

There are many different types of microscopes, all designed to make the very small visible. Microscopes vary in complexity from the simple hand lens to the highly sophisticated electron microscope. The most commonly used microscopes, however, fall somewhere in the middle. These are the light microscopes. Two types of light microscopes are in common use, the compound light microscope and the dissecting microscope. As the name implies, dissecting microscopes are often used to dissect small specimens, such as insects (they are also used for microsurgery). More frequently used in this lab are compound light microscopes, which provide much greater magnification making observation of tissue and some cell structure possible.

Compound light microscopes function by passing light through a specimen and a series of lenses. The lenses bend the light resulting in the magnification of the image that reaches the eye. The precision of the shape and alignment of these lenses determines the resolving power of the microscope and consequent definition of the image. The following overview of the compound microscope provides the minimum that anyone entering into scientific study should know. The compound microscope will be used regularly in this class.

I. Care & Handling of the Microscope:

A. Care of the Microscope

1. Protect from Damage

- a. Always carry microscopes in an upright position close to your body with both hands, one under its base, the other gripping its arm.
- b. Keep the microscope away from the edge of the table. Do not let the electrical cord hang over the edge of the table where it can become a trip hazard. Keep the tabletop as clear as possible.

2. Maintain Cleanliness

- a. Clean lenses with lens paper only. Never clean lenses with anything other than the lens paper designed for this task.

Keeping the microscope and associated items clean is important to effectively use and get the most out of your time with the microscope. Lenses should be cleaned regularly before and sometimes during use. Slides should also be cleaned prior to use (use kimwipes not lens paper to clean slides).

3. Proper Storage

Always remove the slide, return to the low power objective and coil the cord properly (see the diagram on the cabinet) before putting the microscope away.

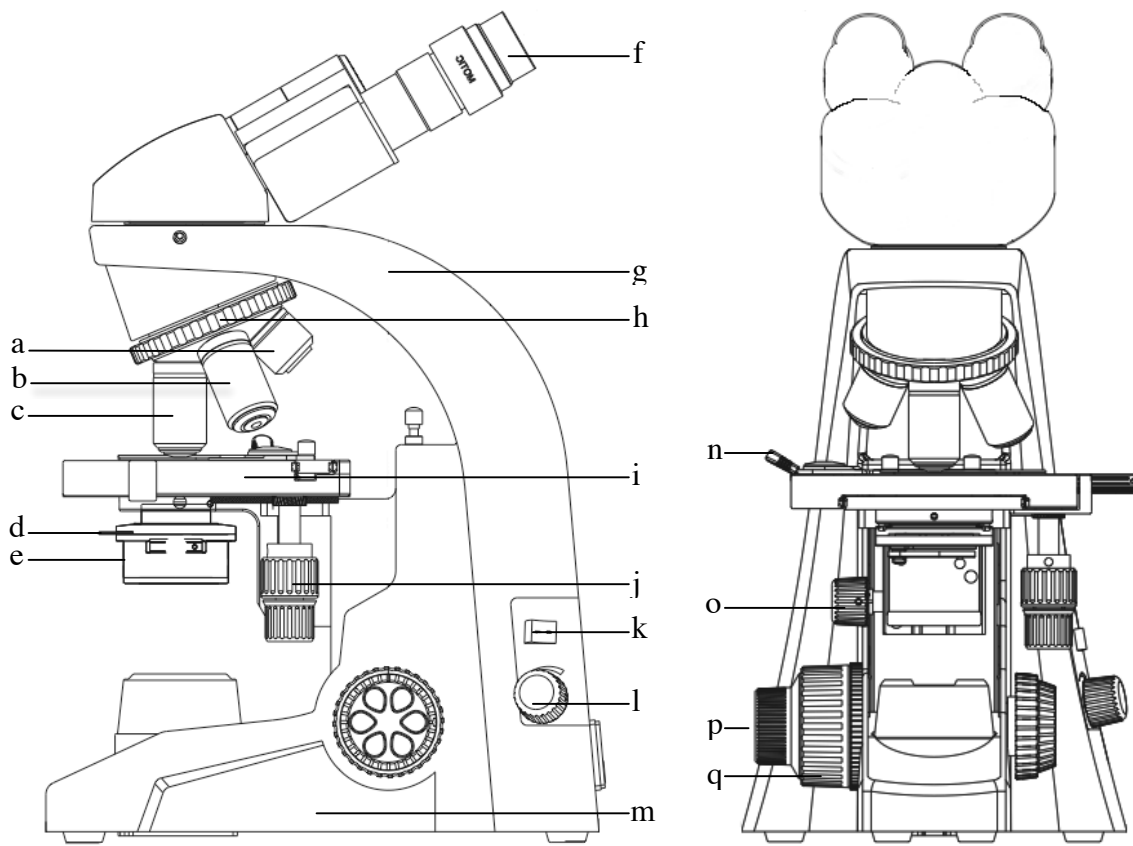
- a. Remove slides and wipe down the microscope.
- b. Be sure lowest power objective lens is locked in viewing position.
- c. Adjust coarse focus so that stage and objective lenses are as far apart as possible (without contacting the condenser).
- d. Be sure arms of mechanical stage system are not sticking out.
- e. Coil cord neatly and store microscope in appropriate shelf (or with dust jacket in place).

B. General Rules of Microscope Use:

1. Always begin with the lowest power objective lens.
2. Look from the side while adjusting the focus so that the low power objective lens is as close to the slide as possible (do not make large adjustments to the focus while looking through the microscope).
3. Always “focus away” when viewing a slide. Adjust the focus so that the objective lens is moving away from the slide while viewing.
4. Look from the side to ensure that there is clearance when changing objective lenses.
5. Only use the fine focus knob to focus anytime the high power objective is in use.
6. Always use a cover slip with a wet mount slide.

II. Components of the Microscope (know location and function)

A. Identification of Microscope Components



Identify the labeled parts of the microscope

a.	g.	m.
b.	h.	n.
c.	i.	o.
d.	j.	p.
e.	k.	q.
f.	l.	

B. Function of Microscope Components

1. Body (body tube, aka head piece) - mounting for the lenses and encloses the path of light from the objective lenses to the ocular lens
2. Arm - supports the body and associated lenses
3. Base - supports the arm and light source, provides stability
4. Stage (Mechanical) - platform onto which slides with specimens are placed for viewing
5. Mechanical Stage System
 - a. Mechanical Retainer Clips - clips that securely hold the slide on the stage
 - b. Adjustment Knobs - two knobs which control the movement of the retaining clips thus providing a means of precisely moving the slide over the stage
6. Nosepiece - the revolving disk at the base of the microscope body to which the objective lenses are mounted, rotating the nosepiece permits changing which objective lens is in the light path
7. Lenses
 - a. Ocular Lens (eyepiece) - the last lens through which the light passes before entering the observers eye (10x)
 - b. Objective Lenses (all) - the first lens through which the light from the specimen passes
 - i. scanning (4x) - not present on most microscopes used in this class
 - ii. low power (10x) - used for searching and overview of the specimen, often low power is sufficient for most observations
 - iii. high power (47x) - used when viewing greater detail is desired
 - iv. oil immersion (100x) - not present on most microscopes used in this class, requires the use of oil between the objective lens and the specimen to minimize refraction and chromatic aberration
8. Focusing Knobs - move stage closer or farther from the objective lenses to bring specimen into focus (once focused objective lenses may be changed with minimal need to adjust the focus).
 - a. Course Focus Knob (large, on either side of arm) - moves stage in large increments
 - b. Fine Focus Knob (small, on either side of arm) - moves stage in small increments
9. Substage Light - light source that illuminates specimen
10. Condenser - adjustable substage lenses that focus the light onto the specimen (light quality)
11. Iris Diaphragm - adjustable substage opening through which light shines to illuminate the specimen (opening and closing the iris diaphragm increases and decreases the amount of light respectively = light quantity). **Note:** regulation of the light is one of the most important skills in microscopy, practice adjusting the condenser and iris diaphragm to optimize resolution and contrast.

III. Concepts of Microscopy

1. Field (field of view) - the round area visible when looking through the microscope (appears to be the same size at different magnifications but is, in fact, smaller at higher magnifications).
 2. Depth of Field - the thickness of the specimen that is in clear focus.
 3. Working Distance - the distance between the specimen and the objective lens.
 4. Resolution (resolving power) - measure of clarity, defined as the ability to distinguish two points as separate points (the closer together the higher the resolution).
 5. Magnification - the increase in the apparent size of the specimen being viewed (total magnification is determined by taking the product of the objective and the ocular lens magnifications).
6. Slide Types
- a. Wet Mount slide - a temporary preparation of a specimen (often in water on the slide, may or may not be covered by a cover slip).
 - b. Prepared Slide - a permanent preparation of a specimen (specimen is fixed and bonded to the slide under a cover slip).
7. Parfocal - characteristic of microscope design that allows changing objective lenses without a significant change in focus.
 8. Parcenter - characteristic of microscope design that allows changing objective lenses without a significant change in the position of the center of the field of view.

Questions / Review

Lab 2.0

In addition to reading and understanding all of the material presented in the lab manual you should be able to answer the following types of questions. These questions are designed to help you focus your studies, how you use them and answer them will determine how much you get from them.

I. Locate all of the parts on your microscope and familiarize yourself with their use.

II. Magnification - The total magnification of the microscope is the magnification of the eyepiece times the magnification of the objective lens being used.

- a. What is the magnification of the eyepiece? _____
- b. What is the magnification of the low power objective? _____
- c. What is the magnification of the high power objective? _____
- d. What is the total magnification of an object being examined under low power? _____
- e. What is the total magnification of an object being examined under high power? _____

III. Basic Set-Up

Use a prepared slide of the letter “e” (alternately prepare a wet mount of the letter “e”). Practice focusing, adjusting the light, moving the slide while viewing.

Swing the low power objective (green band) into place. Place the letter “e” slide right side up at the center of the stage over the stage opening. Use the coarse adjusting knob to bring the stage as close to the objective lens as possible. Then, while looking through the eyepiece very slowly turn the coarse adjusting knob away from you until the letter comes into view. Open the iris diaphragm and then close it down until you find a level of light intensity that is comfortable for you.

IV. Orientation of the image - The appearance and movement of objects when viewed through a microscope is different than when viewed with the naked eye.

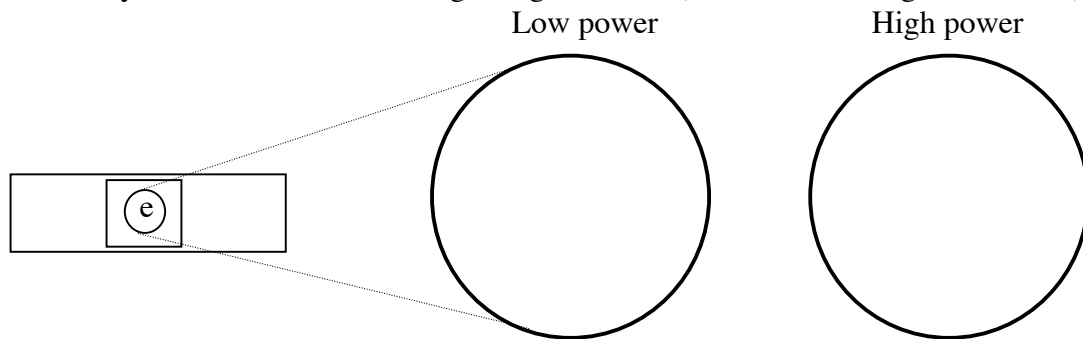
- a. Move the slide using the stage adjustment knobs.
- b. Move the slide to the right. Does the slide appear to be moving in the same direction?
- c. Move the slide away from you. Does the slide appear to be moving in the same direction?

V. Use of the Low and High Power Objectives

Without changing the focus, change to the high power objective (yellow band). Then clarify your view with the fine focus adjusting knob. Adjust the light intensity as needed.

- a. Do you need more or less light when using the high power objective lens?
- b. Which focusing knob do you NEVER use under HIGH power? Explain.

- c. Under what conditions would you use low power?
- d. Under what conditions would you use high power?
- e. Draw what you observe at low and high magnification (note that the image is inverted).



VI. Depth of Field

In the laboratory you will need to examine specimens that are several cells thick. You will need to be able to focus upon each layer separately. The characteristic of the microscope that allows the examination of each separate layer is called the depth of field (sometimes called the depth of focus) or how much of the specimen is in focus at one time.

Obtain a prepared slide of “crossed threads” and examine it under low power. Be sure that you use the proper contrast and center the point where the threads cross in your field of view.

- a. Can all three colored threads be brought into clear focus at the same time?
- b. Can you determine which one is on top, in the middle, on the bottom?

Now switch to the high power objective and adjust the light.

- d. Can all three colored threads be brought into clear focus at the same time?
- e. How can you determine the position of each thread?

VII. Clean Up - when you have finished with the microscope for the day be sure to prepare it properly for storage.

- a. Remove slides and put them away
- b. Wipe dust and debris off of the microscope and clean the lenses.
- c. Be sure lowest power objective lens is locked in viewing position.
- d. Adjust coarse focus so that stage and objective lenses are as far apart as possible (without contacting the condenser).
- e. Center the mechanical stage so that the arms of mechanical stage system are not sticking out.
- f. Coil cord neatly around the ocular lens and the focusing knobs.
- g. Return the microscope to the appropriate shelf.