exercise 19

The Microscope

With the invention of the microscope, biologists gained a valuable tool to observe and study structures (like cells) that are too small to be seen by the unaided eye. The information gained helped in establishing many of the theories basic to the understanding of biological sciences. This exercise will familiarize you with the workhorse of microscopes—the compound microscope—and provide you with the necessary instructions for its proper use.

Care and Structure of the Compound Microscope

The **compound microscope** is a precision instrument and should always be handled with care. At all times you must observe the following rules for its transport, cleaning, use, and storage:

- When transporting the microscope, hold it in an upright position with one hand on its arm and the other supporting its base. Avoid jarring the instrument when setting it down.
- Use only special grit-free lens paper to clean the lenses. Clean all lenses before and after use.
- Always begin the focusing process with the lowest-power objective lens in position, changing to the higher-power lenses as necessary.
- Use the coarse adjustment knob only with the lowest power lens.
- Always use a coverslip with temporary (wet mount) preparations.
- Before putting the microscope in the storage cabinet, remove the slide from the stage, rotate the lowest-power objective lens into position, and replace the dust cover or return the microscope to the appropriate storage area.
- Never remove any parts from the microscope; inform your instructor of any mechanical problems that arise.

Objectives

1. To identify the parts of the microscope and list the function of each.
2. To describe and demonstrate the proper techniques for care of the microscope.
3. To define *total magnification* and *resolution.*
4. To demonstrate proper focusing technique.
5. To define *parfocal, field,* and *depth of field.*
6. To estimate the size of objects in a field.

Materials

- ❑ Compound microscope
- ❑ Stereomicroscope
- ❑ Millimeter ruler
- ❑ Prepared slides of the letter e or newsprint
- ❑ Immersion oil
- ❑ Lens paper
- ❑ Prepared slide of grid ruled in millimeters (grid slide)
- ❑ Prepared slide of 3 crossed colored threads
- ❑ Clean microscope slide and coverslip
- ❑ Toothpicks (flat-tipped)
- ❑ Physiologic saline in a dropper bottle
- ❑ Methylene blue stain (dilute) in a dropper bottle
- ❑ Filter paper or paper towels
- ❑ Prepared slide of cheek epithelial cells
- ❑ Coins
- ❑ Beaker containing fresh 10% household bleach solution for wet mount disposal
- ❑ Disposable autoclave bag

Note to the Instructor: The slides and coverslips used for viewing cheek cells are to be soaked for 2 hours (or longer) in 10% bleach solution and then drained. The slides and disposable autoclave bag (containing coverslips, lens paper, and used toothpicks) are to be autoclaved for 15 min at 121°C and 15 pounds pressure to ensure sterility. After autoclaving, the disposable autoclave bag may be discarded in any disposal facility and the slides and glassware washed with laboratory detergent and reprepared for use. These instructions apply as well to any bloodstained glassware or disposable items used in other experimental procedures.

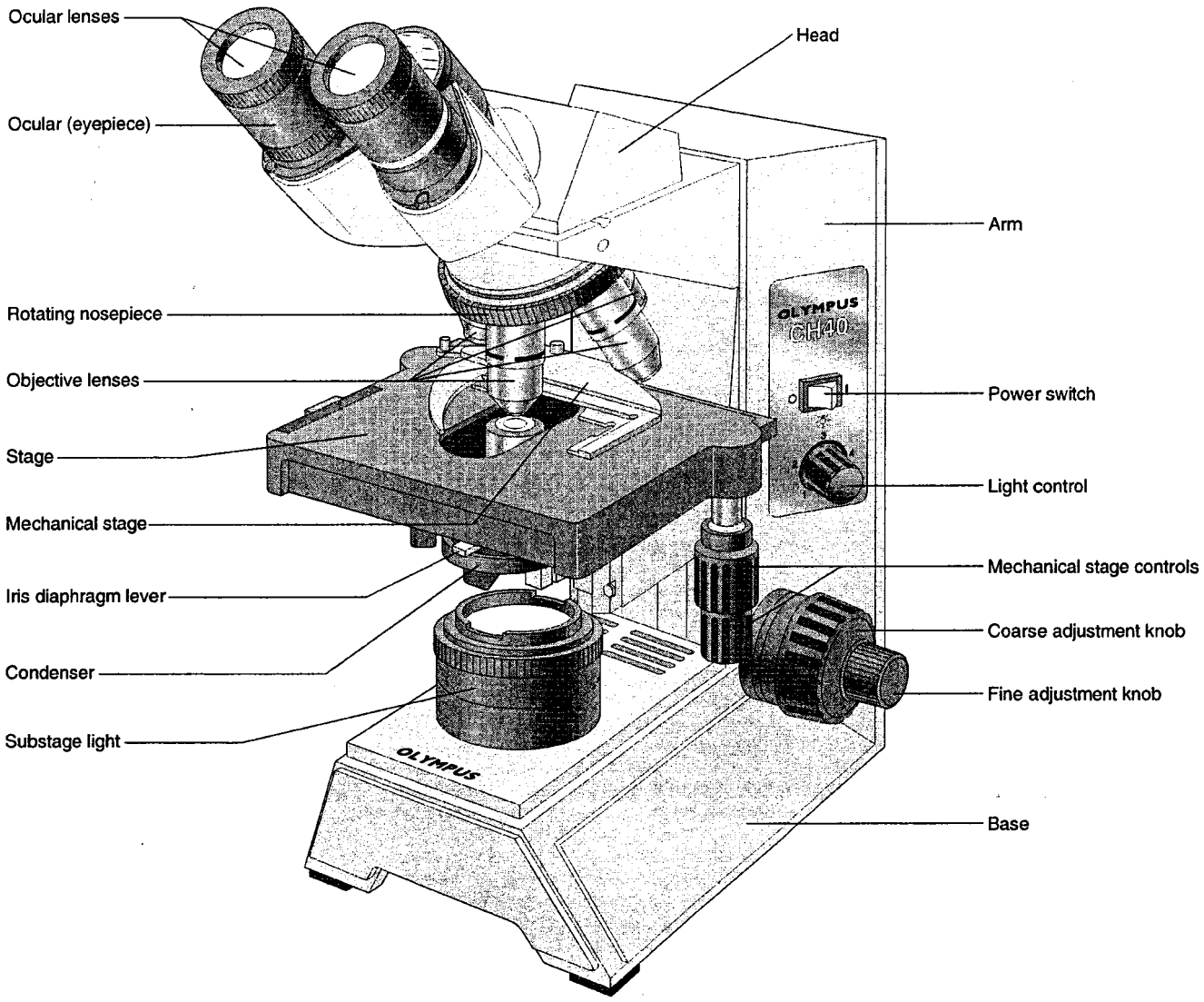


Figure 1 Compound microscope and its parts.

Activity 1:
Identifying the Parts of a Microscope

1. Obtain a microscope and bring it to the laboratory bench. (Use the proper transport technique!)

- Record the number of your microscope in the summary chart.

Compare your microscope with the illustration in Figure 1 and identify the following microscope parts:

Base: Supports the microscope. (Note: Some microscopes are provided with an inclination joint, which allows the instrument to be tilted backward for viewing dry preparations.)

Substage light (or *mirror*): Located in the base. In microscopes with a substage light source, the light passes directly upward through the microscope. If a mirror is used, light must be reflected from a separate free-standing lamp.

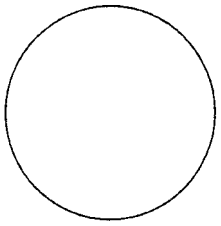
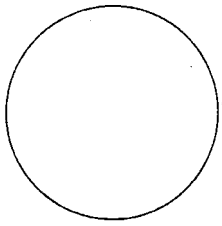
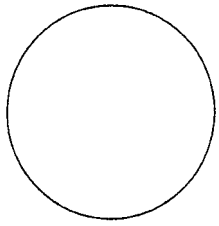
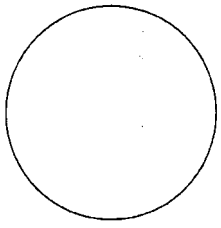
Stage: The platform the slide rests on while being viewed. The stage has a hole in it to permit light to pass through both it and the specimen. Some microscopes have a stage equipped with *spring clips;* others have a clamp-type *mechanical stage* as shown in Figure 1. Both hold the slide in position for viewing; in addition, the mechanical stage permits precise movement of the specimen.

Condenser: Concentrates the light on the specimen. The condenser may have a height-adjustment knob that raises and lowers the condenser to vary light delivery. Generally, the best position for the condenser is close to the inferior surface of the stage.

Iris diaphragm lever: Arm attached to the condenser that regulates the amount of light passing through the condenser. The iris diaphragm permits the best possible contrast when viewing the specimen.

Coarse adjustment knob: Used to focus on the specimen.

Summary Chart for Microscope # _____

	Scanning	Low power	High power	Oil immersion
Magnification of objective lens	×	×	×	×
Total magnification	×	×	×	×
Working distance	mm	mm	mm	mm
Detail observed Letter *e*				
Field size (diameter)	mm μm	mm μm	mm μm	mm μm

Fine adjustment knob: Used for precise focusing once coarse focusing has been completed.

Head or **body tube:** Supports the objective lens system (which is mounted on a movable nosepiece), and the ocular lens or lenses.

Arm: Vertical portion of the microscope connecting the base and head.

Ocular (or *eyepiece*): Depending on the microscope, there are one or two lenses at the superior end of the head or body tube. Observations are made through the ocular(s). An ocular lens has a magnification of 10×. (It increases the apparent size of the object by ten times or ten diameters). If your microscope has a **pointer** (used to indicate a specific area of the viewed specimen), it is attached to one ocular and can be positioned by rotating the ocular lens.

Nosepiece: Generally carries three or four objective lenses and permits sequential positioning of these lenses over the light beam passing through the hole in the stage. Use the nosepiece to change the objective lenses. Do not directly grab the lenses.

Objective lenses: Adjustable lens system that permits the use of a **scanning lens,** a **low-power lens,** a **high-power lens,** or an **oil immersion lens.** The objective lenses have different magnifying and resolving powers.

2. Examine the objective lenses carefully; note their relative lengths and the numbers inscribed on their sides. On many microscopes, the scanning lens, with a magnification between 4× and 5×, is the shortest lens. If there is no scanning lens, the low-power objective lens is the shortest and typically has a magnification of 10×. The high-power objective lens is of intermediate length and has a magnification range from 40× to 50×, depending on the microscope. The oil immersion objective lens is usually the longest of the objective lenses and has a magnifying power of 95× to 100×. Some microscopes lack the oil immersion lens.

- Record the magnification of each objective lens of your microscope in the first row of the chart above. Also, cross out the column relating to a lens that your microscope does not have.

3. Rotate the lowest power objective lens until it clicks into position, and turn the coarse adjustment knob about 180 degrees. Notice how far the stage (or objective lens) travels during this adjustment. Move the fine adjustment knob 180 degrees, noting again the distance that the stage (or the objective lens) moves. ■

Magnification and Resolution

The microscope is an instrument of magnification. In the compound microscope, magnification is achieved through the interplay of two lenses—the ocular lens and the objective lens. The objective lens magnifies the specimen to produce a **real image** that is projected to the ocular. This real image is magnified by the ocular lens to produce the **virtual image** seen by your eye (Figure 2).

The **total magnification** (TM) of any specimen being viewed is equal to the power of the ocular lens multiplied by the power of the objective lens used. For example, if the ocular lens magnifies 10× and the objective lens being used magnifies 45×, the total magnification is 450× (10 × 45).

- Determine the total magnification you may achieve with each of the objectives on your microscope and record the figures on the second row of the chart.

The compound light microscope has certain limitations. Although the level of magnification is almost limitless, the

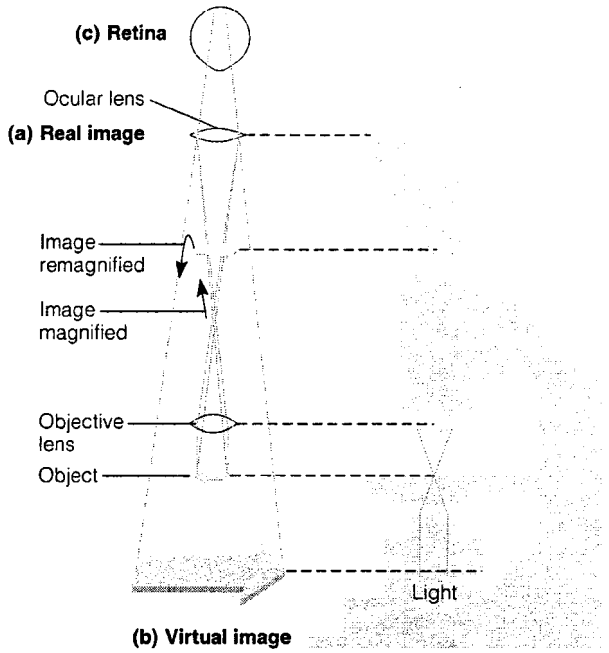


Figure 2 Image formation in light microscopy. (a) Light passing through the objective lens forms a real image. **(b)** The real image serves as the object for the ocular lens, which remagnifies the image and forms the virtual image. **(c)** The virtual image passes through the lens of the eye and is focused on the retina.

resolution (or resolving power), that is, the ability to discriminate two close objects as separate, is not. The human eye can resolve objects about 100 μm apart, but 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 more 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. Thus, you will probably find it necessary to increase the light intensity at the higher magnifications.

Activity 2: Viewing Objects Through the Microscope

1. Obtain a millimeter ruler, a prepared slide of the letter *e* or newsprint, a dropper bottle of immersion oil, and some lens paper. Adjust the condenser to its highest position and switch on the light source of your microscope. (If the light source is not built into the base, use the curved surface of the mirror to reflect the light up into the microscope.)

2. Secure the slide on the stage so that you can read the slide label and the letter *e* is centered over the light beam passing through the stage. If you are using a microscope with spring clips, make sure the slide is secured at both ends. If your microscope has a mechanical stage, open the jaws of its slide retainer (holder) by using the control lever (typically) located at the rear left corner of the mechanical stage. Insert the slide squarely within the confines of the slide retainer. Check to see that the slide is resting on the stage (and not on the mechanical stage frame) before releasing the control lever.

3. With your lowest power (scanning or low-power) objective lens in position over the stage, use the coarse adjustment knob to bring the objective lens and stage as close together as possible.

4. Look through the ocular lens and adjust the light for comfort using the iris diaphragm. Now use the coarse adjustment knob to focus slowly away from the *e* until it is as clearly focused as possible. Complete the focusing with the fine adjustment knob.

5. Sketch the letter *e* in the circle on the summary chart just as it appears in the **field** (the area you see through the microscope).

What is the total magnification? _____ ×

How far is the bottom of the objective lens from the specimen? In other words, what is the **working distance**? Use a millimeter ruler to make this measurement summary.

_____ mm

Record the TM detail observed and the working distance in the summary chart.

How has the apparent orientation of the *e* changed top to bottom, right to left, and so on?

_____

_____

6. Move the slide slowly away from you on the stage as you view it through the ocular lens. In what direction does the image move?

_____

Move the slide to the left. In what direction does the image move?

_____

At first this change in orientation may confuse you, but with practice you will learn to move the slide in the desired direction with no problem.

7. Today most good laboratory microscopes are **parfocal**; that is, the slide should be in focus (or nearly so) at the higher magnifications once you have properly focused. *Without touching the focusing knobs*, increase the magnification by rotating the next higher magnification lens (low-power or

high-power) into position over the stage. Make sure it clicks into position. Using the fine adjustment only, sharpen the focus.* Note the decrease in working distance. As you can see, focusing with the coarse adjustment knob could drive the objective lens through the slide, breaking the slide and possibly damaging the lens. Sketch the letter *e* in the summary chart. What new details become clear?

_____

_____

What is the total magnification now? _____ ×

Record the TM, detail observed, and working distance in the summary chart.

As best you can, measure the distance between the objective and the slide (the working distance) and record it on the chart.

Is the image larger or smaller? _____

Approximately how much of the letter *e* is visible now?

_____

Is the field larger or smaller? _____

Why is it necessary to center your object (or the portion of the slide you wish to view) before changing to a higher power?

_____

Move the iris diaphragm lever while observing the field. What happens?

_____

Is it more desirable to increase *or* decrease the light when changing to a higher magnification?

_____ Why? _____

_____

8. If you have just been using the low-power objective, repeat the steps given in direction 7 using the high-power objective lens.

Record the TM, detail observed, and working distance in the summary chart.

9. Without touching the focusing knob, rotate the high-power lens out of position so that the area of the slide over the

*If you are unable to focus with a new lens, your microscope is not parfocal. Do not try to force the lens into position. Consult your instructor.

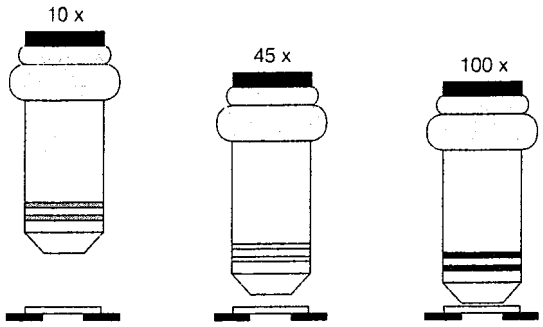


Figure 3 Relative working distances of the 10×, 45×, and 100× objectives.

opening in the stage is unobstructed. Place a drop of immersion oil over the *e* on the slide and rotate the oil immersion lens into position. Set the condenser at its highest point (closest to the stage), and open the diaphragm fully. Adjust the fine focus and fine-tune the light for the best possible resolution.

Note: If for some reason the specimen does not come into view after adjusting the fine focus, do not go back to the 40× lens to recenter. You do not want oil from the oil immersion lens to cloud the 40× lens. Turn the revolving nosepiece in the other direction to the low-power lens and recenter and refocus the object. Then move the immersion lens back into position, again avoiding the 40× lens.

Is the field again decreased in size? _____

What is the total magnification with the oil immersion lens?

_____ ×

Is the working distance less *or* greater than it was when the high-power lens was focused?

_____

Compare your observations on the relative working distances of the objective lenses with the illustration in Figure 3. Explain why it is desirable to begin the focusing process in the lowest power.

_____

_____

_____

10. Rotate the oil immersion lens slightly to the side and remove the slide. Clean the oil immersion lens carefully with lens paper and then clean the slide in the same manner with a fresh piece of lens paper. ■

Table 1 Comparison of Metric Units of Length*

Metric unit	Abbreviation	Equivalent
Meter	m	(about 39.3 in.)
Centimeter	cm	10^{-2} m
Millimeter	mm	10^{-3} m
Micrometer (or micron)	μm (μ)	10^{-6} m
Nanometer (or millimicrometer or millimicron)	nm (mμ)	10^{-9} m
Angstrom	Å	10^{-10} m

*Refer to the "Getting Started" exercise for tips on metric conversions.

Size of the Microscope Field

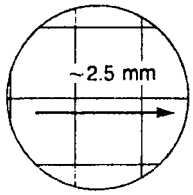
By this time you should know that the size of the microscope field decreases with increasing magnification. For future microscope work, it will be useful to determine the diameter of each of the microscope fields. This information will allow you to make a fairly accurate estimate of the size of the objects you view in any field. For example, if you have calculated the field diameter to be 4 mm and the object being observed extends across half this diameter, you can estimate the length of the object to be approximately 2 mm.

Microscopic specimens are usually measured in micrometers and millimeters, both units of the metric system. You can get an idea of the relationship and meaning of these units from Table 1.

Activity 3:
Determining the Size of the Microscope Field

1. Obtain a grid slide (a slide prepared with graph paper ruled in millimeters). Each of the squares in the grid is 1 mm on each side. Use your lowest power objective to bring the grid lines into focus.

2. Move the slide so that one grid line touches the edge of the field on one side, and then count the number of squares you can see across the diameter of the field. If you can see only part of a square, as in the accompanying diagram, estimate the part of a millimeter that the partial square represents.



Record this figure in the appropriate space marked "field size" on the summary chart. (If you have been using the scanning lens, repeat the procedure with the low-power objective lens.)

Complete the chart by computing the approximate diameter of the high-power and oil immersion fields. The general formula for calculating the unknown field diameter is:

Diameter of field $A\times$ total magnification of field A = diameter of field $B\times$ total magnification of field B

where A represents the known or measured field and B represents the unknown field.

This can be simplified to

$$\text{Diameter of field } B = \frac{\text{diameter of field } A\times \text{ total magnification of field } A}{\text{total magnification of field } B}$$

For example, if the diameter of the low-power field (field A) is 2 mm and the total magnification is 50×, you would compute the diameter of the high-power field (field B) with a total magnification of 100× as follows:

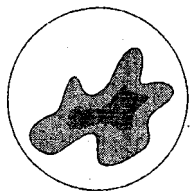
Field diameter B = (2 mm × 50)/100
Field diameter B = 1 mm

3. Estimate the length (longest dimension) of the following microscopic objects. *Base your calculations on the field sizes you have determined for your microscope.*

a. Object seen in low-power field:

approximate length:

_____ mm

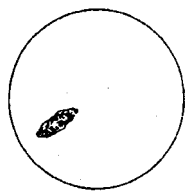


b. Object seen in high-power field:

approximate length:

_____ mm

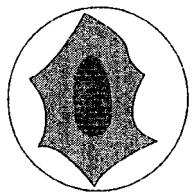
or _____ μm



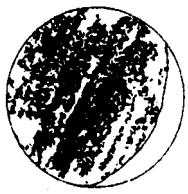
c. ~~Object seen in oil immersion field:~~

~~approximate length:~~

_____ ~~μm~~



4. ~~If an object viewed with the oil immersion lens looked as it does in the field depicted just below, could you determine its approximate size from this view?~~



If not, then how could you determine it? change

I have to have power to see a better view

■

Perceiving Depth

Any microscopic specimen has depth as well as length and width; it is rare indeed to view a tissue slide with just one layer of cells. Normally you can see two or three cell thicknesses. Therefore, it is important to learn how to determine relative depth with your microscope. In microscope work the **depth of field** (the depth of the specimen clearly in focus) is greater at lower magnifications.

Activity 4:
Perceiving Depth

1. Obtain a slide with colored crossed threads. Focusing at low magnification, locate the point where the three threads cross each other.

2. Use the iris diaphragm lever to greatly reduce the light, thus increasing the contrast. Focus down with the coarse adjustment until the threads are out of focus, then slowly focus upward again, noting which thread comes into clear focus first. (You will see two or even all three threads, so you must be very careful in determining which one first comes into clear focus.) Observe: As you rotate the adjustment knob forward (away from you), does the stage rise or fall? If the stage rises, then the first clearly focused thread is the top one; the last clearly focused thread is the bottom one.

If the stage falls, how is the order affected? _____

_____

Record your observations, relative to which thread is uppermost, middle, or lowest:

Top thread _____

Middle thread _____

Bottom thread _____ ■

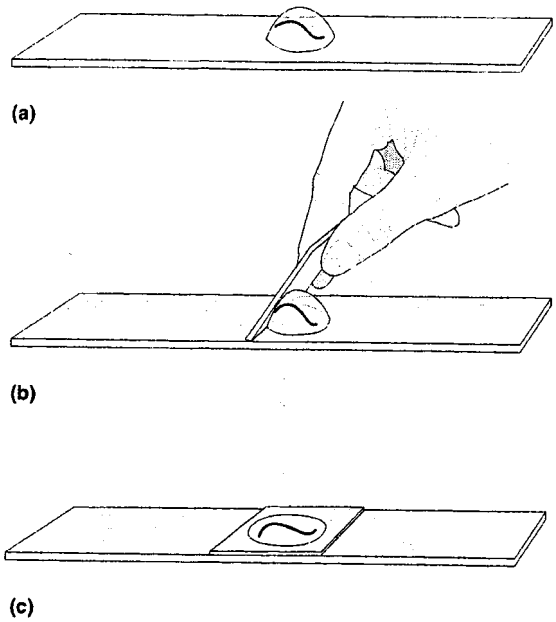


Figure 4 Procedure for preparation of a wet mount. (a) The object is placed in a drop of water (or saline) on a clean slide, **(b)** a coverslip is held at a 45° angle with the fingertips, and **(c)** it is lowered carefully over the water and the object.

Viewing Cells Under the Microscope

There are various ways to prepare cells for viewing under a microscope. Cells and tissues can look very different with different stains and preparation techniques. One method of preparation is to mix the cells in physiologic saline (called a wet mount) and stain them with methylene blue stain.

If you are not instructed to prepare your own wet mount, obtain a prepared slide of epithelial cells to make the observations in step 10 of Activity 5.

Activity 5:
Preparing and Observing a Wet Mount

1. Obtain the following: a clean microscope slide and coverslip, two flat-tipped toothpicks, a dropper bottle of physiologic saline, a dropper bottle of methylene blue stain, and filter paper (or paper towels). Handle only your own slides through-out the procedure.

2. Place a drop of physiologic saline in the center of the slide. Using the flat end of the toothpick, *gently* scrape the inner lining of your cheek. Agitate the end of the toothpick containing the cheek scrapings in the drop of saline (Figure 4a).

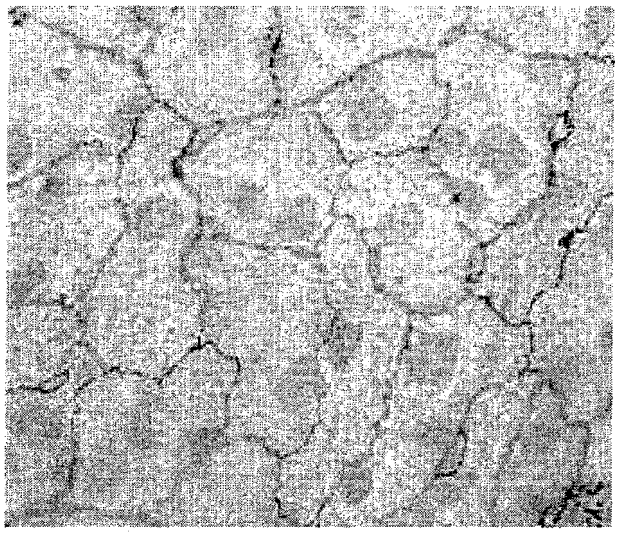


Figure 5 Epithelial cells of the cheek cavity (surface view, 488×).

⚠ *Immediately* discard the used toothpick in the disposable autoclave bag provided at the supplies area.

3. Add a tiny drop of the methylene blue stain to the preparation. (These epithelial cells are nearly transparent and thus difficult to see without the stain, which colors the nuclei of the cells and makes them look much darker than the cytoplasm.) Stir again and then dispose of the toothpick as described above.

4. Hold the coverslip with your fingertips so that its bottom edge touches one side of the fluid drop (Figure 4b), then *carefully* lower the coverslip onto the preparation (Figure 4c). *Do not just drop the coverslip,* or you will trap large air bubbles under it, which will obscure the cells. *A coverslip should always be used with a wet mount* to prevent soiling the lens if you should misfocus.

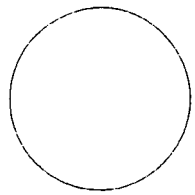
5. Examine your preparation carefully. The coverslip should be closely apposed to the slide. If there is excess fluid around its edges, you will need to remove it. Obtain a piece of filter paper, fold it in half, and use the folded edge to absorb the excess fluid. (You may use a twist of paper towel as an alternative.)

⚠ Before continuing, discard the filter paper in the disposable autoclave bag.

6. Place the slide on the stage and locate the cells in low power. You will probably want to dim the light with the iris diaphragm to provide more contrast for viewing the lightly stained cells. Furthermore, a wet mount will dry out quickly in bright light because a bright light source is hot.

7. Cheek epithelial cells are very thin, six-sided cells. In the cheek, they provide a smooth, tilelike lining, as shown in Figure 5.

8. Make a sketch of the epithelial cells that you observe.



Use information on your summary chart to estimate the diameter of cheek epithelial cells.

_____ mm

Why do *your* cheek cells look different than those illustrated in Figure 5? (Hint: what did you have to *do* to your cheek to obtain them?)

_____

_____

⚠ 9. When you complete your observations of the wet mount, dispose of your wet mount preparation in the beaker of bleach solution, and put the coverslips in an autoclave bag.

10. Obtain a prepared slide of cheek epithelial cells and view them under the microscope.

Estimate the diameter of one of these cheek epithelial cells using information from the summary chart.

_____ mm

Why are these cells more similar to those seen in Figure 5 and easier to measure than those of the wet mount?

_____

_____

11. Before leaving the laboratory, make sure all other materials are properly discarded or returned to the appropriate laboratory station. Clean the microscope lenses and put the dust cover on the microscope before you return it to the storage cabinet. ■

The Stereomicroscope (Dissecting Microscope)

Occasionally biologists look at specimens too large to observe with the compound microscope but too small to observe easily with the unaided eye. The stereomicroscope, sometimes called a dissecting microscope, can be helpful in these situations. It works basically like a large magnifying glass.

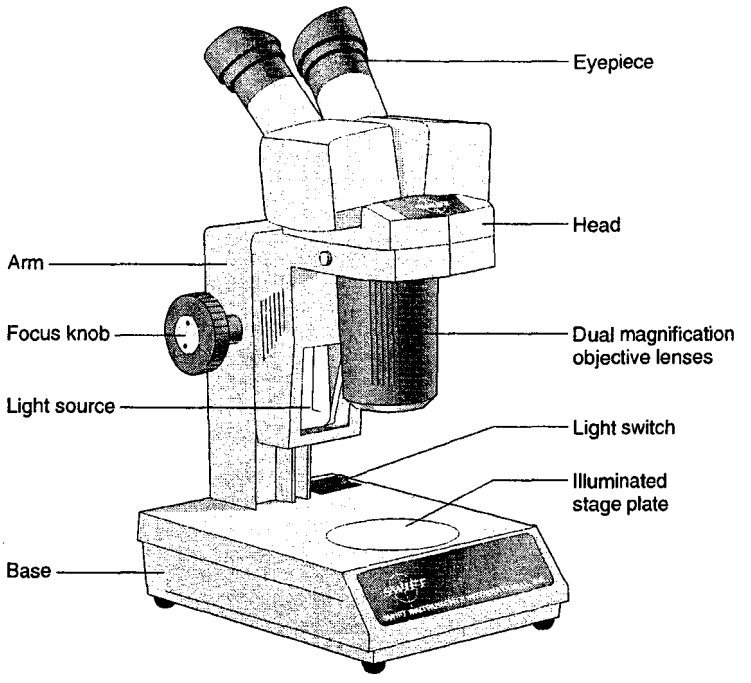


Figure 6 Stereomicroscope and its parts.

Activity 6:
Identifying the Parts of a Stereomicroscope

1. Obtain the microscope and put it on the lab bench.

2. Using what you have learned about the compound microscope and Figure 6, identify the following parts:

Adjustment focus knob: Used to focus on the specimen

Arm: Connects the base to the head of the microscope

Base: Supports the microscope

Eyepieces or **oculars:** Magnify the images from the objective lenses

Head or **body tube:** Supports the ocular and objective lenses

Light control: Switch that allows you to choose transmitted light, reflected light, or both

Objective lenses: Adjustable lens system used to increase or decrease magnification

Stage: Platform that holds the specimen

Substage light: Located in the base; sends light up through the specimen; the source of transmitted light

Upper light source: Located above the stage; directs light onto the surface of the specimen; the source of reflected light ■

Activity 7:
Using the Stereomicroscope

1. Place a coin on the stage of the microscope. Turn on the light source, and adjust the oculars until you can see a single image of the specimen.

2. Focus the microscope on the coin.

3. Experiment with transmitted and reflected light until you get the best image. Which works best in this situation, the transmitted or reflected light?

_____

4. Increase and decrease the magnification to familiarize yourself with the controls.

5. Each coin has a small letter indicating where it was minted. See if you can determine the initial of the mint that produced your coin.

Where was your coin produced? _____

What is the total magnification you used? _____ ■

ILLUSTRATIONS

1, 2–4, 6: Precision Graphics.

PHOTOGRAPHS

5: © Benjamin/Cummings Publishing. Photo by Victor Eroschencko.