The Microscope

PRE-LAB DISCUSSION:
"Micro" refers to tiny, "scope" refers to view or look at. Microscopes are tools used to enlarge small objects so as they can be studied. Microscopes range from a simple magnifying glass to the expensive electron microscope. The compound light microscope is the most common instrument used in education today. It is an instrument containing two lenses, which magnifies, and a variety of knobs to resolve (focus) the picture. It is a rather simple piece of equipment to understand and use. In this lab we are going to learn the proper use and handling of the microscope.

PURPOSE:
- To learn how to use the microscope.
- To demonstrate the proper procedures used in correctly using the compound light microscope.
- To prepare and use a wet mount.
- To determine the total magnification of the microscope.

INTRODUCTION:

A. Parts of the Microscope

Get a microscope from the cabinet. Handle it carefully and bring it to your lab table. Use two hands to carry the microscope, one on the base and one on the stage at all times.

1. Compound Microscope Optics: the glass lenses which modify the light as it passes through the microscope.

The compound microscope has more than one system of lenses; one lens system is the objective lens which is closest to the object being examined and makes the initial magnification. A microscope may have one or more objectives each capable of different magnifications. Depending on your microscope, the lowest power objective lens may magnify four times (4x) or ten times (10x) and the highest power objective can magnify forty-three times (43x). Note what power objectives are on your scope.

The second lens system is the eyepiece or ocular lens. This is located at the top of the body of the microscope. The common eyepiece lens magnifies ten times (10x). To calculate magnification of the object being viewed simply multiply the power of the objective lens by the power of the ocular: e.g., using a 43x objective lens and a 10x ocular, total magnification is 43 x 10 or 430x.

Base: the horizontal, oval-shaped part upon which the microscope rests.

Arm: the vertical member which supports the body.

Body: the portion of the microscope which contains the lenses.

Revolving Nosepiece: the assembly which contains the objective lenses.
Stage: the platform upon which the object to be examined is placed.

Sub-stage Assembly: the parts below the stage; these include the condenser, iris diaphragm, and illuminator. In some scopes this assembly, not including the illuminator, can be adjusted up and down.

Condenser Lens: a lens assembly beneath the stage which concentrates light on the object being examined.

Diaphragm: a series of holes which control the amount of light which reaches the object being examined.

(Iris Diaphragm: an assembly of metal "leaves" which controls the size of opening through which light enters the condenser; therefore it controls the amount of light which reaches the object being examined. It is opened and closed by a small arm to one side of the sub-stage assembly.)

Illuminator: the light source. It may be built into the base or it may not be part of the scope at all.

Coarse and Fine Adjustment Knobs: these knobs adjust the distance between the objective lens and the stage. This distance is called the working distance.

B. Care and Use of the Microscope

1. Carrying the microscope: remember two hands, and keep it upright or the ocular might fall out!

2. Cleaning: be sure that all lenses are clean. Many times water will get onto an objective lens and leaves a film when it dries. This will give you a blurry image. To clean the lenses use the lens paper supplied by the instructor. Do not use a handkerchief when cleaning a lens and do not breathe onto it since this can also leave a film when wiped off. To clean a lens wipe in one direction or gently with a circular motion. If after cleaning the image is not clear, consult your instructor.

3. Focusing: Place the slide in the center of the stage, and start with low power. Looking from the side bring the slide and objective very close together using the coarse adjustment. Then looking through the microscope focus by turning the coarse adjustment knob so that the distance between the slide and objective increases until you see the object in question, then finish the focus with the fine adjustment (the smaller knob). If you don't see anything adjust the light; too much light will wash out the image. Most scopes are parfocal. This means that when you want to change view of the specimen from low power to high power, all that is needed after revolving the nosepiece to high power, is to adjust the focus with only the fine focus knob. You should never focus with the coarse adjustment while using high power, as this leads to broken slides. When changing from low to high power you will notice that more light is also required.

4. Adjustment of light: remember that the condenser controls the quality of light which reaches the slide while the diaphragm controls the quantity of light reaching the slide. If it is too low refraction causes distortion of the object being examined. To control the amount of light reaching the slide adjust only the diaphragm. When the diaphragm is on the largest opening, the greatest resolution of detail is obtained.
while when the diaphragm opening is small, resolution is reduced but contrast is high. Resolution is the ability to see two separate points instead of them combining into one fuzzy point.

**Laboratory Procedures**

**Part 1. Determining Orientation and Adjusting Illumination**

Obtain a "letter e" and place it on a slide. Look at it through the microscope under low power. Is it oriented the same when you see it with the naked eye? Move the slide down while looking through the scope; which way does the e move? Move it to the right; which way does it move as you watch it through the scope. How much of the field of view does the "e" occupy? Change to high power; how much of the field does the e occupy now? Is the light brighter or dimmer? Open the iris a bit. Does this improve the light? Go back to low power; what happens to the light?

**Part 2: The Comics, it’s not just the educational supplement.**

Pick 3 different colors from a comic strip. Make sure one of the colors is either purple or brown. Cut out a small piece of each color and place each on a slide. Observe each color under medium power and record observations in table 1.

**Part 3. Drawings:** Drawings should include certain pertinent information.

- All drawings must be completed in pencil. Colored pencils are optional but preferred. Ink is not permitted.
- Each drawing must be a minimum of 4cm x 6cm in size.
- Drawings should be completed during the lab period and on the specified worksheets.
- All drawings must contain labels.
- All drawings must include a title stating what the drawing is and if necessary where it came from, and how prepared.

**DATA AND OBSERVATIONS:**

**Part 1:**

1. Is the letter "e" oriented the same in the microscope, as it is when you see it with the naked eye?

- Move the slide down while looking through the scope; which way does the "e" move?
- Move it to the right; which way does it move as you watch it through the scope?
- Estimate the percent of the field of view that the "e" occupies at low power.
- Using a clear ruler, measure the size of the "e" at low power.
- Estimate the percent of the field that the "e" occupies at high power.
- Using a clear ruler, measure the size of the "e" at high power.
- Is the light brighter or dimmer at high power?
- What happens when you open the iris diaphragm a bit?
• Go back to low power. What happens to the intensity of the light?

Part 2:

**Table 1**

<table>
<thead>
<tr>
<th>Color you see</th>
<th>Color of the dots</th>
</tr>
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<tbody>
<tr>
<td></td>
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**Calculations:**

**Determining Total Magnification:**
1. Locate the numbers inscribed on the eyepiece and the low power objective and fill in the blanks below.

Eyepiece magnification \( \text{__________} \) \((X)\) Objective magnification = \( \text{__________} \) \( \text{Total Magnification} \) \( \text{__________} \) \( X \)

2. Do the same for the high power objective. \( \text{________________________} \) \( X \).

**Determining Size of Objects**

Size of the “e” at low power \( \text{__________} \) mm
Size of the “e” at high power \( \text{__________} \) mm

Convert the measurement from mm to microns (um) by multiplying by 1000.

Size of the “e” at low power \( \text{__________} \) microns (um)
Size of the “e” at high power \( \text{__________} \) microns (um)

**QUESTIONS FOR DISCUSSION:**
1. Why do we use a microscope when studying biology?
2. Explain the relationship between the movement on the stage of the “e” and how you viewed it thorough the eyepiece.
3. Why are the colored dots you see made up of multiple colors and not a single color?