Microscope Lab and Portfolio

Name _____

In this lab you will become familiar with how a microscope works. The techniques and hints presented in this investigation will help you use your microscope correctly and will enable you to make better and more thorough observations. You will compare the position of an object when viewed through the microscope with its position on the stage, use stains to aid in viewing objects, compare depth of field, and compare how a field of view differs between low, medium, and high power. The final product will be a portfolio of drawings illustrating what you saw and provide feedback on the knowledge you gained. Your sketches are required to be detailed, neat, accurate, and fine illustrations of what you see and how you see it. The student should also refer to the textbook, pp. xxxvi - xxxix in the front of the book.

Part 1. Getting acquainted with your microscope

- On/Off Switch
- Stage
- Stage Clips
- Diaphragm
- Eyepiece and Pointer (if available)
- Three Objectives (lenses)
- Course / Fine Adjustment

a. Turn on the light and turn the objectives until the shortest one (lowest power) is pointing straight down. This sets the scope to the lowest power. **Always start with this lens**.

b. When switching to the next longest (medium) and the longest lenses, the magnification is increasing. Determine the magnifications of each of the three lenses by multiplying the eyepiece magnification (10x on WLHS scopes) by the number stamped on the lens. Write these magnification numbers down on your answer sheet, beginning with the lowest power.

Part 2. Focus, light adjustment, and overlapping objects

While looking through a microscope, you are seeing lots of detail at a small scale. When we look around the room we see things that are close and things that are far. The same is true while looking through a microscope. This exercise will help you with the focus controls as well as adjusting the amount of light, and seeing depth and overlap of objects when viewed through a microscope.

a. Focus: Turn to the shortest lens. *Always* start with the shortest lens (lowest magnification). Choose one of the slides with thick (course) threads. To adjust the focus, turn the coarse adjustment knob (the large knob) until the threads come into focus - they should be fairly clear. Then turn the fine adjustment knob (the small one) until the threads are as crystal clear as possible.

b. Next, adjust the amount of light coming through the lenses and into your eyes. Beneath the stage there is either a solid disk with different sized circles cut into it or a little bar that when moved closes a diaphragm. Look at the slide and adjust the diaphragm to different amounts of light. As you change the diaphragm what gives you the sharpest view of the threads: almost closed, medium, or completely open? Record your answer on the answer sheet.

c. Change to medium power and look at the threads. They will likely be out of focus. To adjust the focus, use only the fine adjustment. NEVER use the course adjustment with the medium or large (longest) lenses because you may break the slide and also get the lens dirty. Adjust the diaphragm again. Which opening gives you the sharpest view now (almost closed, medium, or completely open)?

d. Change to high power and repeat step 'c'.

e. Switch back to low power. Move the slide around until an intersection of threads is in the center of the view. Adjust the focus. Can all threads be seen ***clearly*** and in focus at the same time?

f. Switch to medium power. Look at the intersecting threads, adjusting the focus for the top thread. Can all threads be seen ***clearly*** and in focus at the same time?

g. Switch to high power. Look at the intersecting threads, adjusting the focus for the top thread. Can all threads be seen ***clearly*** and in focus at the same time? Now, while looking through the eyepiece, slowly take the threads out of focus.
   - Which color thread lost focus first? Which is on the bottom of the overlap, which is closest to your eye? Make a decision on the order of threads and record the order from top to bottom on the answer sheet.

h. Sketching. Switch to low power and draw the intersection exactly as you see it. Be as exact as possible. Just scribbling nondescript lines will not be allowed; you must accurately and artistically draw what you see, showing the overlap and twisting of the fibers. Use colored pencils to help show which colored thread is on top of which. Repeat with the same threads on the other two powers. Has the thickness of the threads changed – show it in your drawing. While on high power you might want to move the intersection to be only partially in the center to still see all layers.

i. Choose a slide with thin fibers. Record the number at the top and the letter just above the word "Boreal," e.g. 69276–04 "D". Find the thread overlap just like you did with the thick threads and center it in your view. Using any lens that helps the most, identify which thread is on top, which is in the middle, and which is on the bottom, recording this onto the answer sheet (top to bottom). Move to high power, focus, and draw exactly what you see in the circle. Be detailed and specific.

Part 3. Field of View

The Field of View (FOV) is the area you are able to see within the circle of light viewed through the eyepiece. The amount of area changes as the power of the lens (magnification) increases. For this exercise, you will measure the FOV for each lens beginning with the lowest power lens.

a. Place a clear plastic ruler onto a slide and center it in your FOV. While on low power get a clear focus of the ruler. Now position the ruler so that one vertical line (bar) is just touching the left edge of the FOV and the horizontal line is in the middle. See Figure 1. Switch to each of the other two lenses to see how the FOV and the number and sizes of the bars change. If you don't see any bars then start over and readjust the ruler until you do see them for each lens.

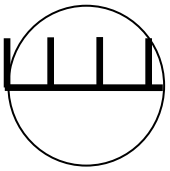
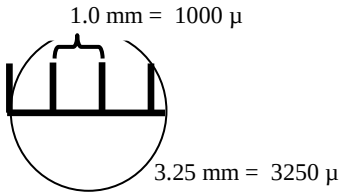


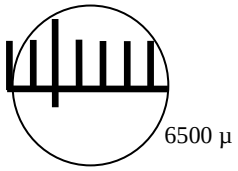
Figure 1

b. Switch to low power. Draw the exact number of bars that you see, proportionately just as it looks through the eyepiece, in the first circle of your answer sheet. Continue with each of the next two lenses, making sure you still see the vertical bars of the ruler. Draw the exact number of bars, proportionately shaped and sized, that you see through each of the lenses.

c. Measure the size (diameter) of the FOV. Each space on the clear ruler is 1 mm, which through the microscope equals 1000 μ (microns or micrometers). What is the diameter in both mm and microns (μ) of each of the FOVs? Record the results on the answer sheet. Use decimal numbers (with two Sig Figs). The unit conversion factor is $\left[\frac{1000\mu}{1mm}\right]$ so for μ, you multiply the ruler measurement you made with this factor.

Examples of two possible microscope FOV measurements are as follows: *[Note, WLHS microscopes are not these measurements]*





Part 4. <u>Practical application of microscopic magnification</u>

In this part, you will practice searching a slide to find a "specimen", continue practicing drawing to proportional sizes, and further your understanding of the concept of the size of the FOV. For many years people have been storing printed information on microfiche, so that the contents of our entire library could be stored in a filing cabinet. Entire books can be stored on a couple of pieces of film the size of a note card. Don't believe me? Let's take a look, I'll show you one.

For this part, take one of the "microword" slides and record the slide number. Using low power, search the white area for the letters that form a science word. There are several combinations of letters, but only one combination will make up a real word. Record this word on the answer sheet.

a. Using low power, *<u>center</u>* the word in the FOV. Write down exactly what you see of the word, drawing it just as it looks.

b. Now switch to medium power, **do not move the slide.** Write down exactly what you see of the word, proportionately and correctly sized.

c. Do the same for high power. Only draw what you see, even if a letter is cut off. Also draw the thickness of the letters proportionately.

Part 5. <u>Wet mounts</u>

Many times we will have to prepare our own slides by making what is called a wet mount. We also have to know how to orient specimens on the slides. This exercise will explain how to do that and will also illustrate how things are actually seen through a microscope.

A wet mount is made by taking an object, setting it on the middle of a slide, adding a drop of water, and carefully putting a cover slip over the top of the object and water. For the last step, putting on the cover slip, it is important to be gentle so that you have few or no air bubbles under the cover slip. Your wise and awesome teacher will demonstrate how to do this. Refer to the following figures:

Prepare a wet mount of a single lowercase 'e' cut from a magazine or newspaper. It should be a standard or normal size letter, not from a headline. Place the letter onto the slide so that the 'e' faces you as it would while reading (right side up). Add one drop of water and gently place a cover slip over the wet paper. Place the slide onto the microscope stage so the 'e' faces you as though you could read it and so it is centered on the equator of the FOV.

Answer each of the following questions on your answer sheet.

a. Observe the letter using low power, focusing with the fine adjustment. How does the orientation of the 'e' viewed through the eyepiece compare with its orientation on the stage?

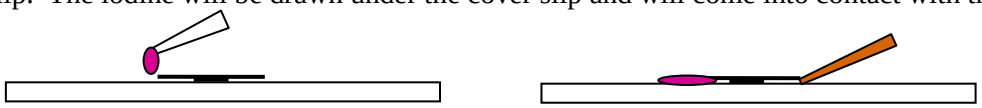
b. While looking through the eyepiece, move the slide slowly from left to right. In what direction does the letter move as seen through the microscope?

c. While looking through the eyepiece, move the slide slowly toward you. In what direction does the letter move when viewed through the microscope?

d. In the circle, draw the letter <u>as you see it through the microscope</u>. The drawing should fill up the FOV circle.

Part 6. <u>Stains to aid viewing</u>

Many objects observed through microscopes are colorless. They appear translucent and difficult to see. Stains are often used in microscope work to color objects for easier and more detailed observation. Stains can be added to a wet mount without disturbing the slide. After the wet mount is prepared add a drop of iodine along one edge of the cover slip. Place a small piece of paper toweling along the opposite edge of the cover slip. The iodine will be drawn under the cover slip and will come into contact with the object.



a. Gently rub a piece of potato on the surface of a slide. You don't need much potato - you should barely see that you have anything; avoid globs. Add one drop of water. Cover with a cover slip. View the slide under low power and focus with the diaphragm open all the way. The little circles you are seeing are starch grains.

b. Switch to high power, focus, and in the first circle on the answer sheet, marked "Unstained", draw any 3 grains with as much detail as you can observe.

c. Remove the slide and stain the starch with iodine. Place the slide back onto the stage and observe under high power. What happened to the starch? Record your answer on the answer sheet.

d. Now draw any 3 grains with as much detail as you can observe in the circle marked "Stained".

Part 7. <u>Area of FOV vs. Numbers of objects in FOV</u>

For this part, you are going to estimate the difference in area of the FOV at low power compared to high power. You are also going to estimate the difference in the number of objects that are visible in low power compared to high power. This part requires counting tiny objects and doing a little bit of math, however, your teacher will show you some "tricks" to making a quick and fairly accurate count. *For this part, use a slide containing stained starch grains that was previously prepared. DO NOT CLEAN slide.*

a. On low power count the number of grains that would fit end to end across the FOV. Record this number in the data table. Record the diameter (in μ) of the FOV (see Part 3-c). Divide the "diameter of the FOV" by the "# of grains". The answer is the approximate diameter of one grain. Do the same for the high power lens. (You may skip the medium power).

b. How do the diameters of the grains compare for each of the lenses? Explain your answer including reasons why they are or are not the same diameter.

c. <u>Comparison of number of objects</u>: For each lens, take the "# of grains end-to-end", divide by 2, and square that number. Record this in the data table as "Total # of grains". Divide the low power 'total # of grains' by the high power 'total # of grains' and find the square root of that answer. How does

the number of grains observed under low power compare with the number seen under high power? For our WLHS microscopes, you should be seeing about 10 times as many *objects* under low power as under high power. Do you? If not, explain why.

d. Comparison of FOV areas: When using low power, the total *area* of your field of view is greater than when using high power. Your WLHS microscope has a magnification of 4x for low power. What is the magnification of the high power? (Look at the number on the objective). Record.

e. The difference in the area can be calculated by dividing the magnification of the high power by the magnification of the low power. Record this number on the answer sheet.

f. How does the final answer for '*e—areas*' compare to the answer for '*c—objects*'? If they are not exactly identical to each other then explain why, giving two or three reasons for the discrepancy.

Part 8. Answer the True-False Questions.

Part 9. Examination of preserved organism specimens

The purpose of this part is to practice finding specimens in prepared slides, judge the correct power to use for examination, and to carefully draw ***details*** of these once living organisms.

a. Choose three different dry mount specimens. When looking at each one, choose an objective lens that best shows the entire specimen, but shows it as large as the FOV will allow and with much detail. The stained colors will help to show details. Draw and color each specimen with all the fine detail that you are able to see. Fill up the area of the circle provided on the answer sheet. Drawings must be specific, detailed, accurate, colorful, and neat. Label the name of the organism and slide number (if numbered).

b. Extra Credit Extension. *After every other part of this lab is completed, you may do one additional drawing, if you like.* You have two choices for this. **Choice 1:** Gently scrape the inside of your cheek with a toothpick. Rub this onto a slide and add one drop of iodine and a cover slip. Observe using high power. Draw these cells using as much detail as you can. Be sure to label (identify) this drawing. **Choice 2:** Make a wet mount slide using 1 drop of aquarium water from the beaker provided by your instructor. Find one living protist – your instructor will help you. Draw that organism with lots of detail so that your instructor can figure out what the organism is simply by looking at your drawing and comparing it to a key of typical organisms. Fill up the space provided. When finished, wash and dry the slide and coverslip.

Rubric and Portfolio Wrap-Up

This will be graded as a 100-point lab report. Make this into a nice word-processed portfolio. All pages must look clean and free of wrinkles and folded corners. All typed text must be 12-point Times Roman Font, double-line spacing. The portfolio will contain the following things, in order:

Cover Page – **7 points**

Provide a nice cover sheet with the title of this Lab, your name, period, date, and instructor's name. In the middle area of the cover page provide a picture of a microscope using your own nice sketch or a picture from the Internet. The picture must be at least 6 inches high and cannot be the microscope diagram (or photocopy of it) that was given to you. It is to be in focus and not pixelized. The following parts must be labeled: stage, eyepiece, three objective lenses, coarse adjustment, fine adjustment, light source, and diaphragm. Extra labels will cost you points.

Introduction – Understanding Your Microscope **10 points**

Get me interested in wanting to read and look at this portfolio. Explain everything you can about the FOV. Tell me what an FOV is. Tell me what a micron is and how big it is. Tell me how a person goes about determining the size of the FOV. How big is the FOV for each lens? How does the FOV change as you change from low to high power lenses? What can you conclude about the difference in numbers of objects and the difference in area as you switch lenses? Explain how the field of view gets smaller but the object is still the same size in reality. You should refer back to the different parts of this lab and the results you obtained. There is much to say about the FOV.

The Studies – The things you learned about the Microscope

Attach the Answer Sheet pages here. They must be in the correct order. The last page must be the drawings of the prepared specimens. Rubric for each part:

- Part 1 – **6 points**
- Part 2 – **10 points**
- Part 3 – **6 points**
- Part 4 – **6 points**
- Part 5 – **5 points**
- Part 6 – **5 points**
- Part 7 – **10 points**
- Part 8 – **8 points**
- Part 9 – **12 points (4 per drawing)**

Conclusion – this will be the last page of the report **10 points**

Each part that you did had a purpose and reason. Make a bullet list of 9 things you learned about the microscope and its use and why those were important. For example, don't just state something like "learned to make stained slides", but also include *how* and *why* you learned how to make and use stains.

Format and Neatness – **5 points**

Provide a professional looking presentation of the report including neatness of the papers, order, and font.

Extra Credit drawing – up to 4 points

Staple the sheets in the upper left corner.