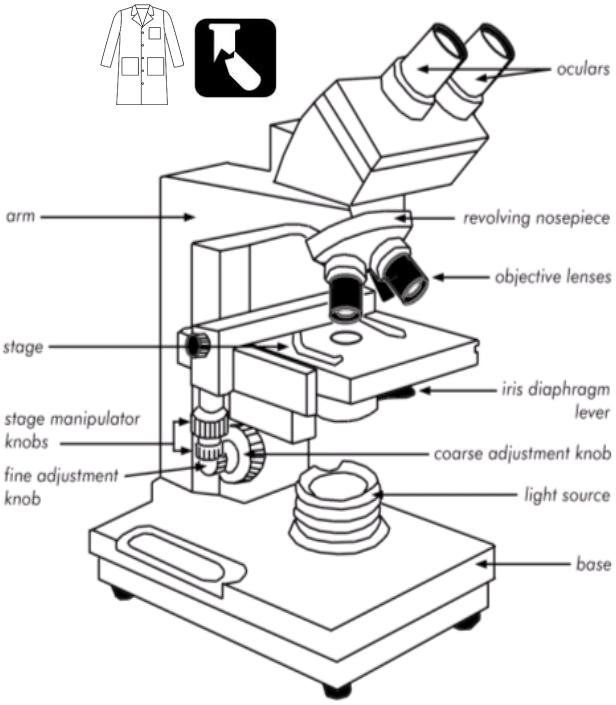
Microscopy Lab #1; SCSh 2, 4



Purpose: The purpose of this lab is to master the use of a compound light microscope and to become familiar with basic slide preparation techniques.

1. Magnification

1. What is the magnification written on the ocular lens? The number that is in mm is the size.

2. What are the four levels of magnification for the various objective lenses? These are the bold numbers. The lowest objective level is often referred to as the “scanning lens” and is always the starting point.

3. Calculate the total magnification for each of the four levels of magnification

4. Carefully read the 100x objective lens. What is different about this lens? Next, read one of the blue information sheets and describe how and when this power of magnification would be used in microscopy.

2. Diaphragm

5. Examine the diaphragm and move the lever left and right. Which setting makes the specimen appear the brightest? What about the darkest?

3. Lenses

6. Look through and turn the ocular lens of a non-digital microscope. What is the purpose of the pointer?

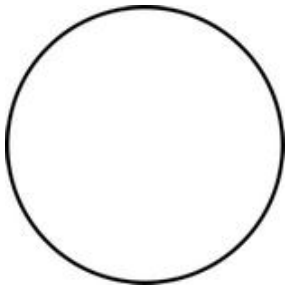
7. Describe what happens to your viewing field if you do not have an objective fully clicked.

4. Viewing a Slide

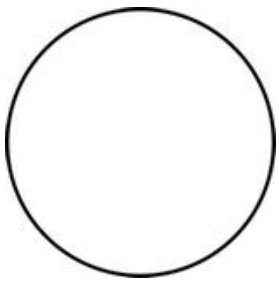
Obtain a prepared "e" slide. Focus the slide first with the scanning objective, then click to lower power and focus again. Finally, focus the slide under high power. Remember, at high power, you should ONLY use the fine adjustment knob.

8. Transcribe the circles below onto your paper. They represent your viewing field. Draw the “e” exactly as it appears in your viewing field for each magnification. The “e” should take up as much space in the drawing as it does in your viewing field while you're looking at it.

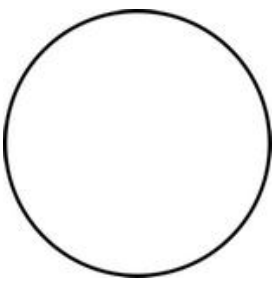
Scanning (4x)



10x Power



40x Power



5. Depth Perception

Obtain a prepared “colored threads” or “cross fibers” slide. You will only need to view it under scanning magnification at this point. Your task is to figure out which thread is on top, which is in the middle, and which is on bottom. You should notice that as you focus the thread, different thread will come into focus at different times. The one that comes into focus the first should be the top thread.

9. What is the color order of your threads or fibers?

As you remove the slide from the stage, note that it is labeled with *w.m.* Read the blue information sheet to determine the different types of slide mounts used in light microscopy.

10. What are the six main types of slide mounts?

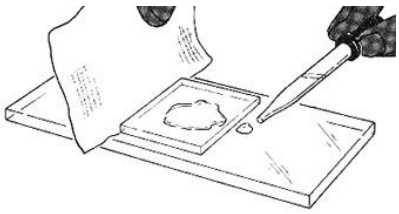
6. Making a Wet Mount of a Slide

Gather a few strands of cotton from a cotton ball using forceps. If your specimen is too thick, then the coverslip will wobble on top of the sample like a see-saw. Place ONE drop of water directly over the specimen. If you put too much water, then the coverslip will float on top of the water, making it hard to draw the specimen, because they might actually float away. Place the coverslip at a 45 degree angle (approximately) with one edge touching the water drop and then gently let go. Performed correctly the coverslip will perfectly fall over the specimen.

11. Transcribe the circles below onto your paper. These represent your field of view. Draw the specimen as it appears in your viewing field at the various levels of magnification.

Scanning (4x) 10x Power 40x Power

7. Staining a Specimen

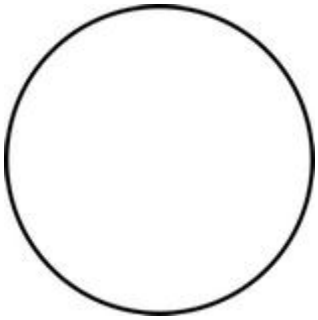


Tear a small corner from a piece of paper and use a pencil to make a mark on it. Make a wet mount as you did with the cotton. And check to make sure you can see your scrap under the microscope. Biologists learn to stain specimens that have already been created. You will not remake this slide, but use a procedure to draw a stain under the coverslip using the capillary action of water.

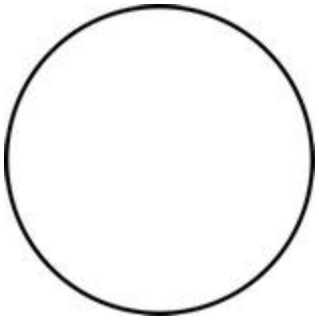
Place one drop of stain (methylene blue or iodine) on the edge of the coverslip you made with the paper. Place the flat edge of a piece of paper towel on the opposite side of the coverslip. The paper towel will draw the water out from under the coverslip, and the cohesion of water will draw the stain under the slide. As soon as the stain has covered the area containing the specimen, you are finished. The stain does not need to be under the entire coverslip. If the stain does not cover as needed, get a new piece of paper towel and add more stain until it does. Be sure to wipe off the excess stain with a paper towel.

12. Draw your specimen as it appears under 4x and 10x power. Use color pencils to show how the stain appears. It may appear darker or lighter in spots. Use shading to show darker and lighter spots.

Scanning (4x)



Low Power (10x)



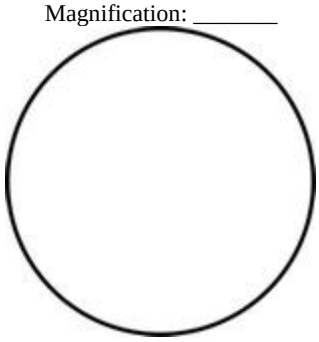
8. Investigation of Microorganisms and Specimens

For these sketches you should use pencil so you can erase and shade areas. All drawings should include clear and proper labels (and be large enough to view details). Drawings should be labeled with the specimen name and magnification. Remember that the circle indicates the viewing field as seen through the eyepiece, specimens should be drawn to scale.

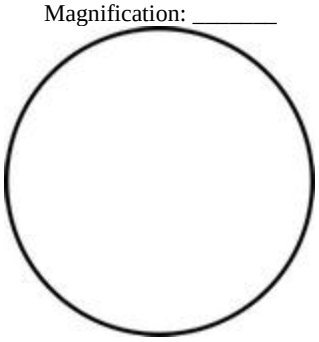
13. Obtain three preserved slides of various microorganisms and specimens that interest you. Draw your specimens and label with the name of the specimen and the magnification.

Preserved Slides of Microorganisms or Specimens

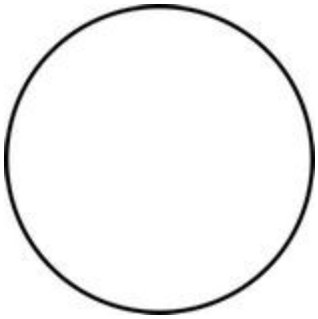
Specimen: _____
Magnification: _____



Specimen: _____
Magnification: _____

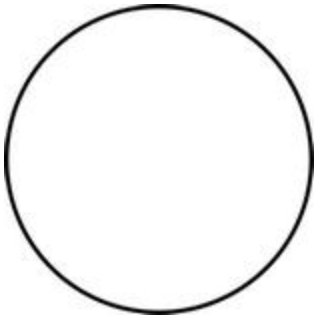


Specimen: _____
Magnification: _____



9. Cheek Cell Smear

Collect some human cheek cells. This can be accomplished by scraping the inside of the cheek with a toothpick and rubbing this into a droplet of methylene blue or iodine to stain the cells of the cheek. Place a cover slip on the droplet. These cells will appear as light blue or brown blobs with dark spots in the center, which are the nuclei. These will be small even at with the 40x objective.



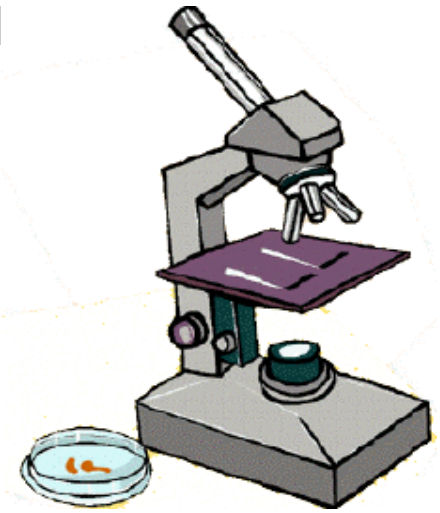
14. Create a wet mount of your cheek cells and find them using the high power (40x) objective. Draw your cells and label the cell membrane and the nucleus.

10. Investigation of Large Specimen with a Stereoscope

Light microscopes are only useful for viewing small thin specimens. In biology, you will need to view larger specimens. In this situation, a stereoscope is the best instrument. Stereoscopes present a larger field of viewing and handle depth much better than the light microscope. The drawback of the stereoscope is that it does not have a high magnification. Examine one or more of the stereoscopes in the room. They will be positioned around the room with specimens. You may need to adjust the light settings. Some stereoscopes have a "darkfield" setting or other light settings. Compare the way the specimen appears using different light settings.

15. Identify and describe the specimen(s) viewed. What is the magnification(s) of the stereoscope? Can the magnification be adjusted?

11. Measuring with a Microscope



Use a clear ruler to determine the width of the viewing field under the scanning (4x) objective. Position the ruler so that the millimeter marks are visible in your viewing field. Remember from the bio-worm computation lab that there are 1000 micrometers in a millimeter.

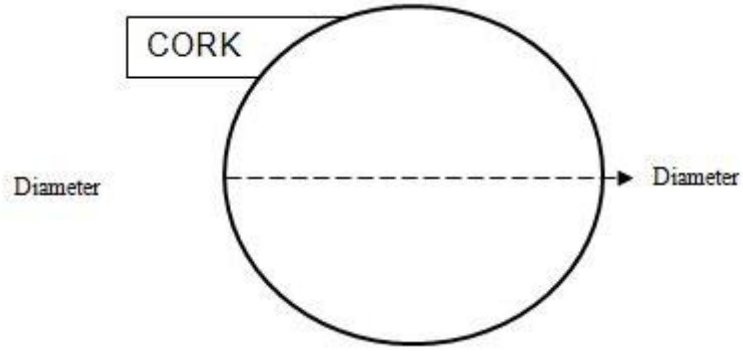
16. Estimate the length (diameter) of your viewing field in micrometers for both the scanning (4x) and the low (10x) magnification levels.

You cannot use this method to determine the diameter under high power (40x). Instead you must use a mathematical proportion method to determine the diameter under high power.

$$\frac{\text{High Power Field of View}}{\text{Low Power Field of View}} = \frac{\text{Low Power magnification}}{\text{High Power Magnification}}$$

17. What is the diameter (in micrometers) of your high power field (40x)? Remember to always show your calculations.

18. Obtain a cork slide and view under 40x power. Indicate the estimated length and width of an individual cork cell. Remember that this is just estimation. Use the template below to sketch and answer this question.



All of the techniques that were used in this lab can also be used with the digital microscope stations located throughout the classroom. The digital microscopes can also capture images and record video. Unlike standard light microscopes, the digital microscopes can also accurately measure specimens under the highest power of magnification.