Microbiology Lab Exercise 1 (20 pt) Name_____

BASIC MICROSCOPY with the OLYMPUS CH-2

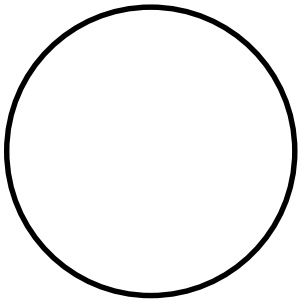
Student Worksheet

Viewing a Ruler under the Microscope

1. Position the ruler on the microscope stage with the number 10 facing you when you are not looking through the microscope. Now, with the 4x objective in place, center the number 10 in the field of view (you will need to do this with your hands since the mechanical stage will not hold the ruler) and examine it through the microscope.
   a. Is the image right side up or upside down (Circle your answer.)?

      right side up upside down

   b. Draw the image that you see through the objective.



   c. Move the ruler forward. Which way does the image move (Circle your answer.)?

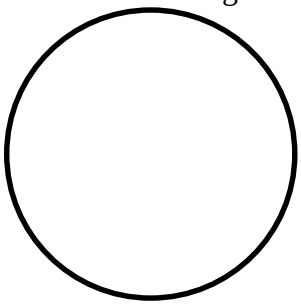
      backwards forward

   d. Move the ruler to the left. Which way does the image move (Circle your answer.)?

      left right

Magnification and Field of View

2. Move the ruler so that you can see the millimeter markings.
   a. Draw the image that you see through the objective.

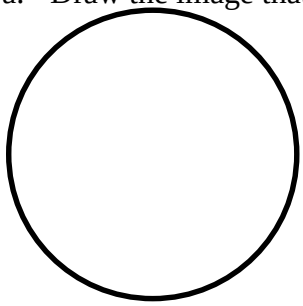


   b. What is the diameter of the field of view (in mm)? (NOTE – The distance between each line is 1 mm.)

   c. What is the diameter of the field of view in µm? (There are 1000 µm in 1 mm. If you are uncertain about how to make this calculation, please ask.)

   d. What is the total magnification at this point? (Multiply the magnification of the objective with the magnification of the 10x ocular.)

3. Switch to the 10x objective and examine the ruler markings again.
   a. Draw the image that you see through the objective.



   b. What is the diameter of the field of view (in mm)? (NOTE – The distance between each line is 1 mm.)

   c. In percent, how wide is the field of view now compared to the previous measure? (Divide the current length of the field of view by the length of the previous field of view and multiply it by 100 to convert it to a percentage.)

   d. What is the total magnification at this point?

   e. In percent, how much larger are objects at this magnification than they were at the previous magnification (from 2d)? (Divide the current magnification by the previous magnification and multiply it by 100 to convert it to a percentage. Yes, the answer is greater than 100%.)

Viewing a Stage Micrometer under the Microscope

4. Remove the ruler and clamp a stage micrometer into place in the mechanical stage. A stage micrometer is a slide that has a miniature (2.00 mm) ruler on it. It allows you to measure distances to the nearest micrometer. Move the slide around with the mechanical stage while focusing with your left hand. The movement of the slide will make it easier to find the micrometer. If you cannot find the micrometer with the 10x objective in place, switch back to the 4x objective to find it, focus on it, and then switch back to the 10x objective.

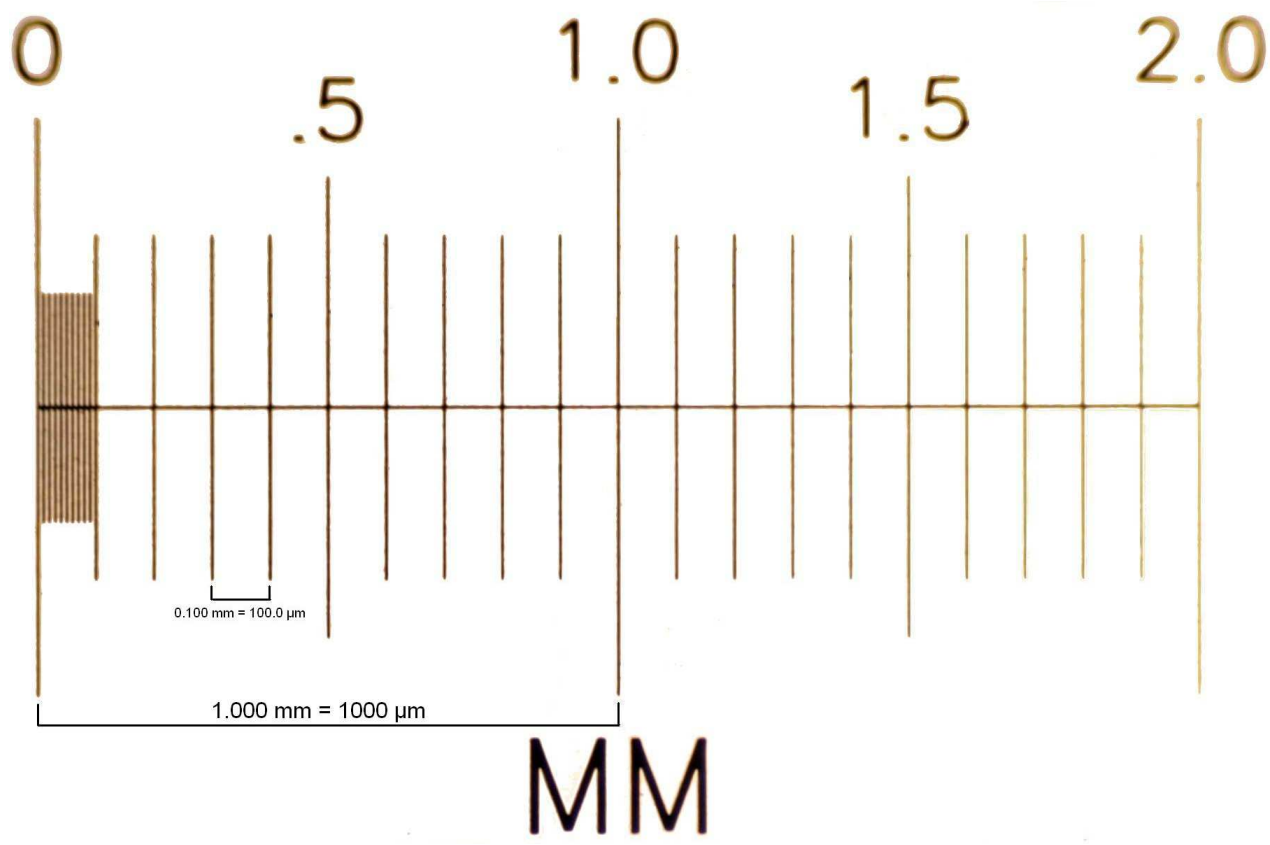
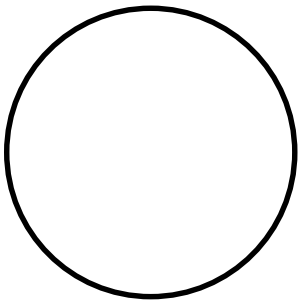


Figure 1: Expanded view of the stage micrometer. Notice that is 2.0 mm long and has divisions every 0.1 mm (or 100 µm).

a. With the 10x objective in place, draw the image that you see through the objective.

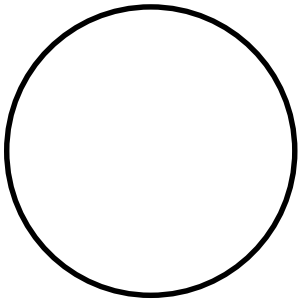


b. What is the diameter of the field of view (in mm)?

c. How close is this new measurement to your previous measurement using a ruler (3b)?

d. What is the diameter of the field of view (in µm)? (There are 1000 µm in 1 mm. If you are uncertain about how to make this calculation, please ask.)

5. Switch to the 40x objective and examine the stage micrometer markings. Adjust the ruler and focus to position and observe the millimeter markings better.
   a. With the 40x objective in place, draw the image that you see through the objective.



   b. What is the diameter of the field of view (in mm)?

   c. What is the diameter of the field of view (in µm)?

6. Switch to the oil immersion objective and examine the stage micrometer markings. Adjust the ruler and focus to position and observe the markings better.

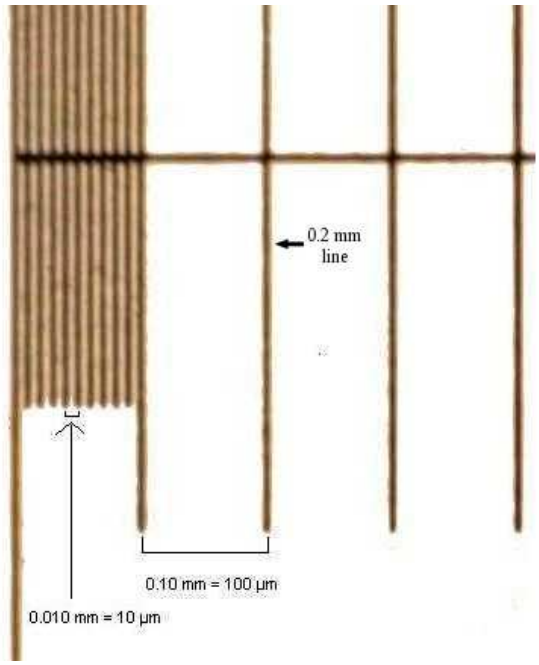
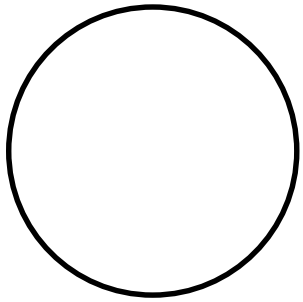


Figure 2: Expanded view of the first 0.4 mm (400 µm) of the stage micrometer. Notice that the first 0.1 mm segment is further divided into 0.01 mm (10 µm) segments. The 0.2 mm line is pointed out.

a. What is the total magnification at this point?

b. In percent, how much larger are objects at this magnification than they were at the previous magnification (from 3d)? (Divide the current magnification by the previous magnification and multiply it by 100 to convert it to a percentage. Yes, the answer is greater than 100%.)

c. Move the stage micrometer so that the 0.2 mm line is on the right edge (see Figure 2). Draw the image that you see through the objective.



d. What is the diameter of the field of view (in µm)

e. In percent, how wide is the field of view now compared to the previous measure? (from 4d)?

7. Is there a relationship between magnification and the diameter of the field of view? Answer (a) and (b) to find out.

   a. Collect the data from the previous pages and fill in this table. To determine the percent change in magnification, divide the current magnification by the total magnification in the 4x objective row and then multiply by 100. To determine the percent change in field of view, divide the current diameter of the field of view by the diameter of the field of view in the 4x row and then multiply by 100.

Objective	**Total Magnification**	**Percent Change in Magnification (compared to 4x)**	**Diameter of the Field of View (in µm)**	**Percent Change in Field of View (compared to 4x)**
4X	2d	100%	2c	100%
10X	3d		4d	
40X			5c	
100X	6a		6d	

   b. Generally, what happens to the diameter of the field of view as the magnification increases (Circle your answer.)?

      increases decreases

Magnification, the Iris Diaphragm and the Depth of Field

8. Turn the 4x objective into place. Remove the stage micrometer, clean the oil off it, and return it to its box at the front of the lab. Pick up one of the prepared slides of three threads and clip it into the mechanical stage. Center on the location where the three strings cross so that you can see all three strings, and then rotate the 10x objective into place.

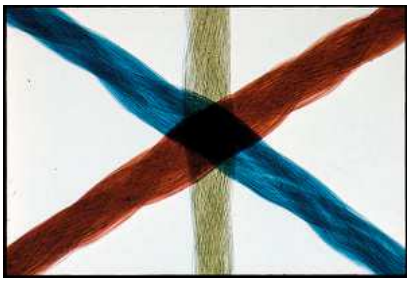


Figure 3 Expanded view of the thread slide. **Sharply** focus on the middle thread (in this case, the blue one). You may need to move slightly off center to just focus on the middle thread, but try to set the image so that the top and bottom threads are still in the field of view.

a. Now move the iris diaphragm all the way to the left near 100x (wide open). What is the maximum number of strings that are in **focus** at one time? (Write a number.)

b. Move the iris diaphragm all the way to the right near 4x (closed down). How many of the strings can you focus on at one time? (Write a number.)

c. Does the amount of light increase or decrease as you close the iris diaphragm, i.e., move the iris diaphragm to the right? (Circle your answer.)

   increase decrease

d. Does the color of the strings become brighter or darker as you close the iris diaphragm, i.e., move the iris diaphragm to the right? (Circle your answer.)

   brighter darker

9. Switch to the 40x objective and use the fine focus adjustment to **clearly focus** the image of the middle thread as sharp as possible. Note that at least one of the threads is in focus.
   a. Now move the iris diaphragm all the way to the left near the 100x symbol (wide open). How may of the three main threads are **in focus** at one time? (Write a number.)

   b. Can you easily determine the colors of the other two strings?

      No Yes

   c. Close the iris diaphragm, i.e, move the lever all the way to the right by the 4x symbol. Sharply focus on the middle thread. How many of the three main threads can you focus on at one time? (Write a number.)

   d. Can you easily determine the color of the other two strings?

      No Yes

SUMMARY

10. Generally, what happens to the intensity of the light (does it increase or decrease) as the iris diaphragm is closed (moved to the right) (Circle your answer.)?

    increases decreases

11. Generally, does the brilliance of the colors become brighter or darker as the iris diaphragm is closed (moved to the right) (Circle your answer.)

    brighter darker

12. Generally, does the depth of field, i.e, to the number of threads that are in sharp focus become larger or smaller as the magnification increases (Circle your answer.)?

    larger smaller

13. Generally, does the depth of field become thicker or thinner as the iris diaphragm is closed (moved to the right)? (Circle your answer.)

    thicker thinner