EXERCISE

3

The Microscope

Objectives

- ☐ Identify the parts of the microscope, and list the function of each.
- ☐ Describe and demonstrate the proper techniques for care of the microscope.
- ☐ Demonstrate proper focusing technique.
- ☐ Define *total magnification, resolution, parfocal, field, depth of field,* and *working distance*.
- ☐ Measure the field diameter for one objective lens, calculate it for all the other objective lenses, and estimate the size of objects in each field.
- ☐ Discuss the general relationships between magnification, working distance, and field diameter.

Materials*

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

Pre-Lab Quiz

1. The microscope slide rests on the _____ while being viewed.
   a. base **c.** iris
   b. condenser **d.** stage
2. Your lab microscope is *parfocal*. What does this mean?
   a. The specimen is clearly in focus at this depth.
   b. The slide should be almost in focus when changing to higher magnifications.
   c. You can easily discriminate two close objects as separate.
3. If the ocular lens magnifies a specimen 10×, and the objective lens used magnifies the specimen 35×, what is the total magnification being used to observe the specimen? _____
4. How do you clean the lenses of your microscope?
   a. with a paper towel
   b. with soap and water
   c. with special lens paper and cleaner
5. Circle True or False. You should always begin observation of specimens with the oil immersion lens.

With the invention of the microscope, biologists gained a valuable tool to observe and study structures, such as cells, that are too small to be seen by the unaided eye. The knowledge they acquired helped establish many of the theories basic to the 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.

* ***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 prepared for use. These instructions apply as well to any bloodstained glassware or disposable items used in other experimental procedures.*

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. Do not swing the instrument during its transport or jar the instrument when setting it down.
- Use only special grit-free lens paper to clean the lenses. Use a circular motion to wipe the lenses, and 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 objective lens.
- Always use a coverslip with 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, wrap the cord neatly around the base, 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.

Activity 1

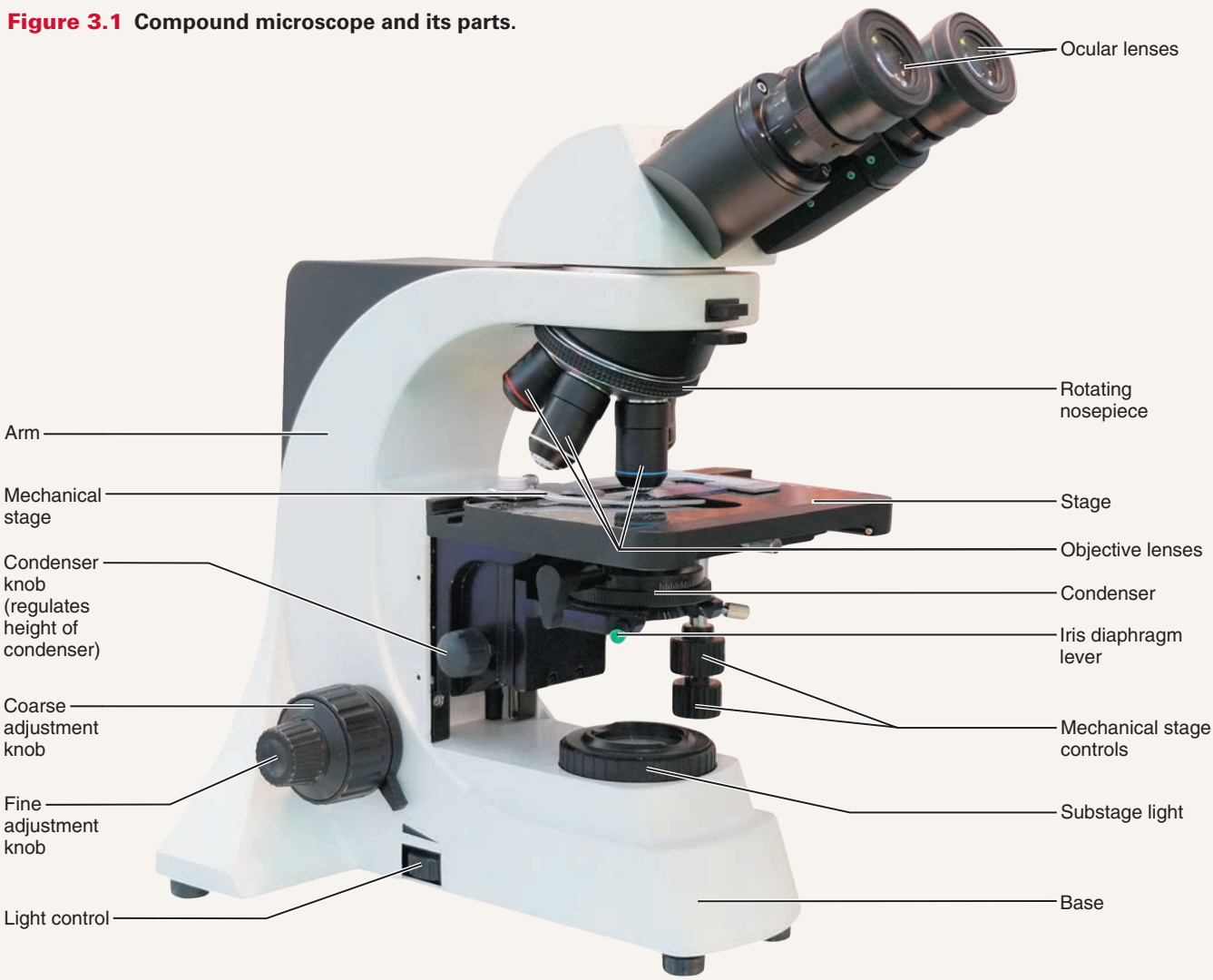
Identifying the Parts of a Microscope

1. Using the proper transport technique, obtain a microscope and bring it to the laboratory bench.

☐ Record the number of your microscope in the **Summary chart** (p. 31).

Compare your microscope with **Figure 3.1**, and identify the microscope parts described in **Table 3.1** on p. 30.

Figure 3.1 Compound microscope and its parts.



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. The low-power objective lens 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 Summary chart (p. 31). Also, cross out any column relating to a lens that your microscope does not have. Plan on using the same microscope for all microscopic studies.

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. With 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** that your eye sees (**Figure 3.2**).

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× (or 10 × 45).

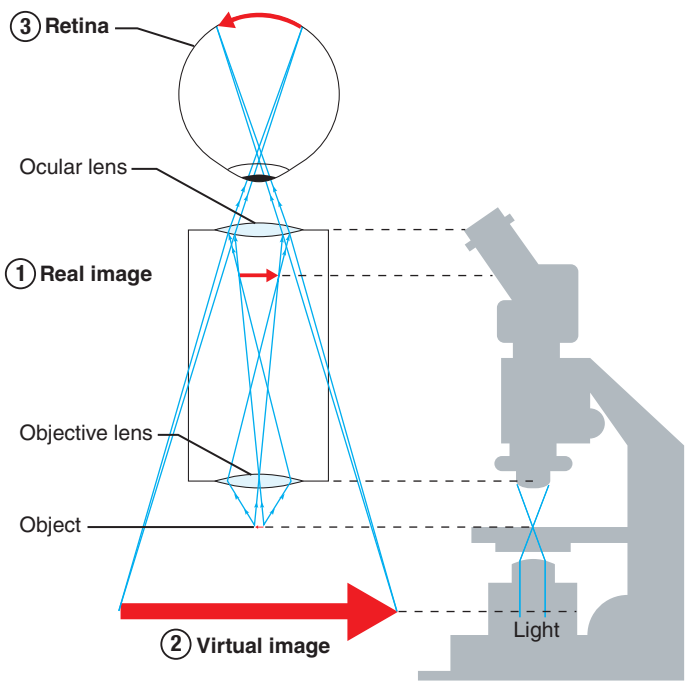


Figure 3.2 Image formation in light microscopy. **Step** ① The objective lens magnifies the object, forming the real image. **Step** ② The ocular lens magnifies the real image, forming the virtual image. **Step** ③ The virtual image passes through the lens of the eye and is focused on the retina.

• Determine the total magnification you may achieve with each of the objectives on your microscope, and record the figures on the third row of the Summary chart.

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

Prepare for lab: Watch the Pre-Lab Video
MasteringA&P®>Study Area>Pre-Lab Videos

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.

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. On the mechanical stage of your microscope, open the jaws of its slide 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 holder.

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.

Text continues on next page. →

Table 3.1 Parts of the Microscope

Microscope part	Description and function
Base	The bottom of the microscope. Provides a sturdy flat surface to support and steady the microscope.
Substage light	Located in the base. The light from the lamp passes directly upward through the microscope.
Light control knob	Located on the base or arm. This dial allows you to adjust the intensity of the light passing through the specimen.
Stage	The platform that the slide rests on while being viewed. The stage has a hole in it to allow light to pass through the stage and through the specimen.
Mechanical stage	Holds the slide in position for viewing and has two adjustable knobs that control the precise movement of the slide.
Condenser	Small nonmagnifying lens located beneath the stage that concentrates the light on the specimen. The condenser may have a knob that raises and lowers the condenser to vary the light delivery. Generally, the best position is close to the inferior surface of the stage.
Iris diaphragm lever	The iris diaphragm is a shutter within the condenser that can be controlled by a lever to adjust the amount of light passing through the condenser. The lever can be moved to close the diaphragm and improve contrast. If your field of view is too dark, you can open the diaphragm to let in more light.
Coarse adjustment knob	This knob allows you to make large adjustments to the height of the stage to initially focus your specimen.
Fine adjustment knob	This knob is used for precise focusing once the initial coarse focusing has been completed.
Head	Attaches to the nosepiece to support the objective lens system. It also provides for attachment of the eyepieces which house the ocular lenses.
Arm	Vertical portion of the microscope that connects the base and the head.
Nosepiece	Rotating mechanism connected to the head. Generally, it carries three or four objective lenses and permits positioning of these lenses over the hole in the stage.
Objective lenses	These lenses are attached to the nosepiece. Usually, a compound microscope has four objective lenses: scanning (4×), low-power (10×), high-power (40×), and oil immersion (100×) lenses. Typical magnifying powers for the objectives are listed in parentheses.
Ocular lens(es)	Binocular microscopes will have two lenses located in the eyepieces at the superior end of the head. Most ocular lenses have a magnification power of 10×. Some microscopes will have a pointer and/or reticle (micrometer), which can be positioned by rotating the ocular lens.

4. Look through the ocular lens and adjust the light for comfort using the iris diaphragm lever. 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 (p. 31) just as it appears in the **field**—the area you see through the microscope.

How far is the bottom of the objective lens from the surface of the slide? In other words, what is the **working distance**? (See Figure 3.3.) Use a millimeter ruler to make this measurement.

Record 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?

_____

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 at the lower magnification. *Without touching the focusing knobs,* increase the magnification by rotating the next higher magnification lens into position over the stage. Make sure it clicks into position. Using the fine adjustment only, sharpen the focus. 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. 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?

_____

_____

As best you can, measure the distance between the objective and the slide.

Record the working distance in the Summary 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 better to increase *or* to 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. What new details become clear?

_____

_____

Record the 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 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. Sketch the letter* e *in the Summary chart. What new details become clear?*

_____

_____

Is the field again decreased in size? _____

As best you can, estimate the working distance, and record it in the Summary chart. 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

Summary Chart for Microscope #____

	Scanning	Low power	High power	Oil immersion
Magnification of objective lens	_____ ×	_____ ×	_____ ×	_____ ×
Magnification of ocular lens	10 ×	10 ×	10 ×	10 ×
Total magnification	_____ ×	_____ ×	_____ ×	_____ ×
Working distance	_____ mm	_____ mm	_____ mm	_____ mm
Detail observed letter *e*				
Field diameter	_____ mm _____ µm	_____ mm _____ µm	_____ mm _____ µm	_____ mm _____ µm

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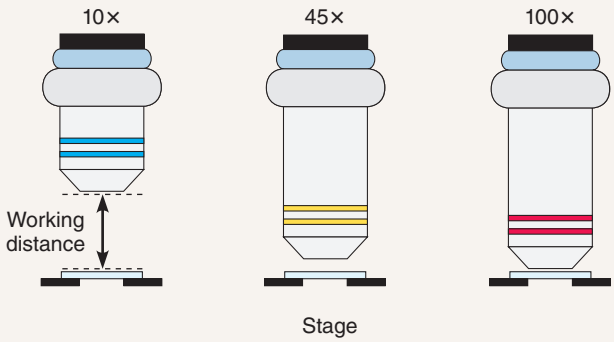


Figure 3.3 Relative working distances of the 10×, 45×, and 100× objectives.

Figure 3.3. Explain why it is desirable to begin the focusing process at 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.

The Microscope Field

The microscope field decreases with increasing magnification. Measuring the diameter of each of the microscope fields will allow you to estimate 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 that the length of the object is 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 3.2**. A more detailed treatment appears in the appendix.

Table 3.2 Comparison of Metric Units of Length

Metric unit	Abbreviation	Equivalent
Meter	m	(about 39.37 in.)
Centimeter	cm	10^{-2} m
Millimeter	mm	10^{-3} m
Micrometer (or micron)	μm (μ)	10^{-6} m
Nanometer	nm (mμ)	10^{-9} m

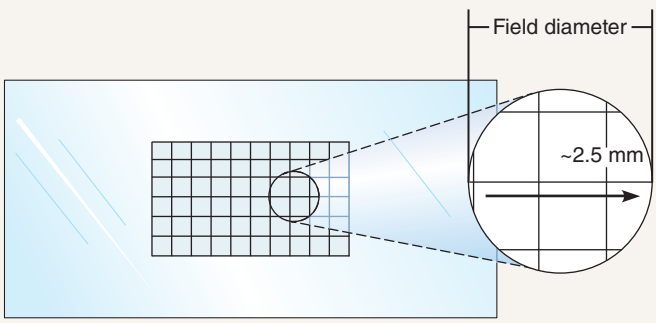
(Refer to the Getting Started exercise on MasteringA&P for tips on metric conversions.)

Activity 3

Estimating the Diameter of the Microscope Field

1. Obtain a grid slide, which is 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 diameter" on the Summary chart (p. 31). (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* × total magnification of field *A* = diameter of field *B* × 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:

$$\text{Field diameter } B = (2 \text{ mm} \times 50)/100$$

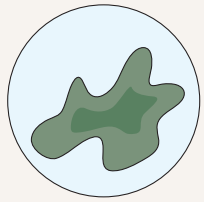
$$\text{Field diameter } B = 1 \text{ mm}$$

3. Estimate the length (longest dimension) of the following drawings of microscopic objects. *Base your calculations on the field sizes you have determined for your microscope and the approximate percentage of the diameter that the object occupies.*

Object seen in low-power field:

approximate length:

_____ mm

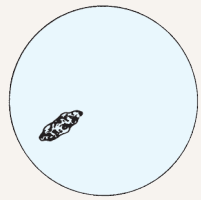


Object seen in high-power field:

approximate length:

_____ mm

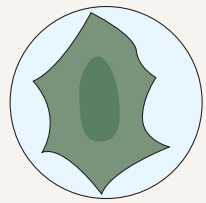
or _____ µm



Object seen in oil immersion field:

approximate length:

_____ µm



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 thickness of the plane that is clearly in focus) is greater at lower magnifications. As magnification increases, depth of field decreases.

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. 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 descends, how is the order affected?_____

_____

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

Top thread _____

Middle thread _____

Bottom thread _____

Viewing Cells Under the Microscope

There are various ways to prepare cells for viewing under a microscope. One method is to mix the cells in physiological saline (called a *wet mount*) and stain them.

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 physiological saline, a dropper bottle of iodine or methylene blue stain, and filter paper (or paper towels). Handle only your own slides throughout the procedure.

2. Place a drop of physiological saline in the center of the slide. Using the flat end of the toothpick, *gently* scrape the inner lining of your cheek. Transfer your cheek scrapings to the slide by agitating the end of the toothpick in the drop of saline (**Figure 3.4a** on p. 34).

Immediately discard the used toothpick in the disposable autoclave bag provided.

3. Add a tiny drop of the iodine or methylene blue stain to the preparation. (These epithelial cells are nearly transparent and thus difficult to see without the stain, which

Text continues on next page. →

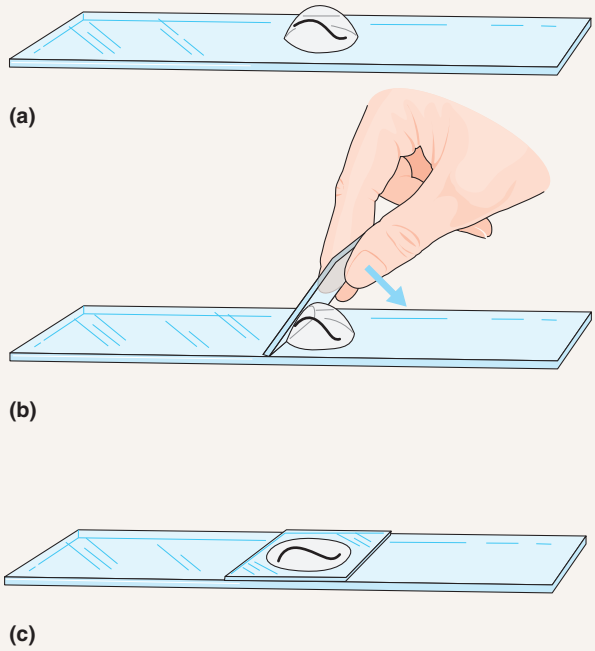


Figure 3.4 **Procedure for preparation of a wet mount.** **(a)** Place the object in a drop of water (or saline) on a clean slide; **(b)** hold a coverslip at a 45° angle with the fingertips; and **(c)** lower the coverslip slowly.

colors the nuclei of the cells.) Stir again, using a second toothpick.

Immediately discard the used toothpicks in the disposable autoclave bag provided.

4. Hold the coverslip with your fingertips so that its bottom edge touches one side of the drop (Figure 3.4b), then *slowly* lower the coverslip onto the preparation (Figure 3.4c). *Do not just drop the coverslip,* or you will trap large air bubbles under it, which will obscure the cells. *Always use a coverslip with a wet mount* to protect the lens.

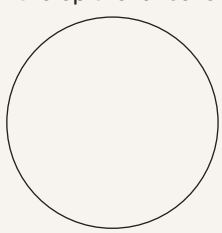
5. Examine your preparation carefully. The coverslip should be tight against the slide. If there is excess fluid around its edges, you will need to remove it. Obtain a piece of filter paper, fold it in half, and use the folded edge to absorb the excess fluid.

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

6. Place the slide on the stage, and locate the cells at the lowest power. You will probably want to dim the light to provide more contrast for viewing the lightly stained cells.

7. Cheek epithelial cells are very thin, flat cells. In the cheek, they provide a smooth, tilelike lining (**Figure 3.5**). Move to high power to examine the cells more closely.

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



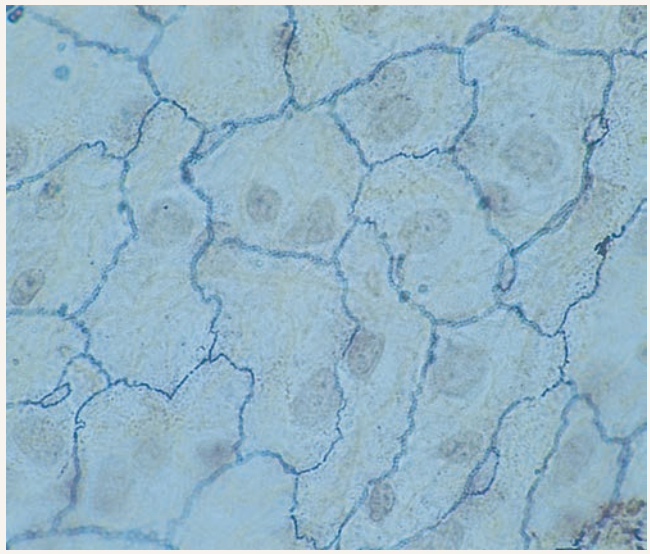


Figure 3.5 **Epithelial cells of the cheek cavity (surface view, 600×).**

Use information on your Summary chart (p. 31) to estimate the diameter of cheek epithelial cells. Record the total magnification (TM) used.

_____ μm _____ × (TM)

Why do *your* cheek cells look different than those in Figure 3.5? (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 (p. 31).

_____ μm _____ × (TM)

Why are these cells more similar to those in Figure 3.5 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 return the microscope to the storage cabinet.

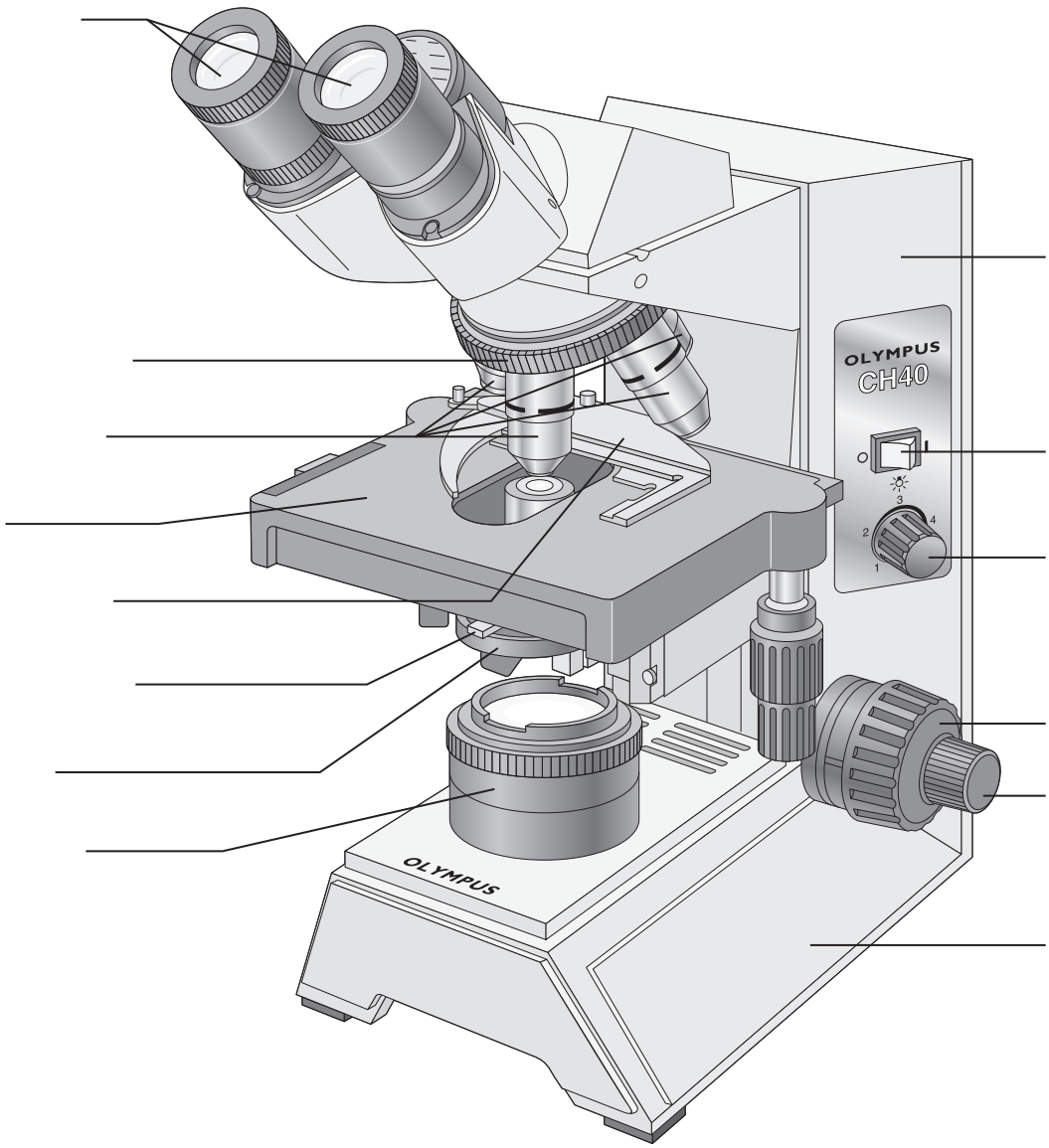
EXERCISE 3

REVIEW SHEET
The Microscope

Name _____ Lab Time/Date _____

Care and Structure of the Compound Microscope

1. Label all indicated parts of the microscope.



2. Explain the proper technique for transporting the microscope.

3. The following statements are true or false. If true, write *T* on the answer blank. If false, correct the statement by writing on the blank the proper word or phrase to replace the one that is underlined.

_____ 1. The microscope lens may be cleaned <u>with any soft tissue</u>.

_____ 2. The microscope should be stored with the <u>oil immersion</u> lens in position over the stage.

_____ 3. When beginning to focus, use the <u>lowest-power</u> lens.

_____ 4. When focusing on high power, always use the <u>coarse</u> adjustment knob to focus.

_____ 5. A coverslip should always be used <u>with wet mounts</u>.

4. Match the microscope structures in column B with the statements in column A that identify or describe them.

Column A

_____ 1. platform on which the slide rests for viewing

_____ 2. used to adjust the amount of light passing through the specimen

_____ 3. controls the movement of the slide on the stage

_____ 4. delivers a concentrated beam of light to the specimen

_____ 5. used for precise focusing once initial focusing has been done

_____ 6. carries the objective lenses; rotates so that the different objective lenses can be brought into position over the specimen

Column B

a. coarse adjustment knob
b. condenser
c. fine adjustment knob
d. iris diaphragm lever
e. mechanical stage
f. nosepiece
g. objective lenses
h. ocular lens
i. stage

5. Define the following terms.

virtual image: _____

_____

resolution: _____

Viewing Objects Through the Microscope

6. Complete, or respond to, the following statements:

_____ 1. The distance from the bottom of the objective lens to the surface of the slide is called the _____.

_____ 2. Assume there is an object on the left side of the field that you want to bring to the center (that is, toward the apparent right). In what direction would you move your slide? _____.

_____ 3. The area of the slide seen when looking through the microscope is the _____.

_____ 4. If a microscope has a 10× ocular lens and the total magnification at a particular time is 950×, the objective lens in use at that time is _____ ×.

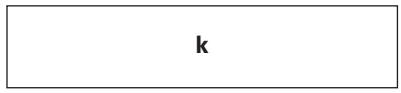
_____ 5. Why should the light be dimmed when looking at living (nearly transparent) cells?

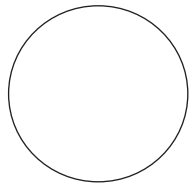
_____ 6. If, after focusing in low power, you need to use only the fine adjustment to focus the specimen at the higher powers, the microscope is said to be _____.

_____ 7. You are using a 10× ocular and a 15× objective, and the field diameter is 1.5 mm. The approximate field size with a 30× objective is _____ mm.

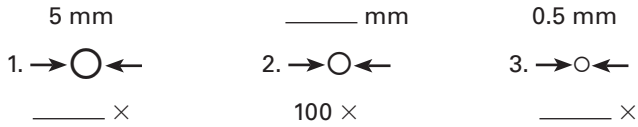
_____ 8. If the diameter of the high-power field is 1.2 mm, an object that occupies approximately a third of that field has an estimated diameter of _____ mm.

7. You have been asked to prepare a slide with the letter *k* on it (as shown below). In the circle below, draw the *k* as seen in the low-power field.





8. Calculate the magnification of fields 1 and 3, and the field diameter of 2. (*Hint:* Use your ruler.) Note that the numbers for the field diameters below are too large to represent the typical compound microscope lens system, but the relationships depicted are accurate.



9. Say you are observing an object in the low-power field. When you switch to high power, it is no longer in your field of view.

Why might this occur? _____

_____

What should you do initially to prevent this from happening? _____

_____

10. Do the following factors increase or decrease as one moves to higher magnifications with the microscope?

resolution: _____ amount of light needed: _____

working distance: _____ depth of field: _____

11. A student has the high-power lens in position and appears to be intently observing the specimen. The instructor, noting a working distance of about 1 cm, knows the student isn't actually seeing the specimen.

How so? _____

12. Describe the proper procedure for preparing a wet mount.

_____

_____

_____

13. Indicate the probable cause of the following situations during use of a microscope.

a. Only half of the field is illuminated: _____

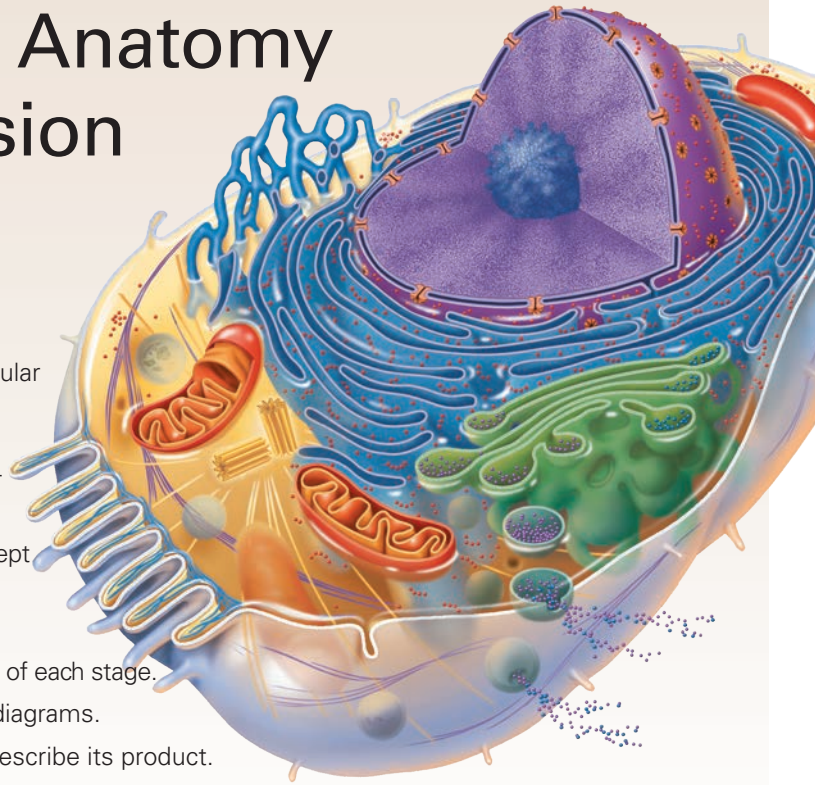
_____

b. The visible field does not change as the mechanical stage is moved: _____

_____

EXERCISE

4 The Cell: Anatomy and Division



Objectives

- ☐ Define *cell, organelle,* and *inclusion.*
- ☐ Identify on a cell model or diagram the following cellular regions and list the major function of each: nucleus, cytoplasm, and plasma membrane.
- ☐ Identify the cytoplasmic organelles and discuss their structure and function.
- ☐ Compare and contrast specialized cells with the concept of the "generalized cell."
- ☐ Define *interphase, mitosis,* and *cytokinesis.*
- ☐ List the stages of mitosis, and describe the key events of each stage.
- ☐ Identify the mitotic phases on slides or appropriate diagrams.
- ☐ Explain the importance of mitotic cell division, and describe its product.

Materials

- Three-dimensional model of the "composite" animal cell or laboratory chart of cell anatomy
- Compound microscope
- Prepared slides of simple squamous epithelium, teased smooth muscle (l.s.), human blood smear, and sperm
- Animation/video of mitosis
- Three-dimensional models of mitotic stages
- Prepared slides of whitefish blastulas
- Chenille sticks (pipe cleaners), two different colors cut into 3-inch pieces, 8 pieces per group

Note to the Instructor: *See directions for handling wet mount preparations and disposable supplies (p. 34, Exercise 3). For suggestions on the animation/video of mitosis, see the Instructor's Guide.*

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For related exercise study tools, go to the Study Area of **MasteringA&P**. There you will find:

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Pre-Lab Quiz

1. Define *cell.* _____

   _____
2. When a cell is not dividing, the DNA is loosely spread throughout the nucleus in a threadlike form called:
   a. chromatin **c.** cytosol
   b. chromosomes **d.** ribosomes
3. The plasma membrane not only provides a protective boundary for the cell but also determines which substances enter or exit the cell. We call this characteristic:
   a. diffusion **c.** osmosis
   b. membrane potential **d.** selective permeability
4. Proteins are assembled on these organelles.

   _____
5. Because these organelles are responsible for providing most of the ATP that the cell needs, they are often referred to as the "powerhouses" of the cell. They are the:
   a. centrioles **c.** mitochondria
   b. lysosomes **d.** ribosomes
6. Circle the correct underlined term. During cytokinesis / interphase, the cell grows and performs its usual activities.
7. Circle True or False. The end product of mitosis is four genetically identical daughter nuclei.
8. How many stages of mitosis are there? _____
9. DNA replication occurs during:
   a. cytokinesis **c.** metaphase
   b. interphase **d.** prophase
10. Circle True or False. All animal cells have a cell wall.

The **cell,** the structural and functional unit of all living things, is a complex entity. The cells of the human body are highly diverse, and their differences in size, shape, and internal composition reflect their specific roles in the body. Still, cells do have many common anatomical features, and all cells must carry out certain functions to sustain life. For example, all cells can maintain their boundaries, metabolize, digest nutrients and dispose of wastes, grow and reproduce, move, and respond to a stimulus. This exercise focuses on structural similarities found in many cells and illustrated by a "composite," or "generalized," cell (**Figure 4.1a**) and considers only the function of cell reproduction (cell division).

Anatomy of the Composite Cell

In general, all animal cells have three major regions, or parts, that can readily be identified with a light microscope: the **nucleus,** the **plasma membrane,** and the **cytoplasm.** The nucleus is near the center of the cell. It is surrounded by cytoplasm, which in turn is enclosed by the plasma membrane. See the diagram (Figure 4.1a) representing the fine structure of the composite cell. An electron micrograph (Figure 4.1b) reveals the cellular structure, particularly of the nucleus.

Nucleus

The nucleus contains the genetic material, DNA, sections of which are called *genes*. Often described as the control center of the cell, the nucleus is necessary for cell reproduction. A cell that has lost or ejected its nucleus is programmed to die.

When the cell is not dividing, the genetic material is loosely dispersed throughout the nucleus in a threadlike form called **chromatin.** When the cell is in the process of dividing to form daughter cells, the chromatin coils and condenses, forming dense, rodlike bodies called **chromosomes**—much in the way a stretched spring becomes shorter and thicker when it is released.

The nucleus also contains one or more small spherical bodies, called **nucleoli,** composed primarily of proteins and ribonucleic acid (RNA). The nucleoli are assembly sites for ribosomes that are particularly abundant in the cytoplasm.

The nucleus is bound by a double-layered porous membrane, the **nuclear envelope.** The nuclear envelope is similar in composition to other cellular membranes, but it is distinguished by its large **nuclear pores.** They are spanned by protein complexes that regulate what passes through, and they permit easy passage of protein and RNA molecules.

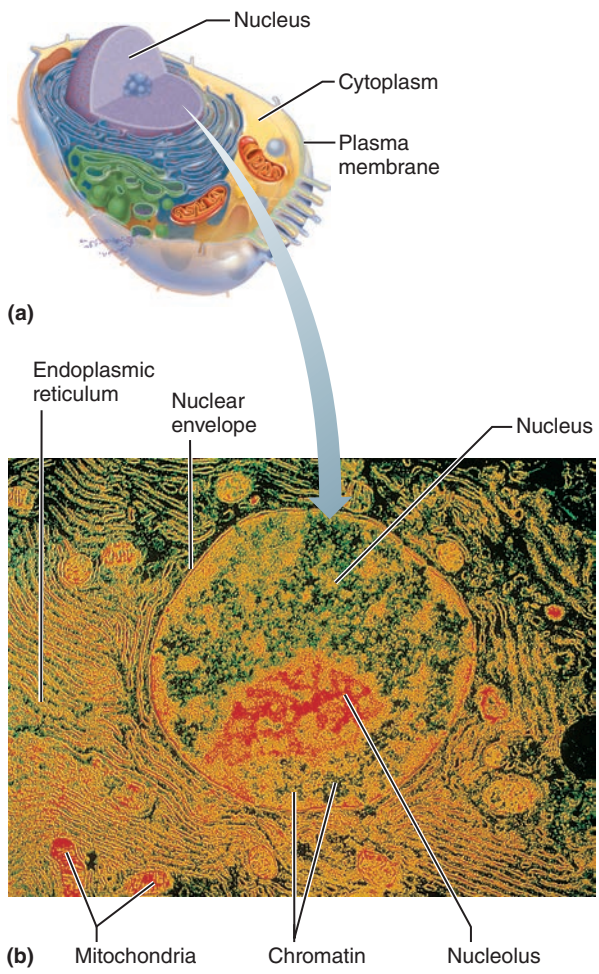


Figure 4.1 Anatomy of the composite animal cell. **(a)** Diagram. **(b)** Transmission electron micrograph (5000×).

Activity 1

Identifying Parts of a Cell

Identify the nuclear envelope, chromatin, nucleolus, and the nuclear pores in Figure 4.1a and b and Figure 4.3.

Plasma Membrane

The **plasma membrane** separates cell contents from the surrounding environment, providing a protective barrier. Its main structural building blocks are phospholipids (fats) and globular protein molecules. Some of the externally facing proteins and lipids have sugar (carbohydrate) side chains attached to them that are important in cellular interactions (**Figure 4.2**). As described by the fluid mosaic model, the membrane is a bilayer of phospholipid molecules in which the protein molecules float. Occasional cholesterol molecules dispersed in the bilayer help stabilize it.

Because of its molecular composition, the plasma membrane is selective about what passes through it. It allows nutrients to enter the cell but keeps out undesirable substances. By the same token, valuable cell proteins and other substances are kept within the cell, and excreta, or wastes, pass to the exterior. This property is known as **selective permeability.**

Additionally, the plasma membrane maintains a resting potential that is essential to normal functioning of excitable cells, such as neurons and muscle cells, and plays a vital role

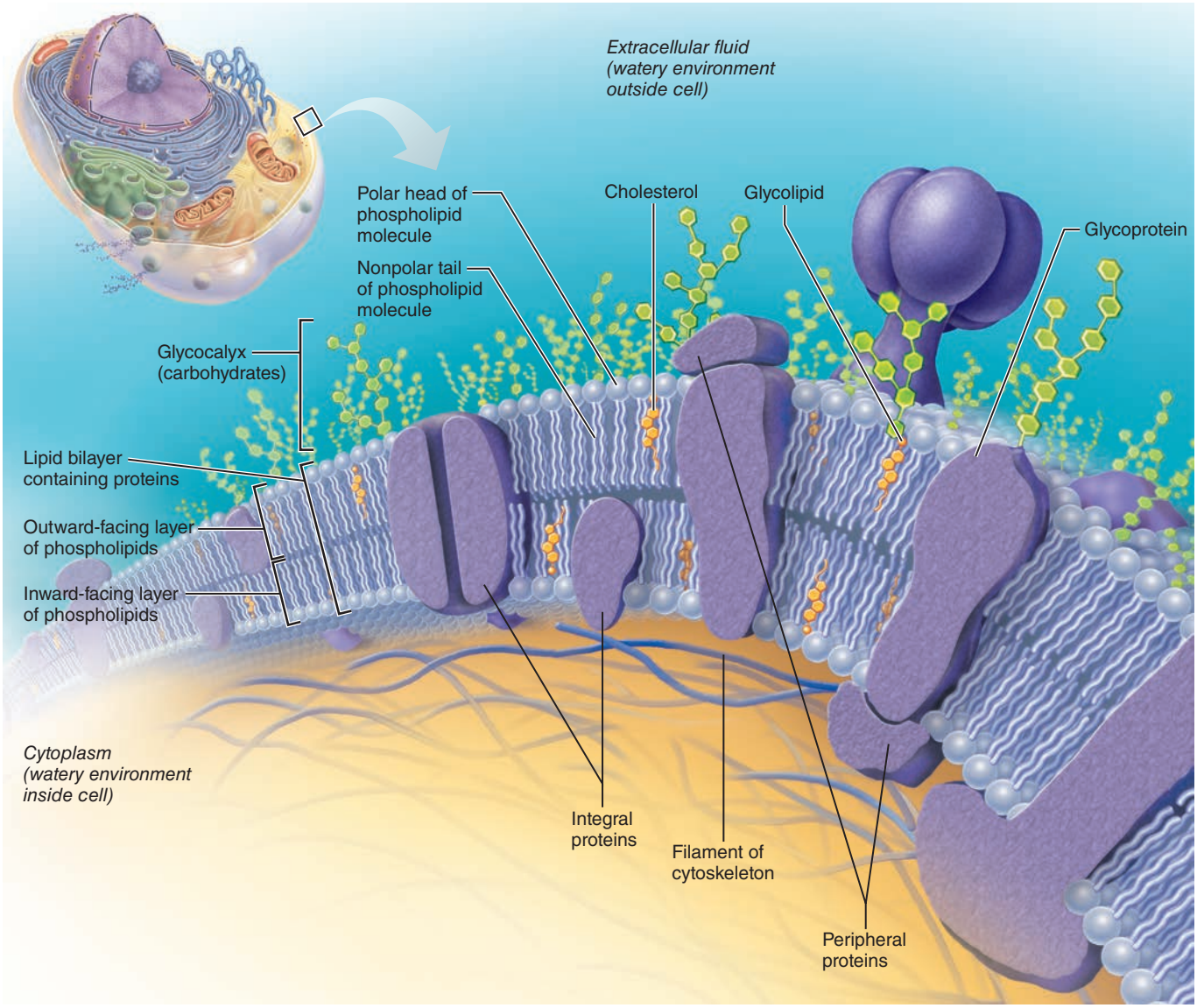


Figure 4.2 **Structural details of the plasma membrane.**

in cell signaling and cell-to-cell interactions. In some cells, the membrane is thrown into tiny fingerlike projections or folds called **microvilli** (**Figure 4.3**, p. 42). Microvilli greatly increase the surface area of the cell available for absorption or passage of materials and for the binding of signaling molecules.

Activity 2

Identifying Components of a Plasma Membrane

Identify the phospholipid and protein portions of the plasma membrane in Figure 4.2. Also locate the sugar (*glyco* = carbohydrate) side chains and cholesterol molecules. Identify the microvilli in the generalized cell diagram (Figure 4.3).

Cytoplasm and Organelles

The cytoplasm consists of the cell contents between the nucleus and plasma membrane. Suspended in the **cytosol,** the fluid cytoplasmic material, are many small structures called **organelles** (literally, "small organs"). The organelles are the metabolic machinery of the cell, and they are highly organized to carry out specific functions for the cell as a whole. The cytoplasmic organelles include the ribosomes, smooth and rough endoplasmic reticulum, Golgi apparatus, lysosomes, peroxisomes, mitochondria, cytoskeletal elements, and centrioles.

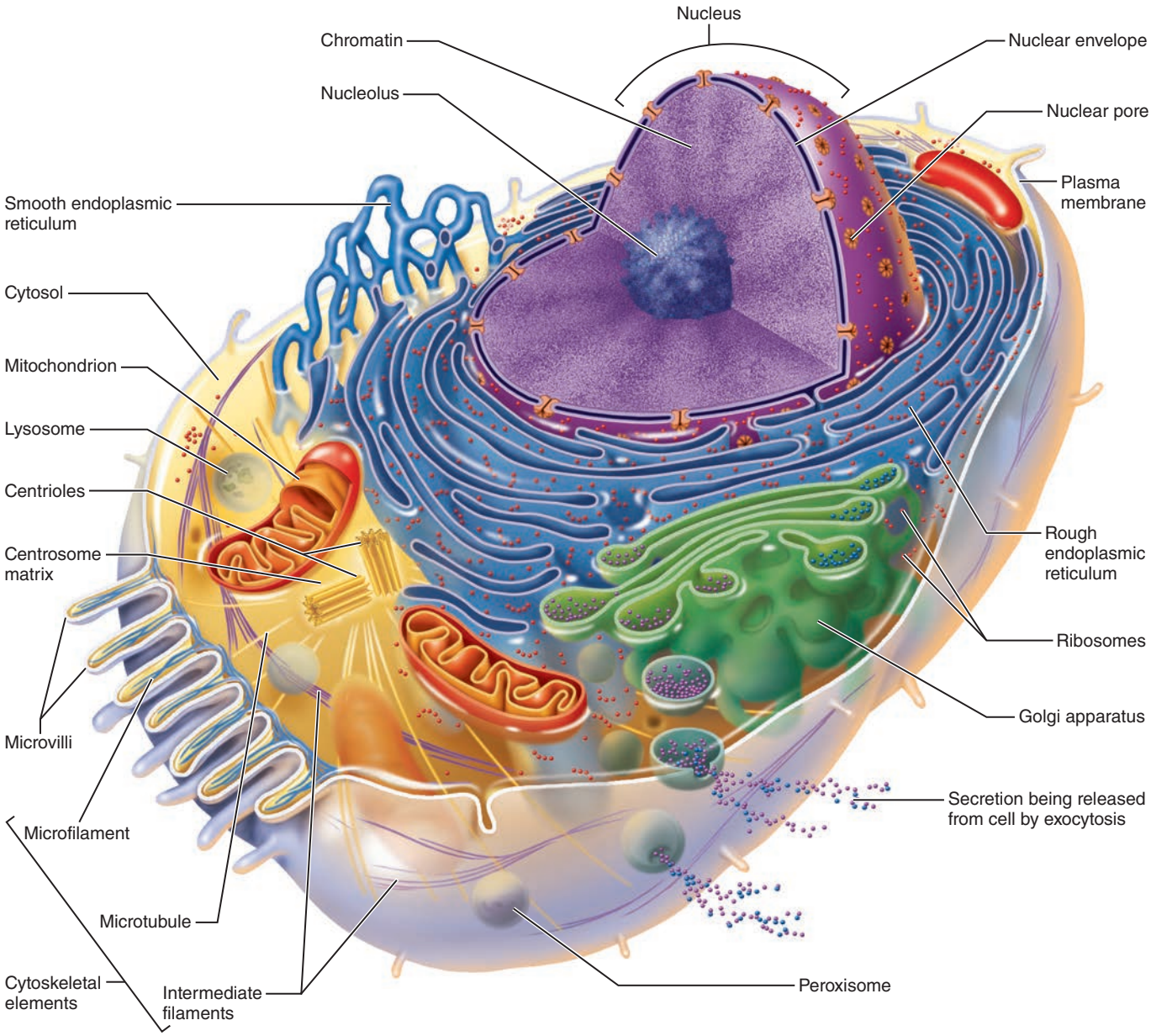


Figure 4.3 Structure of the generalized cell. No cell is exactly like this one, but this composite illustrates features common to many human cells. Not all organelles are drawn to the same scale in this illustration.

Activity 3

Locating Organelles

Each organelle type is described in **Table 4.1**. Read through the table, and then, as best you can, locate the organelles in Figures 4.1b and 4.3.

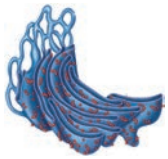
The cell cytoplasm may or may not contain **inclusions.** Examples of inclusions are stored foods (glycogen granules and lipid droplets), pigment granules, crystals of various types, water vacuoles, and ingested foreign materials.

Activity 4

Examining the Cell Model

Once you have located all of these structures in the art (Figures 4.1b and 4.3), examine the cell model (or cell chart) to repeat and reinforce your identifications.

Table 4.1 Summary of Structure and Function of Cytoplasmic Organelles

Organelle		Location and function
Ribosomes		Tiny spherical bodies composed of RNA and protein; floating free or attached to a membranous structure (the rough ER) in the cytoplasm. Actual sites of protein synthesis.
Endoplasmic reticulum (ER)		Membranous system of tubules that extends throughout the cytoplasm; two varieties: rough and smooth. Rough ER is studded with ribosomes; tubules of the rough ER provide an area for storage and transport of the proteins made on the ribosomes to other cell areas. Smooth ER, which has no function in protein synthesis, is a site of steroid and lipid synthesis, lipid metabolism, and drug detoxification.
Golgi apparatus		Stack of flattened sacs with bulbous ends and associated small vesicles; found close to the nucleus. Plays a role in packaging proteins or other substances for export from the cell or incorporation into the plasma membrane and in packaging lysosomal enzymes.
Lysosomes		Various-sized membranous sacs containing digestive enzymes including acid hydrolases; function to digest worn-out cell organelles and foreign substances that enter the cell. Have the capacity of total cell destruction if ruptured and are for this reason referred to as "suicide sacs."
Peroxisomes		Small lysosome-like membranous sacs containing oxidase enzymes that detoxify alcohol, free radicals, and other harmful chemicals. They are particularly abdundant in liver and kidney cells.
Mitochondria		Generally rod-shaped bodies with a double-membrane wall; inner membrane is shaped into folds, or cristae; contain enzymes that oxidize foodstuffs to produce cellular energy (ATP); often referred to as "powerhouses of the cell."
Centrioles		Paired, cylindrical bodies that lie at right angles to each other, close to the nucleus. Internally, each centriole is composed of nine triplets of microtubules. As part of the centrosome, they direct the formation of the mitotic spindle during cell division; form the bases of cilia and flagella and in that role are called *basal bodies*.
Cytoskeletal elements: microfilaments, intermediate filaments, and microtubules		Form an internal scaffolding called the *cytoskeleton*. Provide cellular support; function in intracellular transport. Microfilaments are formed largely of actin, a contractile protein, and thus are important in cell mobility, particularly in muscle cells. Intermediate filaments are stable elements composed of a variety of proteins and resist mechanical forces acting on cells. Microtubules form the internal structure of the centrioles and help determine cell shape.

Differences and Similarities in Cell Structure

Activity 5

Observing Various Cell Structures

1. Obtain a compound microscope and prepared slides of simple squamous epithelium, smooth muscle cells (teased), human blood, and sperm.

2. Observe each slide under the microscope, carefully noting similarities and differences in the cells. See photomicrographs for simple squamous epithelium (Figure 3.5 in Exercise 3) and teased smooth muscle (Figure 6.7c in Exercise 6). The oil immersion lens will be needed to observe blood and sperm. Distinguish the boundaries of the individual cells, and notice the shape

Text continues on next page. →

4

Simple squamous epithelium
Diameter _____

Sperm cells
Length _____

Human red blood cells
Diameter _____

Teased smooth muscle cells
Length _____

and position of the nucleus in each case. When you look at the human blood smear, direct your attention to the red blood cells, the pink-stained cells that are most numerous. The color photomicrographs illustrating a blood smear (Figure 29.3 in Exercise 29) and sperm (Figure 43.3 in Exercise 43) may be helpful in this cell structure study. Sketch your observations in the circles provided above.

3. Measure the length or diameter of each cell, and record below the appropriate sketch.

4. How do these four cell types differ in shape and size?

_____

_____

_____

_____

How might cell shape affect cell function?

_____

_____

_____

_____

_____

Which cells have visible projections? _____

_____

How do these projections relate to the function of these cells?

_____

_____

Do any of these cells lack a plasma membrane? _____

A nucleus? _____

In the cells with a nucleus, can you discern nucleoli?

_____

Were you able to observe any of the organelles in these cells? _____ Why or why not? _____

_____

_____

_____

_____

Cell Division: Mitosis and Cytokinesis

A cell's *life cycle* is the series of changes it goes through from the time it is formed until it reproduces. It consists of two stages—**interphase,** the longer period during which the cell grows and carries out its usual activities (Figure 4.4a), and **cell division,** when the cell reproduces itself by dividing. In an interphase cell about to divide, the genetic material (DNA) is copied exactly via DNA replication. Once this important event has occurred, cell division ensues.

Cell division in all cells other than bacteria consists of two events called mitosis and cytokinesis. **Mitosis** is the

division of the copied DNA of the mother cell to two daughter nuclei. **Cytokinesis** is the division of the cytoplasm, which begins when mitosis is nearly complete. Although mitosis is usually accompanied by cytokinesis, in some instances cytoplasmic division does not occur, leading to the formation of binucleate or multinucleate cells.

The product of **mitosis** is two daughter nuclei that are genetically identical to the mother nucleus. This distinguishes mitosis from **meiosis,** a specialized type of nuclear division (covered in Exercise 43) that occurs only in the reproductive organs (testes or ovaries). Meiosis, which yields four daughter nuclei that differ genetically in composition from the mother nucleus, is used only for the production of gametes (eggs and sperm) for sexual reproduction. The function of cell division, including mitosis and cytokinesis in the body, is to increase the number of cells for growth and repair.

The phases of mitosis include **prophase, metaphase, anaphase,** and **telophase.** The detailed events of interphase, mitosis, and cytokinesis are described and illustrated in **Figure 4.4** on pp. 46–47.

Mitosis is essentially the same in all animal cells, but depending on the tissue, it takes from 5 minutes to several hours to complete. In most cells, centriole replication occurs during interphase of the next cell cycle.

At the end of cell division, two daughter cells exist—each with a smaller cytoplasmic mass than the mother cell but genetically identical to it. The daughter cells grow and carry out the normal spectrum of metabolic processes until it is their turn to divide.

Cell division is extremely important during the body's growth period. Most cells divide until puberty, when adult body size is achieved and overall body growth ceases. After this time in life, only certain cells carry out cell division routinely—for example, cells subjected to abrasion (epithelium of the skin and lining of the gut). Other cell populations—such as liver cells—stop dividing but retain this ability should some of them be removed or damaged. Skeletal muscle, cardiac muscle, and most mature neurons almost completely lose this ability to divide and thus are severely handicapped by injury.

Activity 6

Identifying the Mitotic Stages

1. Watch an animation or video presentation of mitosis (if available).
2. Using the three-dimensional models of dividing cells provided, identify each of the mitotic phases illustrated and described in Figure 4.4.
3. Obtain a prepared slide of whitefish blastulas to study the stages of mitosis. The cells of each *blastula* (a stage of embryonic development consisting of a hollow ball of cells) are at approximately the same mitotic stage, so it may be necessary to observe more than one blastula to view all the mitotic stages. A good analogy for a blastula is a soccer ball in which each leather piece making up the ball's surface represents an embryonic cell. The exceptionally high rate of mitosis observed in this tissue is typical of embryos, but if it occurs in specialized tissues it can indicate cancerous cells, which also have an extraordinarily high mitotic rate. Examine the slide carefully, identifying the four mitotic phases and the process of cytokinesis. Compare your observations with the photomicrographs (Figure 4.4), and verify your identifications with your instructor.

Activity 7

"Chenille Stick" Mitosis

1. Obtain a total of eight 3-inch pieces of chenille stick, four of one color and four of another color (e.g., four green and four purple).
2. Assemble the chenille sticks into a total of four chromosomes (each with two sister chromatids) by twisting two sticks of the same color together at the center with a single twist.

What does the twist at the center represent? _____

_____

3. Arrange the chromosomes as they appear in early prophase.

Name the structure that assembles during this phase.

_____

Draw early prophase in the space provided in the Review Sheet (question 10, p. 51).

4. Arrange the chromosomes as they appear in late prophase.

What structure on the chromosome centromere do the growing spindle microtubules attach to? _____

What structure is now present as fragments? _____

_____

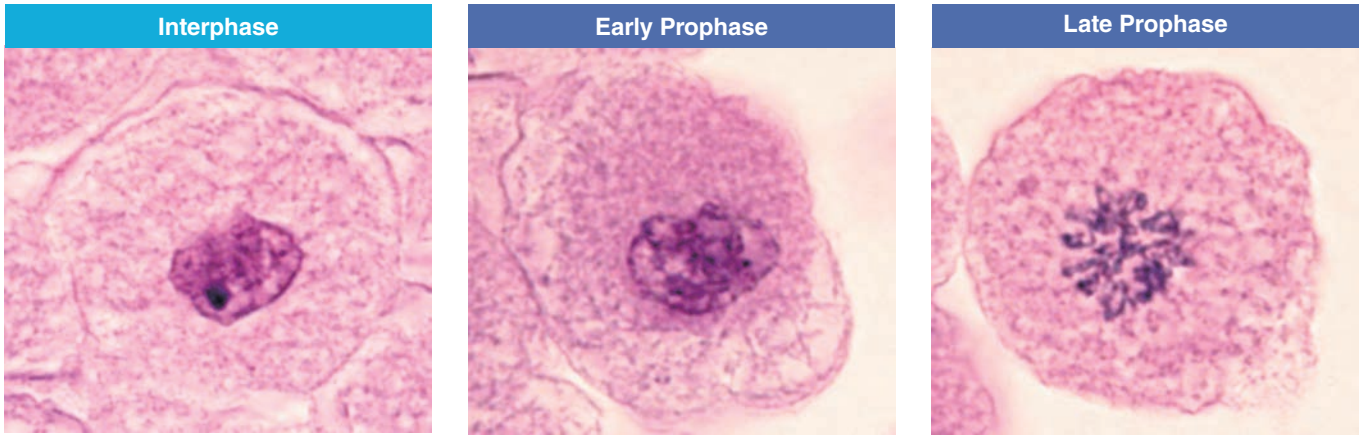
Draw late prophase in the space provided on the Review Sheet (question 10, p. 51).

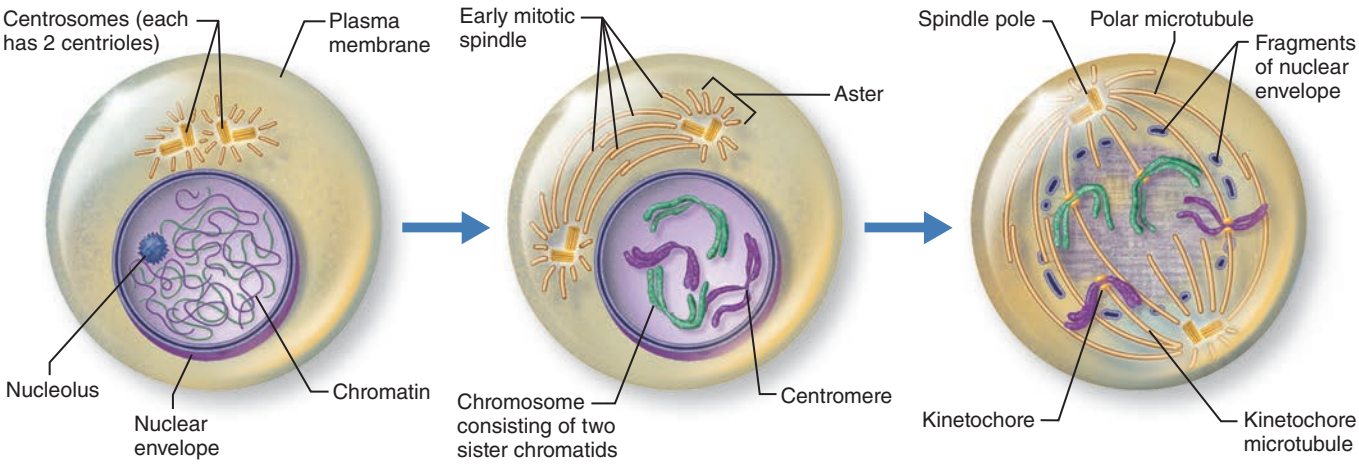
5. Arrange the chromosomes as they appear in metaphase.

What is the name of the imaginary plane that the chromosomes align along? _____

Draw metaphase in the space provided on the Review Sheet (question 10, p. 51).

Text continues on page 48. →





Interphase

Interphase is the period when the cell carries out its normal metabolic activities and grows. Interphase is not part of mitosis.

- During interphase, the DNA-containing material is in the form of chromatin. The nuclear envelope and one or more nucleoli are intact and visible.
- There are three distinct periods of interphase:
  G_1: The centrioles begin replicating.
  S: DNA is replicated.
  G_2: Final preparations for mitosis are completed, and centrioles finish replicating.

Prophase—first phase of mitosis

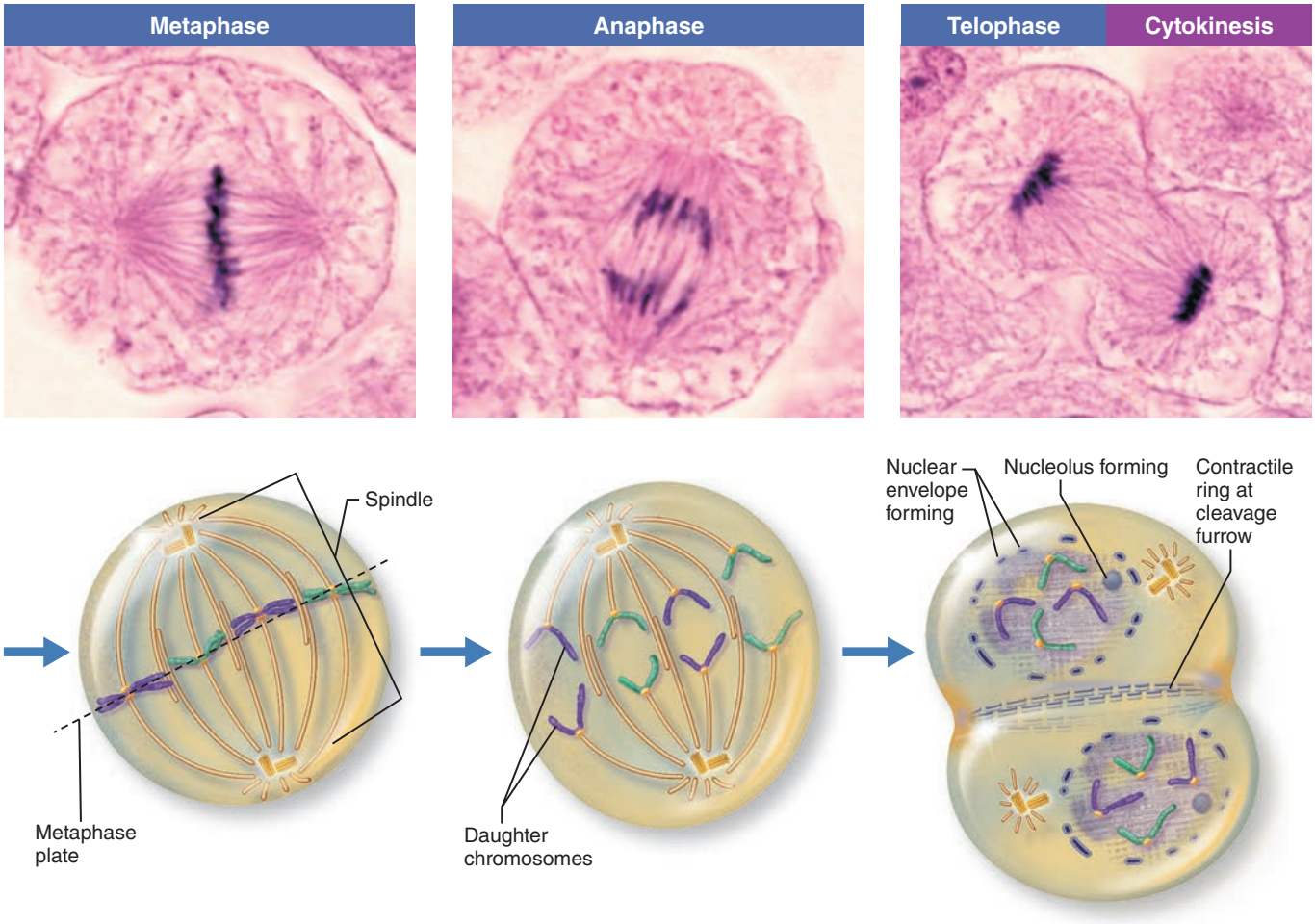
Early Prophase

- The chromatin condenses, forming barlike chromosomes.
- Each duplicated chromosome consists of two identical threads, called **sister chromatids**, held together at the **centromere**. (Later when the chromatids separate, each will be a new chromosome.)
- As the chromosomes appear, the nucleoli disappear, and the two centrosomes separate from one another.
- The centrosomes act as focal points for growth of a microtubule assembly called the **mitotic spindle**. As the microtubules lengthen, they propel the centrosomes toward opposite ends (poles) of the cell.
- Microtubule arrays called **asters** ("stars") extend from the centrosome matrix.

Late Prophase

- The nuclear envelope breaks up, allowing the spindle to interact with the chromosomes.
- Some of the growing spindle microtubules attach to **kinetochores**, special protein structures at each chromosome's centromere. Such microtubules are called **kinetochore microtubules**.
- The remaining spindle microtubules (not attached to any chromosomes) are called **polar microtubules**. The microtubules slide past each other, forcing the poles apart.
- The kinetochore microtubules pull on each chromosome from both poles in a tug-of-war that ultimately draws the chromosomes to the center, or equator, of the cell.

Figure 4.4 The interphase cell and the events of cell division. The cells shown are from an early embryo of a whitefish. Photomicrographs are above; corresponding diagrams are below. (Micrographs approximately 1600×.)



Metaphase—second phase of mitosis

- The two centrosomes are at opposite poles of the cell.
- The chromosomes cluster at the midline of the cell, with their centromeres precisely aligned at the **equator** of the spindle. This imaginary plane midway between the poles is called the **metaphase plate**.
- Enzymes act to separate the chromatids from each other.

Anaphase—third phase of mitosis

The shortest phase of mitosis, anaphase begins abruptly as the centromeres of the chromosomes split simultaneously. Each chromatid now becomes a chromosome in its own right.

- The kinetochore microtubules, moved along by motor proteins in the kinetochores, gradually pull each chromosome toward the pole it faces.
- At the same time, the polar microtubules slide past each other, lengthen, and push the two poles of the cell apart.
- The moving chromosomes look V shaped. The centromeres lead the way, and the chromosomal "arms" dangle behind them.
- The fact that the chromosomes are short, compact bodies makes it easier for them to move and separate. Diffuse threads of chromatin would trail, tangle, and break, resulting in imprecise "parceling out" to the daughter cells.

Telophase—final phase of mitosis

Telophase begins as soon as chromosomal movement stops. This final phase is like prophase in reverse.

- The identical sets of chromosomes at the opposite poles of the cell uncoil and resume their threadlike chromatin form.
- A new nuclear envelope forms around each chromatin mass, nucleoli reappear within the nuclei, and the spindle breaks down and disappears.
- Mitosis is now ended. The cell, for just a brief period, is binucleate (has two nuclei), and each new nucleus is identical to the original mother nucleus.

Cytokinesis—division of cytoplasm

Cytokinesis begins during late anaphase and continues through and beyond telophase. A contractile ring of actin microfilaments forms the **cleavage furrow** and pinches the cell apart.

Figure 4.4 *(continued)* **The events of cell division.**

6. Arrange the chromosomes as they appear in anaphase.

What does untwisting of the chenille sticks represent?

_____

_____

Each sister chromatid has now become a _____.

Draw anaphase in the space provided on the Review Sheet (question 10, p. 51).

7. Arrange the chromosomes as they appear in telophase.

Briefly list four reasons why telophase is like the reverse of prophase.

_____

_____

_____

Draw telophase in the space provided on the Review Sheet (question 10, p. 51).

EXERCISE 4

REVIEW SHEET

The Cell: Anatomy and Division

Name _____ Lab Time/Date _____

Anatomy of the Composite Cell

1. Define the following terms:

organelle: _____

_____

cell: _____

2. Cells have differences that reflect their specific functions in the body, but what functions do they have in common?

_____

3. Identify the following cell structures:

_____ 1. external boundary of cell; regulates flow of materials into and out of the cell; site of cell signaling

_____ 2. contains digestive enzymes of many varieties; "suicide sac" of the cell

_____ 3. scattered throughout the cell; major site of ATP synthesis

_____ 4. slender extensions of the plasma membrane that increase its surface area

_____ 5. stored glycogen granules, crystals, pigments; present in some cell types

_____ 6. membranous system consisting of flattened sacs and vesicles; packages proteins for export

_____ 7. control center of the cell; necessary for cell division and cell life

_____ 8. two rod-shaped bodies near the nucleus; associated with the formation of the mitotic spindle

_____ 9. dense nuclear body; packaging site for ribosomes

_____ 10. contractile elements of the cytoskeleton

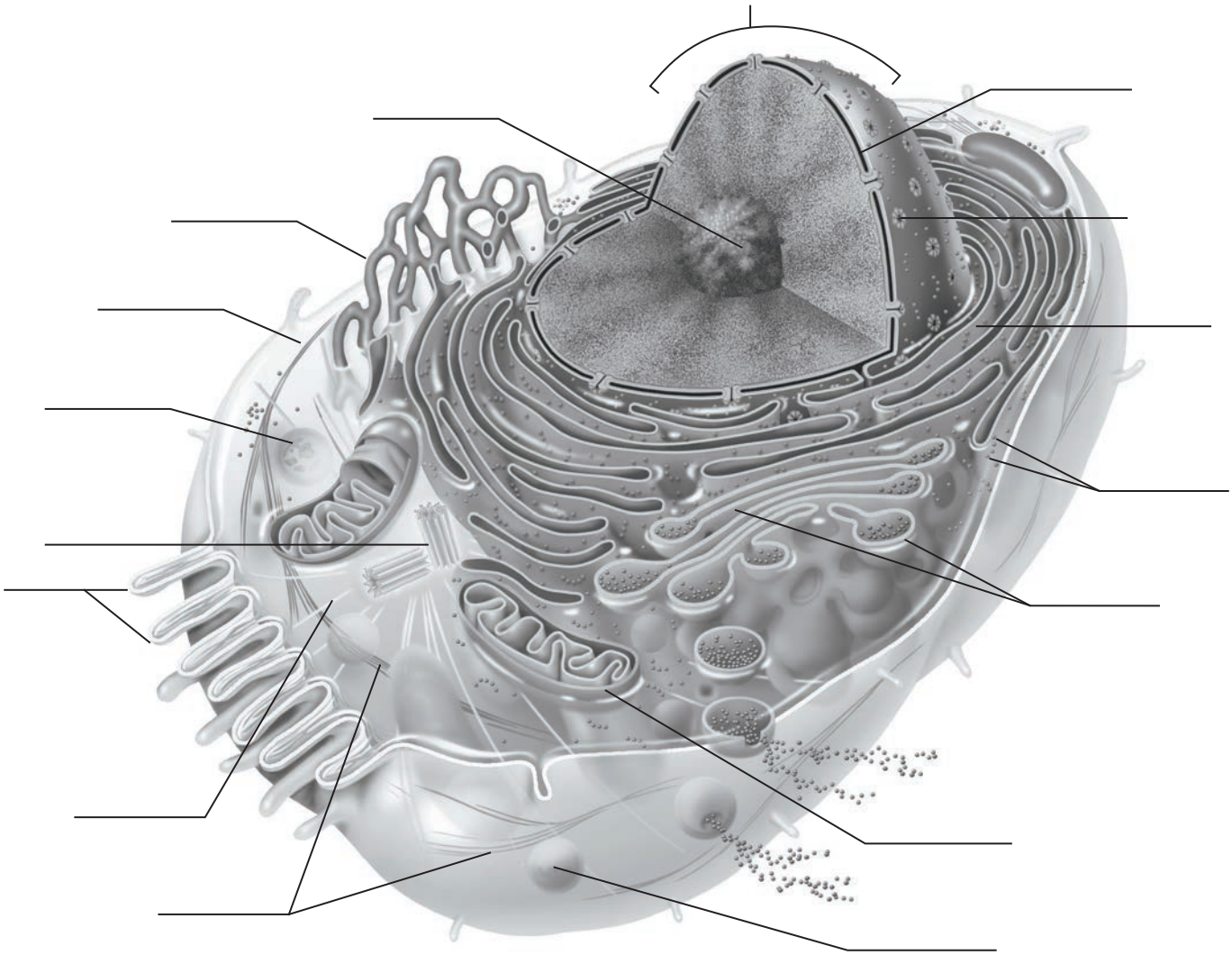
_____ 11. membranous tubules covered with ribosomes; involved in intracellular transport of proteins

_____ 12. attached to membrane systems or scattered in the cytoplasm; site of protein synthesis

_____ 13. threadlike structures in the nucleus; contain genetic material (DNA)

_____ 14. site of free radical detoxification

4. In the following diagram, label all parts provided with a leader line.



Differences and Similarities in Cell Structure

5. For each of the following cell types, list (a) *one* important structural characteristic observed in the laboratory, and (b) the function that the structure complements or ensures.

squamous epithelium	a.	_____
	b.	_____
sperm	a.	_____
	b.	_____
smooth muscle	a.	_____
	b.	_____
red blood cells	a.	_____
	b.	_____

6. What is the consequence of the red blood cell being anucleate (without a nucleus)? _____

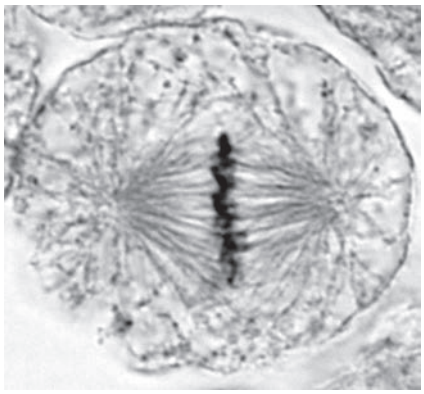
_____

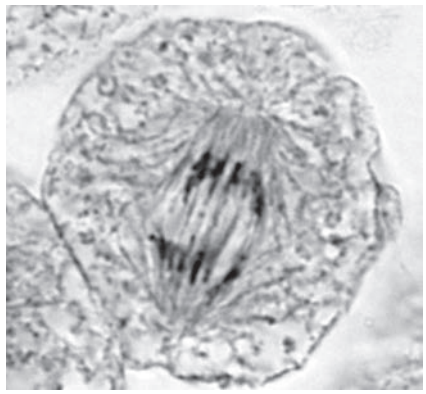
Did it ever have a nucleus? (Use an appropriate reference.) _____ If so, when? _____

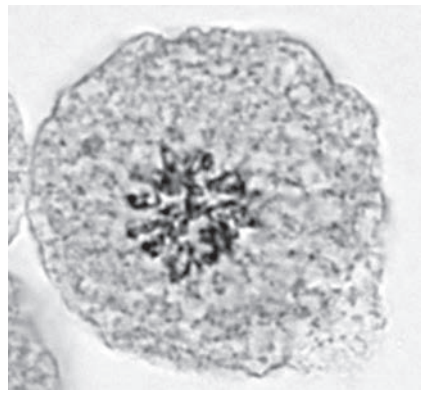
7. Of the four cells observed microscopically (squamous epithelial cells, red blood cells, smooth muscle cells, and sperm), which has the smallest diameter? _____ Which is longest? _____

Cell Division: Mitosis and Cytokinesis

8. Identify the three phases of mitosis in the following photomicrographs.







a. _____ b. _____ c. _____

9. What is the function of mitotic cell division? _____

_____

10. Draw the phases of mitosis for a cell that contains four chromosomes as its diploid, or 2*n*, number.

11. Complete or respond to the following statements:

Division of the __1__ is referred to as mitosis. Cytokinesis is division of the __2__. The major structural difference between chromatin and chromosomes is that the latter are __3__. Chromosomes attach to the spindle fibers by undivided structures called __4__. If a cell undergoes mitosis but not cytokinesis, the product is __5__. The structure that acts as a scaffolding for chromosomal attachment and movement is called the __6__. __7__ is the period of cell life when the cell is not involved in division. Three cell populations in the body that do not routinely undergo cell division are __8__, __9__ and __10__.

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10. _____

12. Using the key, categorize each of the events described below according to the phase in which it occurs.

Key: a. anaphase b. interphase c. metaphase d. prophase e. telophase

_____ 1. Chromatin coils and condenses, forming chromosomes.

_____ 2. The chromosomes are V shaped.

_____ 3. The nuclear envelope re-forms.

_____ 4. Chromosomes stop moving toward the poles.

_____ 5. Chromosomes line up in the center of the cell.

_____ 6. The nuclear envelope fragments.

_____ 7. The mitotic spindle forms.

_____ 8. DNA replication occurs.

_____ 9. Centrioles replicate.

_____ 10. Chromosomes first appear to be duplex structures.

_____ 11. Cleavage furrow forms.

_____ and _____ 12. The nuclear envelope is completely absent.

13. What is the physical advantage of the chromatin coiling and condensing to form short chromosomes at the onset of mitosis?

_____

_____