5A Using the Microscope

Because cells are small, you must make them appear larger than they really are in order to see and study them. To view cells closely, you will use a compound light microscope (**Figure 1**). It employs two lenses and a light source to make the object appear larger. The object is magnified by a lens near your eye, the ocular lens (sometimes called the eyepiece), and again by a second lens, the objective lens, which is just above the object. The comparison of the actual size of the object with the size of its image is referred to as magnification.

The parts of the microscope are described in **Table 1**.

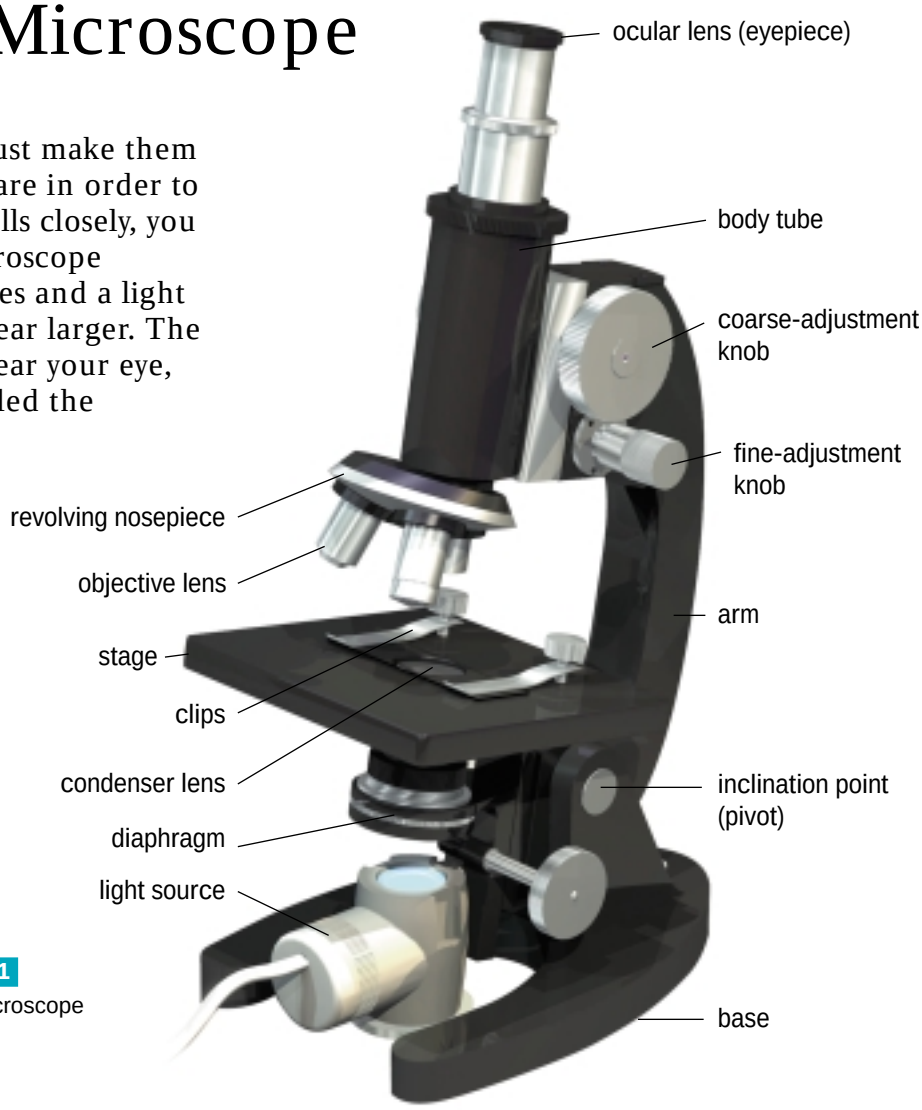


Figure 1
The microscope

Table 1 **Parts of the Microscope**

Structure	Function
body tube	Contains ocular lens, supports objective lenses.
clips	Found on the stage and used to hold the slide in position.
coarse-adjustment knob	Moves the body tube up or down so you can get the object or specimen into focus. It is used with the low-power objective lens only.
condenser lens	Directs light to the object or specimen.
diaphragm	Regulates the amount of light reaching the object being viewed.
fine-adjustment knob	Moves the tube to get the object or specimen into sharp focus. It is used with medium- and high-power magnification. The fine-adjustment knob is used only after the object or specimen has been located and focused under low-power magnification using the coarse adjustment.
objective lenses	Magnifies the object. Usually three complex lenses are located on the nosepiece immediately above the object or specimen. The smallest of these, the low-power objective lens, has the lowest magnification, usually four times (4X). The medium-power lens magnifies by 10X, and the long, high-power lens by 40X.
ocular lens	Magnifies the object, usually by 10X. Also known as the eyepiece, this is the part you look through to view the object.
revolving nosepiece	Rotates, allowing the objective lens to be changed. Each lens clicks into place.
stage	Supports the microscope slide. A central opening in the stage allows light to pass through the slide.

Care of the Microscope

The microscope is an important and expensive scientific instrument. It should always be used with care and patience. Here are some points to remember when using your microscope.

1. Always keep a microscope in an upright position.
2. When carrying a microscope, grasp its arm with one hand and support its base with the other (**Figure 2**).

Figure 2
How to carry a microscope

3. Once you have the microscope at your workstation, turn the coarse adjustment knob to lower the objective lens to its lowest point. Focus by using the knob to move the objective lens slowly upward to bring an object into focus. You must always remember to use both knobs when adjusting your microscope or you will strip the adjustment gears.
4. Microscope lenses are made of optical glass, which is soft and scratches easily. Use special lens paper to remove any dust or dirt.
5. When you complete an investigation using the microscope, follow these steps:
   - Rotate the nosepiece to the low-power objective lens.
   - Remove the slide and cover slip (if applicable).
   - Clean the slide and cover slip and return them to their appropriate location.
   - Return the microscope to the storage area.

Basic Microscope Skills

The skills outlined below are presented as sets of instructions. This will enable you to practice these skills before you are asked to use them in the activities and investigations in this course.

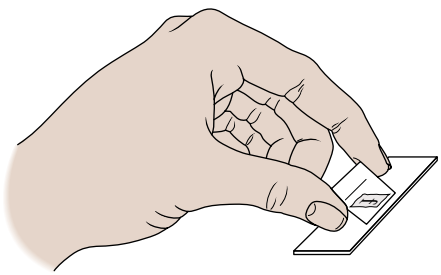
Materials

- newspaper that contains lower-case letter "*f*" or similar small object
- scissors
- microscope slide
- cover slip
- dropper (pipet)
- water
- compound microscope
- thread
- compass or petri dish
- pencil
- transparent ruler

Preparing a Dry Mount

This method of preparing a microscope slide is called a dry mount, because no water is used. A dry mount can be used for any specimen that won't dry out while you are examining it.

1. Find a small, flat object, such as a lower-case letter "*f*" cut from a newspaper.
2. Place the object in the center of a microscope slide.
3. Hold a cover slip between your thumb and forefinger. Place the edge of the cover slip to one side of the object. Gently lower the cover slip onto the slide so that it covers the object.



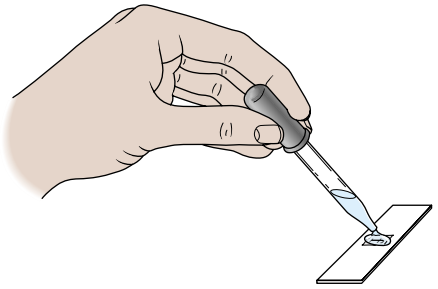
Preparing a Wet Mount

This method of preparing a microscope slide is called a wet mount, because water is used.

A wet mount is used for specimens that can dry out while you are examining them. For instance, when you use a specimen from a living thing, like an onion, the specimen must be very thin. This means that it can lose moisture rapidly: it needs to be kept moist or it will begin to shrivel and become more and more difficult to examine.

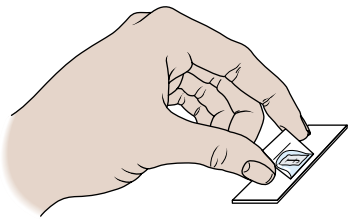
1. Find a small, thin, flat object.
2. Place two drops of water on the slide.

Step 2



3. Place the object in the center of the water drop on the microscope slide.
4. Holding the cover slip with your thumb and forefinger, touch the edge of the surface of the slide at a 45º angle. Gently lower the cover slip, allowing the air to escape.

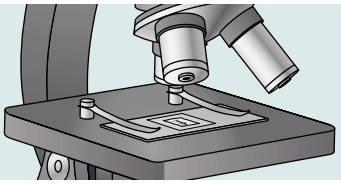
Step 4



Positioning Objects Under the Microscope

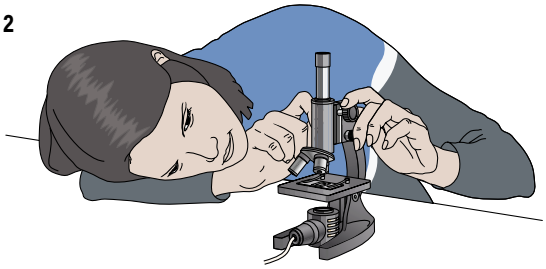
1. Make sure the low-power objective lens is in place on your microscope. Then put either the dry or wet mount slide in the centre of the microscope stage. Use the stage clips to hold the slide in position. Turn on the light source.

Step 1



2. View the microscope stage from the side. Using the coarse-adjustment knob, bring the low-power objective lens and the object as close as possible to one another. Do not allow the lens to touch the cover slip.

Step 2



3. View the object through the eyepiece. Slowly move the coarse-adjustment knob so the objective lens moves away from the slide, to bring the image into focus. Note that the object is facing the “wrong” way and is upside down.
4. Using a compass or a petri dish, draw a circle in your notebook to represent the area you are looking at through the microscope. This area is called the field of view. Look through the microscope and draw what you see. Make the object fill the same amount of area in your diagram as it does in the microscope.
5. While you are looking through the microscope, slowly move the slide away from your body. Note that the object appears to move toward you. Now move the slide to the left. Note that the object appears to move to the right.
6. Rotate the nosepiece to the medium-power objective lens. Use the fine-adjustment knob to bring the object into focus. Note that the object becomes larger.

Never use the coarse-adjustment knob with the medium or high-power objective lenses.

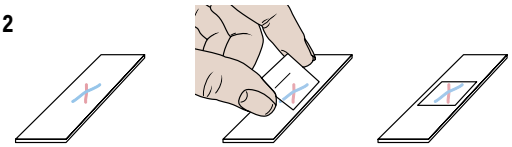
7. Adjust the object so that it is directly in the center of the field of view. Rotate the nosepiece to the high-power objective lens. Use the fine-adjustment knob to focus the image. Note that you see less of the object than you did under medium-power magnification. Also note that the object seems closer to you.

Investigating Depth of Field

The depth of field is the amount of an image that is in sharp focus when it is viewed under a microscope.

1. Cut two pieces of thread of different colors.
2. Make a temporary dry mount by placing one thread over the other in the form of an X in the center of a microscope slide. Cover the threads with a cover slip.

Step 2



3. Place the slide on the microscope stage and turn on the light.
4. Position the low-power objective lens close to, but not touching, the slide.
5. View the crossed threads through the ocular lens. Slowly rotate the coarse-adjustment knob until the threads come into focus.
6. Rotate the nosepiece to the medium-power objective lens. Focus on the upper thread by using the fine-adjustment knob. You will probably notice that you cannot focus on the lower thread at the same time. The depth of the object that is in focus at any one time represents the depth of field.
7. Repeat step 6 for the high-power objective lens. The stronger the magnification, the shallower the depth of field.

Determining the Field of View

The field of view is the circle of light seen through the microscope. It is the area of the slide that you can observe.

1. With the low-power objective lens in place, put a transparent ruler on the stage. Position the millimeter marks on the ruler immediately below the objective lens.
2. Using the coarse-adjustment knob, focus on the marks on the ruler.
3. Move the ruler so that one of the millimeter markings is just at the edge of the field of view. Note the diameter of the field of view in millimeters, under the low-power objective lens.

Step 3

4. Using the same procedure, measure the field of view for the medium-power objective lens.
5. Most high-power lenses provide a field of view that is less than one millimeter in diameter, so it cannot be measured with a ruler. The following steps can be followed to calculate the field of view of the high-power lens.

   Calculate the ratio of the magnification of the high-power objective lens to that of the low-power objective lens.

$$\text{Ratio} = \frac{\text{magnification of high-power lens}}{\text{magnification of low-power lens}}$$

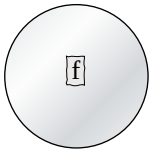
Use the ratio to determine the field of diameter (diameter of the field of view) under high-power magnification.

$$\text{Field diameter (high power)} = \frac{\text{field diameter (low power)}}{\text{ratio}}$$

Estimating Size

1. Measure the field of view, in millimeters, as shown above.
2. Remove the ruler and replace it with the object under investigation.

Step 2



3. Estimate the number of times the object could fit across the field of view.
4. Calculate the width of the object:

$$\text{width of object} = \frac{\text{width of field of view}}{\text{number of objects across field}}$$

Remember to include units.