3. MICROSCOPY

OBJECTIVES

1. Identify the parts and tell how to focus the dissecting microscope.
2. Identify and give the function of the basic parts of the compound microscope.
3. List, in proper order, the steps for bringing an object into focus with the compound microscope.
4. Describe how to prepare wet mount slides.

INTRODUCTION

Understanding the physical world around us isn’t hard to do when we can see it. However, humans cannot see things that are very small or things that are very far away. The invention of the microscope and the telescope has greatly enhanced our ability to observe the worlds of the very small and the very distant. Because of these inventions, biology and astronomy have become major arenas of scientific investigation.

RULES FOR PROPER HANDLING

Microscopes are expensive, please observe the following cautions:

1. Always carry a microscope upright, with two hands—one hand holding the arm of the microscope and the other hand supporting the base of the microscope.
2. Never remove the eyepiece. Removing the eyepiece can put its lenses out of alignment and admits dust onto the internal optical surfaces; both of these situations require repairs.
3. Never remove an objective. This again admits dust and creates the risk of dropping the expensive objective.
4. Never use force to move a microscope part. All microscope parts that are intended to move, will move easily. If they do not, then notify the instructor.
5. Never touch lenses with your fingers. Fingerprints are bad for the lens coating and blur the image.
6. Use only lens paper to clean lenses. Other tissue paper will scratch lenses.
7. Keep all parts of the microscope dry. Promptly and thoroughly clean up any spills.

A. THE DISSECTING MICROSCOPE

The dissecting microscope is used to view small three-dimensional objects. Although dissecting microscope does not have as high a magnification as the compound microscope, the dissecting microscope does have several advantages:

1. It can be used to view whole specimens (whereas the compound microscope requires that a thin slice of the specimen be prepared).
2. The dissecting does not require that the specimen be mounted onto a microscope slide.
3. The dissecting microscope is usually easier to focus than the compound microscope.
3. MICROSCOPY

PARTS OF THE DISSECTING MICROSCOPE

Take the dissecting microscope out from your cabinet and place it on your lab bench. Label each of the following items on the drawing of the dissecting microscope below.

- Stage
- Objective
- Paired Eyepieces
- Magnification control knob
- Foot and Stand
- Stage Clip

A. __________________________________________
B. __________________________________________
C. __________________________________________
D. __________________________________________
E. __________________________________________
F. __________________________________________

EYEPieces

Since there are two eyepieces on these microscopes, you will have to adjust the distance between the two eyepieces until you see one central circle.

The two eyepiece lenses of the dissecting microscope are slightly different. Notice that one of them (usually the left one) has an indented area on it that can be turned, while the other eyepiece does not. This is called a diopter adjustment and use of it will allow you to adjust the microscope so that it is customized for your eyes. If this is out of adjustment, one of your eyes will see a blurry image – no matter how much you try to focus the microscope. Since many people use these microscopes, you will need to make this adjustment every time you use the microscope.

If your microscope has an adjustable left eyepiece, look in the scope with your left eye closed. Use the focus knob to get a clear image in view with your right eye. Now close your right eye and look in the scope with your left eye only – DO NOT ADJUST THE FOCUS KNOB. Turn the left eyepiece until the image becomes clear. Now open both eyes and you will see a clear image that is in focus with both eyes.
3. Microscopy

**Magnification**

1. What is the magnification of the eyepiece lens (also called ocular lens)?

Locate the magnification control knob.

2. What is the magnification range of this microscope?

Look at your finger by placing it flat on the stage under the dissecting microscope. Turn on the illuminator (a flashlight-like device) and adjust its angle so that it shines on your finger. Bring your fingerprint into view by using the focus adjustment knob on the arm of the microscope to increase the magnification. Adjust the focus as necessary.

3. Draw a picture of your fingerprint that shows the pattern of ridges.

![Fingerprint Image]

Magnification: _________

4. Compare your fingerprint to others at the table. Are there any that are similar? Why?

**Orientation of the Image**

5. Place your finger under the dissecting microscope again. Move your finger to the right. Which way does the image move in the microscope?

6. Move your finger toward you. Which way does the image move in the microscope?

**Size of Field of View**

As you look through the eyepiece you can see a circle of light. This is the field of view. The object on a slide will be observed in the field of view.

Obtain a piece of graph paper. View the paper under the dissecting microscope.

7. Under low magnification, how many squares do you count in one row across the diameter of the field?

8. Increase the magnification. How many squares can you count?

9. As the magnification increases, what happens to the size of the field of view?
3. MICROSCOPY

DEPTH OF FIELD

The depth of field of a microscope describes how much of the specimen is in focus at one time. Obtain a triangle with markings every millimeter. Stand the triangle on its shortest side and focus on the longest side. Using low magnification, focus on the number 4.

10. Set the magnification to 0.7 and slowly focus up and down the ruler. Notice that only some of the lines are in focus as you do this. How many lines can you get in focus at the same time?

Depth of field is approximately ________ mm.

11. Change the magnification to 1.5, focus up and down and again determine how many lines you can get in focus at the same time.

Depth of field is approximately ________ mm.

12. Increase the magnification to 3.0, and for the last time focus up and down and determine how many lines you can get in focus at the same time.

Depth of field is approximately ________ mm.

13. As the magnification increases, what happens to the depth of field?

PROCEDURE

1. Exit the lab room and collect an outdoor specimen to view. The specimen can be anything you choose, such as a leaf, a mushroom, an insect or worm.

2. Place the specimen under the dissecting microscope. If your specimen is moving, place it in a glass dish to keep it from crawling away.

3. Turn on the illuminator (a flashlight-like device) and adjust its angle so that it shines on the specimen.

4. Locate the magnification adjustment knob. Set the magnification for as low a possible to start with. Look through the ocular lenses and adjust the focus.

5. Use the magnification adjustment knob to zoom in on any areas of the specimen that look interesting to you. Sketch a picture of your biological specimen in the circle below.

What is it? ______________________

Depth of field is approximately ________ mm.

Magnification:_______________________

6. If your specimen was an animal, please return it to where you found it when done viewing.

7. Put the dust cover back over your dissecting scope and return it to the correct cabinet.
3. MICROSCOPY

B. COMPOUND LIGHT MICROSCOPE

The parts of the compound microscope are shown in Figure 3.1. The name “compound microscope” indicates that the microscope contains two main lens systems. Light passes through the specimen and then through the first lens system (the objective), which magnifies the specimen and forms an image within the body of the microscope. This image is then further magnified by the second lens system (the eyepiece).

Obtain a compound microscope from the cabinet at the back of the classroom. Pick up the microscope and carry it back to your lab bench with two hands. Place one hand on the arm of the microscope and one hand under the base. Do not plug in the microscope or turn it on until you are instructed to do so.

Figure 3.1
The Compound Light Microscope

- Eyepieces - contain the ocular lenses (usually 10X magnification).
- Viewing head - holds the ocular lenses.
- Nosepiece - Revolving device that holds objectives.
- Objectives - magnification is stamped on each:
  - 4X: scanning power
  - 10X: low power
  - 40X: high power
- Arm - Supports the upper parts and provides a carrying handle.
- Stage - Holds and supports microscope slides.
- Condenser - Lens system below the stage used to focus the beam of light on the object being viewed.
- Iris Diaphragm - adjusts the amount of light admitted to the condenser
- Power switch - Turns microscope on and off.
- Rheostat - Adjusts light intensity.
- Stage controls - Moves stage for accurate positioning of the slide.
- Coarse-adjustment knob - Knob used to bring object into approximate focus, used only with low power objective.
- Fine-adjustment knob - Knob used to bring object into final focus.
- Light source - An attached lamp that directs a beam of light through the object.
- Base - The flat surface of the microscope that rests on the table.
3. Microscopy

**Magnification**

The total magnification of the microscope is the magnification of the eyepiece times the magnification of the objective lens being used.

1. What is the magnification of the eyepiece?

2. What is the magnification of the low power objective?

3. What is the magnification of the high power objective?

4. What is the total magnification of an object being examined under low power?

5. What is the total magnification of an object being examined under high power?

6. Fill in the Table below.

<table>
<thead>
<tr>
<th>Objective #1 (lowest magnification)</th>
<th>Magnification Produced by Objective</th>
<th>Total Magnification of Specimen</th>
</tr>
</thead>
</table>

**Preparing a Wet Mount**

1. Using a pair of scissors, cut out a letter “e” from your piece of newspaper. Cut the smallest letter you can find. Position the “e” in the center of a clean glass slide.

2. Use a medicine dropper to place one drop of water on the letter “e”.

3. Hold a coverslip in your fingers. Make sure the bottom edge of the coverslip is in a drop of water.

4. Slowly lower the coverslip onto the wet paper. Slowly lowering the coverslip prevents air bubbles from being trapped between the slide and the coverslip.

5. Water should be present under the entire cover slip. If there is not enough or barely enough water under the cover slip, add a drop to the edge of the slip—the drop will flow under the slip. If there is too much water, absorb the excess with a tissue or bit of paper towel. *There should be no water on top of the cover slip!*

6. The type of slide that you have just made is called a **wet mount**. You will be making many wet mount slides in this class, so practice your technique until you can make wet mount slides without trapping any air bubbles on the slide.
1. Check to be sure the rheostat (light intensity) knob is turned down to the lowest setting.
2. Plug in the microscope cord.
3. Turn on the microscope. Adjust the rheostat to 2/3 brightness. Use the iris diaphragm lever to adjust the amount of light and contrast. If there is too much light, it is like looking into a spotlight—the contrast is poor and you will see very little.
4. Swing the low power objective (smallest objective) into place. Always start with the lowest power objective!
5. Place the slide you just made of the letter “e” right side up at the center of the stage over the stage opening.
6. Use the coarse adjusting knob to bring the stage as close to the objective lens as possible. Then, while looking through the eyepiece, very slowly turn the coarse adjusting knob away from you until the letter comes into view.

**NEVER USE THE COARSE ADJUSTMENT KNOB TO FOCUS AN IMAGE AT HIGHER POWER.**

7. Adjust the light intensity until you find a level that is comfortable for you.

1. Sketch what you see.

Without changing the focus, change to the next highest objective. Then clarify your view with the **fine focus adjusting knob**. Adjust the light intensity as needed. Repeat until you get to the highest power objective (largest objective).

2. Do you need more or less light?

3. Sketch what you can see. How has it changed?

Magnification: ______________

4. Which focusing knob do you NEVER use under HIGH power?

5. Under what conditions would you use low power?

6. Under what conditions would you use high power?

Magnification: ____________
3. MICROSCOPY

3. Microscopy

The appearance and movement of objects when viewed through a microscope is different than when viewed with the naked eye.

1. Move the slide to the right while viewing it through the low powered objective. In which direction does the image move?

2. Now move the slide away from you. In which direction does the image move?

**Important Note:** Always return to the shortest objective (low power) before removing the microscope slide from the stage! This avoids scratching the objective lens because the short scanning objective provides the most working distance. **Working distance** is the distance between the microscope slide’s cover slip and the lens at the end of the objective. High-power objectives have a working distance of only about 1 mm.

**Depth of Field**

In the laboratory you will need to examine specimens that are several cells thick. A leaf for example, has different types of cells on the surface than it does in the interior. You will need to be able to focus upon each layer separately. The characteristic of the microscope that allows the examination of each separate layer is called the depth of field (sometimes called the depth of focus) or how much of the specimen is in focus at one time.

Obtain a prepared slide of “crossed threads” and examine it under low power. Be sure that you use the proper light intensity and center the point where the threads cross in your field of view.

1. Can all three threads be brought into focus at the same time?

2. What is the color of the thread on the:
   - Top
   - Middle
   - Bottom

3. Draw what you observe:

4. Now switch to the high power objective and adjust the light.

5. Can all three colored threads be brought into clear focus at the same time?

6. How can you determine the position of each thread?
Make a wet mount slide of a leaf from the Elodea plant by placing the leaf from the Elodea plant on a glass slide with a drop of water and cover with a cover slip. Using the compound microscope at low power, focus on the cells. Switch to high power and draw ONE, SINGLE cell and label at least two parts of the cell. Make the cell large enough so your instructor can see the cell parts.

6. Drawing of Elodea cell.

Magnification: __________

7. Using the high power objective, very slowly move the fine focus knob back and forth. Are you able to tell that there is more than a single layer of cells? How many layers of cells do you see?

8. Is the relationship between depth of field and magnification the same as in the dissecting microscope?

9. Select two different biological specimens from the lab cart to view on the compound microscope. Observe these specimens and sketch them below.

What is it? ________________

Magnification:_______________

What is it?__________________

Magnification:_______________

What is it?__________________

Magnification:_______________
3. Microscopy

INSTRUCTIONS FOR PUTTING AWAY COMPOUND MICROSCOPE

1. Turn down the rheostat (light intensity).
2. Turn off the power.
3. Unplug the cord by pulling on the plug, not the cord.
4. Wrap the cord around the cord holder.
5. Put the 4X lens in place.
6. Make sure there is no water or stain on the microscope (wipe off the stage).
7. Cover with plastic dust cover.
8. Use two hands to carry the microscope to the cabinet.
9. Carefully return the microscope to the assigned space, with the stage on the inside and the neck of the scope toward you.

C. Which Microscope to Use

Fill in the microscope comparison table below and then answer the questions that follow based on the information in the table.

<table>
<thead>
<tr>
<th></th>
<th>Dissecting Microscope</th>
<th>Compound Microscope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnification range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative working distance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Image orientation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nature of light source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative Size/Thickness of specimen in relation to light source used</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of specimen that can be observed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

You are in charge of a laboratory that investigates crime scenes. In order to carry out your investigations you often have to use microscopes. For each of the situations listed below indicate which type of microscope you would use and why.

1. If you believed that a twenty dollar bill was counterfeit, which microscope would you use to prove your case? Why?
2. If you wanted to determine if the handwriting on a note used in a bank robbery was the same as the handwriting on a note found in a suspect’s car, which microscope would you use? Why?

3-10
3. If you wanted to determine if there were cells present at the base of a hair of a specimen, which microscope would you use? Why?

4. If you wanted to compare the markings on two bullets to determine if they were fired from the same gun, which microscope would you use? Why?

**Quick Reference Guide to Correct Microscope Usage**

1. When carrying the microscopes, put one hand on the arm/body tube and one hand under the base of the microscope. Pretend it is a football and keep it close to your body.

2. Proper steps for all viewing:
   a. Lower the stage
   b. Make sure rheostat (light intensity) is turned down
   c. Turn on the microscope and turn the rheostat up about 2/3 of full intensity
   d. Rotate in the low power objective, if it is not already in position
   e. Center the slide on the stage
   f. Raise the stage to the objective and slowly move the slide away from the objective to focus.
   g. Increase the magnification
   h. Focus with the fine adjustment knob.

3. Always wipe slides with a kim wipe before and after use.

4. Only use lens paper on the microscope lenses.

5. Proper steps for putting away the microscope:
   a. Rotate the low power objective into place
   b. Lower the stage completely
   c. Turn down the light source using rheostat knob
   d. Turn off the microscope
   e. Remove the slide
   f. Clean the slide
   g. Wrap the cord around the cord holder
   h. Cover with plastic cover
   i. Use 2 hands to carry the microscope to the cabinet

6. Carefully return the microscope to the cabinet, with the stage on the inside and the neck of the scope toward you.

7. When in doubt, ask your instructor how to use the microscope!