

Care and Use of the Microscope Laboratory

Objectives

When you finish this laboratory you should be able to:

1. Define these terms and use them in an appropriate context:
 - a. diopter value
 - b. FOV
 - c. interpupillary distance
 - d. parfocal
 - e. resolution
2. Carry a microscope safely to your desk.
3. Name the parts of the microscope and their functions.
4. Discuss the advantages and disadvantages of using compound microscopes and dissecting scopes.
5. Calculate interpupillary and diopter values.
6. Prepare dry and wet mounts for use with the compound microscope.
7. Correctly focus the microscope on tissue and be able to calculate cell size.
8. Clean the microscope and correctly prepare it for storage.

Introduction

Many organisms are too small to be observed with the naked eye. These organisms are multicellular as well as single celled. Representatives of all three Domains – Bacteria, Archaea and Eukarya – are microscopic in size. Microbes are less than 1 millimeter in size and usually measured in micrometers ($1\ \mu\text{m} = 1/1000\ \text{mm}$.) While there are many types of microscopes available to biologists today, the most commonly used for observing cells and small organisms are the dissecting microscope and the compound light microscope. Your instructor may introduce you to the dissecting microscope (see Appendix 1.). Today's exercise, however, will focus on the care and use of the compound light microscope as this is the one you will encounter most in your biology career..

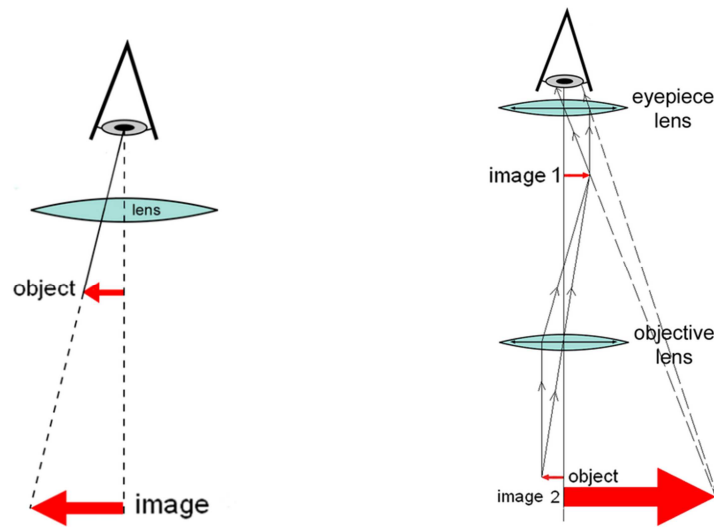
Introduction to the Compound Light Microscope

The microscope pictured in Figure 2 is referred to as a compound light microscope. The term *light* refers to the method by which light transmits the image to your eye. *Compound* deals with the microscope having more than one lens. *Microscope* is the combination of two words; "micro" meaning small and "scope" meaning view.

Early microscopes, like Anton van Leeuwenhoek's original microscopes (about 1660-1670), were called simple because they only had one lens. Simple scopes work like magnifying glasses that you have seen and/or used. These early microscopes had limitations to the amount of magnification no matter how they were constructed.

The creation of the compound microscope (generally credited to Zacharias Janssen around 1600), and the ability to create better quality lenses, helped to advance the field of microscopy light years ahead of where it had been only just a few years earlier. The addition of the second lens allows for magnification of the image of the primary (or first) lens, reduced chromatic aberration (or image distortion) and exchangeable objective lenses to adjust the magnification. (See Figure 1.)

Figure 1.



a) A simple microscope

b) A compound microscope

Simple light microscopes of the past could magnify an object to 266X as in the case of Leeuwenhoek's microscope. Modern compound light microscopes, under optimal conditions, can magnify an object from 1000X to 2000X (times) the specimen's original diameter.

Identification of the Parts of a Compound Light Microscope (See Figure 2.):

Arm: The pan that you grasp to carry the instrument. The second hand supports the scope beneath the base. It supports the body tube.

Base: It gives the microscope support.

Ocular lens: Magnifies ten times. Adjustment of these two lenses is important to avoid eye-strain. This will be discussed further shortly.

Objective lens: Magnifies the object. Scanning objective has a magnification of 4X. Low power has a magnification of 10X. High power has a magnification of 40X. Oil immersion lens has a magnification of 100X.

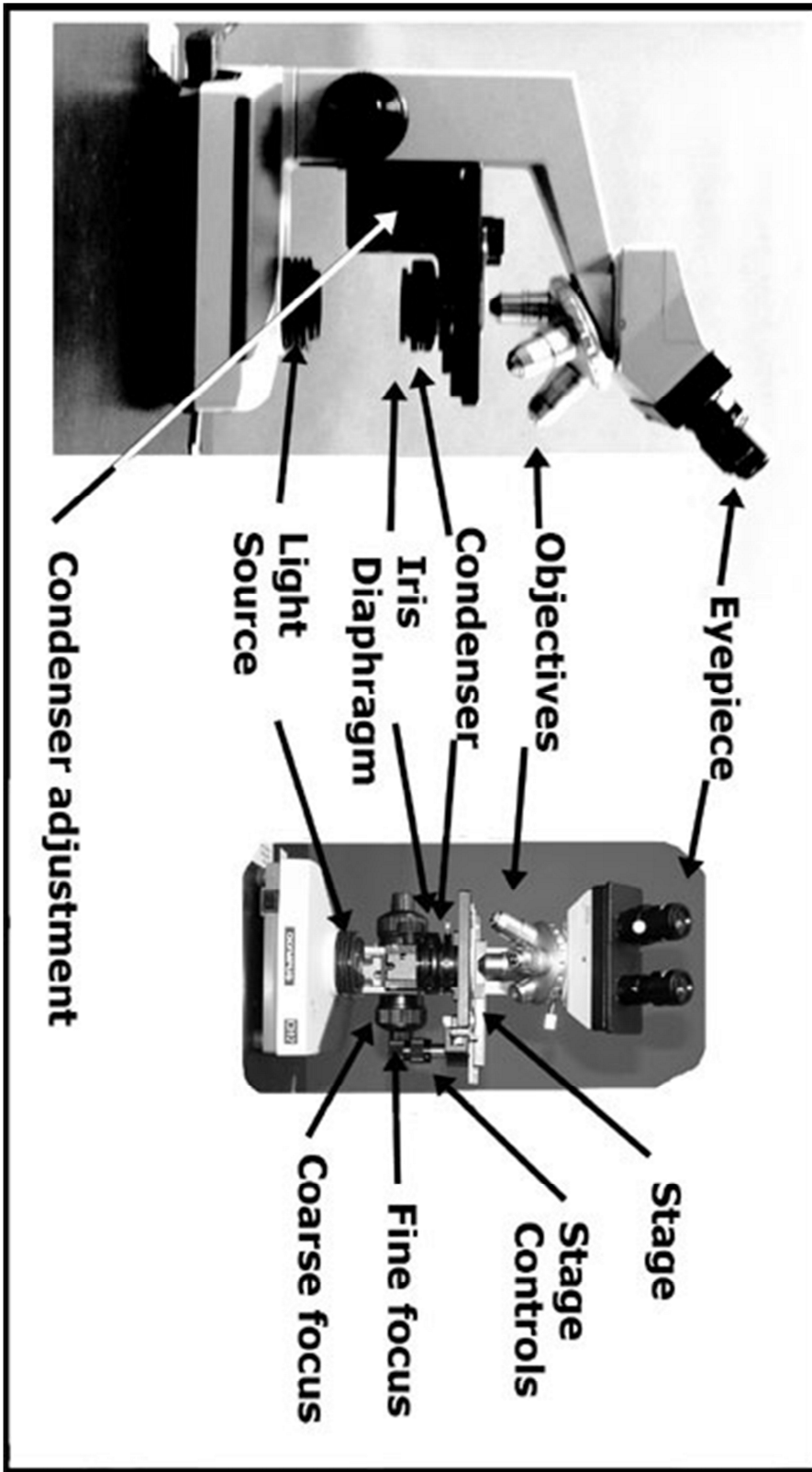
Nosepiece: The revolving pan to which the objectives are attached.

Body tube: Joins the ocular lens and the nosepiece.

Stage: A flat area that supports the slide. A hole in the stage allows the light to be focused on the tissue or specimen.

Focus adjustment knobs: The large knob is the coarse focus adjustment knob. It is always used first when focusing on a specimen. The fine focus adjustment knob is the smaller knob and is used to more finely adjust the focus. If the microscope is parfocal, one should only have to adjust the fine focus adjustment knob when moving up in magnification.

Figure 2.



Light source: Often a small electric light beneath the stage. It is controlled by a light switch and often a rheostat.

Iris diaphragm: Regulates the amount of light that goes through the specimen. A lever controls this. Light rays are deflected from the edges of the diaphragm and enter the slide at oblique angles. This scattered light makes materials appear darker because some rays of light take more time to reach the eye, thus giving the specimen greater contrast.

Condenser: Located above the diaphragm, this lens concentrates light before it passes through the tissue. The position is controlled by an adjustable knob.

Mechanical Stage Drive Knobs: Two knobs below the stage that will move the slide on the stage, giving the observer a total field as he/she looks through the eyepiece.

Care and Use of the Compound Microscope

Cleaning the Lenses

Your instructor will demonstrate the correct method for cleaning the lenses but you should remember that nothing should touch the microscope lenses except the special lens paper and appropriate solvent.

The lenses may be cleaned with clean lens paper. Lens cleaner must be used to clean grease or oil on the lenses. Handle solvents carefully and wash your hands after use.

Calculating Interpupillary Distance and Diopter Values.

Before viewing a slide one should calculate the interpupillary distance using the following steps:

1. Focus on a specimen using the low power objective.
2. Sit back from the microscope and slowly move the oculars apart.
3. While attempting to look through the oculars, notice that you can see two images. Carefully move the oculars together until the split image is viewed as one.
4. Repeat the procedure.
5. Locate the scale on the microscope between the oculars. Record the number in your lab book.

The diopter values must also be calculated:

1. After the interpupillary distance has been determined, locate the diopter adjustment rings on the ocular lenses.
2. Turn the diopter rings so that the mark on each ring aligns with the midpoint of the microscope scale on the ocular.
3. Close the left eye, and use the fine focus to find the clearest possible image.
4. Open the left eye, and close the right eye.
5. Do not change the fine focus, but instead, turn the diopter ring on the left lens from one extreme to the other to find the best viewing place.
6. Repeat the procedure, and record the diopter values in your lab book.

(Note: Your interpupillary and diopter values will apply to any other microscopes in the lab.)

Viewing a Slide

The simplest specimen preparation for light microscopy is called the dry mount. As the name suggests no liquid is used in mounting a specimen on a microscope slide. Prepare a dry mount by placing a small piece of newsprint on a glass slide and covering it with a cover slip. Place the slide on the stage, using the stage clips to hold the slide securely. Adjust the light source to provide 75% of the total light. Always begin slide viewing with the lowest power objective available. Click the scanning objective in place and turn the coarse focus adjustment knob until the letters come into focus. Then use the fine focus adjustment knob to refine the image. Adjust the amount of light coming through the newspaper print using the light rheostat and the iris diaphragm lever.

As you examine the print, is the letter reversed? _____

While you are looking at the stage move the adjustment knobs to observe whether the objective is moving closer or further from the stage.

Turn the mechanical stage drive knobs, one at a time. Note the direction that they move the stage when turned clockwise and counterclockwise. _____

Focus on the letter, change objectives by moving to the next highest objective. **The microscope is parfocal, therefore it is not necessary to adjust the view with the coarse adjustment knob. Use only the fine focus adjustment knob from this point.** Note that the field of view has gotten smaller as the magnification increased. The depth of field also is decreased – this is seen in the fact that only a thin layer of tissue is in focus at higher magnifications. By focusing up and down you can view different parts of a single cell or different cell layers.

The specimen must be centered over the hole in the stage through which light passes.

Computing Magnification

Magnification refers to the degree of enlargement of a specimen in appearance but not in physical size. It is important in examination of cells and tissues. It is not the only concern in observation. Objects must not only appear larger, but also clearer. **Resolution** or resolving power measures how clearly details can be seen. It is the smallest distance between two points which can be separately distinguished as different points.

Total magnification using the light compound microscope can be calculated by multiplying the magnification of the ocular lens by the magnification of the specific objective.

Measuring Objects Using the Microscope

Remember: as you increase the magnification you are decreasing the FOV (Field of View). If you know the FOV and the magnification when viewing an object you can calculate the size of the cell or organelle that you are observing. There is a mathematical relationship between the magnification and the diameter of the FOV.

$$\frac{X_1}{X_2} = \frac{D_2}{D_1} \quad \text{Where } X_1 = \text{Magnification with a specific objective.}$$

X_2 = Magnification using a different objective.

D_1 = Diameter of FOV at magnification 1.

D_2 = Diameter of FOV at magnification 2.

We can therefore calculate any of these values if the other three are available. Such as:

$$D_2 = \frac{D_1 X_1}{X_2}$$

Record the diameters of the FOV for all objectives that you are using today in Table 1.

Table 1.

Objective lens	Ocular Magnification	Objective Magnification	Total Magnification	Diameter of FOV (μm)
Scanning lens				
Low power lens				
High power lens				
Oil immersion lens				

Measuring cells.

Today you will observe cell types presented by your instructor. They may be prepared slides or wet mounts that you have prepared with materials provided for you. Calculate cell size by estimating the number of cells that would fit across the FOV and then divide the diameter of the FOV by the number of cells.

$$\text{cell size} = \frac{\text{diameter of the FOV}}{\text{number of cells}}$$

As we estimate the size of the cell that we are viewing we must take into consideration the objective that we are using to view the object with and the diameter of the field of view (FOV.) Using the scanning objective, the diameter of the FOV is 4,500μm. The diameter of the FOV for low power is 1,800μm, the diameter of the FOV for high power is 450μm and the diameter of the FOV for oil-immersion is 180μm.

For example: if you count 5.5 cells that would fit across the diameter of the field of view using the scanning objective, you would divide 4,500μm by 5.5 cells giving a cell length of 818.181.... We can round off our answer to 820μm.

One question students frequently ask is “What objective lens should I use?”. There is no one correct answer to this question as it depends on what you are trying to do. Instead you should choose the objective lens that gives you the best view of the cell for the task you are trying to accomplish. It is not always best to use the highest power objective. (However you are likely to estimate more accurately and you don't have to count as many cells.)

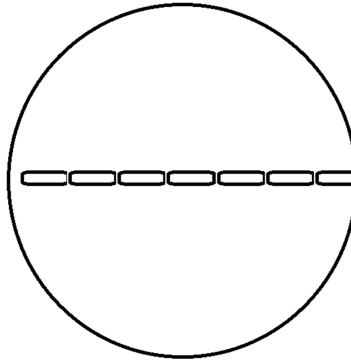
Example: 12 cells fit across the diameter of the FOV using low power. Calculate cell size.

$$\text{cell size} = \frac{\text{diameter of FOV}}{\text{number of cells across the diameter of the FOV}}$$

$$\text{cell size} = \frac{1800\mu\text{m}}{12.0 \text{ cells}} = 150\mu\text{m cell}$$

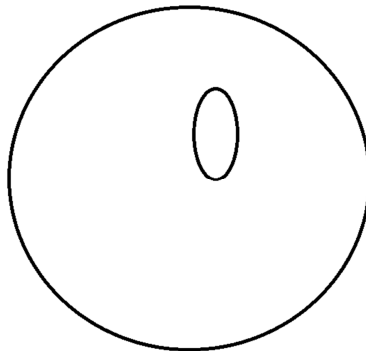
Using low power, calculate the cell size in the following diagram:

$$\text{cell size} = \frac{\text{diameter of FOV}}{\text{number of cells across diameter of FOV}}$$



Calculate cell size in the following diagram of a single organism viewed under high power. (Remember that you must estimate how many cells would fit across the diameter of the FOV.)

$$\text{cell size} = \frac{\text{diameter of the FOV}}{\text{number of cells across diameter of FOV}}$$



Preparation of a wet mount.

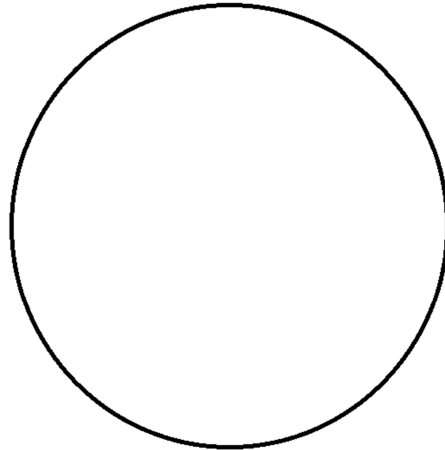
The most commonly used specimen preparation in biology labs is the wet mount. Wet mounts are useful in observing living tissues or cells for characteristics such as movement, color or behavior. To prepare a wet mount follow these steps:

1. Obtain a slide, coverslip and a toothpick.
2. Place a drop of water, or stain if necessary, and place your specimen in the liquid. If the cells or tissue are suspended in solution, place a drop of the solution on your slide.
3. Touch the coverslip to one margin of the drop, support the other edge with the toothpick, and slowly lower it.

The slide should not have bubbles if you perform this carefully, but they do give you something to focus on!

Obtain a thin section of onion membrane by snapping a layer of onion in half (your instructor will demonstrate how to do this) and transfer the transparent thin film to a slide. Apply one or two drops of I₂KI (Logol's Solution) to stain it. Place the edge of the coverslip slowly on the slide over the stained material at an angle rather than dropping it on the slide from a parallel position.

In the space below draw a diagram that is representative of what you can observe in the microscope. Remember that all drawings based on microscope observations must include the total magnification used and an estimate of the cell size (usually the long axis).



Use the remaining time in lab to view as many specimens as possible and fill in the following chart to record your calculations of the sizes of up to 5 different cells.

Table 2.

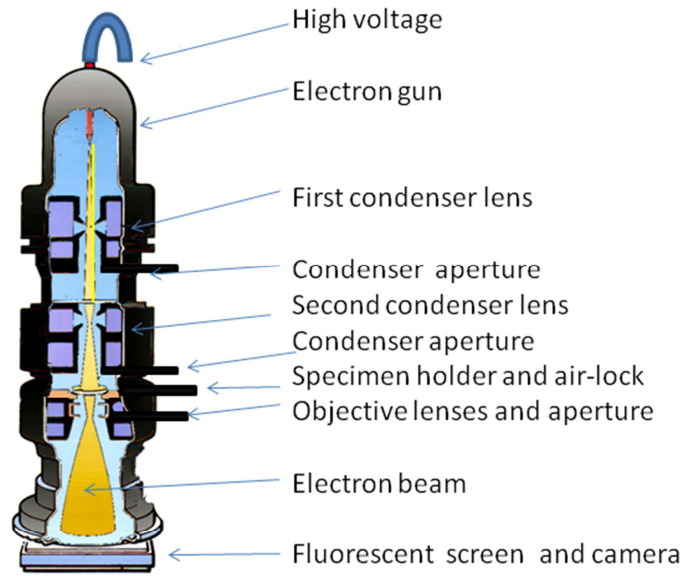
Cell type	Objective lens used	Total magnification	Diameter of FOV (μm)	No. of cells that fit across the FOV	Estimated cell size (μm)

Electron Microscopes

Light microscopes are limited in magnification (and resolution) by the wavelength of visible light. To achieve useful magnifications above 2000 times, another energy source is needed. Electron microscopes use a beam of electrons as an illumination source. Electrons, like light, exhibit two natures – particles and waves. Electron beams have wavelengths 100,000 times shorter than visible light and allow

magnifications up to 10 million times and resolutions measured in 10^{-12} meters (picometers). The basic principle of the electron microscope is the same as the light microscope (see Figure 3.). However, the lenses are magnets and the microscope column is under a near-perfect vacuum. Consequently, live specimens cannot be observed in electron microscopes and sample preparation takes days (or weeks) rather than minutes.

Figure 3.



Transmission Electron Microscope

Various types of electron microscopes allow viewers to increase the magnification without losing the resolution of the picture. The view does not become fuzzy with increased magnification. Transmission electron microscopes, scanning electron microscopes and probe electron microscopes are all available for more sophisticated study of cell structure and structure of macromolecules. The use of these has increased greatly for study of cell structure and identification.

Electron microscopes are expensive to build and maintain and must be housed in stable buildings (sometimes underground) with special services such as magnetic field cancelling systems to achieve high resolutions. Electron microscopes are not generally available to introductory biology students but you may have the opportunity to observe the use of one. Most hospital Pathology departments have electron microscopists on site to examine surgical specimens for the presence of pre-cancerous and cancerous cells and other cellular abnormalities.

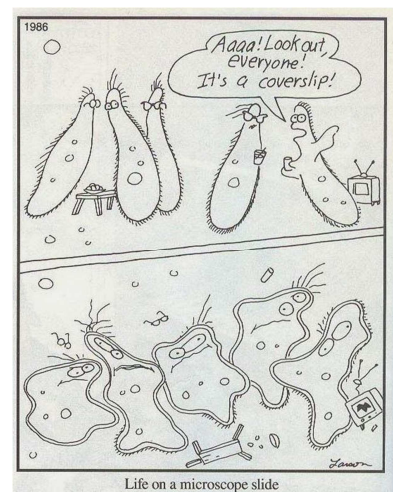
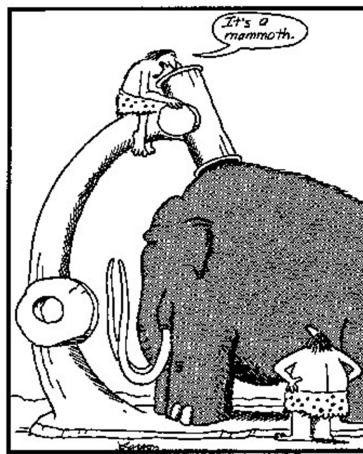
Student Questions

1. Discuss the difference between magnification and resolution.
2. Discuss the relationship between: magnification, diameter of FOV and relative brightness for all objectives.
3. Discuss how you would calculate cell size of a single celled organism.
4. Compare and contrast the dissecting and light microscopes.

Rules for Living

You are accountable for the condition of your microscope when you place it in the storage cabinet. Make sure you complete the following tasks before putting away your microscope:

1. Clean ocular lens and all objectives with lens paper and cleaner.
2. Place plant and animal tissues as well as cover slips in the trash.
3. Remove slides from the stage, clean (wash with soap and dry) and return to the correct trays.
4. Clean stage if needed.
5. Turn revolving nosepiece so that scanning objective is in line with ocular lens.
6. Turn light switch to off position and light dial to lowest setting.
7. Place mechanical stage drive apparatus at the back of stage and center it.
8. Wrap the electric cord in the direction that it emanates from the microscope and then around the cord holder or around the back of the arm/base juncture and the light source.
9. Clean your station and show your microscope to your professor.



The LAB-NESS MONSTER

Appendix One

Use of the Dissecting Microscope

Dissecting microscopes are designed for low magnification observation of a sample, typically using light reflected from the surface of an object rather than transmitted through it. The degree of magnification of objects is similar to that of a magnifying glass. The field of view for this device is large and the depth of field that is in focus at one time is also large. Light can be directed from above the object or below. Magnification is controlled by a zoom knob.

Carry the dissecting microscope by its arm with one hand supporting the base. After placing it on the bench name the parts and associated functions.

Parts of the dissecting scope (Figure 4.):

Base with illuminator.

Light switch: Turns on the illuminator.

Illuminator control knob: Two positions for one switch may alternately select lighting from above or below the stage or both lighting sources may be used simultaneously.

Oculars: Move them together or apart until you feel comfortable looking into the microscope. A single circle of light should be observed

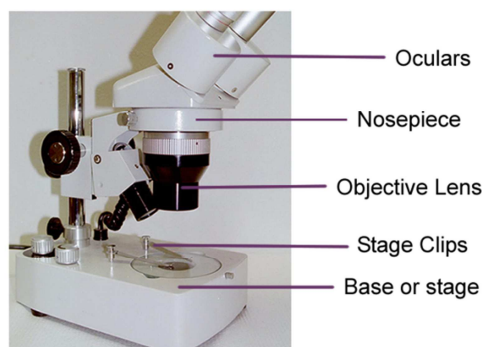
Zoom knob: Controls magnification of the specimen. Rotate it to increase or decrease magnification.

Stage: The flat area where the specimen is placed.

Focus knob: A knob that you should turn until an object on the stage is in focus.

Figure 4.

Dissecting Microscope



Your instructor will provide objects of biological interest for you to examine with the dissecting scope. Place the object on a slide or watch glass. Using a small ruler, calculate the size of various objects.

When finished with the use of this scope, clean the lens, wrap the cord carefully around the base and safely store the microscope.

Oil Immersion

Oil immersion objectives are used only at very large magnifications that require high resolving power. The resolution of the image is increased by filling the space between the slide and the lens with oil. Oil has a much higher index of refraction than air and contributes to the magnification of the image. Using an oil immersion lens without immersion oil will result in unusable images and can cause irreversible damage to the first glass element of the lens located at the tip of the lens barrel. **Your instructor may show you how to use an oil immersion lens. However, if you are not shown how to use it correctly DON'T!!**

1. Locate the object and center it with scanning lens.
2. Move to low, then to high power; refocus while using the fine adjustment knob only.
3. Swing the high power lens away from the slide. Place a drop of immersion oil on the center of the coverslip and move the oil immersion lens into position. Refocus using the fine adjustment knob only.
4. When you are finished, swing the lens away. Do not put any other lens in position.
5. Remove the slide and wipe most of the oil off with paper, then with lens cleaner.
6. Clean the lens also with lens cleaner and then dry lens paper.