

**Prelab for Exercise 3-1
The Microscope**

Name _____

Match the microscope structures given in the left column with the statements in the right column that identify or describe them.

Key:

- | | |
|---------------------------|------------------------|
| a. coarse adjustment knob | f. turret or nosepiece |
| b. condenser | g. objective lenses |
| c. fine adjustment knob | h. stage |
| d. iris diaphragm | i. ocular |
| e. mechanical stage | |

1. _____ platform on which the slide rests for viewing
2. _____ eye piece
3. _____ secures(s) the slide to the stage
4. _____ delivers a concentrated beam of light through the slide specimen
5. _____ used for precise focusing once initial focusing has been done
6. _____ carries the objective lenses
7. _____ used to control the amount of light passing through the specimen

8. Explain the proper technique for transporting the microscope.

9. The microscope most frequently in our labs is a compound light microscope. Why this name?

10. Name one advantage and one disadvantage of using the stereoscopic microscope (also called a dissecting microscope) instead of a compound light microscope.

11. Define the term resolution.

12. Express the maximum resolution of compound microscopes in terms of micrometers (μm).

13. What feature of an electron microscope allows it to achieve resolution at much higher magnifications compared to a compound microscope?

14. When will the 100x objective lens be used in this class?

Exercise 3-1 Microscope

Introduction

Both light and electron microscopes are used extensively in biological research. The light microscope allows us to "see" details of cells and tissues of living organisms. With the electron microscope objects as tiny as individual protein molecules may be observed.

The purpose of this laboratory is to introduce you to the microscopes that you will use to observe many biological structures and processes during this course. The familiarity that you gain with this exercise will allow you to spend your time in subsequent labs more effectively.

The microscope used most frequently in the biology lab is the compound light microscope. The compound microscope is used to magnify and resolve fine detail within a transparent specimen (one through which light can pass). The compound light microscope is so called because it has two separate lens systems; an objective lens, located near the specimen, which magnifies the specimen a certain amount, and an ocular lens, or eyepiece, which further magnifies the image formed by the objective lens. The total magnification observed by the human eye is the product of the magnification of the two lenses (objective X ocular).

The resolution, or resolving power, of the microscope is just as important as its ability to magnify an object. Resolving power is the ability to discriminate two close objects as separate. To give you an idea of what resolving power means, the human eye can distinguish two objects as separate when they are at least 0.1 mm apart (objects closer than this will appear as one fuzzy image). A compound light microscope has the ability to resolve objects 1000 times closer than the human eye can, while an electron microscope can distinguish objects as small as 0.2 nanometers. In general the more light delivered to the objective lens, the greater the resolution.

Activity 1: Identifying the Parts of the Microscope

In order to use a microscope properly you must be familiar with the optical and mechanical parts of the microscope. You must also learn the appropriate care of this delicate and expensive instrument. The microscope gives you the opportunity to see a world of things you may not have seen before, but you must know how to use it to enjoy the experience.

Remove a microscope from the cabinet by its arm, supporting the base of the microscope with your free hand. Take the microscope to your desk continuing to support the base with one hand and the arm of the microscope with your other hand. The following diagram and list of terms and illustrations will help you to become familiar

with the microscope. As you read through the list locate each of the parts on your microscope. If you don't understand how something functions or can't find it on your microscope, check with your instructor.

Once you have read through and located each of the features of the scope, point to and name each of the microscope parts to your lab partner.

a. Adjustment Knobs

Your microscope has two adjustment knobs which are used to focus the specimen to be studied. The largest is the coarse adjustment knob. This is used for rapid (or coarse) focusing of the specimen when using the scanning objective lens and the low power lens. The coarse objective knob is rotated until the specimen is roughly in focus and then left alone. The fine adjustment knob controls precise focusing of the object. Only the fine adjustment knob should be used with the high magnification lenses (high power and the oil immersion objective lenses). Moving the fine adjustment knob also helps you to determine the third dimension (depth) of the specimen you are studying.

b. Stage

The stage holds the slide to be observed. The center of the stage has an aperture or hole through which light passes to illuminate the specimen on the slide. Moving the coarse and fine adjustment knobs changes the working distance between the specimen and the objective lens.

c. Mechanical Stage

Your slide is fixed into position on the stage with the mechanical stage. The slide is fastened into the mechanical stage by using a small lever located on the mechanical stage. Two knobs located on the side of the mechanical stage are used to move the slide around to locate your specimen. One knob moves the slide from side to side and the other moves the slide forward and backward. The mechanical stage permits precision movements of your slide, without you touching the slide itself.

d. Condenser

The condenser, located below the stage, contains a system of lenses that directs light from a light source through the slide specimen. The condenser may be raised or lowered using the condenser knob. Most microscopes have a built-in light source.

e. Iris Diaphragm

The iris diaphragm is located on the condenser. The lever of the iris diaphragm is used to adjust the amount of light striking the object being studied. It is critical that you know the proper use of the condenser and iris diaphragm. A common problem with microscope use is having too much light which obliterates the object (more or less like trying to see something while looking directly at the sun). You will have better contrast if you reduce light to a minimum.

f. Objective Lenses

The pattern of light formed by the specimen is focused into a real image by the objective lens. When proper illumination is provided the resolving power of a microscope depends on the quality of its objective lenses. Our microscopes are provided with 4 objective

lenses which are mounted on a rotating turret or nosepiece. As you rotate the turret you will feel the lens "click" into position for proper viewing. If a lens is not properly locked in position you will observe a dark area as you look into the microscope.

The 4 objective lenses of your microscope and their magnifications are:

Scanning lens	4x magnification
Low Power Lens	10x magnification
High Power Lens	40x, 43x or 45x magnification
*Oil Immersion Lens	100x magnification

*** Note: The magnification of the oil immersion lens requires using the lens with a special immersion oil for proper resolution. Never use oil immersion lens without immersion oil. Serious damage might result. The oil immersion lens will not be used in BIOL 101.**

g. Ocular

The ocular lens, or eyepiece, further magnifies the image formed by the objective lens. It does not improve resolution. Your microscope will probably have a monocular system (one ocular lens). A few of the microscopes have adjustable binocular systems (two ocular lenses). The magnification of the ocular is 10x.

The ocular may be equipped with a pointer or a numerical scale. They are useful in pointing out specific structures or determining the real dimensions of the specimen you are observing.

Additional Microscope Features

In addition to the parts of the microscope you have just identified, the following are a few features of the microscope which will be useful to know. Be sure you can describe these terms.

a. Parfocal

Most objective lens systems are parfocal, which means that once you have an object in focus at one magnification, it will stay reasonably in focus when you change objectives. When the microscope is parfocal only fine adjustment is required when changing magnification.

b. Parcentered

This term means that when you have your specimen in the center of the field of view at one magnification it will remain centered as you change magnification. It is most important to center your specimen when using the lower power objectives to avoid "losing" it from the field of view as you change to higher magnification.

c. Field of View

The visible area seen through the microscope when a specimen is in focus is the field of view. The greater the magnification the smaller the field of view.

d. Depth of Field

The depth of field is the thickness of the specimen which can be seen when in focus. As you increase magnification the depth of field decreases.

e. Working Distance

The space between the front mount (bottom) of the objective lens and the top of the coverslip of the microscope slide is the working distance. The working distance decreases as the objective lens magnification increases.

If a slide preparation is too thick it will be impossible to focus with the high power objectives because of the short working distance. In addition, there is a great risk of crushing the specimen, breaking the coverslip or slide, or damaging the objective lens with the shorter working distance. Accidents can be avoided if you never use the coarse adjustment knob while focusing with high power objective lens.

f. Total Magnification

The total magnification of the lens system is the product of the magnification of the ocular times the magnification of the objective lens being used (ocular X objective).

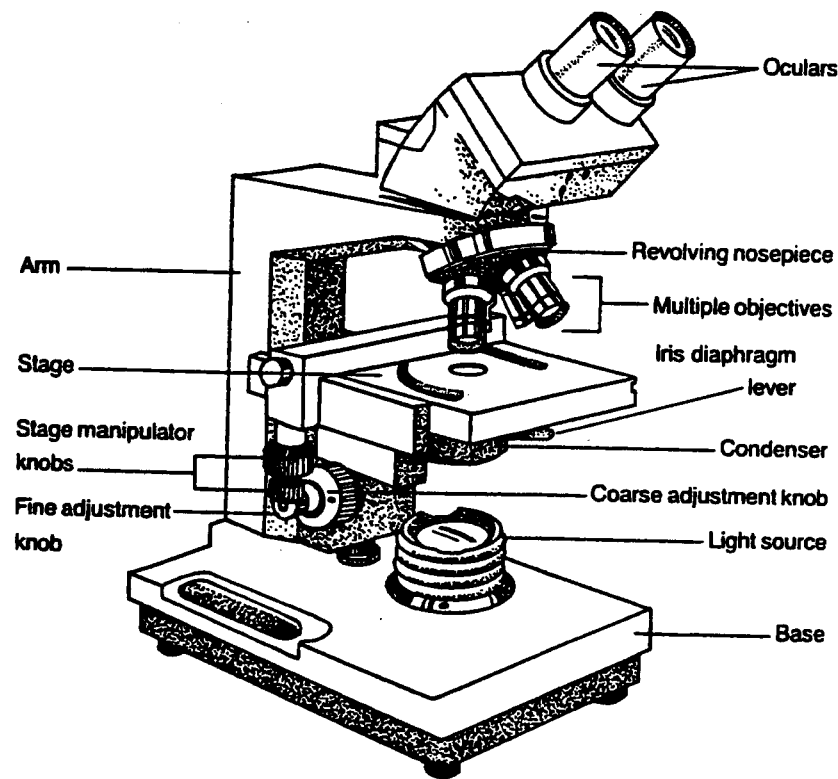
Record below the magnification for each of the lens systems of your microscope. Then record the total magnification achieved for each of the lenses.

Lens	Objective Magnification	Ocular Magnification	Total Magnification
Scanning			
Low Power			
High Power			
Oil Immersion			

Care of the microscope

- Always carry the microscope in an upright position using both hands: one hand on the arm and the other hand supporting the base.
- Keep the microscope away from the edge of the table and place the cord out of the way where it will not catch on something.
- Use only lens paper to clean your microscope (this is special grit-free paper). The objective lenses, ocular lens, and top of the condenser, require frequent cleaning to remove smudges. Clean prepared microscope slides with lens paper as well.
- The most common cause of not being able to focus a specimen is a dirty lens or slide. A fingerprint that you may not be able to see can prevent a crisp focus.
- Keep the stage dry at all times. A wet stage will prevent the slide from being accurately positioned. Liquids may also corrode the microscope.

- f. When returning your microscope to its proper place in the cabinet always:
- Remove the slide from the mechanical stage.
 - Clean all lens surfaces and the stage.
 - Rotate the turret so that the scanning lens is in place.
 - Secure the cord so that it does not hang down.



Binocular Compound Light Microscope.

Activity 2: Using the Microscope

The purpose of this exercise is to provide you with some experience in using the microscope. The slides you will be studying are ones that illustrate specific features of microscope use.

Materials Required

Compound microscope
 Prepared slides (permanent)*
 leaf cross section
 stem cross section
 Blank slides, cover slips and lens paper
 Newspaper and scissors

*Please note: there are usually not enough permanent slides for each student to use one at the same time, so please only have the slide at your desk while you are viewing it, then immediately return it to its box on the equipment cart.

When viewing objects through the microscope please use the following steps:

- Always start with the scanning lens. Rotate the nosepiece to swing the scanning lens into position over the aperture (make sure it clicks into its locked position). This provides a larger field of view to help you find the sample. It also provides maximum clearance (larger working distance) between the objective and the stage for placement of the slide.
- Place the slide on the stage of the microscope and use the mechanical stage mechanism or spring clips to secure the slide. Move the slide so the specimen is over the stage aperture.
- Use the coarse adjustment knob to move the scanning (low power) lens to its lowest position (bringing the objective and stage as close together as possible).
- Next, look into the ocular and adjust the light for comfort. Now use the coarse adjustment knob to focus the image by slowly raising the lens away from the specimen.
- To examine part of the specimen at a higher magnification, center that part of the image over the aperture before changing to a higher-magnification objective lens. This keeps the specimen in the smaller field of view at the high magnification.
- As you increase magnification the iris diaphragm should be opened so that just enough light for resolution, but not so much that contrast is lost.
- Do not move the focusing knobs before increasing magnification. Most microscopes are parfocal and are designed to stay in focus when a different objective lens is selected.
- After changing to the high objective lens, use only the fine focus knob to adjust the lens.
- When you have completed your observations return the microscope to the cabinet and the materials to their appropriate locations. Make sure that everything at your table is clean and all lab supplies have been put away

Part A: Slide Preparation and observation

Permanent Slides (prepared slides): Permanent slides have been professionally prepared. They have often been stained to show better contrast of structure. A permanent slide may be a whole mount of the entire specimen or a section of the material. You should have only one permanent slide at your table at a time. Permanent slides should be cleaned only with lens paper.

Procedure:

- Obtain a stem cross section slide.
- On a separate piece of paper draw a sketch of the tissue at low (4x) power.
- Obtain a leaf cross section slide.
- On a separate piece of paper draw a sketch of the tissue at high (40x) power.

Wet Mount Slides: Wet mounts are temporary slides that you prepare yourself.

Prepare Slide:

- Obtain a slide, a coverslip, and newspaper and scissors.
- Take your newspaper and from the regular small print cut either a single letter (preferably an “a” or an “e”) or a small strip of letters.
- Add a small drop of water to the center of the slide. Place the letter in the drop of water.
- Hold the cover slip upright so that one edge of the slip touches the edges of the drop of water.
- Gently lower the cover slip over the drop of water. The coverslip will keep the lenses dry. Use a paper towel to absorb excess water that has leaked out from under the coverslip.

Observe Slide:

1. Click the scanning objective into place and place the slide on the stage. Move the slide until the newspaper print is directly over the aperture of the stage. Make sure the coarse adjustment is turned to its lowest point (the objective closest to the stage).
2. Look through the ocular and very slowly rotate the coarse adjustment up so that the objective is gradually moving away from the stage. The letter should gradually come into focus.
3. You may adjust the iris diaphragm if the light is too intense. Is the image of the letter oriented in the same direction in which it is placed on the slide (right side up, upside down, forward, backward)?
4. Move the slide a little to the left. Did the image move in the same direction?.

5. Now observe the letter "e" with the 10x objective. Is the image still in focus? Is the "e" still in the center of the field of view? You may have to adjust the focus and re-center the image.

6. Finally rotate the 40x objective into position. Sharpen the focus with the fine adjustment knob. Remember -- never use the coarse adjustment knob with the high magnification objectives -- you might cause damage to the slide or to the lens.

As you increased magnification what happened to the apparent size of the image? The brightness? The area of the field of view?

7. Before removing the slide rotate the 4x objective into position.

Why do you always start your microscope observations with the 4x objective in position?

After you have made your observations the slide and coverslip should be washed and dried for the next observation

Part B: Depth of Field Observation

Although a slide specimen may appear very thin, it usually has many layers of cells. Depth of field is the portion of a specimen in focus, the layer of the slide currently in focus. This focal depth is greatest at low power and decreases as magnification increases. To show depth of field (the thickness of the specimen which can be seen when in focus) you will examine a prepared slide of overlapping threads or a wet mount of overlapping hairs. Notice how the threads or hairs are layered and how much of the slide is in focus at each magnification. This is an important technique to learn. No matter how thinly we cut a specimen, it will always have some thickness, i.e. a three dimensional object that we are looking at in two dimensions.

Procedure:

1. Take one hair from your head and one hair from someone else's head (different colors are helpful). Prepare a wet mount with these hairs crossing in the middle.
2. Using correct microscope technique, view your slide.
3. Using the 4x objective focus down with the coarse adjustment until the hairs are out of focus, then slowly focus upward again, noting which hair comes into clear focus first. The hair that comes into focus first is the lowest layer on the field. Continue to focus upward until the uppermost thread is clearly focused. This hair is the highest position on the field. Repeat this with the 10x and 40x objectives using the fine adjustment knob.

Activity 3 - The Dissecting Microscope

Materials Required

- Dissecting Microscope
- Prepared slide of flea or other insect
- Small culture dish containing moss
- Small culture dish containing salt crystals

The dissecting microscope allows you to magnify objects too large or too thick for the compound microscope. The effective magnification of the dissecting microscope is limited. Most provide magnifications in the range of 4x - 50x.

A. Using the Dissecting Microscope

1. Remove a dissecting microscope from the cabinet. If your dissecting microscope does not have a built in light source, get a lamp also.
2. Identify the parts of the dissecting microscope as shown in the diagram. Your microscope will have a magnification knob located on the side, with a magnification range of 0.7x to 3x, and a focusing knob. The oculars have a magnification of 10x. Some microscopes have set magnifications, others have a zoom lens which works like a zoom on a camera. What is the range of total magnification possible with your dissecting microscope?

Adjust the interpupillary distance (the distance between the 2 eyepieces) for your eyes by moving the ocular lenses towards or away from each other.

3. Observe the flea slide, moss specimen and salt crystals with the microscope. How does the dissecting microscope compare with the compound microscope? You may wish to examine other things.
4. When you have completed your observations return the microscope to the cabinet and the materials to their appropriate locations. Make sure that everything at your table is clean and all lab supplies have been put away.

Compound microscopes and dissecting microscopes are but two of many different types of microscopes used by biologists. Much of the research in cell biology involves working with electron microscopes, both transmission electron microscopes and scanning electron microscopes. Electron microscopes focus a beam of electrons rather than light waves and can achieve resolution at much higher magnifications.

Activity 4 - Estimating Sizes

Materials Required:

- Clear plastic metric rulers
- Stage micrometers
- Dissecting and Compound Light Microscopes

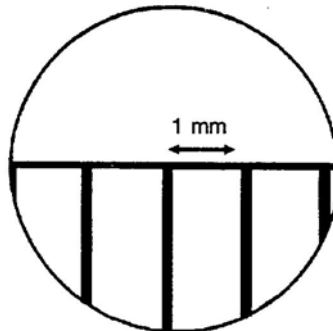
You will frequently be asked to estimate the size of the specimens you observe in lab. To do this you must know the approximate diameter of your field-of-view for your particular microscope. Knowing this for each objective lens, you can compare the size of the specimen against the known field diameter and make a reasonable estimate of size.

Determining Your Field-of-View Diameter

Use the worksheet at the end of this lab to record your data.

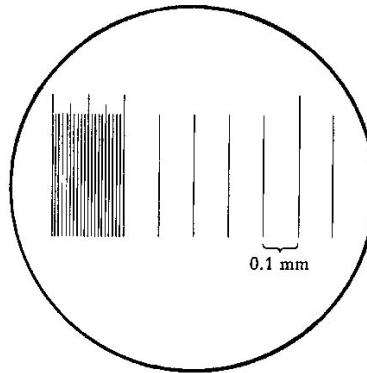
1. Obtain a clear metric ruler and position it on the stage of your microscope.
2. Bring the scale on the ruler into focus with the lowest power objective (on the compound microscope) or with all objectives on the dissecting microscope.

The scale bars are in increments of **1 mm**. Each line is actually quite thick when viewed through the microscope. The distance from the left side of one line to the left side of the next line is one millimeter.



3. Adjust the ruler so that the left side of one line is just tangent to the lighted field-of-view at the diameter. It should look something like the diagram above.
4. Starting at that edge, count how many bars and spaces it takes to cross the field-of-view. You will probably have to estimate the last fraction of a space or bar.
5. Record your microscope's field diameter with the 4X objective in the table below.
6. Now obtain a stage micrometer from the cart at the front of the room.

The scale bars with the smallest distance between them are **0.01 mm** (10 μm) apart.



7. Follow the directions given above to measure the field-of-view for the 10X and the 40X objectives. While doing this be very careful to follow the directions for use of all the objectives.

8. Use the information you have gathered to calculate the area of the visual field for each objective.

$$\text{Area of circle} = \pi \times \text{radius}^2 \quad (\pi = 3.14)$$

Activity 4 Work Sheet

Name _____

A. Determining Field-of-View Diameter:

Dissecting Microscope:

Total Magnification (objective X ocular)	Diameter of Visual Field (Reminder: mm x 1000 = μm)		Area	
	mm	μm	mm ²	μm^2

Compound Microscope:

Total Magnification (objective X ocular)	Diameter of Visual Field (Reminder: mm x 1000 = μm)		Area	
	mm	μm	mm ²	μm^2

B. Making measurements using the field diameter.

Measure the longest dimension of the following cells by estimating their size relative to the field of view. Fill in the table with your size estimates (be sure to use units) and compare your answers with those of your classmates.

Cell All three are one-celled organisms (protists) commonly found in pond water	Measured longest dimension (in μm)	Two size estimates (in μm) from other students for each organism student names estimated sizes	
<i>Paramecium</i>			
<i>Euglena</i>			
<i>Stentor</i>			

Explain differences in measurement assuming all measurements were correct.