The Compound Microscope

The **compound microscope** is a basic tool of the microbiology laboratory. This precision instrument contains a series of lenses allowing a specimen to be magnified up to a thousand times (1,000x). Mechanical adjustments and supportive features of the microscope afford a broad range of possibilities for viewing various types of microorganisms.

The microscope combines the principles of an optical system and an illumination system to achieve magnification in a bright field. Light, projected toward an object, passes through the object and is collected by the objective lens (near the object) to form a magnified image. This magnified image becomes an object for a second lens, the ocular lens (near the eye), which magnifies it further and forms an image visible to the observer.

An understanding of certain aspects of microscopy is essential for optimal use of the microscope. For example, the **resolving power** determines the size of the smallest object that can be seen clearly under specified conditions. Another aspect, the **working distance,** refers to the proximity of the slide to the bottom of the objective lens. A third factor, the **refractive index,** pertains to the light-bending ability of glass, oil, and air–media through which light must pass during image formation.

A. Parts of the Microscope and Their Use

PURPOSE: to learn the parts of the light microscope and understand their functions.

In this exercise, the basic features of the microscope will be explored and the function of the parts explained. Experience will be gained in using the instrument through the observation of various objects.

Special Materials

- Compound microscope
- Glass slides and coverslips
- Various samples for viewing

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Procedure

DO NOT use paper towels or tissues to clean the lenses. Use lens paper only.

1. The instructor will outline certain precautions to be followed when using the microscope, including the proper method for transporting it. When carrying the microscope, hold it upright with two hands: one hand holding the arm of the microscope, the other supporting its base. Each student should secure a microscope for use and place it on the desk. **Lens paper** should be used to clean the lenses and stage area before work is begun. Coarser types of tissue are not useful because they leave lint and may scratch the lens.

2. The instructor will point out the major parts of the microscope and their functions. These parts include the **ocular lens** (the eyepiece), the various **objective lenses**, the **stage** and **slide holder** (mechanical stage), the **condenser** and **diaphragm,** the **coarse** and **fine adjustments**, and the light source. Note the position of each of these parts in **Figure 1**, and enter the functions as explained by the instructor in **Table 1** in the Results section. Binocular microscopes should have a mechanism for adjusting the distance between the eyes.

3. The **total magnification** of the lens system is calculated by multiplying the ocular magnification by the objective magnification. The total magnification possible with each objective should be determined and recorded in the Results section in **Table 2**.

4. To begin your microscopic work, prepare a **wet mount** of a hair fiber as follows: Place a small drop of water on a clean glass slide, add a hair fiber, and cover the preparation with a coverslip. Remove any excess water by touching a piece of paper toweling at the edge of the coverslip. Your instructor may substitute another specimen for viewing.

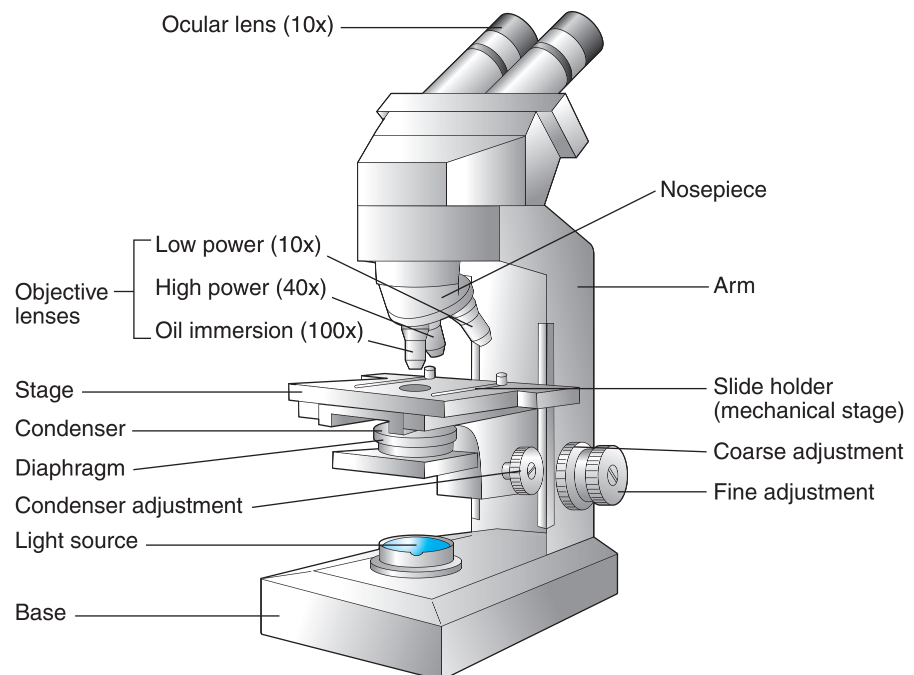

FIGURE 1
The parts of the compound microscope

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5. Place the slide in the slide holder or mechanical stage of the microscope. Now move the slide to place the specimen directly over the hole in the stage.

   Raise the stage all the way up and then, using the coarse adjustment knob, slowly lower the stage to locate the hair fiber with the scanning or low power objective lens (as directed by the instructor). Moving the slide slightly while focusing helps in locating the object. Use the fine adjustment knob to sharpen the focus. Adjust the **diaphragm lever** to achieve optimal lighting, noting that optimal lighting is not always the most light.

   Looking in the oculars, you should see a single circular area called the **field**. Both eyes should be open if a binocular microscope is used. If your microscope is monocular, practice viewing with both eyes open, using the left eye to look into the microscope (if you are right-handed) or the right eye (if you are left-handed). Move the object about for several minutes while training your brain to follow it.

   Draw a representation of the fiber or other specimen in the appropriate space in the Results section. Include the magnification of the image with the representation.

If you break a slide while working, be sure to place it in the designated sharps container in the lab or in the glass receptacle indicated by the instructor.

6. Note the working distance; that is, the distance between the slide and the objective lens. Most modern microscopes are **parfocal,** meaning an object in view under one objective will still be in view under the other lenses. Therefore, to move to the next objective (low or high power), place the specimen in the center of the field and simply rotate the nosepiece to swing the next objective into position. A minor adjustment with the fine adjustment knob may be required. Scan the object, then enter a representation of the fiber when your observations are complete. Again, note the working distance.

DO NOT adjust the focus with the coarse adjustment knob.

7. Do not use the oil immersion (100x) objective lens with this specimen.
8. To remove the specimen, rotate the nosepiece back to the scanning or low power (10x) objective. Lower the stage and then remove the slide.
9. Clean or recycle the slide and coverslip as directed by your instructor.

B. Observations of Prepared Slides

You now have gained some experience with the microscope and observing specimens. However, much of your microscope observations will entail use of the oil immersion objective lens (100x) because many microorganisms, especially bacteria, are so small.

PURPOSE: **to observe specimens with the oil immersion objective lens.**

Special Materials

- Compound microscope
- Prepared slides of microorganisms
- Immersion oil

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Procedure

1. Obtain a **prepared slide** of stained microorganisms. These are commercially prepared permanent slides of dead organisms. Clean the slide with soap and water, and dry it with paper towels. Remove any remaining "towel lint" with a piece of lens paper.

2. Place the slide on the slide holder or mechanical stage and center the specimen over the hole in the stage. Adjust the diaphragm lever and focus on the specimen with the low power lens.

3. At these magnifications, do not expect to see individual cells. Most bacteria are far too small to be resolved. Rather focus on the areas containing stain used to stain the cells.

4. Once you have located stained material and have focused on it, center the specimen in the field. Rotate the high power (40x) objective lens into position. Use the fine focusing knob to sharpen the focus.

5. With the high power (40x) lens, you may be able to see individual bacteria. Still, most are too small to be observed or their size, shape, and arrangement to be determined. The oil immersion lens (100x) must be used. Center the specimen in the field before proceeding.

6. To use the **oil immersion lens** and achieve the magnification it affords, the air between the slide and lens must be replaced by a special type of synthetic oil called immersion oil. **Immersion oil** has the same refractive index (or light-bending ability) as glass. The oil thus keeps light in a straight line as it passes from the glass slide to the oil and then to the glass of the objective lens. Immersion oil improves the resolving power of the microscope by providing enough light to let you see clearly.

7. To use the oil immersion lens, swing away the high power objective (40x), and apply a drop of immersion oil on the slide area being viewed. Open the diaphragm fully, and swing the oil immersion objective into position. To bring the object into view, a minor adjustment with the fine adjustment knob may be needed.

8. Once the microorganisms have been located, it is important that you spend a few minutes scanning the slide. Scanning allows you to determine the general pattern of the microorganisms as well as their shape, size, and arrangement. It also helps your mind eliminate the debris that may be mixed in with the smear; and it helps you locate a thin area most suitable for your drawing. Using a sharp pencil, enter representations of the microorganisms in the Results section along with the magnifications used. Drawings and documentation of the specimens viewed are almost always made at 1000x total magnification. Consult with your instructor on any special preferences he or she may have.

9. A rough estimate of the size of an object can be made in one of two ways. Your microscope may have an **ocular micrometer** mounted in one ocular. At 1000X total magnification, each ocular division is equal to one micrometer (µm). For example, if the length of a bacterial cell spans three divisions, it is 3 µm long; if a spherical yeast cell is 6 divisions, it is 6 µm in diameter.

Quick Procedure
Microscopic Examination of Prepared Slides

1. Place prepared or stained slide on microscope stage and center specimen.
2. Focus at low power (10x) on the specimen or areas containing stain.
3. Center specimen in the field and rotate the high power (40x) lens into position.
4. Use the fine focus to resolve the specimen and center it in the field.
5. Rotate the 40x lens away from the specimen and add a drop of immersion oil onto the slide surface.
6. Rotate the 100x lens into position and use the fine focus to resolve the specimen.
7. Make a drawing or record results from your observations.

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If an ocular micrometer is not provided, consider that the distance across the field of view with the low power lens is approximately 1600 micrometers. With the high power lens, it is approximately 400 micrometers. And with the oil immersion lens, it is approximately 160 micrometers. By determining how wide the object is relative to the diameter of the field of view, you can estimate the object's size.

10. Additional practice with the microscope may be obtained with other specimens supplied by the instructor. A drop of **yeast cells,** a drop of **hay infusion,** and a few **cotton fibers** in a drop of water are all useful specimens for developing a facility with the microscope.

11. *See it–Draw it.* This exercise will give you an appreciation of how difficult it is to accurately describe microscopic organisms to someone else without the benefit of a detailed drawing. It requires the participation of two persons and one microscope. One person is the viewer, the other person the recorder. The first person locates a smear of bacteria under oil immersion. Now he or she describes the view to the second person, who makes a representation of the bacteria on a sheet of drawing paper (preferably hidden from the view of the first person). When completed, compare the drawing with the microscope image. It will soon become apparent that drawing an accurate picture is far superior to trying to explain what bacteria look like.

12. *Microscopic troubleshooting.* Beginning students as well as experienced ones often encounter difficulties with the microscope that can be resolved by relatively simple adjustments or procedures. Here is a list of ten possible problems and solutions you might find useful:

1. Problem: The object is observed clearly under low power, but you lose it when moving to high power.
   Solution: Be sure you place the object in the center of the field before moving to the next higher objective. The microscope field becomes smaller as the magnification increases, so you should always center your object before switching objectives.
2. Problem: You see a black half-moon or quarter-moon off to the side of the microscope field.
   Solution: The objective must be "clicked" into position before using it. Check to see that it is in position.
3. Problem: You can see the objects at 400X, but they cannot be found when you move to oil immersion (1000X).
   Solution: After fine focusing at 1000X without finding the objects, rotate the nosepiece forward to the low power (10X) objective. **Do not go backward to the 40X lens, as the lens will touch the oil: never get oil on the 40X lens.** Re-find the objects at 10X and then rotate the nosepiece backward to the 100X oil immersion lens. Use the fine focus to find the objects. (See also Problem 4).
4. Problem: You cannot focus down to the level where the specimen is.
   Solution: Check the slide to ensure that it is not upside down on the stage.
5. Problem: You constantly encounter a fuzzy image under high power.
   Solution: Someone using the microscope before you may have left oil on the lens. Moisten a piece of lens tissue with water and vigorously clean the objective, then dry it with a clean piece of lens tissue.

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6. Problem: The image is too dark to see clearly.
   Solution: Try opening the diaphragm to let in more light. If the results are not satisfactory, adjust the condenser up or down until the quality and quantity of light improve.
7. Problem: You have cleaned the ocular and objective lenses thoroughly, and still the image is not clear.
   Solution: Try cleaning the lens of the condenser where it meets the opening of the stage. There may be a layer of oil on the lens.
8. Problem: You are not sure whether the lint and debris you see are on the ocular or objective.
   Solution: As you view through the microscope, rotate the ocular; the dirt will rotate if it is on the ocular. If so, wipe the glass of the oculars with lens paper.
9. Problem: You wonder whether you should wear your glasses while using the microscope.
   Solution: Most microscopes are adjusted to compensate for the viewer's glasses. Indeed, the most comfortable viewing point without glasses is farther back than it is with glasses. Also, wearing glasses while you view allows you to move from the microscope to your notebook and back without putting on or removing your glasses.
10. Problem: You have a binocular microscope, and you have trouble focusing with two eyes open.
    Solution: You should check your oculars to see which one has an ocular adjustment (not a focus adjustment); if it is the right ocular, then close the right eye, and while looking with the left eye, focus the ocular adjustment on the right ocular (do the reverse if the adjustment is on the left ocular); now use the knob between the oculars to adjust the distance between your pupils.

13. When your work with the microscope is completed, rotate the nosepiece to bring the low power (10x) lens into position. Remove the slide and remove the oil from the oil immersion lens by wiping it with lens paper. The other lenses and parts of the microscope should also be cleaned well. For microscope storage, move the stage to the safety stop, and wind the cord neatly around the base or arm of the instrument.

Questions

1. Explain the problem encountered when magnifying an object with the oil immersion lens and indicate how immersion oil helps solve the problem.

2. Why do you use only the fine adjustment knob when viewing specimens with the high power (40x) objective and with the oil-immersion (100x) objective?

3. Describe the important steps that should be taken to care for the microscope during and after use in the laboratory.

4. What does the adjective *parfocal* mean when applied to a microscope? How is it a valuable asset in the use of the microscope?

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5. Explain resolving power and working distance as they apply to the microscope. Determine the resolving power of your microscope, using the oil immersion objective. Can a spherical bacterium measuring 3 μm in diameter be seen with the oil immersion lens? Explain.

$$\text{The resolving power (RP)} = \frac{\lambda \text{ (wavelength of light)}}{2 \text{ NA (numerical aperture)}}$$

Assume $\lambda = 500$ nm and NA $= 1.25$

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Name

Date | Section

Exercise Results

The Compound Microscope

A. Parts of the Microscope and Their Use

Table 1. Function of the Microscope Parts

Microscope Part	Function
Ocular	
Objective	
Stage	
Condenser	
Diaphragm	
Coarse adjustment	
Fine adjustment	

Table 2. Calculating Total Magnification

Objective Lens	Objective Lens Magnification	Ocular Lens Magnification	Total Magnification
Scanning	4X		
Low			
High			
Oil immersion			

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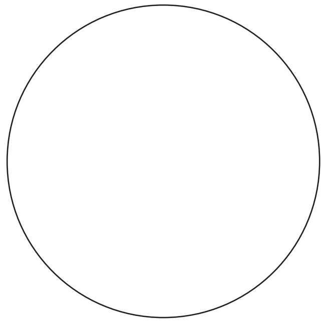
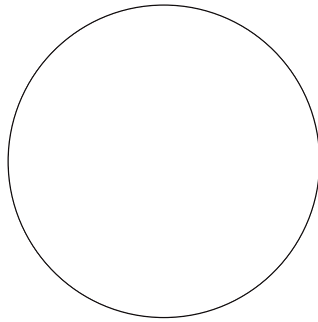
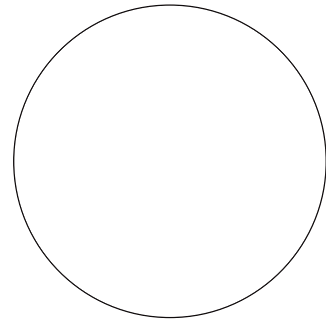
Observation of Wet Mount

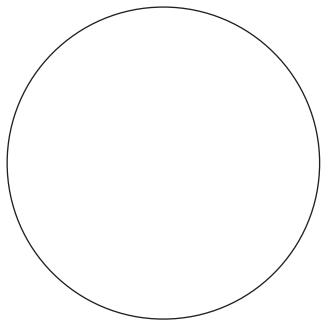
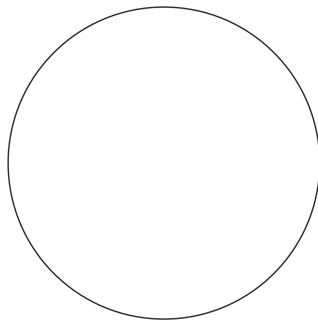
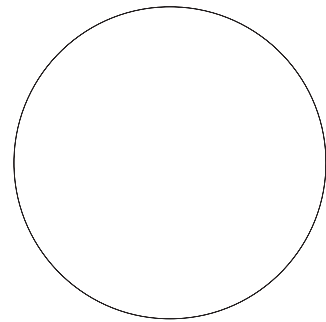




Specimen: ____________ ____________ ____________

Magnif.: ____________ ____________ ____________

Cell size (μm): ____________ ____________ ____________

B. Observations of Prepared Slides

Specimen: ____________ ____________ ____________

Magnif.: ____________ ____________ ____________

Cell size (μm): ____________ ____________ ____________

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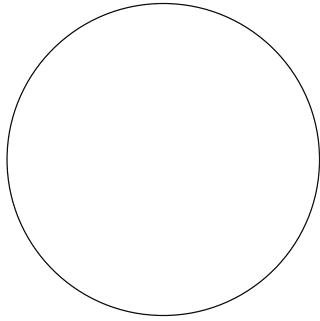
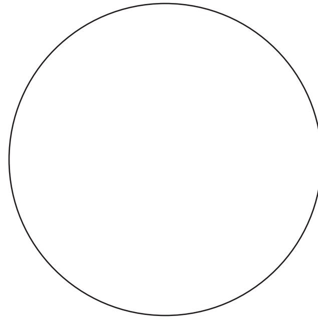
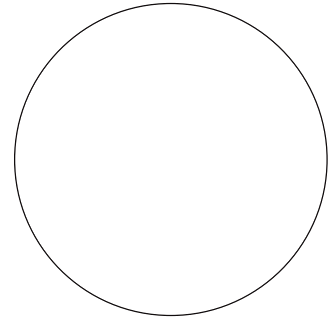
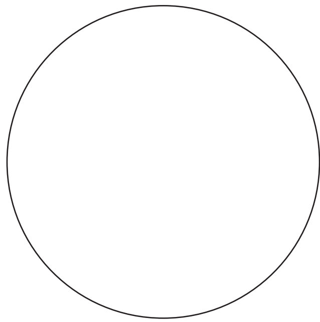
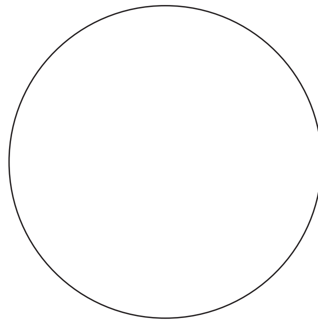
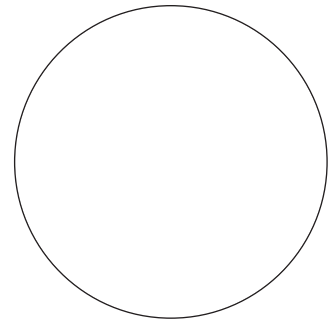




Specimen: ______________ ______________ ______________

Magnif.: ______________ ______________ ______________

Cell size (μm): ______________ ______________ ______________

Specimen: ______________ ______________ ______________

Magnif.: ______________ ______________ ______________

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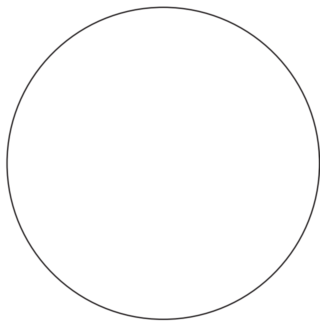
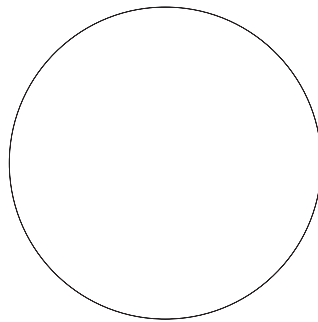
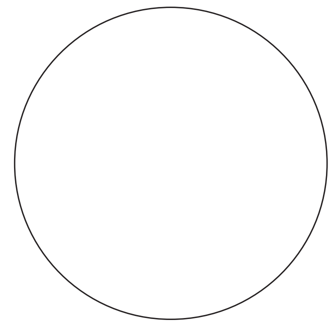

Specimen: ______________ ______________ ______________

Magnif.: ______________ ______________ ______________

Cell size (μm): ______________ ______________ ______________

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