Advanced Microscope Techniques

Name:_____ Period:_____

Background: Most of the objects you view under the compound microscope are smaller than two millimeters. Obviously, measuring these microscopic objects could prove to be quite difficult and inexact if millimeters are used as the unit of measure. To solve this problem scientists divide the millimeter into 100 smaller units called micrometers (μm). Tiny objects can then be accurately measured in micrometers.

Purpose: In this lab you will learn how to estimate the size of the tiny organisms you view under your compound microscope. Also you will pick up some additional techniques on using the diaphragm, measuring, and focusing that will increase your skill using the microscope.

Materials : Microscope slides, medicine droppers, tap water, coverslips, compound microscopes, prepared slides of cells, forceps, dissecting needles, cotton balls, thread (one dark and one light color), scissors, transparent plastic centimeter ruler

Procedures:

Adjusting the Diaphragm

1. Obtain a microscope and the supplies needed to make a wet mount of several cotton fibers. Make a wet mount of the cotton fibers.

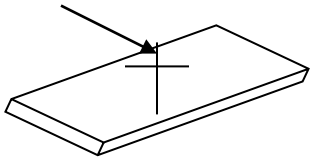
2. Look at the fibers under low power while slowly opening and closing the diaphragm. Which diaphragm opening provides the sharpest, clearest view of the cotton fibers? (a)_____

3. Look at the cotton fibers under high power while slowly opening and closing the diaphragm. Which diaphragm opening provides the sharpest, clearest view of the cotton fibers? (b)_____

Locating Various Depths of Field

1. Make a wet mount of two threads crossed to form an X as shown below. Use one dark thread and one light thread.

threads



2. Look at the slide under low power where the two threads come cross forming an X. Adjust the diaphragm to give the sharpest view. Use the fine adjustment to focus. Are both threads clearly visible at the same time under low power? (c)_____

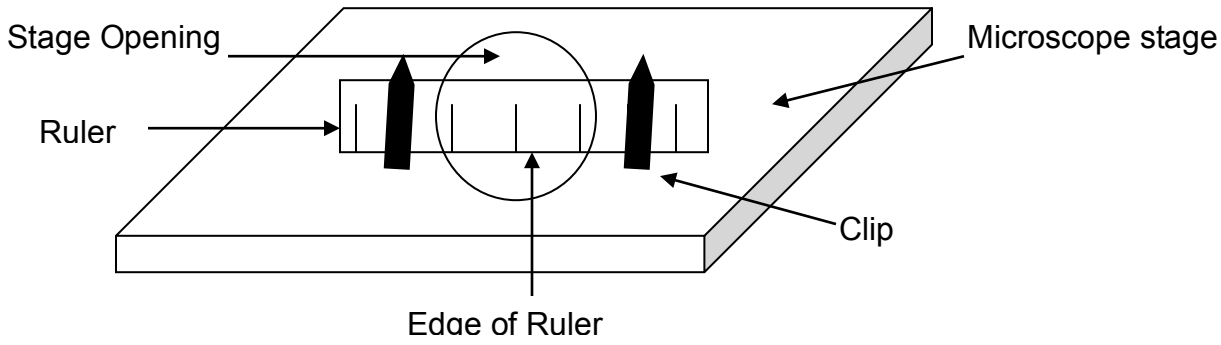
3. Look at the slide under high power where the two threads cross threads forming the X. Adjust the diaphragm to give the sharpest view. Use the fine adjustment to focus. Are both threads clearly visible at the same time under high power? (d)_____

4. Describe what you see as you fine focus the threads. (e)_____

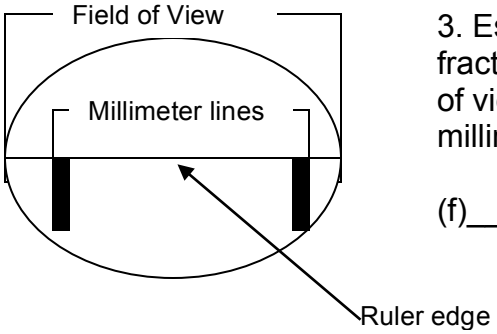
_____

Figuring the Field Diameter of Your Microscope

1. Place the transparent plastic ruler on the stage so that the ruler's edge is centered in your field of view under low power. This is shown below.



2. Position the ruler so one of the millimeter markings is just visible to the left in your field of view as shown below. Notice that the distance between the marking on the left and the next mark is one millimeter.



3. Estimate the remaining distance in decimal fractions of millimeters across the diameter of the field of view. What is you total field of view size in millimeters under low power?

(f)_____

4. What is your field of view under low power in millimeters or microns? (HINT: remember that 1000 micrometers equals 1 millimeter.)

(g)_____

5. Switch to the high power objective. Look at the markings on the ruler. You will find that the high power field of view is less than one millimeter or 1000 micrometers. For that reason, it is difficult to estimate the diameter of the field of view using the same technique you used for low power. However, you can determine the field of view under high power by doing a simple calculation using the following formula:

$$\frac{\text{High power magnification}}{\text{Low power magnification}} = \frac{\text{Low power field diameter}}{\text{High power field diameter}}$$

6. To use the formula you will need the following data: What is your microscope's high power magnification? (h)_____

What is your microscope's low power magnification? (i)_____

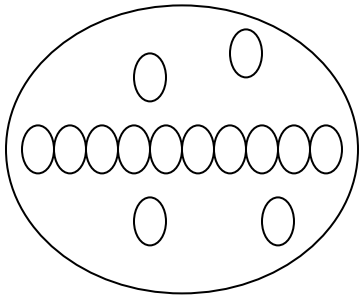
What was your microscope's estimated field of view diameter under low power in micrometers? (see letter (g) in step 4 on the previous page) (j)_____

7. Insert the numbers from (h), (i), and (j) into the formula to determine the high power field diameter. What is your calculated high power field diameter?

(k)_____

Estimating the Size of Objects Viewed Under the Compound Microscope

1. Now that you know the diameter of your field size under both high and low power, you can use that information to estimate the size of objects you examine across the field of view. For example in the field of view below, 10 circular objects fit across. The field of view is 2000 micrometers in diameter.



Since each object takes up $1/10^{th}$ of the 2000 micrometers field of view diameter, the size of each object is 200 micrometers. You can use this method to estimate size of objects you view under your microscope once you know your microscope's field of view diameter. If two organisms fit across the field of view that has a diameter of 1000 micrometers, how large is each organism?

(l)_____

2. Obtain prepared slide of various organisms and practice estimating their lengths. Write the name of the organism or part you examine and its estimated size in the chart below.

Name of Organism of Part of Organism	Estimated Size in Micrometers

Analysis Questions:

1. In general, how would you have to adjust the diaphragm after switching from low to high power?

2. Of what importance is knowing that fields of different depths exist when you are observing objects under high power? (HINT: think about the 2 different colored strings you looked at.)

3. Do you observe more or less area in your field of view when under high power compared to low power?

4. If a microscope has a low power magnification of 100X and a high power magnification of 500X, and a low power field diameter of 1500 micrometers, what is the high power field diameter in micrometers?

5. If 20 objects fit across the diameter of a low power field of view whose field diameter is 4000 micrometers, what would be the approximate size of each object?

6. How has the development of the microscope aided the development of Cell Theory?

Conclusions: Please explain 3 things that you learned in complete sentences.