the stage with the low power objective lens in place. Center the letter in the field of view.
16. Bring the "e" into focus under low power.
17. Draw the "e" as you see it through the eyepiece with the low power lens in place.
18. Bring the "e" into focus under high power.
19. Draw the "e" as you see it through the eyepiece with the high power lens in place.

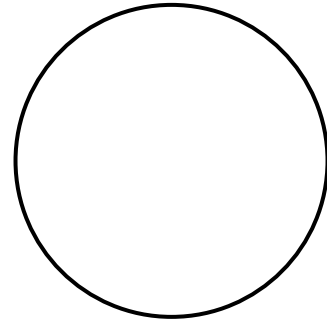
**e viewed without
Microscope**



**e viewed under
Low Power**



**e viewed Under
High Power**



20. With the low power objective lens in place, move the slide to the right while watching the image through the microscope.

In what direction does the image move? _____

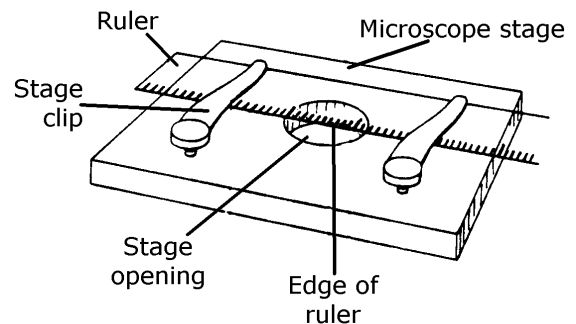
21. Move the slide away from you. In what direction does the image move?

What is the relationship between the movement of the image and the movement of the object?

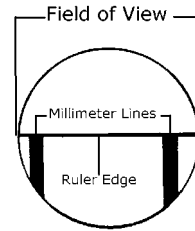
E: MICROSCOPE MEASUREMENT

Most of the objects you view under the compound microscope are smaller than two millimeters. Obviously, measuring these microscopic objects could prove to be quite difficult and inexact if millimeters are used as the unit of measure. To solve this problem scientists divide the millimeter into 1000 smaller units called micrometers (μm). Tiny objects can then be accurately measured in micrometers. In this section you will learn how to estimate the size of the tiny organisms you view under the compound microscope.

22. Obtain a transparent plastic ruler from the supply area.
23. Place the plastic ruler on the stage so that the ruler's edge is centered in your field of view under low power. Make sure you use the millimeter side of the ruler. Use the diagram below for help.



24. Position the ruler so one of the millimeter marks is just visible to the left in your field of view. Use the diagram at the right for help. Notice the distance between the mark on the left and the next mark is one millimeter. Estimate the remaining distance in decimal fractions of a millimeter across the diameter of the field of view.



What is your total field of view size in millimeters under low power? _____

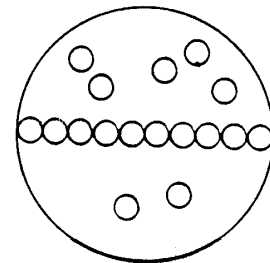
25. What is the low field diameter in micrometers (μm)? ($1 \text{ mm} = 1000 \mu\text{m}$)

26. Switch to high power. Look at the marks on the ruler. You will find that the high power field of view is less than 1mm or $1000\mu\text{m}$. For that reason, it is difficult to estimate the diameter of the field of view using the same technique used for low power. However, you can determine the field of view under high power by using the formula below:

$$\frac{\text{High Power Magnification}}{\text{Low Power Magnification}} = \frac{\text{Low Power Field Diameter}}{\text{High Power Field Diameter}}$$

What is the microscope's calculated high power field diameter in μm ?

27. Now that you know the diameter of your field size under both high and low power, you can use that information to estimate the size of objects you observe under the microscope. For example, in the diagram at the right, 10 circular objects fit across the field of view. The field of view is $2000\mu\text{m}$ in diameter. Since each object takes up $1/10$ of the $2000\mu\text{m}$ field diameter, the size of each object is $200\mu\text{m}$. You can use this method to estimate size of objects you view under your microscope once you know your microscope's field diameter.



28. Obtain the prepared slides for this section from the supply area.
29. Focus under low or high power to view each specimen and then estimate the size of each. Record your observations in the table below.

Specimen	Viewed under Low or High power	Field Diameter	# of specimens that fit across field	Estimated Specimen Size
Leaf blade (thickness)				
Oedogonium (thickness)				
Whitefish blastula (diameter)				
Artery wall (thickness)				

30. Return all slides to the supply area.

F: DEPTH OF FIELD

31. Obtain a microscope slide of silk fibers from the supply area.

32. Look at the slide under low power where the threads cross. Adjust the diaphragm to give the sharpest view. Are all three thread colors equally visible under low power?

33. Look at the slide under high power where the threads cross. Adjust the diaphragm to give the sharpest view and fine focus. Are all three thread colors equally visible under high power?

34. Slowly fine focus up and down to determine the order of the thread colors.

Which color is on top? _____

Which color is on the bottom? _____

How did you determine the order of the thread colors? _____

35. Return the slide to the supply area.

PART II: QUESTIONS

36. Describe how a compound microscope should be held and carried.

37. How is the total magnification of a microscope determined?

38. If the eyepiece on a microscope has a magnification of 10X, what is the total magnification with a 15X objective?

39. If the eyepiece on a microscope has a magnification of 15X, what is the total magnification with a 45X objective?

40. A microscope gives a total magnification of 1500X, but the image is too blurry to be useful. What might be the problem with the microscope?

41. An image is located in the lower right hand corner of the field of view. How would you move the slide to center the image?

42. Objects viewed under a compound microscope are frequently lost when switching from low to high power. Give one reason why this happens.

43. How did the light intensity change when you switched from low power to high power objective?

44. In general, how would you have to adjust the diaphragm after switching from low to high power?

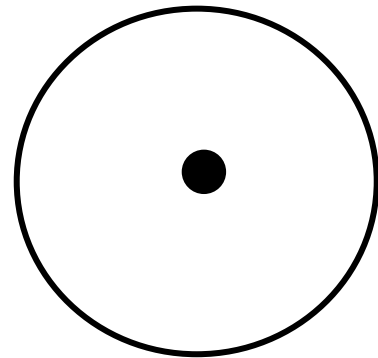
45. Do you observe more or less area in your field of view when under high power compared to low power?

46. If a microscope has a low power magnification of 100X and a high power magnification of 500X, and a low power field of 1500 μ m, what is the high power field in μ m?

47. If 20 objects fit across the diameter of a low power field of view whose field diameter is 4000 μ m, what would be the approximate size of each object?

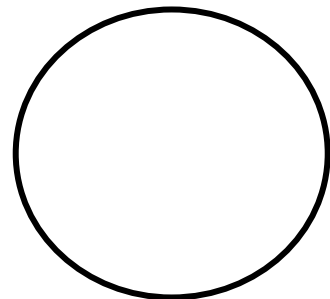
48. Why is it more difficult to measure the diameter of the high power field of view than the low power field of view?

49. The circle at the right represents a microscope's field of view with a black dot under 10X magnification. Draw how large the dot would appear under 40X magnification. Also, draw a circle to indicate the size of the field of view under 40X magnification.



50. Sketch the number 4 as it appears through the lenses of the compound microscope.

How has the lens system of the compound microscope changed the orientation of the numeral?



51. A student focuses on a specimen at low power and carefully centers it before changing to high power. At high power, however, he doesn't see the part of the specimen he was interested in. What might be the problem?

52. Inspired by her biology lab, a student decides to make a closer study of the food she eats. She uses a razor blade to make a very thin section from a raw potato and mounts it in a drop of water on a slide. To her disappointment, she can barely make out the cells under the microscope. What might she do to improve her results?

53. What are two methods used to study cells?

54. How is magnification different from resolving power?

Magnification	Resolving Power

55. What are the advantages and limitations of studying cells using light microscopy?

Advantages	Limitations

56. Compare and contrast transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

	TEM	SEM
Similarities		
Differences		

57. What are the advantages and limitations of studying cells using electron microscopy?

Advantages	Limitations

58. List the steps in cell fractionation.

59. What are the advantages and disadvantages of studying cells using cell fractionation?

Advantages	Disadvantages