LAB TOPIC 2

Microscopes and Cells

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Identify the parts of compound and stereoscopic microscopes and be proficient in their correct use in biological studies.
2. Describe procedures used in preparing materials for electron microscopy and compare these with procedures used in light microscopy.
3. Identify cell structures and organelles from electron micrographs and state the functions of each.
4. Describe features of specific cells and determine characteristics shared by all cells studied.
5. Compare the structure of animal and plant cells as seen in both light and electron microscopy.
6. Distinguish between eukaryotic and prokaryotic cells.
7. Discuss the evolutionary significance of increasing complexity from unicellular to multicellular organization and provide examples from the lab.

For a 2-hour lab: Omit the electron microscope and micrographs study (Exercise 2.4) and the unknowns (Exercise 2.5, Lab Study D), although the latter is a highlight of the lab. The final discussion can be reduced or left to the discussion questions at the end of the lab topic.

Introduction

According to cell theory, the *cell* is the fundamental biological unit, the smallest and simplest biological structure possessing all the characteristics of the living condition. All living organisms are composed of one or more cells, and every activity taking place in a living organism is ultimately related to metabolic activities in cells. Thus, understanding the processes of life necessitates an understanding of the structure and function of the cell.

The earliest known cells found in fossilized sediments 3.5 billion years old (called **prokaryotic** cells) lack nuclei and membrane-bound organelles. Cells with a membrane-bound nucleus and organelles (**eukaryotic** cells) do not appear in the fossil record for another 2 billion years. But the eventual evolution of the eukaryotic cell and its internal compartmentalization led to enormous biological diversity in single cells. The evolution of loose aggregates of cells ultimately to colonies of connected cells provided for specialization, so that groups of cells had specific and different functions. This early division of labor included cells whose primary function was locomotion or reproduction. The evolution of multicellularity appears to have originated more than once in eukaryotes and provided an opportunity for extensive adaptive radiation as organisms specialized and diversified, eventually giving rise to fungi, plants, and animals. This general trend in increasing complexity and specialization seen in the history of life will be illustrated in Lab Topic 2.

Given the fundamental role played by cells in the organization of life, one can readily understand why the study of cells is essential to the study of life. Cells, however, are below the limit of resolution of the human eye. We cannot study them without using a microscope. The microscope has probably contributed more than any other instrument to the development of biology as a science and continues today to be the principal tool used in medical and biological research. There are four types of microscopes commonly used by biologists. You will learn how to use two of these microscopes, the compound microscope and the stereoscopic microscope, in today's laboratory. Both of these microscopes use visible light as the source of illumination and are called light microscopes. Two other microscopes, the scanning electron microscope and the transmission electron microscope, use electrons as the source of illumination. Electron microscopes are able to view objects much smaller than those seen in a light microscope. Although these microscopes are not used in this laboratory, you will be given the opportunity to learn more about them in Exercise 2.4.

Microscopes are used by biologists in numerous subdisciplines: genetics, molecular biology, neurobiology, cell biology, evolution, and ecology. The knowledge and skills you develop today will be used and enhanced throughout this course and throughout your career in biology. It is important, therefore, that you take the time to master these exercises thoroughly.

EXERCISE 2.1
The Compound Light Microscope

Materials

compound microscope

Introduction

The microscope is designed to make objects visible that are too difficult or too small to see with the unaided eye. There are many variations of light microscopes, including phase-contrast, darkfield, polarizing, and UV. These differ primarily in the source and manner in which light is passed through the specimen to be viewed.

The microscopes in biology lab are usually compound binocular or monocular light microscopes, some of which may have phase-contrast attachments. **Compound** means that the scopes have a minimum of two magnifying lenses (the ocular and the objective lenses). **Binocular microscopes** have two eyepieces, **monoculars** have only one eyepiece, and **light** refers to the type of illumination used, that is, visible light from a lamp.

Your success in and enjoyment of a large portion of the laboratory work in introductory biology will depend on how proficient you become in the use of the microscope. When used and maintained correctly, these precision instruments are capable of producing images of the highest quality.

Although there are many variations in the features of microscopes, they are all constructed on a similar plan (Figure 2.1). In this exercise you will be introduced to the common variations found in different models of compound microscopes and asked to identify those features found on your microscope.

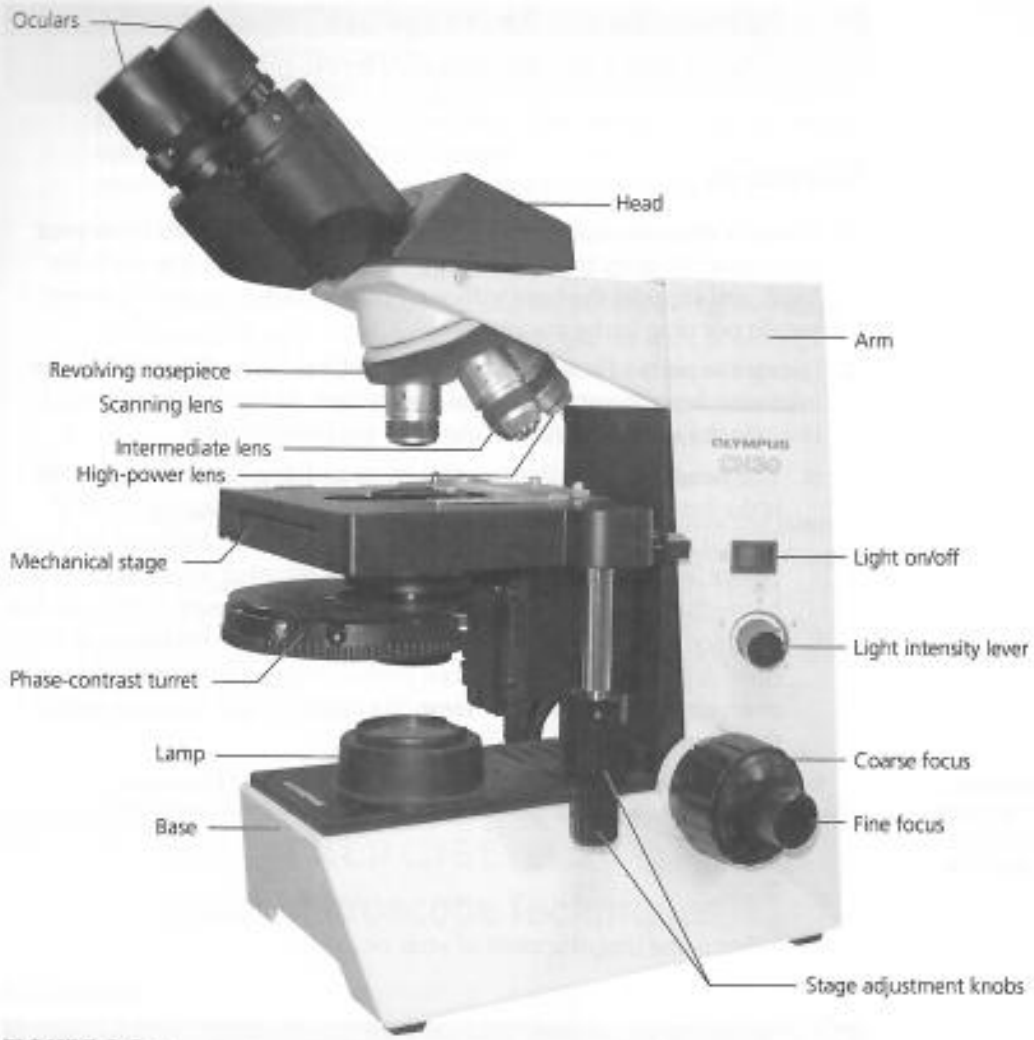


FIGURE 2.1a

The compound binocular light microscope. Locate the parts of your microscope described in Exercise 2.1 and label this photograph. Indicate in the margin of your lab manual any features unique to your microscope.

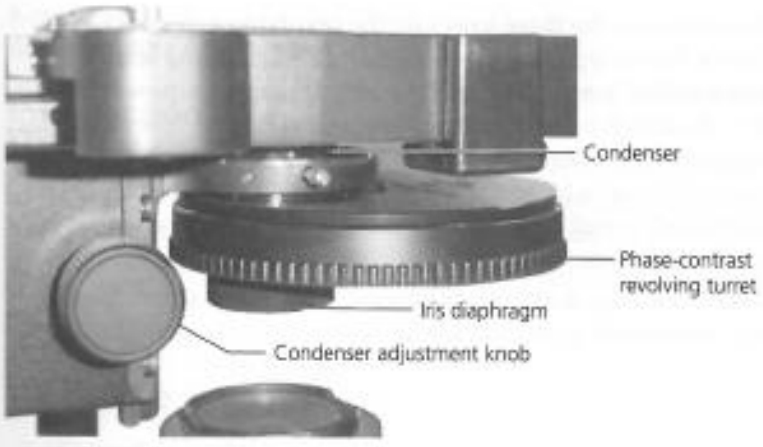


FIGURE 2.1b

Enlarged photo of compound light microscope as viewed from under the stage. This microscope is equipped with phase-contrast optics. Locate the condenser, condenser adjustment knob, phase-contrast revolving turret, and iris diaphragm on your microscope (if present) and label them on the diagram.

Please treat these microscopes with the greatest care!

Procedure

1. Obtain a compound light microscope, following directions from your instructor. To carry the microscope correctly, hold the arm with one hand, and support the base with your other hand. Remove the cover, but do not plug in the microscope.
2. Locate the parts of your microscope, and label Figure 2.1. Refer to the following description of a typical microscope. In the spaces provided, indicate the specific features related to your microscope.
   a. The **head** supports the two sets of magnifying lenses. The **ocular** is the lens in the eyepiece, which typically has a magnification of 10×. If your microscope is binocular, the distance between the eyepieces (**interpupillary distance**) can be adjusted to suit your eyes. Move the eyepieces apart, and look for the scale used to indicate the distance between the eyepieces. Do not adjust the eyepieces at this time. A pointer has been placed in the eyepiece and is used to point to an object in the **field of view,** the circle of light that one sees in the microscope.

      Is your microscope monocular (one eyepiece) or binocular (two eyepieces)?

      What is the magnification of your ocular(s)?

If you are using binocular microscopes, demonstrate the movement of the oculars, and indicate the scale for interpupillary distance. Students should not adjust the oculars at this time.

Although the eyepiece may be removable, it should not be removed from the microscope.

If your students' microscopes have an oil immersion objective, you may want to point it out and demonstrate its correct use in the next section. Oil immersion is not required for any of the exercises in this manual.

   b. **Objectives** are the three lenses on the **revolving nosepiece.** The shortest lens is typically 4× and is called the **scanning lens.** The **intermediate lens** is 10×, and the longest, the **high-power lens,** is 40× (the fourth position on the nosepiece is empty). It is important to clean both the objective and ocular lenses before each use. Dirty lenses will cause a blurring or fogging of the image. Always use lens paper for cleaning! Any other material (including Kimwipes®) may scratch the lenses.

      What is the magnification of each of your objectives? List them in order of increasing magnification.

   c. The **arm** supports the stage and condenser lens. The **condenser lens** is used to focus the light from the **lamp** through the specimen to be viewed. The height of the condenser can be adjusted by an **adjustment knob.** The **iris diaphragm** controls the width of the circle of light and, therefore, the amount of light passing through the specimen.

If your microscope has phase-contrast optics, the condenser may be housed in a **revolving turret.** When the turret is set on 0, the normal optical arrangement is in place. This condition is called **bright-field microscopy.** Another position of the turret sets phase-contrast optics in place. To use phase-contrast, the turret setting must correspond to the magnifying power of the objective being used.

Is your microscope equipped with phase-contrast optics?

The **stage** supports the specimen to be viewed. A mechanical stage can be moved right and left and back and forth by two **stage adjustment knobs.** With a stationary stage, the slide is secured under stage clips and moved slightly by hand while viewing the slide. The distance between the stage and the objective can be adjusted with the **coarse** and **fine focus adjustment knobs**.

Does your microscope have a mechanical or stationary stage?

d. The **base** acts as a stand for the microscope and houses the lamp. In some microscopes, the intensity of the light that passes through the specimen can be adjusted with the **light intensity lever.** Generally, more light is needed when using high magnification than when using low magnification. Describe the light system for your microscope.

EXERCISE 2.2
Basic Microscope Techniques

Materials

clear ruler
coverslips
prepared slides: letter and crossed thread
lens paper
blank slides
Kimwipes®
dropper bottle with distilled water

Introduction

In this exercise, you will learn to use the microscope to examine a recognizable object, a slide of the letter *e*. Recall that microscopes vary, so you may have to omit steps that refer to features not available on your microscope. The following procedure will allow you to practice adjusting your microscope to become proficient in locating a specimen, focusing clearly, and adjusting the light for the best contrast.

Procedure

1. Clean microscope lenses.

   Each time you use the microscope, you should begin by cleaning the lenses. Using lens paper moistened with a drop of distilled water, wipe the ocular, objective, and condenser lenses. Wipe them again with a piece of dry lens paper.

Use only lens paper on microscope lenses. Do not use Kimwipes®, tissues, or other papers.

2. Adjust the focus on your microscope.
   a. Plug your microscope into the outlet.
   b. Turn on the light. Adjust the light intensity to mid-range if your microscope has that feature.
   c. Rotate the 4× objective into position using the revolving nosepiece ring, not the objective itself.
   d. Take the letter slide and wipe it with a Kimwipes® tissue. Each time you study a prepared slide, you should first wipe it clean. Place the letter slide on the stage, and center it over the stage opening.

Slides should be placed on and removed from the stage only when the 4x objective is in place. Removing a slide when the higher objectives are in position may scratch the lenses.

   e. Look through the ocular and bring the letter into rough focus by slowly focusing upward using the coarse adjustment.
   f. For binocular microscopes, looking through the oculars, move the oculars until you see only one image of the letter *e*. In this position, the oculars should be aligned with your pupils. In the margin of your lab manual, make a note of the **interpupillary distance** on the scale between the oculars. Each new lab day, before you begin to use the microscope, set this distance.
   g. Raise the condenser to its highest position, and fully close the iris diaphragm.
   h. Looking through the ocular, slowly lower the condenser just until the graininess disappears. Slowly open the iris diaphragm just until the entire field of view is illuminated. This is the correct position for both the condenser and the iris diaphragm.
   i. Rotate the 10× objective into position.
   j. Look through the ocular and slowly focus upward with the coarse adjustment knob until the image is in rough focus. Sharpen the focus using the fine adjustment knob.

Do not turn the fine adjustment knob more than two revolutions in either direction. If the image does not come into focus, return to 10x and refocus using the coarse adjustment.

k. For binocular microscopes, cover your left eye and use the fine adjustment knob to focus the fixed (right) ocular until the letter *e* is in maximum focus. Now cover the right eye and, using the diopter ring on the left ocular, bring the image into focus. The letter *e* should now be in focus for both of your eyes. Each new lab day, as you begin to study your first slide, repeat this procedure.

l. You can increase or decrease the contrast by adjusting the iris diaphragm opening. Note that the maximum amount of light provides little contrast. Adjust the aperture until the image is sharp.

m. Move the slide slowly to the right. In what direction does the image in the ocular move?

left

n. Is the image in the ocular inverted relative to the specimen on the stage?

yes

o. Center the specimen in the field of view; then rotate the 40× objective into position while watching from the side. *If it appears that the objective will hit the slide, stop and ask for assistance.*

Most of the microscopes have **parfocal** lenses, which means that little refocusing is required when moving from one lens to another. If your scope is *not* parfocal, ask your instructor for assistance.

p. After the 40× objective is in place, focus using the fine adjustment knob.

Never focus with the coarse adjustment knob when you are using the high-power objective.

q. The distance between the specimen and the objective lens is called the **working distance.** Is this distance greater with the 40× or the 10× objective?

10x

3. Compute the total magnification of the specimen being viewed. To do so, multiply the magnification of the ocular lens by that of the objective lens.

a. What is the total magnification of the letter as the microscope is now set?

400x

b. What would be the total magnification if the ocular were 20× and the objective were 100× (oil immersion)? This is the magnification achieved by the best light microscopes.

2,000×

4. Measure the diameter of the **field of view.** Once you determine the size of the field of view for any combination of ocular and objective lenses, you can determine the size of any structure within that field.

a. Rotate the 4× objective into position and remove the letter slide.

b. Place a clear ruler on the stage, and focus on its edge.

Students must estimate the distance between mm marks on the ruler. These may not be in sharp focus.

c. The distance between two lines on the ruler is 1 mm. What is the diameter (mm) of the field of view?

4 mm

If your microscopes have a mechanical stage, you can tape a section cut from a clear ruler to a microscope slide. Try this with your microscope first.

d. Convert this measurement to micrometers (μm), a more commonly used unit of measurement in microscopy (1 mm = 1,000 μm).

e. Measure the diameters of the field of view for the 10× and 40× objectives, and enter all three in the spaces below to be used for future reference.

4× = 4,000 μm 10× = 1,900 μm 40× = 800 μm

f. What is the relationship between the size of the field of view and magnification?

As the magnification increases, the field of view decreases.

5. Determine spatial relationships. The **depth of field** is the thickness of the specimen that may be seen in focus at one time. Because the depth of focus is very short in the compound microscope, focus up and down to clearly view all planes of a specimen.

Encourage students to adjust the light so that they have good contrast for the fibers in the threads.

a. Rotate the 4× objective into position and remove the ruler. Take a slide of crossed threads, wipe it with a Kimwipe®, and place the slide on the stage. Center the slide so that the region where the two threads cross is in the center of the stage opening.

b. Focus on the region where the threads cross. Are both threads in focus at the same time?

yes

c. Rotate the 10× objective into position and focus on the cross. Are both threads in focus at the same time?

no

Does the 4× or the 10× objective have a shorter depth of field?

10×

d. Focus upward (move the stage up) with the coarse adjustment until both threads are just out of focus. Slowly focus down using the fine adjustment. Which thread comes into focus first? Is this thread lying under or over the other thread?

under

Use a mixture of slides in the lab that vary for the color of thread on top.

e. Rotate the 40× objective into position and slowly focus up and down, using the fine adjustment only. Does the 10× or the 40× objective have a shorter depth of field?

40×

6. At the end of your microscope session, use the following procedures to store your microscope.
   a. Rotate the 4× objective into position.
   b. Remove the slide from the stage.
   c. Return the phase-contrast condenser to the 0 setting if you have used phase-contrast.
   d. Set the light intensity to its lowest setting and turn off the power.
   e. Unplug the cord and wrap it around the base of the microscope.
   f. Replace the dust cover.
   g. Return the microscope to the cabinet using two hands; one hand should hold the arm, and the other should support the base.

These steps should be followed every time you store your microscope.

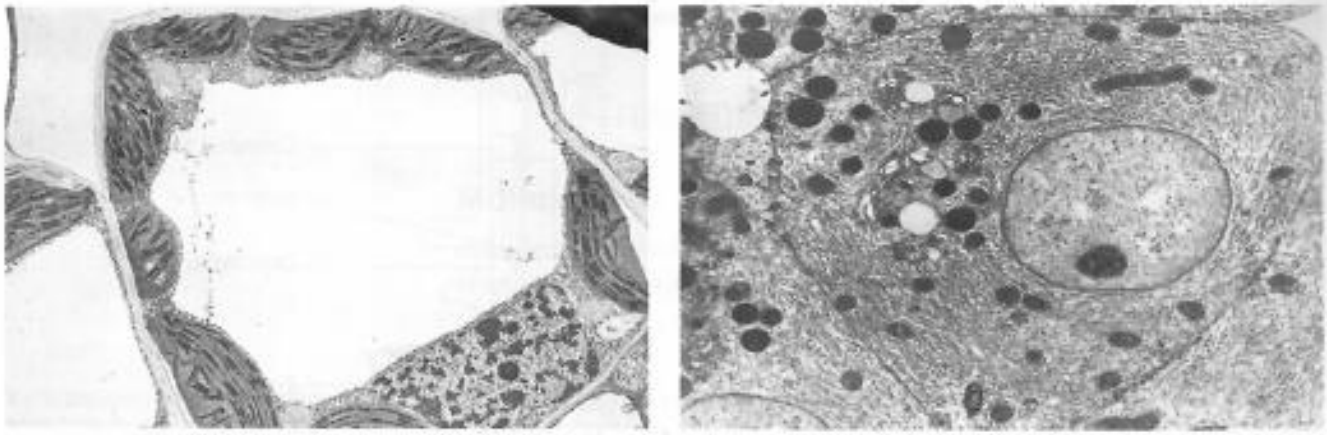
EXERCISE 2.3
The Stereoscopic Microscope

Materials

stereoscopic microscope
dissecting needles
living *Elodea*
microscope slides
droppers of water
coverslips

Introduction

The stereoscopic (dissecting) microscope has relatively low magnification, 7× to 30×, and is used for viewing and manipulating relatively large objects. The binocular feature creates the stereoscopic effect. The stereoscopic microscope is similar to the compound microscope except in the following ways: (1) The depth of field is much greater than with the compound microscope, so objects are seen in three dimensions, and (2) the light source can be directed down onto as well as up through an object, which permits the viewing of objects too thick to transmit light. Light directed down on



a. b.

FIGURE 2.5
Cells as seen in a transmission electron microscope. (a) Electron micrograph of a plant cell. (b) Electron micrograph of a rat pancreas cell. The large dark bodies in the cytoplasm are secretory globules.

5. When a transmission electron microscope is used, cells are usually studied using electron micrographs, photographs taken of the image seen on the fluorescent screen. Observe the electron micrographs in Figure 2.5a and b, respectively, a plant cell and an animal cell. Other micrographs may be on demonstration in the laboratory, and also check your lecture text for examples. Working with your lab partner, see if you can identify and label the following organelles and structures in Figure 2.5 and in other micrographs on demonstration in the laboratory.

   plasma membrane, cell wall, nucleus, chloroplast, mitochondria, large central vacuole, Golgi apparatus, lysosome, endoplasmic reticulum, ribosome

   Predict which of these organelles will *not* be visible when studying plant and animal cells using the *light* microscope. *Underline* those structures in the above list. Return to this activity after you have completed Exercise 2.5, Lab Study C, to confirm your predictions.

EXERCISE 2.5
The Organization of Cells

In this exercise, you will examine the features common to all eukaryotic cells that are indicative of their common ancestry. However, you will observe that all cells are not the same. Some organisms are **unicellular** (single-celled), with all living functions (respiration, digestion, reproduction, and excretion) handled by that one cell. Others form random, temporary **aggregates,** or clusters, of cells. Clusters composed of a consistent and predictable number of cells are called **colonies.** Simple colonies are clusters of cells of similar types with a predictable structure, but the cells

have no physiological connections. More complex colonies have cells of different types. In some colonial algae the cells are called *somatic cells* (cells that are not reproductive) and *reproductive cells* (cells that specialize in reproduction). In these colonies, if *either* type of cell is isolated from the colony, it may be reproductive, dividing and producing new colonies.

Other algae may contain both cell types, somatic and reproductive, but their somatic cells *never* become reproductive, even when isolated. Furthermore, their reproductive cells cannot persist independently, but must be associated with somatic cells to live. These algae are described as **multicellular.** They demonstrate the following two defining features:

- Multicellular organisms consist of two or more types of cells with specialized structure and function.
- If any one of the cell types of the organism is isolated, it is not capable of perpetuating the species in nature.

In more complex algae, fungi, plants, and animals, specialized cell types may be organized into *tissues* that perform particular functions for the organism. Tissues, in turn, may combine to form *organs*, and tissues and organs combine to form a coordinated single *organism*.

In this exercise, you will examine selected unicellular, aggregate, colonial, and multicellular organisms.

Lab Study A. Unicellular Organisms

Materials

microscope slides
culture of *Amoeba*
living termites
forceps
coverslips
dissecting needles
insect Ringers

Introduction

Unicellular eukaryotic organisms may be **autotrophic** (photosynthetic) or **heterotrophic** (deriving food from other organisms or their by-products). These diverse organisms, called protists, will be studied in detail in Lab Topic 14.

Procedure

1. Examine a living *Amoeba* (Figure 2.6) under the compound microscope. Amoebas are aquatic organisms commonly found in ponds. To transfer a specimen to your slide, follow these procedures:
   a. Place the culture dish containing the amoeba under the dissecting microscope, and focus on the bottom of the dish. The amoeba will appear as a whitish, irregularly shaped organism attached to the bottom.

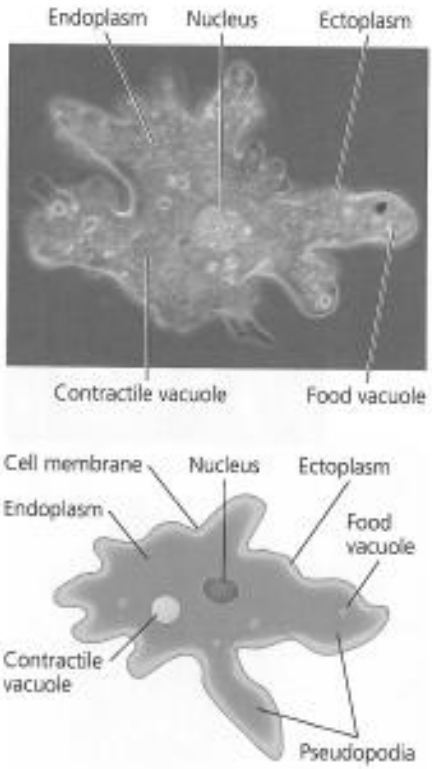


FIGURE 2.6
Amoeba. An amoeba moves using pseudopodia. Observe the living organisms using the compound microscope.

b. Using a clean pipette (it is important not to interchange pipettes between culture dishes), transfer a drop with several amoebas to your microscope slide. To do this, squeeze the pipette bulb *before* you place the tip under the surface of the water. Disturbing the culture as little as possible, pipette a drop of water with debris from the *bottom* of the culture dish. You may use your stereoscopic microscope to scan the slide to locate amoebas before continuing.

c. Cover your preparation with a clean coverslip.

d. Under low power on the compound scope, scan the slide to locate an amoeba. Center the specimen in your field of view; then switch to higher powers.

e. Identify the following structures in the amoeba:

The **cell membrane** is the boundary that separates the organism from its surroundings.

Ectoplasm is the thin, transparent layer of cytoplasm directly beneath the cell membrane.

Endoplasm is the granular cytoplasm containing the cell organelles.

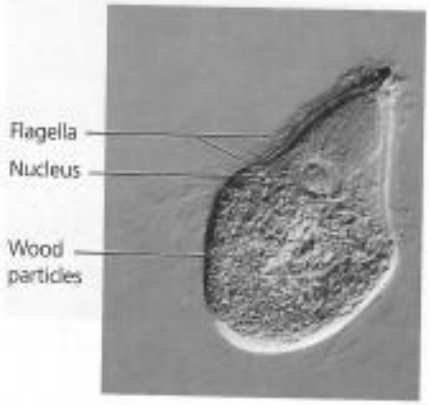
The nucleus is the grayish, football-shaped body that is somewhat granular in appearance. This organelle, which directs the cellular activities, will often be seen moving within the endoplasm.

Contractile vacuoles are clear, spherical vesicles of varying sizes that gradually enlarge as they fill with excess water. Once you've located a vacuole, watch it fill and then empty its contents into the surrounding environment. These vacuoles serve an excretory function for the amoeba.

Food vacuoles are small, dark, irregularly shaped vesicles within the endoplasm. They contain undigested food particles.

Pseudopodia ("false feet") are fingerlike projections of the cytoplasm. They are used for locomotion as well as for trapping and engulfing food in a process called **phagocytosis**.

Amoebas appear gray and may be near debris. The fast-swimming protozoans are food. Students having trouble locating an amoeba should look at other students' specimens so they have a good "search image." If a microscope/camera/TV setup is available, use a successful student's slide to show an amoeba to all students.



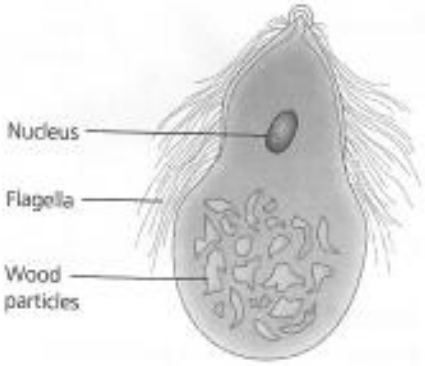


FIGURE 2.7
Trichonympha. A community of microorganisms, including *Trichonympha*, inhabits the intestine of the termite. Following the procedure in Exercise 2.5, Lab Study A, disperse the microorganisms and locate the cellular structures in *Trichonympha*.

MB Student Media Videos—Ch. 28: Amoeba; Amoeba Pseudopodia

2. Examine *Trichonympha* under a compound microscope. You will first have to separate the *Trichonympha* (Figure 2.7) from the termite with which it lives in a symbiotic relationship. *Trichonympha* and other organisms occupy the gut of the termites, where they digest wood particles eaten by the insect. Termites lack the enzymes necessary to digest wood and are dependent on *Trichonympha* to make the nutrients in the wood available to them. *Trichonympha* has become so well adapted to the environment of the termite's gut that it cannot survive outside of it.

To obtain a specimen:

a. Place a couple of drops of **insect Ringers** (a saline solution that is isotonic to the internal environment of insects) on a clean microscope slide.

b. Using forceps or your fingers, transfer a termite into the drop of Ringers.

c. Place the slide under the dissecting microscope.

d. Place the tips of dissecting needles at either end of the termite and pull in opposite directions.

e. Locate the long tube that is the termite's intestine. Remove all the larger parts of the insect from the slide.

f. Using a dissecting needle, mash the intestine to release the *Trichonympha* and other protozoa and bacteria.

g. Cover your preparation with a clean coverslip.

h. Transfer your slide to the compound microscope and scan the slide under low power. Center several *Trichonympha* in the field of view and switch to higher powers.

Several types of protozoans and bacteria will be present in the termite gut.

i. Locate the following structures under highest power.

Flagella are the long, hairlike structures on the outside of the organism. The function of the flagella is not fully understood. Within the gut of the termite, the organisms live in such high density that movement by flagellar action seems unlikely and perhaps impossible.

The **nucleus** is a somewhat spherical organelle near the middle of the organism.

Wood particles may be located in the posterior region of the organism.

Lab Study B. Aggregate and Colonial Organisms

Materials

microscope slides
dissecting needles
forceps
coverslips
cultures of *Protococcus* and *Scenedesmus*

Introduction

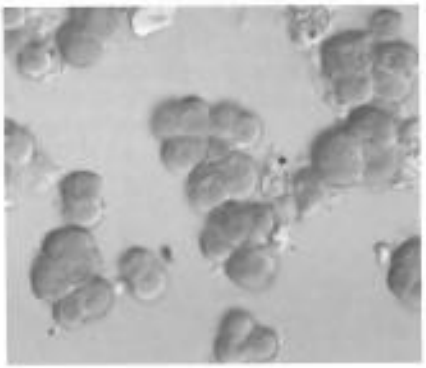
Unlike unicellular organisms, which live independently of each other, colonial organisms are cells that live in groups and are to some degree dependent on one another. The organisms studied in this exercise show an increasing degree of interaction among cells.

Procedure

1. Examine *Protococcus* under the compound microscope. *Protococcus* (Figure 2.8) is a terrestrial green alga that grows on the north sides of trees and is often referred to as "moss."

   a. To obtain a specimen, use a dissecting needle to brush off a small amount of the green growth on the piece of tree bark provided into a drop of water on a clean microscope slide. Avoid scraping bark onto the slide. Cover the preparation with a clean coverslip.

Protococcus cells are small and clumped. Students should disperse the cells well and view only a few green cells.



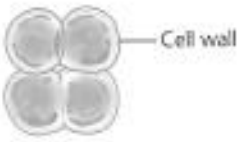


FIGURE 2.8
Protococcus. *Protococcus* is a terrestrial green alga that forms loose aggregates on the bark of trees.

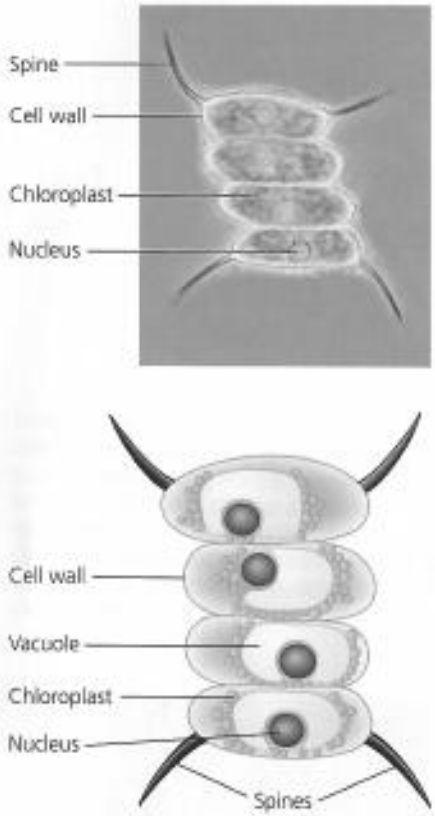


FIGURE 2.9
Scenedesmus. *Scenedesmus* is an aquatic alga that usually occurs in simple colonies of four cells connected by the cell wall.

b. Observe at highest power that these cells are **aggregates:** The size of the cell groupings is random, and there are no permanent connections between cells. Each cell is surrounded by a cell membrane and an outer **cell wall**.

c. Observe several small cell groupings and avoid large clumps of cells. Cellular detail may be obscure.

2. Examine living *Scenedesmus* under the compound microscope. *Scenedesmus* (Figure 2.9) is an aquatic green alga that is common in aquaria and polluted water.

a. To obtain a specimen, place a drop from the culture dish (using a clean pipette) onto a clean microscope slide, and cover it with a clean coverslip.

b. Observe that the cells of this organism form a **simple colony:** The cells always occur in groups of from four to eight cells, and they are permanently united.

c. Identify the following structures.

The **nucleus** is the spherical organelle in the approximate middle of each cell.

Vacuoles are the transparent spheres that tend to occur at either end of the cells.

Spines are the transparent projections that occur on the two end cells.

Cell walls surround each cell.

Lab Study C. Multicellular Organisms

Materials

microscope slides
dropper bottles of water
toothpicks
coverslips
Elodea
methylene blue
finger bowl with disinfectant
broken glass chips
Volvox cultures

Introduction

Review the criteria for characterizing an organism as multicellular in the introduction of Exercise 2.5 on pp. 46–47. Recall that multicellular organisms have two or more cell types with specialized structure and function that cannot persist when isolated from other cells in the organism. If these cells are isolated, they are not capable of perpetuating the species. In this lab study, you will examine an example of a green alga, a plant, and an animal to investigate the criteria for multicellularity and observe cells that compose basic tissue types.

Procedure

Volvox

Volvox (Figure 2.10) is an aquatic green alga that is common in aquaria, ponds, and lakes. In older literature this organism was described as colonial and was not considered to be multicellular. Today, however, scientists have concluded that it is more accurate to call *Volvox* multicellular. In this activity you will look for evidence that supports this conclusion.

1. Examine living *Volvox* under the compound microscope. To obtain a specimen, prepare a wet mount as you did for *Scenedesmus* with the following addition: Before placing a drop of the culture on your slide, place several glass chips on the slide. This will keep the coverslip from crushing these spherical organisms.
2. Observe that the cells of this organism lie in a transparent matrix forming a large hollow sphere. The approximately 500 to 50,000 (depending on the species) nonreproductive somatic cells are permanently united by cytoplasmic connections. These cells have chloroplasts for photosynthesis and flagella that beat in a coordinated motion to move the colony like a ball. During asexual reproduction, certain cells in the sphere (reproductive cells) enlarge and migrate inward to become daughter colonies.
3. Identify the following structures: **somatic cells** with **cytoplasmic connections** and **flagella.** Depending on the magnification of your microscope, you may be able to distinguish **cell walls** and **nuclei** in the cells. **Daughter colonies** are smaller spheres within the larger colony. These are released when the parent colony disintegrates.

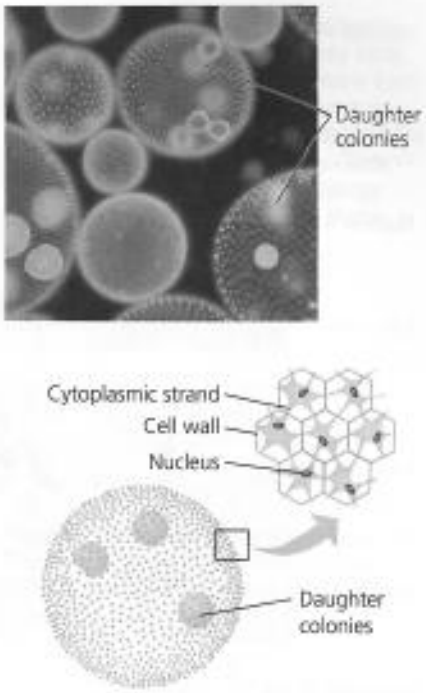


FIGURE 2.10

Volvox. In this organism, the individual cells are interconnected by cytoplasmic strands to form a sphere. Small clusters of cells, called daughter colonies, are specialized for reproduction.

MB Student Media Video—Ch. 28: *Volvox* Colony

Plant Cells

1. The major characteristics of a typical plant cell are readily seen in the leaf cells of *Elodea*, a common aquatic plant (Figure 2.11). Prepare a wet mount and examine one of the youngest (smallest) leaves from a sprig of *Elodea* under the compound microscope.
2. Identify the following structures.

   The **cell wall** is the rigid outer framework surrounding the cell. This structure gives the cell a definite shape and support. It is not found in animal cells.

   Protoplasm is the organized contents of the cell, exclusive of the cell wall.

   Cytoplasm is the protoplasm of the cell, exclusive of the nucleus.

   The **central vacuole** is a membrane-bound sac within the cytoplasm that is filled with water and dissolved substances. This structure serves to store metabolic wastes and gives the cell support by means of turgor pressure. Animal cells also have vacuoles, but they are not as large and conspicuous as those found in plants.

   Chloroplasts are the green, spherical organelles often seen moving within the cytoplasm. These organelles carry the pigment chlorophyll that is involved in photosynthesis. As the microscope light heats up the cells, cytoplasm and chloroplasts may begin to move around the central vacuole in a process called *cytoplasmic streaming*, or *cyclosis*.

   The **nucleus** is the usually spherical, transparent organelle within the cytoplasm. This structure controls cell metabolism and division.
3. What three structures observed in *Elodea* are unique to plants?

   chloroplast, cell wall, large central vacuole

The nucleus may be difficult to see because the chloroplasts are abundant in the cytoplasm, obstructing the view of the nucleus. The majority of the cell is filled by the clear vacuole. Strands of grainy cytoplasm may be visible in cells with few chloroplasts. The nucleus will appear as a clear oval, often in a corner of the cell.

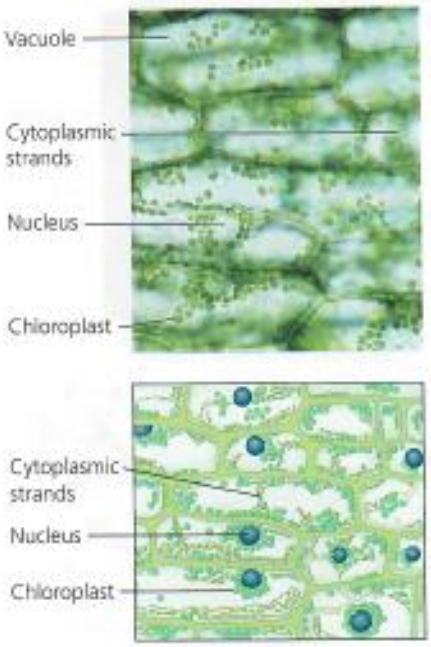


FIGURE 2.11
Elodea. *Elodea* is an aquatic plant commonly grown in freshwater aquaria. The cell structures may be difficult to see because of the three-dimensional cell shape and the presence of a large central vacuole.

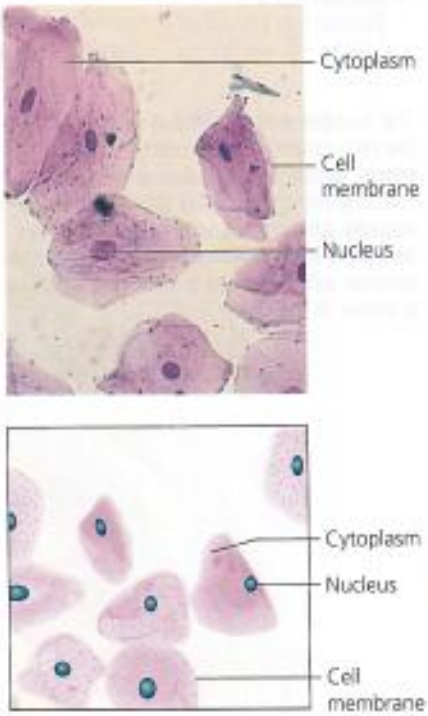


FIGURE 2.12
Human epithelial cells. The epithelial cells that line your cheek are thin, flat cells that you can remove easily from your cheek by scraping it with a toothpick.

4. Compare your observations of *Elodea* using the compound scope with those made in Exercise 2.3 using the stereoscopic scope. List the structures seen with each:

   Stereoscopic:

   cell outlines; chloroplasts as green pigment, but probably cannot distinguish

   Compound:

   cell walls, nuclei, central vacuole, chloroplasts

MB Student Media Video—Ch. 6: Cytoplasmic Streaming

Animal Cells

1. Animals are multicellular heterotrophic organisms that ingest organic matter. They are composed of cells that can be categorized into four major tissue groups: epithelial, connective, muscle, and nervous tissue. In this lab study, you will examine epithelial cells. Similar to the epidermal cells of plants, **epithelial cells** occur on the outside of animals and serve to protect the animals from water loss, mechanical injury, and foreign invaders. In addition, epithelial cells line interior cavities and ducts in animals. Examine the epithelial cells (Figure 2.12) that form the lining of your inner cheek. To obtain a specimen, follow this procedure:
   a. With a clean toothpick, gently scrape the inside of your cheek several times.
   b. Roll the scraping into a drop of water on a clean microscope slide, add a small drop of methylene blue, and cover with a coverslip. Discard the used toothpick in disinfectant.
   c. Using the compound microscope, view the cells under higher powers.
2. Observe that these cells are extremely flat and so may be folded over on themselves. Attempt to locate several cells that are not badly folded, and study their detail.
3. Identify the following structures.

   The **cell membrane** is the boundary that separates the cell from its surroundings.

   The **nucleus** is the large, circular organelle near the middle of the cell.

   Cytoplasm is the granular contents of the cell, exclusive of the nucleus.

Lab Study D. Unknowns

Materials

microscope slides
coverslips
pond water or culture of unknowns

Introduction

Use this lab study to see if you have met the objectives of this lab topic. As you carry out this lab study, (1) think carefully about using correct

microscopic techniques; (2) distinguish organisms with different cellular organization or configuration (unicellular, colonial, etc.); (3) note how the different organisms are similar yet different; and (4) note cell differences.

All of the cells studied to this point in this lab topic have been examples of **eukaryotic** cells. As you examine drops of pond water as described in the following procedure, you may observe examples of prokaryotic cells in colonies or filaments. Eukaryotic cells have a true nucleus containing chromosomes with genetic material separated from the remainder of the cell by a nuclear envelope. All cellular organelles are also bound by membranes. In prokaryotic cells genetic material is not bound by a nuclear envelope, and no membrane-bound organelles are present. Prokaryotic cells will be studied in more detail in Lab Topic 13 Bacteriology.

Ask students to bring their own pondwater samples. This is the students' favorite study. Mixed protozoan cultures are available from supply houses for those students who are unsuccessful with their own cultures. See the Preparation Guide. The Preparation Guide can be downloaded at masteringbiology.com in Instructor Resources, Lab Media.

Procedure

1. Examine several drops of the culture of pond water that you collected, or examine the unknown culture provided by the instructor.
2. Record in Table 2.1 the characteristics of at least four different organisms.
3. Determine if a well-defined nucleus and organelles are present (eukaryote).

Identifying organisms is not important; however, some students will be curious. Provide resources or diagrams of common organisms.

TABLE 2.1 Characteristics of Organisms Found in Pond Water

Unknown	Means of Locomotion	Cell Wall (+/–)	Chloroplasts (+/–)	Cellular Organization	Eukaryote (yes or no)
1					
2					
3					
4					
5					

Reviewing Your Knowledge

1. Describe at least two types of materials or observations that would necessitate the use of the stereoscopic microscope.

   small insects, small flower structures, surface features of plants and animals

2. What characteristics do all eukaryotic cells have in common?

   nucleus, cytoplasm, plasma membrane, mitochondria, etc.

3. a. What cellular features differentiate plants from animals?

   chloroplast, cell wall, large central vacuole

   b. How are the structures that are unique to plants important to their success?

   The chloroplast is necessary for photosynthesis; the cell wall provides the rigid support provided in animals by a skeleton; the large vacuole provides for storage and flexible support by turgor pressure.

4. Return to Step 5, p. 46. Based on your observations in today's laboratory, *circle* those organelles that are visible in the light microscope. Compare your observations with your initial predictions.
5. Review the criteria used to distinguish between colonial and multicellular organisms. Why is *Volvox* now considered multicellular?

Volvox has two cell types (somatic and reproductive) specialized for different functions. Isolated differentiated somatic cells are not capable of perpetuating the species.

Applying Your Knowledge

1. In your own words, describe the evolutionary trend for increasing organismal complexity, using examples from this lab to illustrate your answer.

In the history of life, the organization of cells and organisms has become increasingly complex. The first eukaryotic organisms that evolved were single-celled, represented in this lab by the amoeba. Aggregates (loose clusters of unconnected cells) existed later, represented by *Protococcus*. Simple colonies evolved with structural connections (*Scenedesmus*), and eventually more complex colonies and multicellularity evolved. Multicellularity evolved several times, giving rise to plants (*Elodea*), animals (humans), and fungi.

2. We often imply that multicellular organisms are more advanced (and therefore more successful) than unicellular or colonial organisms. Explain why this is not true, using examples from this lab or elsewhere.

Complex organisms should not be thought of as advanced in the sense of better adapted; rather, they originated later in the history of life than simple organisms. Extant organisms, such as humans and amoebas, are obviously adapted to a successful way of life today.

3. Following is a list of tissues that have specialized functions and demonstrate corresponding specialization of subcellular structure. Match the tissue with the letter of the cell structures and organelles listed to the right that would be abundant in these cells. Use your text for a description of any organelles or structures that are unfamiliar to you.

Tissues

- Enzyme (protein)-secreting cells of the pancreas

  c, e, h
- Insect flight muscles

  b
- Cells lining the respiratory passages

  f
- White blood cells that engulf and destroy invading bacteria

  i
- Leaf cells of cacti

  d, g

Cell Structures and Organelles

a. plasma membrane
b. mitochondria
c. Golgi apparatus
d. chloroplast
e. endoplasmic reticulum
f. cilia and flagella
g. vacuole
h. ribosome
i. lysosome