MICROSCOPE LAB 1

THE MICROSCOPE
The microscope is one of the principal tools of the biologist. Without the microscope, many of the great discoveries of biology would never have been made. The light compound microscope, illustrated in Figure 1, is the type of microscope most commonly used. Proper, comfortable use of the instrument demands practice. The practice afforded you in this exercise depends upon familiarity with the parts of the microscope and with their interactions.

MAGNIFICATION AND RESOLUTION
In using the microscope it is important to know how much you are magnifying an object. To compute the total magnification of any specimen being viewed multiply the power of the eye piece (ocular lens) by the power of the objective lens being used. For example, if the eye piece magnifies 10X and the objective lens magnifies 40X, then 10 x 40 gives a total magnification of 400X.

The compound microscope has certain limitations. Although the level of magnification is almost limitless, the resolution (or resolving power) is not. Resolution is the ability to discriminate two objects close together as being separate. The human eye can resolve objects about 100 µm apart. The compound microscope has a resolution of 0.2 µm under ideal conditions. Objects closer than 0.2 µm are seen as a single fused image.

Resolving power is determined by the amount and physical properties of the visible light that enters the microscope. In general, the greater the amount of light delivered to the objective lens, the greater the resolution. The size of the objective lens aperture (opening) decreases with increasing magnification, allowing less light to enter the objective lens. Thus, you will probably find it necessary to increase the light intensity at the higher magnifications.

DEPTH PERCEPTION AND THE MICROSCOPE
Any microscopic object viewed has depth as well as length and width. While the lens of your eye fully adjusts to focus on an object being viewed and provides you with a three dimensional interpretation, the lenses of a microscope are focused mechanically and can only “see” in two dimensions (length and width). For example, if the specimen you are examining has three layers of cells, you will only be able to focus on one cell layer at a time. In order to perceive the relative depth of your specimen use the fine adjustment to focus through the different planes (the three cell layers) individually to build a three dimensional picture or interpretation of your specimen.
THE FIELD OF VIEW: A CIRCLE
When you view an object under the microscope you will observe that it lies inside a circular field of view. Each different magnification has a different sized field of view. If you determine the diameter of the field of view you can estimate the size of an object seen in that field. As you increase the magnification, the field of view (and diameter) gets proportionately smaller. As a consequence, a critter that appears small under scanning power may appear large under high power. The actual size of the critter did not change, only the space in which you placed it for viewing.

CARE OF THE MICROSCOPE
Your microscope is an expensive instrument that must be given proper care. Always follow these general instructions when using a microscope.
1. Always carry the microscope with both hands, one hand under the base, and the other on the arm. When getting ready to put the microscope away, always return it to the low-power or scanning-power setting.
2. When setting the microscope on a table, always keep it away from the edge. It is generally best to clear your lab table of items that are not being used.
3. The lenses of the microscope cost almost as much as all of the other parts together. Never clean them with anything other than lens paper.
4. Please report any microscope damage or irregularity in its operation at the beginning of the period so that the repair costs will not be charged to you. You are responsible for the microscope while you are using it.
SETTING UP & VIEWING OBJECTS THROUGH THE MICROSCOPE

1. **Place the low-power objective in position** if it is not so fixed. In changing from one objective to another, you will hear a click when the objective is set in proper position. Make certain that the lenses are clean.

2. **Check preliminary lighting**
   a. Turn on the substage light.
   b. Position the condenser as high as it will go by turning the substage adjustment. This provides for a maximum of light.
   c. Open the iris diaphragm by means of the lever beneath the condenser, which is below the stage of the microscope.

3. **Place the slide for viewing**
   a. Make certain that the lower-power objective is in place.
   b. Lower the stage (away from the objective) with the coarse adjustment.
   c. Place a properly prepared slide (see below) on the stage and secure with the stage clips or mechanical stage depending upon which is present on your microscope.
   d. Place the slide with the object directly above the brightly illuminated substage condenser.

4. **Focus: Proper focusing technique**
   a. Viewing the stage from the side, use the **coarse adjustment knob** to raise the stage until the stop is reached that will prevent further movement of the stage.
   b. Looking through the eye piece, or ocular, **lower the stage slowly by turning the coarse adjustment knob** counter-clockwise until the object is in focus.
   c. Use the **fine adjustment** to bring the object into sharp focus.
   d. If necessary readjust the amount and intensity of light for comfortable viewing.

5. **Increasing Magnification: Switching from Low to High Power**
   a. First, be sure your slide is focused under low-power.
   b. Leave the slide where it is and switch to high-power. Watch from the side to make sure that the objective lens does not touch the slide. The object should be in focus.

   **NOTE:** Most microscopes are “parfocal”; that is, the slide should be in focus (or nearly so) when you switch from low-power to high-power. If you have properly focused your microscope at low-power and your slide is properly prepared (i.e. the object is flattened by a coverslip), you should be able to switch automatically from low-power to high without fear of having the high-power objective lens scraping or touching the slide.

   An important factor in using the microscope is the distance between the object viewed and the objective lens of the microscope. This is called the **working distance**. As you increase magnification the working distance becomes less.

6. **Re-Focus under High Power**
   a. **ONLY USE THE FINE ADJUSTMENT.** When the high-power objective is being used, never use the coarse adjustment.

7. **Remove the slide**
   a. Switch the objective to either scanning or low-power.
   b. Lower the stage using the coarse adjustment.
c. Remove the slide from the stage.
   
   **Note:** Never remove a slide while under high-power.

### PREPARING A TEMPORARY SLIDE OR A WET MOUNT

In this lab all the objects, cells, or live specimens you prepare for observation will be viewed by making a wet mount slide. A temporary slide, or wet mount, is prepared as follows:

a. Place a drop of water on a clean slide with a dropper.

b. Put the object in the water drop.

c. Lower one edge of the coverslip to the edge of the water drop as shown in the illustration (Figure 2). Lower the coverslip slowly to avoid air bubbles. A gentle tapping will usually remove any bubbles that may be present. Blot any excess water. More water can be added with a dropper at the edge of the coverslip. Do not let your specimen dry out.

![Figure 2. Wet Mount Preparation](image)

### PROCEDURES

As you follow the procedures outlined below, answer the questions on your lab worksheet.

**Part A: Letter E** (questions 1-10 on the lab worksheet)

Make a wet mount of a letter “e” (lower case letter), which has been cut from a newspaper. Observe this under low and high-power.

**Part B: Defining Resolution and Determining the Total Magnification**

Using the information given in the section “Magnification and Resolution”, determine the total magnification of the scanning, low, high, and oil immersion fields of view. Record these in the Summary Chart (#11). Then, apply your knowledge of total magnification and resolution to answer questions 12-14.
Part C: Determining the Size of the Microscopic Field of View
You will often want to know the size of the objects you are observing under the microscope. Because these objects are usually too small to permit direct measurement, it will be convenient for you to learn a method to indirectly measure them. Determining the diameter of the field of view for the different objective magnifications will enable you to determine indirectly the approximate size of objects viewed under the microscope.

The diameter of the field of view for low-power is given to you in Figure 3. If you would like to verify this diameter, do the following: Be sure the low-power objective is in position. Place the graduated edge of a plastic metric ruler across the midline (diameter) of the field of vision. Bring the ruler into focus under low power. Record the diameter in millimeters. Now record the diameter in micrometers. A micrometer (um) equals 0.001 mm. (See Figure 4). This is the most common unit of measurement in microscope work. Once you have determined the diameter for the low power field of view you can calculate the diameters of the fields of view for the rest of the objective lenses (consult Figure 3). For example, to calculate the diameter of the high-power field knowing the diameter of the low-power field, first find the number on each objective that indicates the magnification. Then divide the high-power by the low-power magnification to get a factor that indicates how much smaller the high power field is. For example, if the low-power objective reads 15X and the high-power 45X, dividing 45 by 15 gives a factor of 3. If the diameter of the low-power field were 1.5 mm, then the diameter of the high-power field would be 1.5 divided by 3 or 0.5 mm. Armed with this knowledge answer question 15-16, and fill in the appropriate sections of Summary Chart #11 on your lab worksheet.

Once you know the field of view diameter, you should be able to estimate the size of any organism found within that field. Question #17 on you lab worksheet provides you with practice size estimation problems.

Part D: Depth Perception
It is important to remember that by using the coarse and fine adjustments you bring the microscope into focus at many different levels. At each setting you can see clearly only one plane of the object. To see other planes clearly, you must change the focus. Obtain a small piece of nylon hose approximately 1 cm square. Prepare a wet mount of this material. Examine under low and high-power. Notice as you rotate the fine adjustment knob back and forth slowly you can see the fibers at different depths. You get a three-dimensional (i.e. length, width, and depth) interpretation of the cloth. Use this information to help answer #18 on your lab worksheet.

Optional: Obtain a prepared slide with three colored cross threads (or make a wet mount if a prepared slide is not available.) Focusing with low magnification, locate
the point where the three threads cross each other. Using proper focusing technique as outlined in the beginning of this lab determine which thread comes into focus first.

**Part E: Staining** - In this part of the exercise you will learn a simple staining technique used to observe organisms under the microscope. The stain you will be using is I₂KI (iodine-potassium-iodide). You will use the following procedure for staining each of the ‘specimens’ called for in this section of the lab:

1. Place a small drop of iodine-potassium-iodide (I₂KI) solution at the edge of one side of the cover slip.
2. Obtain a small piece of paper towel or tissue paper and place its edge in contact with the water at the opposite edge of the cover slip from the I₂KI drop.

Water will be absorbed by the paper towel or tissue paper. As water is removed at one edge of the mount, I₂KI solution will be drawn under the cover slip at the opposite edge. Continue until the I₂KI solution is drawn about halfway across the space under the cover slip. I₂KI solution will then continue to spread slowly throughout the mount. The result will be that you will have ‘specimens’ exposed to different concentrations of I₂KI solution. So, by examining different regions of the mount, you can observe the effects of different concentrations of the I₂KI solution on the ‘specimens’ observed.

1. **Mounting, Staining, and Observing Starch Grains**

Obtain a clean slide and place it on the flat surface of your lab table. Obtain a small piece of white potato and place it on the center of the slide. Apply pressure to the potato until a small amount of juice is forced out of the potato and onto the slide. Distribute this juice in a more or less even layer over the center of the slide by moving your piece of potato in a circular pattern. Discard your potato. Immediately add a drop of water and a clean cover slip to the slide.

Examine your preparation under low-power. Adjust your contrast and lighting so that you can clearly see the starch grains. Move the slide on the stage until you have located a group of well separated grains. Center this group in the field of view and examine them again under high-power.

Describe the shape of an individual starch grain. Can you see any internal structures in these grains? If you can, describe what you observe.

When you have finished with these observations return to low-power. You will now stain the starch grains using the procedure given above.
Examine the slide first under low-power and then under high-power. What changes do you observe in the starch grains exposed to relatively high concentrations of I₂KI? What differences do you observe between these grains and others exposed to relatively low concentration of I₂KI? Can you observe internal structures more clearly in the stained grains than the unstained grains? If so describe the structures you can see. When you have completed your observations, remove and clean your slide. Use these observations to answer questions 19-20 on your lab worksheet.

2. **Examining Yeast Cultures** (Use this information to answer questions 21-22.)
The culture of yeast you are observing in this portion was prepared a day or so ago by placing a few grains of dried yeast in a solution of molasses and water.

Prepare a wet mount of the yeast culture by placing a small drop of the culture on a clean slide and applying a coverslip. Examine the preparation under low-power and then under high-power. Describe the shape of the yeast organisms.

Study the arrangement of a small group of yeast. From your observations, can you come to any conclusions about how new yeast organisms are formed? What internal structures, if any can you see in the yeast? Sketch any such structures that you observe.

Now, using the I₂KI solution, stain the yeast in the same way as you did the starch grains. Compare the effects of the I₂KI solution on the yeast organisms with its effects on starch grains. Can you see any structures that were not visible in the unstained yeast organisms? If so, describe them. Clean up your slide when you are finished.

3. **Procedures for Examining a Mixed Culture of Microorganisms**
Use these observations to answer questions 23-25 on your worksheet.
Using a medicine dropper, remove a small amount of liquid from the upper part of the mixed culture of microorganisms. Place one drop in the center of a clean glass slide and add a cover slip. Focus with low-power and adjust the substage diaphragm to obtain good contrast. Move the slide around on the stage so that all areas under the cover slip are examined. A variety of critters should be visible. Study these with particular attention to variations in size, shape, movement (twisting, wiggling, crawling, etc.) and internal structure. As water evaporates from the slide the cover slip will be drawn closer to the surface of the slide. As this occurs, the movements of larger organisms will be more and more restricted. Locate one or more of the organisms that have been either slowed down or stopped. Turn to high-power and observe your critter. As organisms move about, they not only change position in the field of view, but they also move in and out of focus. Why? If you do not constantly readjust the focus, you will see only part of the detail to be observed.
When seen under high power do the organisms appear to move more rapidly or more slowly than when seen under low power? Now stain the mount, repeating the procedures you used earlier with the starch grains and yeast. Do moving organisms continue to move when exposed to I₂KI solution? What changes in color do you observe? Can you observe any changes in internal structure as staining proceeds? If so, record these in sketches. When finished clean up your slides and cover slips.

At the end of the lab period, clean all the slides and cover slips. Place the microscope in its proper storage place following the procedures discussed earlier.