

2b

Name : _____

Microscope

Objectives :

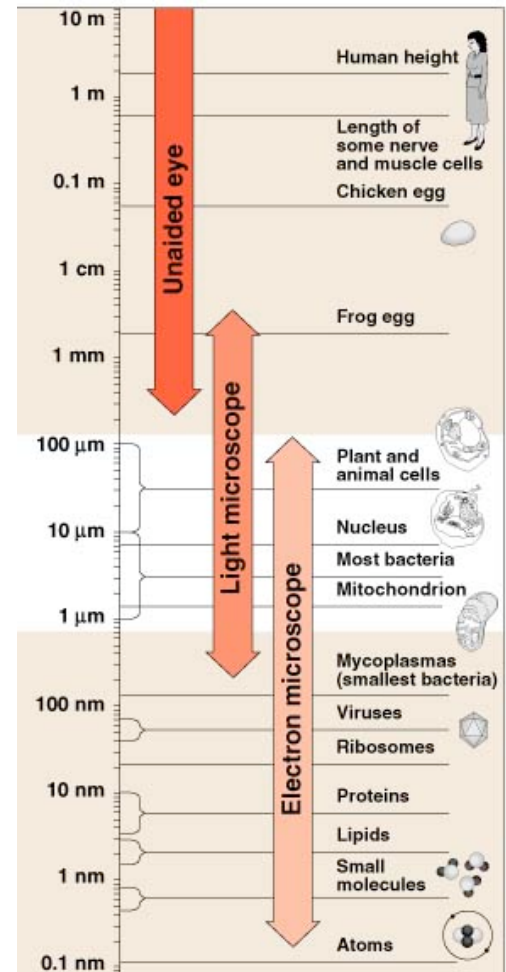
1. Understand the difference between Magnification and Resolution
2. Identify the major microscopic components & demonstrate proper microscope use
3. Be able to prepare and view wet mount slides

1A. Microscopy :

"The evolution of a science often parallels the invention of instruments that extend human senses to new limits." (Campbell 2002)

The microscopic study of anatomy is an excellent example supporting this statement. Without the aid of microscopes, our study and acquisition of knowledge about organisms and cells smaller than 0.1 mm (limit of the human eye) would never have been possible. The first microscope constructed and used for the examination of cells was the light microscope in 1665 by Sir Robert Hooke. He utilized the magnifying power of the simple yet powerful light microscope to describe for the first time the general structure of cells, symmetrical repeating patterns of cubes which reminded him of small monastery rooms called cells, hence the name. This began the wondrous journey into the unknown world of cells, launching the field of study known as *Cytology*. Light microscopes function by passing visible light through a specimen & series glass lenses. The lenses function to bend & change the course of the light as it passes toward the eye. The result is the production of a magnified specimen image.

Light microscopes are effective in magnifying images up to 1000x. Even today, the light microscope is the most commonly used scientific instrument. It is for this reason that anyone entering into scientific study should become familiar with the use and care of microscopes. Remember, just as the invention of instruments can extend our human senses, the ignorance of their proper use can hinder scientific discovery.



1B. LIGHT MICROSCOPY :

Two important parameters of microscopy are :

1. **Magnification:** The enlargement of a specimen's image size beyond actual size or the ration of an object's image to it's actual size
2. **Resolving Power (Resolution):** A measure of the images clarity; it is the minimum distance that two points can be clearly seen as distinct and separate points.

The limiting factor of most microscopes is the resolving power. For example, light microscopes use light to illuminate images. The smallest wavelengths of visible light are too large to resolve images smaller than 0.25 micrometers (um). This means that when images are enlarged greater than 1,000x, they will start to appear blurry. Therefore, the greater the magnification, the lower the resolving power. In order to clearly see objects smaller than 0.25um, a media with a much smaller wavelength must be utilized. In the 1950's the development of the electron microscope incorporated the electron technology, focusing a beam of electrons through a specimen or onto its surface. Electron microscopes have the ability to magnify & resolve objects as small as 0.1 nanometers (nm) (Approximately a million times smaller than the period at the end of this sentence) and hundreds of times better than the light microscope.

1C. Components of the Microscope

The microscope is the most basic and widely used instrument in both clinical and research laboratories. In order to make the microscope an effective and useful scientific instrument; you should become familiar with the names and functions of the following microscope parts listed below and diagramed in figure 1:

1. **Ocular Lenses (Eyepiece):** Last lens through light passes through before entering the eye. The ocular lens has the ability to magnify images **10x**. Most microscopes have two ocular lenses microscopes (Binocular Scopes). The binocular microscopes are generally preferred to the monocular microscopes because they provide a greater depth perception.
2. **Arm :** Supports the body and associated lenses.
3. **Base :** Supports the arm & provides stability to the microscope.
4. **Revolving Nosepiece :** Mounts the objective lenses to the body. By rotating the nosepiece, each objective lens can be brought into the path of light & used for magnification.
5. **Objective Lenses :** Function to differentially magnify images by specified degrees (10x, 43x, 98x).
 - a. Scanning (4x) - not present on microscopes used in this class
 - b. Low power (10x) – used during initial focusing; used for searching and gaining an overview of the a slide or specimen, often low power is sufficient for most observations
 - c. High power or high dry (40x) - used when viewing greater detail is desired
 - d. Oil immersion (100x) - not present on most microscopes used in this class; Increases the resolving power of the 98x objective by decreasing the refraction of light at the glass air interface of the slide & lens.
6. **Stage :** Flat surface below objectives upon which slides are placed for viewing.
7. **Stage Clips and Adjustment Knobs:**
 - a. Stage Clips (Stage Apparatus or Slide Holder) - clamps which hold the slide on the stage
 - b. Stage Clip Adjustment Knobs - two knobs control the movement of the clamps thus providing a means of precise movement the slide over the stage
8. **Course & Fine Focus :** Focusing Knobs - move stage closer or farther from the objective lenses to bring specimen into focus. Microscopes contain parfocal lenses. This means that once an image is brought into focus through one objective, it will be in focus in all other objectives with just minimal fine focusing.
 - a. Fine Focus Knob (smaller outer knob on either side of arm) - moves stage in small increments

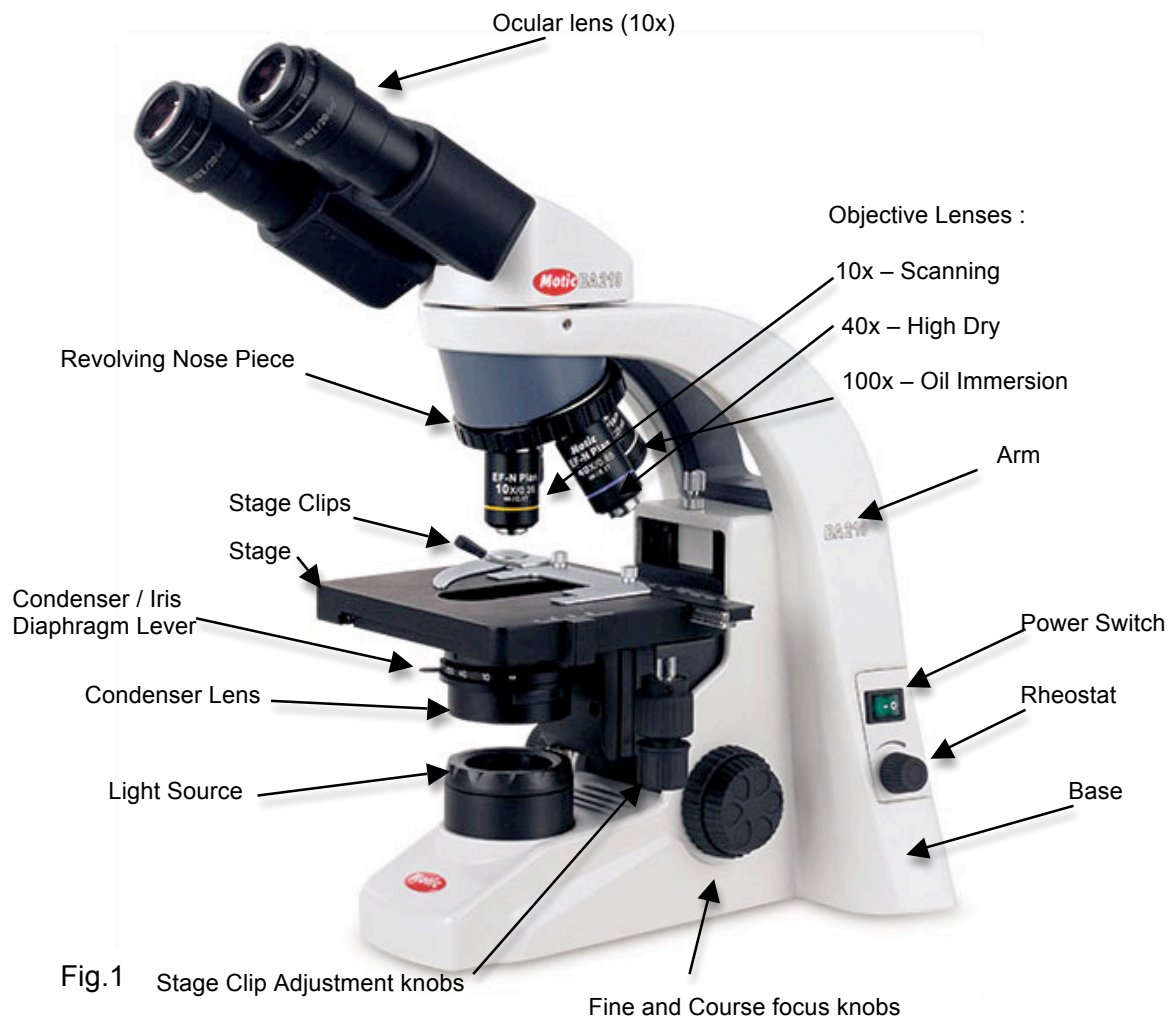
b. Course Focus Knob (larger inner knob, on either side of arm) - moves stage in large increments

9. Condenser : Adjustable sub-stage lens which functions to focus (or "Condense") light onto the specimen. When viewing specimens with little contrast the amount of light passing through the condenser should be decreased, but when viewing specimens at the higher magnifications more light is typically required.

a. Condenser (Substance) Adjustment : Moves the condenser up or down, thereby focusing light. The condenser is in relatively good focus when the condenser is positioned just slightly below the stage (Usually a ½ turn down from its highest position).

10. Condenser or Iris Diaphragm Lever : Adjustable sub-stage opening which functions to control the amount of light illuminating the specimen. By adjusting the condenser diaphragm an images' *contrast* can be increased or decreased. Decreasing the condenser diaphragm is essential when viewing specimens which are not highly pigmented and have little contrast.

Listed below are the main microscope parts. You should be able to locate and describe the function of all microscope parts listed.



1D. Handling and Care:

The microscopes in this laboratory are stored in numbered cubby holes corresponding to the microscope's numbers and in the cabinets at some of the student desks. Microscopes must be returned to their assigned cubby upon return. The following is a check list detailing the manner in which the microscopes should be handled and returned.

1. Always carry the microscope with two hands close to your body
2. ONLY *clean* microscope lenses (objective lenses) with LENS paper (stored in lab table drawer)
3. When putting away the microscopes :
 - a. Remove all slides from the stage
 - b. Move lowest power objective into viewing position
 - c. Move stage lowest position (furthest from objective lenses)
 - d. Center the stage
 - e. Coil electrical cord around *the bracket on the rear of the arm* or around the ocular lenses
 - f. Return microscope to cabinet cubby

★ *Each student is responsible for his/her own microscope.*

1E. Initial Focusing and Correct Use of the Microscope :

In order to effectively use the microscope it is important that the user be able to find and focus on images with relative ease. The following is a list of steps which should be followed EVERYTIME you put a slide on the stage. Using these simple steps will eliminate most of the frustration which generally accompanies microscopy.

1. Plug microscope into closest outlet, position cord so that it does not hang over the edge of the table.
2. Turn on microscope by rolling the "on switch" backwards (or push on button).
3. Make sure the lowest power objective is in the viewing position.

(ALWAYS START WITH THE *LOWEST POWER OBJECTIVE*)

4. Secure the slide to be viewed in mechanical retainer clamps (stage apparatus). Be sure that the slide's inferior surface is not wet, this will inhibit the slides movement.
5. While looking from the side, center the slide over (or the specimen to be viewed) the stage aperture (opening).
6. Using the course adjustment knob, move the stage upward toward the objective lenses (if using low power objective the slide will NOT hit the objective lens)
7. In order to focus, Look through the ocular lens and use the COURSE adjustment to move the stage DOWNWARD (away from the objectives) continue moving the course adjustment until the image comes into focus, then use the fine adjustment knob to make any minor adjustments in clarity. If you do not see the image come into focus, you have probably moved past the focal point. Reposition the stage to its initial position (moved all the way up) and start over.

(ALWAYS FOCUS AWAY FROM THE OBJECTIVES)

8. ONLY when the specimen is in focus on the low power should you then switch to a higher magnification. Use the FINE adjustment ONLY to sharpen the image.
9. ONLY use the *FINE adjustment* whenever you are using a *HIGH power objective* or oil immersion lens.
10. Always use a cover slip with wet mount slides.

1F. Important Terminology :

Like any instrumentation, the light microscope has specialized features and properties which must be defined. The following is a list of features and properties which are important for the understanding and correct use of a microscope.

1. **Magnification** : When viewing images through a microscope, it is important to be able to convey how much larger the image is than the actual specimen size. By convention the magnification is always expressed as the “Total magnification” of the scope (not the magnifying power of the lens being used). The total magnification of the microscope can be easily determined by multiplying the magnifying power of the ocular lens (10x) by the magnifying power of the objective lens being used (4x, 10x, 40x, 100x).

i.e. : The total magnification of the microscope (image) when using the 40x objective is 400x.

2. **Field (Field of view)** : The area of the slide which is visible when viewing through the microscope. The size of the field of view decreases with increasing magnification.

3. **Depth of Field** : The clearly visible distance *into* the slide (remember a slide is 3 dimensional). The depth of field decreases with increasing magnification.

4. **Parfocal Microscope** : Microscope designed to allow changing of the objectives without significant changes in focus.

5. **Working Distance** : The distance between the specimen and the objective lens.

6. **Wet mount slide** : A temporary slide preparation where the specimen is suspended in a liquid (often under a cover-slip)

7. **Prepared Slide** : A permanent slide preparation where the specimen is fixed and bonded under a cover slip

1G. Image Orientation

When using a microscope it is important to be aware of the relationship between specimen and the image’s orientations. Because light microscopes pass the image of the viewed specimen through several lenses the image of the specimen and the actual orientation of the specimen are different. Light microscopes function to invert the image into a position ultimately *upside down and backwards* relative to the actual specimen.

1H. Wet Mount Slide Preparation

Often when working with living material, wet mount slide preparation is often required in order to examine the microscopic anatomy of the specimens. In this case the experimenter or technician will need, microscope, clean glass slide, cover slips, holding container for fluids, and a transfer pipette. In order to prepare a wet mount follow the direction below:

1. Place slide on a flat surface.
2. Using a transfer pipette, place a drop of fluid on the slide.
3. Hold the coverslip by its sides and lay its bottom edge on the slide close to the edge of the fluid drop. Holding the coverslip at a 45o angle helps.
4. Slowly lower the coverslip so that it spreads the fluid out.
5. If you get air bubbles (looking like little black doughnuts), gently press on the coverslip to move them to the edge.
6. If there are dry areas under the coverslip, add a little more fluid at the edge of the coverslip.
7. Too much water can be dabbed off with a piece of paper towel or you can start over with a new slide.

2b

Name: _____

Microscope

Complete the following exercises. If you need more practice there are microscopes and prepared slides in the student study room.

Exercise 1: Preparing a Wet Mount (Sheep's blood)

- Obtain a clean glass slide, cover slip, small glass test tube, 0.9% saline and a plastic transfer pipette. (ONE test tube of blood per table).
- Put 3 mls of saline into test tube. Put ONE drop of sheep's blood into test tube (ONE per Table).
- Agitate tube to mix blood. The tube should be a pale cloudy red.
- Transfer one drop of diluted blood to the center of your clean slide
- Place a small square cover slip over the blood. (note you should not have fluid rushing off the slide. If you do you have TOO much fluid and should start over.
- When you are finished viewing your slides, dispose of the used slides in the 10% bleach water.
- When all students at your table are finished with the sheep's blood test tube, empty the blood into the 10% bleach water and place the test tube in the used test tube rack.

Exercise 2: Focusing on Wet Mount Slide;

- Using the correct steps to focusing, focus on the sheep's blood first on low power (10x) and then on high power (40x).
- Note the appearance of the sheep's erythrocytes (red blood cells).

Questions:

- Do sheep erythrocytes have a similar anatomy to human erythrocytes?
- Can you see leukocytes? Why or why not?
- Are the blood cells moving? If so what is causing the motion?
- Are the cells alive or dead?

Exercise 3: Using a clear ruler to approximate size:

- Place a clear plastic ruler with the mm marking on top of the stage.
- Using the lower power objective (10x) approximate the distance of the field of view.

_____mm

- Using the high power objective (40x) approximate the distance of the field of view.

_____mm

Questions:

- How might this information be useful?

Exercise 4: Summary & Clean-up

- b. Be sure you are familiar with the microscope parts and functions
- c. Make sure you clean up your area, put away all materials and the microscope correctly.