LABORATORY EXERCISE #2

INTRODUCTION TO THE COMPOUND LIGHT MICROSCOPE (Nedwidek revised)

INTRODUCTION
One of the biologist's most important laboratory tools is the *compound light microscope* (Greek: micron = small and scopos = aim) which consists of two converging lens systems: the objective and the eyepiece. It is an instrument that is used to magnify objects that are too small to be seen with the unaided eye.

STUDENT OBJECTIVES
1. Learn proper handling of the compound microscope.
2. Know and label the names and functions of the parts of the microscope.
3. Prepare a slide.
4. Acquire skills in using low and high-power magnification.
5. Estimate sizes of specimens.

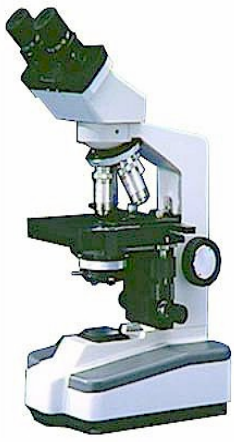
PRE-LAB QUESTION
1. Explain the difference between magnification and resolution. Which is more important?
2. Explain the function(s) of each of the following parts of the compound light microscope:
    a. Ocular lens
    b. Nosepiece
    c. Wide angle objective lens
    d. Low power objective lens
    e. High power objective lens
    f. Diaphragm
    g. Coarse adjustment knob
    h. Fine adjustment knob.

MATERIALS
Microscope, glass slide, cover slips, lens paper, slide with a small clear metric ruler, pieces of newspaper, tweezers

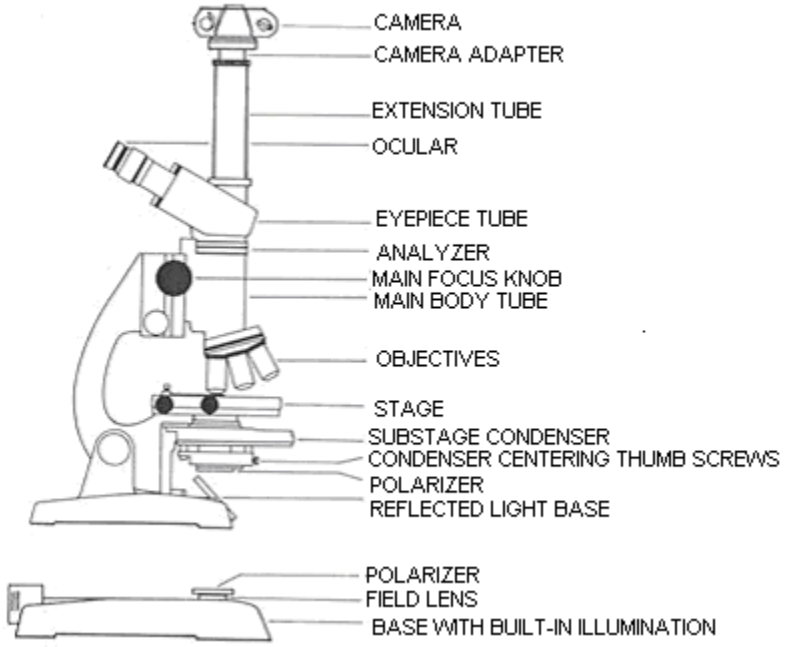
PROCEDURE
Last lab you received a "LAB BENCH NUMBER." This number corresponds to your seat at your lab bench. It is also the number on your microscope and other materials that you will use during the term. You are responsible for items with this number. When instructed, go to the microscope cabinet and grasp your microscope with two hands. Bring it back to your bench and wait for further instructions.

I. Parts of the Microscope
1. As your teacher reviews the parts of the microscope and their uses, you should label the diagram of the microscope.

    The following are common compound microscopes in the Biology Department:



You can find the names of the parts of the microscope from the diagram below:



2. The following rules for the proper use of the microscope should be remembered:
   a. Always use two hands when carrying a microscope. One hand should be grasping the arm of the microscope while the other hand should be under the base. Always keep the base parallel to the floor.
   b. Place the microscope on the desk with the arm away from you.
   c. **ONLY use lens paper** to clean the lenses and the mirror before use.

II. Using the microscope

1. Setting up the microscope.

Remember that ocular x objective is total magnification. Oculars on most of our scopes are 10x.

   a. Revolve the **scanning objective (4X)** until it is centered over the hole in the stage. You should feel a click when it is in place.
   b. Turn on the light (some microscopes may have a concave mirror) and open the diaphragm to produce a bright circle of light. This circle is your field of vision (FOV).
   c. After you have prepared the slide to be viewed you will continue with the following.

2. Focusing the microscope.
   a. Place the slide on the carrier on the stage between the prong and the spring clip so that it nestles against the inside corner of the carrier. DO NOT put the slide under the metal carrier. Using the knobs at the side of the stage, adjust the carrier so that the slide is centered over the hole in the stage.
   d. Using the coarse adjustment knob, bring the stage as close as possible to the objective. For most of our microscopes, the stages rises to meet the objective).
   b. To focus, always start with the coarse adjustment. Focus by moving the stage down slowly. If you have moved the focus a long way and nothing has appeared, you may have to move the stage slowly up until it is a little less than 1 cm from the objective. Move the stage down again until you find the material on your slide. Remember to center your specimen using the knobs at the side of the stage.
   c. To examine the specimen under **low power objective (10X),** gently swivel the objective into place. Focus using the **coarse adjustment knob first, and then fine fine adjustment knob.** Move the slide around to get the best view in the center of the FOV.

d. To examine the specimen under the **high power objective (40X)** gently swivel the objective into place. ONLY FOCUS WITH THE FINE ADJUSTMENT KNOB.
e. When finished, swivel the nosepiece into the "neutral" position (no objective is in place) and carefully remove the slide from the stage.

III. Examining the letter "e"

1. Preparation and examination of the LOWER CASE letter "e" slide.
   a. Prepare slide for observation by placing a piece of newspaper with a word containing the letter "e" on a slide (the rectangular piece of glass) in an upright position. Cover it slowly with a coverslip (the small plastic square). (Your teacher may require you to use a drop of water on the specimen to make a WET MOUNT.)
   b. Put the slide in the slide holder and move it so that letter e is over the hole on the stage. Keep the "e" in the upright position.
   c. Follow the instructions in procedure II for focusing under first the **scanning objective (4X)** and then the **low-power objective (10X). Remember to center your letter "e" in the FOV**
2. Observation of the letter "e".
   a. Under the low-power objective, note the orientation of your letter "e". Move the slide gently to the right and then left, noting the direction in which the letter moves. Move the slide away from you and towards you, noting the direction in which the letter moves.
   b. On your answer paper, draw a circle about 5 cm in diameter to represent the circle of light (field of vision) that you observe in the microscope. In this circle, DRAW AND LABEL the letter "e" under the low power objective. Observe the way the ink clings to the paper and the texture of the paper. Try to capture these features in your drawing.
   c. Switch to the **high power objective** and think about what has happened to the image. In a second circle, DRAW AND LABEL the letter "e" under high power.

IV. Measurement of the size of the field.: 40x (scanning), 100x (low), 400x (high).

1. Switch the nosepiece into the neutral position.
2. Remove the slide of the letter "e".
3. Place a slide of a clear metric ruler onto the stage and adjust it over the light. Carefully switch to the **low-power objective.** Focus with the coarse adjustment knob. Position the ruler's bottom edge along the diameter so you can see at least one mm mark. **ESTIMATE** the size, in micrometers (μm), of the field of vision and record your answer on the answer page.
4. Magnification has an **INVERSE** relationship to the diameter of the field of vision. If the high-power magnification is approximately 4 times that of the low power, what would be the size of the high-power objective FOV in proportion to the low-power objective? Determine the actual size, in micrometers (μm), of the scanning, low and high-power fields of vision for your answer page.
5. In a third circle, DRAW AND LABEL the ruler under the **low power objective**.
6. In a fourth circle, DRAW AND LABEL the "e" with ruler under low power (100x).