

Microscopy

Today we will learn how to use one of the most important tools a biologist has, the microscope. This lab will probably be combined with another lab that requires you to use the microscope to make observations.

Objectives:

1. You should be able to correctly operate a compound microscope, know its basic parts, and be able to put it away properly. This will be review for many of you.

Synopsis of the Lab Exercise:

You should read through the introductory material below *before* lab. You should also read pages 42-47 in your textbook (Lewis et al. 2002). There is a **pre-lab assignment** on page 9 of this handout to complete **before** coming to lab.

1. You will be asked to identify the name and function of the various parts of the compound microscope and to learn proper microscopic technique.
2. You will familiarize yourself with the use of the microscope initially by viewing a slide containing a permanently mounted typed letter.

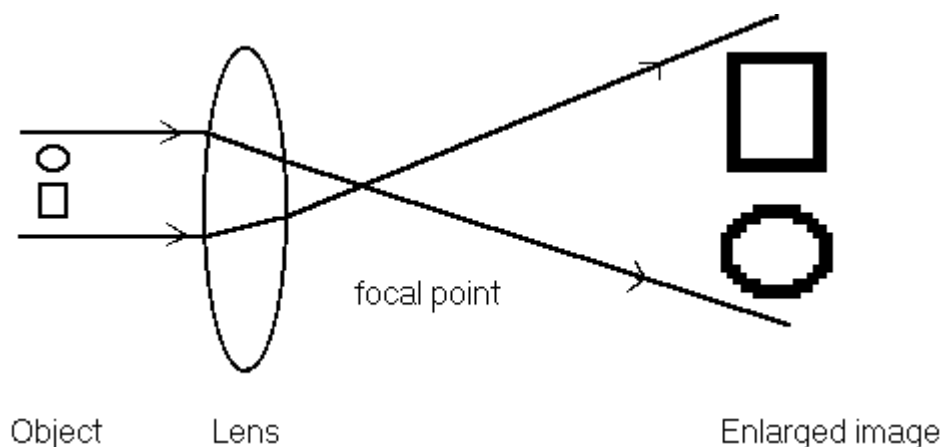
Bring your textbook to lab.

MICROSCOPY

The microscope is the biologist's greatest and most powerful observational tool, and has provided a view of nature that is otherwise hidden from human eyes. Remarkably, the very existence of microorganisms remained unknown throughout most of human history, and their existence only became known through the microscopic observations of Antoni van Leeuwenhoek between 1660 and 1675. It was during this same period of time that the cellular structure of plants and animals was first unveiled by Robert Hooke using a primitive compound microscope. Today, microscopes are not just the tools of biologists, but also of scientists working in many other fields ranging from electronics to chemistry. However, even in this age of advanced electron microscopes that allow visualization of the very atomic structure of molecules, light microscopes that are the descendants of those used by van Leeuwenhoek and Hooke retain their central importance to biological research.

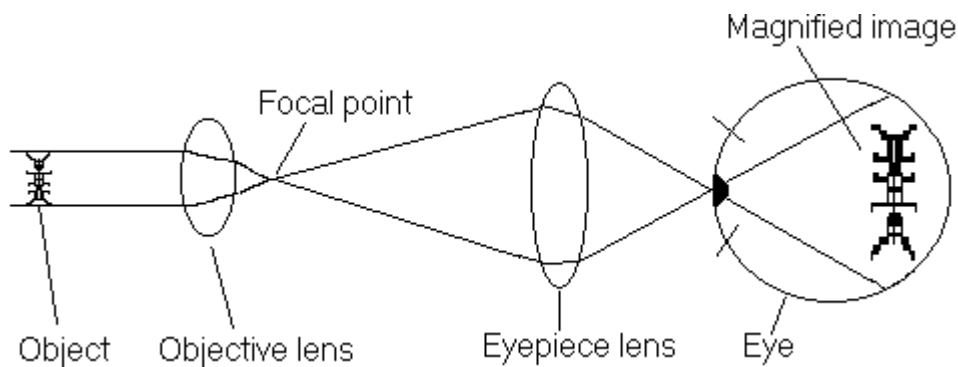
For the vast majority of biologists, the **LIGHT MICROSCOPE** remains the most useful type of microscope for routine observations. A light microscope functions by enlarging the image of an object illuminated by sunlight or an electric bulb. This enlargement is achieved when glass **LENSES** cause bending of the light rays that carry the image. Bending of light rays also occurs when light passes from air into water, and causes submerged objects to appear displaced from their actual positions. The rays that carry an image into a convex-shaped lens are **FOCUSED** at a position on the opposite side of the lens, and create beyond the focal point an enlarged, inverted image of the object. The magnification of an image by a single lens is shown in Figure 1, below.

Figure 1. Magnification of an image by a single glass lens.



During this lab period you will learn how to use a modern **COMPOUND MICROSCOPE**. In a compound microscope, the image passes through two lenses arranged in series. This provides much greater magnification than a single lens alone. Figure 2 shows the fundamental structure of a compound microscope.

Figure 2. Magnification of an image by a compound microscope.



I. PRACTICAL USE OF THE MICROSCOPE

1. Parts of the microscope

The various parts of a light microscope and their functions will be discussed by the instructor at the beginning of the lab period. You should be able to identify the parts of the microscope illustrated in Figure 3. Your pre-lab assignment is to fill in the names of the parts of the microscope in Figure 3 (page 10 of this section).

All modern microscopes are called **COMPOUND MICROSCOPES** because they possess two lens systems. The **OBJECTIVE LENS** focuses the light after it passes through the object on the microscope slide. The objective lens creates a magnified image in essentially the same way as a slide projector, but instead of projecting this image onto a screen, the image is projected onto the second lens system called the **EYE PIECE, or OCULAR**. The ocular further magnifies the image and then projects it into the eye. Although we have a few different types of microscopes in the laboratory, all possess the same functional components. The goal of this exercise is for you to be able to identify the essential components of any compound microscope. If you are unable to identify any of these parts on the microscope present at your laboratory bench, ask for assistance from the instructor.

2. Some important concepts you should know.

There are certain theoretical and practical aspects of light microscopy that must be understood to be able to properly use a microscope. The quality of a microscopic image is determined by the characteristics of the image referred to as **MAGNIFICATION, RESOLUTION, and CONTRAST**. Proper adjustment of the microscope must be performed to optimize these characteristics to obtain good image quality, and also to minimize eye-strain while making microscopic observations.

MAGNIFICATION. *The apparent increase in size of an object viewed through a microscope is called "magnification".* The total magnification produced by a compound microscope is calculated by **MULTIPLYING** the magnifications of the **OBJECTIVE lens** and the **EYEPIECE lens**. The total magnification of a microscope can be varied by rotating into place different objective lenses. The common objective lenses found on most compound microscope are referred to as **SCANNING (4x)**, **LOW (10x)**, and **HIGH (45x)**. The eye piece commonly has a magnification of 10x. **In Table 1 (page 11) write in** the magnification of the objectives and eyepiece of your microscope and calculate the total magnifications under each objective.

RESOLUTION. An essential function of all microscopes is to increase resolution as well as magnification. *Resolution is the ability to distinguish closely spaced objects.* Surprisingly, most of the technological advances in lens design have been directed toward improving resolution, rather than magnification. Obviously, magnification is critical to the ability to see small objects, however, if the resolution is not improved along with magnification then the objects will appear blurry and distorted. The factors that affect the

resolution of a microscope include proper adjustments of the light source and substage diaphragm; and cleanliness of the lenses, the microscope slide, and the coverglass. Thus, your ability to properly use the microscope will be an important factor that determines the quality of the images that you view.

CONTRAST. *Contrast is simply the difference in intensity between an object and its surroundings,* and is most conveniently adjusted controlling the amount of light entering the microscope by use of the **SUB-STAGE DIAPHRAGM** located below the condenser lens. The diaphragm works like the iris of your eye or a camera lens. Decreasing the aperture of the diaphragm (and thus the amount of light) will increase the contrast. As you increase magnification (by switching to a stronger objective), the diaphragm will need to be opened to increase the amount of light entering the objective. A frequent difficulty encountered by students when trying to locate or focus on an object is improper adjustment of the sub-stage diaphragm.

Another important characteristic of a microscope lens is called the **WORKING DISTANCE** -- the distance between the upper surface of the slide and the front of the objective lens when the specimen is in focus. The working distance of high magnification lenses can be quite small (less than a millimeter), and thus, *extreme care must be exercised to keep an objective lens from hitting the slide.* This can damage the objective lens, which may cost hundreds of dollars to replace. The best way to avoid such an accident is to remember that the objective lenses on our microscopes are **PARFOCAL**. This means that when a specimen is in sharp focus under a low magnification lens, the next higher power objective lens can be rotated into place without hitting the slide and brought into focus with only minor adjustment of the fine focus knob.

3. Operation of the microscope.

The following steps and helpful suggestions are provided to guide you in the steps that are necessary to find and view objects on a specimen slide.

1. Carry the microscope with both hands - one on the arm of the microscope and the other supporting the microscope from below. At your table, inspect your microscope before use. Make sure the microscope surfaces are clean of dust and lint. **YOU ARE RESPONSIBLE FOR THE PROPER HANDLING OF YOUR MICROSCOPE DURING THE LAB PERIOD. IF YOU FIND ANY DAMAGE TO THE MICROSCOPE UPON YOUR ARRIVAL IN LAB, YOU SHOULD REPORT IT IMMEDIATELY TO THE INSTRUCTOR.**

2. Before putting a slide on the stage, rotate the scanning power (4x) objective into position. Using the coarse focus adjustment knob, position the 4x objective so that it lies approximately 1 cm (1/4 inch) above the stage. For most microscopes, movement of the objective will stop before the low power objective hits the stage, but this is not always true. Some microscopes focus by moving the objective lenses, others by moving the stage, so when using a microscope for the first time, be careful to determine which component moves. The cardinal rule of focusing a microscope is: **always watch from**

the side when you are moving the stage and the objective lens closer together. You should look through the microscope when focusing only if you are moving the stage and the eyepiece apart or when you are using the fine adjustment knob.

3. The light source (ILLUMINATOR) should be turned on. If your microscope has an adjustment for the light intensity on the base, then adjust this to about 3/4 full intensity.

4. The sub-stage diaphragm should be adjusted by moving its lever to give the **minimum** comfortable brightness. When initially locating a specimen under low power, high contrast usually is more important than good resolution.

5. When using a permanently prepared slide, clean the top and bottom surface with lens paper; when preparing your own wet mount, be sure to use a perfectly clean cover glass (no dust or fingerprints, which lower resolution)

6. Holding the specimen slide between your fingers, visually locate the area on the slide where the object to be viewed is located. Place the microscope slide under the slide clamps on the microscope stage, and roughly center the object below the 4x objective lens.

7. Bring the specimen into focus by racking the objective lens **away** from the slide using the COARSE FOCUS KNOB. Note: the focusing knobs of some microscopes operate by moving the objective nosepiece, while others move the stage platform.

8. The specimen should be brought into sharp focus using the fine focus adjustment knob, and then the diaphragm and/or mirror should be adjusted to optimize illumination.

9. IF YOU CANNOT LOCATE THE SPECIMEN, visually check to see that the slide is positioned correctly; next try closing the SUBSTAGE DIAPHRAGM further to increase contrast. If you still don't see anything, move the slide over to another area (as you move the slide around you often notice shadows of out-of-focus objects); repeat the focusing process from the beginning (step 2).

10. To view a specimen under the 10x lens, first move the slide so that the object is centered in the field of view of the 4x lens. Next, **while watching from the side**, rotate the 10x objective into viewing position. Since these objectives are parfocal, only minor adjustment of the focus (using the fine focus knob) will be necessary. The substage diaphragm should be adjusted after changing objectives to obtain the best contrast and resolution.

11. To view a specimen under the high power lens, repeat the above procedures, and rotate the high power lens into position.

12. Before removing a microscope slide from the stage, always rotate the lowest power objective into position first!

4. CLEANING OF MICROSCOPE LENSES

A dirty lens will cause distortion of the image of a specimen. Dirt or a smudge can occur on the eye piece, objective, condenser lenses, or the microscope slide itself. It is usually possible to identify the location of the dirt or smudge by following a simple procedure:

1. Rotate the eyepiece. If the dirt is on the ocular, it will rotate also.
2. Change the objective lens. If the distortion disappears, then the objective lens is dirty.
3. If the above steps fail, remove the slide and check the condenser lens and the microscope slide.

UTMOST CARE MUST BE USED WHEN CLEANING LENSES, AND YOU SHOULD ASK FOR THE INSTRUCTORS HELP BEFORE DOING SO. Only the special "lens paper" provided in the lab should be used for this purpose since the glass of which lenses are made is relatively soft and is easily scratched.

5. STEPS IN PUTTING AWAY THE MICROSCOPE

1. Rotate the scanning (4x) objective into position.
2. Remove the slide.
3. Clean the microscope surfaces free of water and dirt.
4. Only clean the lenses if they are known to be dirty, and then only using lens paper.
5. Reposition the slide hold down clamps on the microscope stage.
6. Place the microscope back in its proper place in the cabinet.

Report any damage to your microscope to the instructor. **YOU ARE RESPONSIBLE FOR ANY DAMAGE THAT OCCURS TO YOUR MICROSCOPE DUE TO CARELESSNESS OR NOT PROPERLY FOLLOWING THE ABOVE INSTRUCTIONS.**

Today's Lab

I. Examining a permanently mounted "letter slide" (your instructor may make this optional)

Although some of you may have had previous experience using a microscope, almost everyone initially has difficulty scanning the slide while looking through the eyepiece. You will need to remember that the image viewed through the microscope is upside-down and backwards relative to the actual orientation of the object. For example, in order to examine an object located near the right-hand side of the field of view, you will have to move the slide toward the left.

Use the letter slide to acquaint yourself with this relationship. Place the slide on the microscope stage so that the letter appears in its normal orientation when viewed with the unaided eye, and, in the space provided below, draw the letter as it appears to the unaided eye. Now focus on the letter under low power and adjust the illumination for best viewing. Draw the letter as it now appears. Refocus under the high power objective and draw the letter again as it now appears.

Appearance of Letter Slide

unaided eye

low power

high power

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Under low power, move the slide itself, first to the left and then to the right. How does the orientation of the letter move as viewed through the microscope?

II. Examination of Pond Water:

After getting to know your microscope, you should turn your attention to the pond water cultures in the laboratory. Examination of these cultures will help you learn to distinguish between microorganisms and will also expose you to a wide variety of fascinating creatures. You should try to identify every organism you see, however, be aware that even an expert will have trouble doing so since such a fantastic diversity of life exists even in a single drop of pond water. We will keep a running tally of the different species observed; *you should try to observe each of these organisms and draw a picture of it.*

BE SURE TO MAKE DETAILED DRAWINGS OF YOUR OBSERVATIONS ON PAGE 12 AT THE END OF THIS LAB EXERCISE.

Pre - Lab Assignment

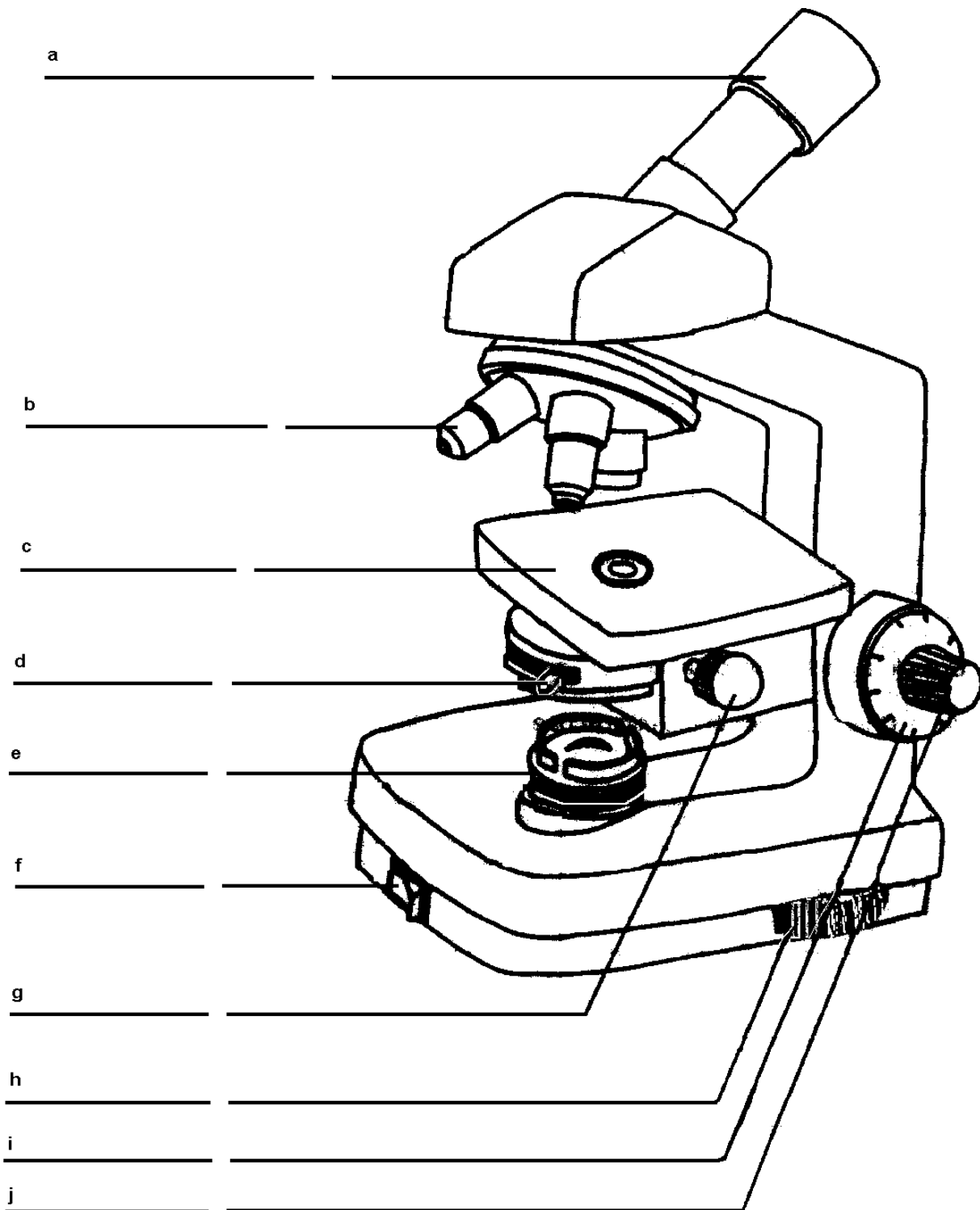
-----> Name _____

Complete the following *before* lab:**QUESTIONS**

1. What PROPERTY of a microscopic IMAGE is described by the terms:
 - a. Magnification
 - b. Resolution
 - c. Contrast

2. On the following page, name the indicated structures on the microscope.

Figure 3 - A typical compound microscope



In - Lab Assignment

-----> Name _____

Complete the following *during* lab:

1. Examination of the letter slide:

Draw the letter slide as viewed with the unaided eye, under low power, and under high power:

Appearance of Letter Slide :

unaided eye

low power

high power

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Under low power, move the slide itself, first to the left and then to the right. How does the orientation of the letter move as viewed through the microscope?

2. Complete the following Table:

Table 1. Characteristics of the microscope and objective lenses of your microscope.

Objective	Objective Magnification	Ocular Magnification	Total Magnification
Scanning			
Low			
High			

Pond Water

Running list of organisms in pond water:	Drawings:
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	
11.	
12.	
14.	
15.	
16.	
17.	
18.	
19.	
20.	