Microscope Activity

On the following pages you will find a copy of a microscope lab I do with my Bio 100 honors students. There have been a few, but not many modifications in this version I have given you. The purposes of this lab are:

1) To familiarize you with different types of microscopes
2) To give you practice in finding live organisms and prepared organisms on the microscope.
3) To teach you how to properly adjust a microscope for contrast and brightness
4) To give you experience in calibrating a microscope and then measuring objects under the microscope.
5) To assure that you feel comfortable using microscopes in your classroom.
6) To demonstrate safety practices for microscope use.
7) To introduce you to some current technology available for projecting microscope images through a TV or data projector.

The Lab

Rules for microscope use

Microscopes are very expensive to replace; therefore, be particularly careful when handling. Follow these simple rules and you can protect your microscope.

- Use both hands to carry the microscope.
- Always **hold it upright** so that the ocular lens does not slip out.
- Use only clean, dry lens paper to clean dust off of the glass lenses. Do **not** use wet paper, paper towels, or other materials that may scratch these lenses.
- The lowest power objective should be in place at both the beginning and end of use.
- Keep the stage clean and dry to prevent slides from sticking to the stage. When slides stick the mechanical stage will not be able to move the slide around during viewing. This could break the mechanism of the mechanical stage.
- Do **not** remove parts of the microscope unless directed to do so by Dr. Firestone.
- Always replace the microscope to the properly numbered shelf in the microscope cabinet.
- **Report any malfunction to Dr. Firestone IMMEDIATELY.** DO NOT simply trade the defective scope for a new one.
- Try **not** to get the lenses wet during microscope use. If they do get wet, Wipe them clean with a **piece of lens tissue ONLY**.

Care of slides:

- Slides and coverslips are glass. Be careful not to cut yourself when using them.
- Carefully clean slides before you use them, because dust or fingerprints will interfere with your ability to see a specimen. Paper towels may be used to clean **slides ONLY**.
- Dispose of broken glass or organic materials as indicated by the instructor.
Pre-lab Preparation:
You should complete the following activities before coming to lab.

- Read through this entire exercise and make sure you understand what will be happening in lab.
- Study the diagram of both microscopes and familiarize yourself with the labeled parts, see if you can name them using the terms given in Objective #1 below.

Objectives:
1. Be able to identify and use the following parts of the microscope correctly:
   - coarse focus knob
   - fine focus knob
   - mechanical stage
   - stage
   - iris diaphragm
   - ocular lens
   - objective lens
   - condenser lens
   - revolving nosepiece
   - illuminator
   - arm
   - condenser lens
   - condenser lens knob
2. Define the function of the following terms in microscopy
   - resolving power
   - contrast
   - depth of focus
   - field of view
   - parfocal capability
   - micrometer
   - calibration
3. Be able to properly adjust the microscope for proper contrast using three different types of adjustment.
4. Be able—when given a slide, coverslip, and specimen—to construct a temporary wet mount and focus on the specimen in the center of the field of view, using high power.
5. Be able to determine the approximate size of an object under low power or high power using the ocular micrometer.
6. Be able to determine the compounded magnification of a set of lenses, given the magnification of each separate lens.
7. Be able to properly clean a microscope and keep it in good working order.
8. Understand and be able to name the differences between a compound and a dissecting microscope.
9. List the purposes of a dissecting microscope and recognize situations in which a compound microscope is preferred over a dissecting microscope and vice versa.
**Introduction:**

Biological study includes the microscopic examination of many organisms. During the course of this semester, we will be examining organisms and parts of organisms that will require the use of the microscope. In this lab you will learn the correct terminology and techniques needed for efficient utilization of the light microscope. In addition to giving you training in proper microscope usage, this exercise will provide an opportunity to practice using a microscope.

<table>
<thead>
<tr>
<th>MICROSCOPE PART</th>
<th>FUNCTION</th>
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<td>Coarse focus knob</td>
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<td>Fine focus knob</td>
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<td>Mechanical stage</td>
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<td>Stage</td>
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<td>Iris diaphragm</td>
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<td>Condenser lens</td>
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Exercise #1

Identifying and describing parts of a light microscope.

Label the parts of the microscope below and describe the function of each in the table following:

Figure 3.1 Labeled Photograph of Graf Apsco microscope.
EXERCISE #1 – PARTS OF THE MICROSCOPE

Identifying and describing the parts of a light microscope.
Label the parts of the microscope below and describe the function of each in the table following.

Figure 3.2 Diagram of the Swift M400D binocular microscope
Exercise #2

MAGNIFICATION THROUGH THE MICROSCOPE

Your microscope is capable of four powers of magnification, based on the four objective lenses found on your microscope. You have objective lenses of 3x; 10x; 44x or 40x and 100x. Your ocular lens, or eyepiece, has a magnification of 10x. In order to calculate the total magnification when viewing a specimen, you simply multiply the ocular lens magnification by the objective lens magnification. For example, when the shortest, or 3.5x objective, is in place you will be viewing the specimen at a magnification of 35x (3.5 * 10).

Calculate the power for each of your objective lenses and list them below:

3.5x objective ___35x__
10x objective _______
40 OR 44x objective _______
100x objective _______

Exercise #3 only properly cleaned microscopes will give proper results.

Activity

Cleaning and care of your microscope

Take a few minutes right now to clean your microscope as best you can so that you will be sure to have a properly functioning microscope. Do the following tasks in order.

- As it is demonstrated by your instructor, unscrew each objective lens one at a time and carefully wipe the lens with a lens paper. We will ONLY DO THIS ON THE FIRST USE OF THE SEMESTER, after that it will only be done as needed, AFTER OBTAINING PERMISSION TO DO SO FROM THE INSTRUCTOR.
- Check that the mechanical stage is moving smoothly in both directions.
- Clean off the stage with a damp paper towel and then dry it off thoroughly.
- Check that the condenser lens is clean by bringing it up as close to the stage as possible and looking through the hole in the stage. Clean it with a dry lens paper ONLY, if necessary.
- Make sure that the condenser raises and lowers smoothly and stays in position where you leave it without sinking down away from the stage on its own.
- Check your ocular lens for visible dust or dirt and wipe it off with a clean, dry piece of lens tissue.

Report any microscope problems to Dr. Firestone immediately.

Exercise #4

PROPER USE OF THE MICROSCOPE.

Before we begin your practice of using the microscope to examine several objects today, you need to understand a few more terms that refer to the microscope and its proper use. In this exercise we will learn these terms and you will learn how to properly clean and care for your microscope.

In this exercise we will learn about:
- how to view and focus on objects under the microscope
- proper contrast adjustment
Part A. Viewing and Focusing: follow along on your microscope as this is discussed

Before you attempt to view any specimen through the microscope today, you must learn the correct PROCEDURE FOR VIEWING a slide. This procedure should be followed for every new slide you view.

Do all Exercise 4 activities with both the Graf-Apsco and Swift microscopes.

Activity 1

- First, rotate the low-power objective lens (remember this is the shortest lens) into position directly over the opening in the stage.
- Next, watch the stage and objective lens from alongside (not through) the microscope and turn the coarse adjustment knob to bring the lens and stage as close together as possible.
- Based upon your observation of microscope movements, and without having a slide on your stage yet, determine the direction of coarse adjustment knob rotation you should use to move glass away from glass.
- The remainder of these instructions should be followed explicitly when viewing all slides. Each time you change slides follow the remaining steps from the beginning.
- Next place a prepared slide of bacteria types on the stage. Center your specimen over the stage aperture (opening).
- Position the low-power lens over the slide. Make the distance between the specimen and the low-power lens look as small as possible.
- While looking through the ocular lens, turn the coarse adjustment knob to move the objective lens away from the specimen until a part of the specimen comes into focus. (It may be necessary to also move the slide around a little using the mechanical stage so that you can find the specimen). This may take practice. If you have trouble please ask for help!
- Center the specimen in the field of view.
- Switch to high power and sharpen the focus with the fine adjustment knob only. Never use the coarse adjustment knob when viewing slides through the eyepiece. Use only the fine focus knob when using all objectives lenses except the 3.5x or 10x objective lenses.

NOTES:
Most of these microscopes should be parfocal. This means that when you focus on a specimen under low power, you should be able to see it when you switch to a higher power by just a slight adjustment of the fine focus knob. If you have trouble finding specimens on higher power using just the fine focus, notify the instructor or lab assistant for help!

Keep both eyes open even though only one is used in the monocular, compound microscope. After a short while, you can get accustomed to ignoring impressions coming from the free eye. Squinting leads to muscle fatigue.
**Part B. Proper contrast adjustment of the microscope.**

Adjusting the contrast properly allows you to distinguish better between the object you are observing and the background. There are three potential parts of the microscope that can be used to properly adjust the microscope for proper light and contrast of the specimen. Two of these are available on your microscope.

**Activity 2**

**STEP ONE: IRIS DIAPHRAGM ADJUSTMENT.**

Locate the condenser lens underneath the microscope stage. Locate the knob which moves the lens up and down. Raise the condenser lens as far up toward the stage as it will go. You are now ready to adjust the iris diaphragm.

The iris diaphragm regulates the amount of light passing through the stage aperture and specimen. We will first adjust the diaphragm to obtain optimal lighting.

With your slide still on the stage, the 10x objective in place, and the light on, remove the ocular lens from your microscope. Look down through the tube of the microscope at the light. As you are looking at the light move the iris diaphragm lever back and forth and see what happens to the light.

First close the iris diaphragm down so you see only a pinhole of light. To properly adjust the iris diaphragm, slide the lever until you see the microscope tube just fill evenly with light.

**STEP TWO: CONDENSER LENS ADJUSTMENT**

The condenser lens focuses the light up through the stage and onto the specimen. The condenser lens must be properly positioned to obtain maximum contrast when viewing the specimen.

**PROCEDURE FOR PROPER POSITIONING OF CONDENSER LENS IN BLACK SCOPES**

- Focus on a slide using the 10x objective lens.
- Use the condenser lens knob to move the condenser lens as close to the stage as possible.
- While looking through the eyepiece, slowly move the condenser lens away from the stage. As you do this you will see a granular background appear at one point. Continue to move the condenser away slowly just until the graininess disappears. This is the optimum adjustment.

**PROCEDURE FOR PROPER POSITIONING OF CONDENSER LENS IN SWIFT SCOPES**

Unlike the black Graf- Apsco scopes the Swift scopes do not show the graininess as described above. The best way to adjust these scopes is to

1) Move the condenser lens all the way up to the stage
2) Look through the scope at a specimen you already have in focus and SLOWLY move the condenser lens down until you get the sharpest view possible.

**STEP THREE: RHEOSTAT ADJUSTMENT**

A third possible adjustment for light and contrast is the rheostat on the light source itself. Only the Swift microscopes have a rheostat adjustment available.
The rheostat controls the amount of current flowing into the bulb. This allows the light to be dimmed or brightened. This can allow the observer yet another means to control the amount of light reaching the specimen and therefore allow a control over contrast.

After focusing a specimen on the microscope, move the Rheostat adjustment until you have the best contrast possible. Notice that less light usually gives better contrast.

**Part C. Resolving power**

Resolving power is a measure of lens quality. Quality lenses have a high resolving power, which is the capacity to deliver a clear image in fine detail. If a lens has a high magnifying power but a low resolving power, it is of little value. Although the image may be large, it will not be clear enough to show fine detail. Use of a blue filter between the illuminator and the condenser lens helps to increase the resolving power. The higher the resolving power of a lens, the better the lens is at distinguishing two objects from each other when they are close together.

Another factor that influences resolving power is the cleanliness of the lenses. Dirt, water, or oil on the lens may scatter light and reduce the effective resolving power of the microscope. Therefore, lenses should always be kept clean. Use only lens paper to clean the lens.

**Part D. Field of View**

You have already learned that lenses have different magnifying powers, but it is also important to understand that each lens will have a particular field of view. The field of view is the size of the area that can be seen through the microscope. The larger the magnifying power of an objective lens the smaller the area viewed when looking through the microscope. This is sometimes hard to appreciate since to you—the observer—the size of the circle of light you see through the ocular lens appear the same for all powers of objective lenses. When you switch from low power to high power, however, you are actually looking at the central portion of what was visible under low power. Therefore, it is important to center the specimen on low power before making the switch to high power.

**Part E. Depth of field or focus**

The vertical distance that remains in focus at one time is called the depth of focus. Figure 3.3 below demonstrates how focusing on an object at different depths 1, 2, and 3 would produce three different images under the microscope.

*Figure 3.3 What the image would look like if the microscope is focused in at various depths in a three dimensional object.*
Activity 3

Obtain a slide of 3 threads (red, yellow, and blue) which are overlapped on a slide. Using your choice of microscope. Determine the order in which the 3 threads were place on the slide from bottom to top.

Bottom _______________ Middle _______________ Top _______________

Part F. Calibration of your ocular micrometer

The ocular micrometer provides a means to estimate very closely the size of organisms viewed under the microscope. An ocular micrometer is a small glass disk onto which have been etched lines that are an unknown distance apart (The distance between lines on the ocular micrometer will represent different distances depending on the total magnification being used). The ocular micrometer is permanently inserted in the eyepiece of the microscope you will be using today. Before the ocular micrometer can be used to measure anything, it must be calibrated against a stage micrometer. A stage micrometer has lines etched at known distances. Both ocular and stage micrometers are fairly expensive (between $50 and $150 each). Therefore, we will be doing this calibration together as an entire class using a microscope with a camera attached. (As a teacher you could do this for your students explain how it works and then simply explain how they can use this to measure objects under the microscope.) In this Bio 405 class you will have the opportunity to do the calibration yourself after a demonstration is given.

PROCEDURE FOR CALIBRATING AN OCULAR MICROMETER

Refer to Figure 3.4 to see how the ocular and stage micrometers are used in calibration as you read the following description of the procedure.

Calibration steps:

1. Rotate the eyepiece until the lines of the ocular micrometer are parallel to those on the stage micrometer.
2. Align lines at the left edges by moving the stage micrometer and rotating the ocular lens.
3. Find a place where the ocular lines and the stage lines line up perfectly. For an example see Figure 3.4 c. This figure shows (by the dotted line) that the 22nd line on the ocular micrometer lines up exactly with the 13th line on the stage micrometer.
4. On the teacher demonstration using the 3.5x objective lens, count how many spaces on the stage micrometer fit precisely into a given number of spaces on the ocular micrometer. Record the values below:
   # of ocular spaces ______________  # of stage spaces ______________
5. The smallest space on a stage micrometer is equal to 0.01 mm. To calculate the distance between the lines on the ocular micrometer at this magnification power, use the following formula:
   \[ \text{# ocular spaces (mm/space)} = \text{# stage spaces (.01mm/space)} \]
   \[ 1 \text{ ocular space (mm)} = \left(\frac{x}{y}\right) (.01\text{mm}) \]
6. Calibrate the ocular micrometer for each of the objective lenses on your microscope.
   - 10x lens  ocular spaces = ____________ stage spaces = ____________
   - 40x or 44x lens  ocular spaces = ____________ stage spaces = ____________

Can you determine the calibration for a 100 power objective lens using the above information?

Do it! Show your work. →
Exercise # 5 Practice using the microscope

Practice in using the microscope.
In this exercise you will have the opportunity to practice using the microscope, preparing “wet mount” slides, and measuring items with your ocular micrometer. You will examine a leaf from a water plant - *Elodea*
Proper Wet-mount slide preparation

Procedure for observing Elodea. Make a wet-mount slide of a piece of an Elodea leaf.

1. Place one Elodea leaf from near the tip of the stem on a slide in a drop of water.
2. Add a coverslip as shown above. Try to avoid trapping air bubbles on the leaf surface.
3. Focus on the Elodea cells at scanning (35) power, then at low power and finally at 440 power.
4. With the 44x objective in place, move the fine focus knob up and down to see through the different planes of focus and to examine the entire thickness of a cell.
5. Find a single chloroplast inside one of the leaves and measure its length using your ocular micrometer. Magnification used ______________ Number of ocular spaces per chloroplast ____ Actual length of chloroplast _______________.

Figure 3.5. Two views of the Elodea leaf under a compound microscope. Left a view at approximately 100x and on the right one cell viewed at approximately 400-500x.
Exercise #6 Dissecting or stereoscopes
In this lab you will have 2 types of dissecting microscopes available.

Activity 1
Examine both microscopes and identify the following:
Ocular lenses
objective lenses
light source
  incident vs transmitted light
magnification or magnification range available

Activity 2
Take the flower provided and dissect it under the Meiji dissecting microscope. Is it easier to use incident, transmitted or both types of light? ________________

Draw and label a picture of your flower’s structure. Make sure to label all of the following:
  sepals
  petals
  stamens
    filaments
    anthers
  pistil
    carpels
    style
    stigma
  receptacle