LAB TOPIC 4

Using the Microscope

Introduction

The invention of the microscope was the first technological breakthrough in the study of biology. Although anatomists could investigate the basic architecture of organisms, very little could be learned about their composition before the microscope came into use. It made possible the discovery that all living organisms are composed of cells and that all cells are similar in some ways. It also enabled scientists to learn how various types of cells are different from each other. A light microscope can magnify specimens up to 1500×. Since a typical animal cell might be approximately 0.02 mm in diameter and the unaided eye can only distinguish objects as small as about 0.2 mm in diameter, the microscope is an essential tool for studying cells and subcellular components.

You will be using two types of microscopes, the compound light microscope and the stereoscopic dissecting microscope. Effective use of these microscopes is one of the fundamental skills required for studying biology, and it takes some practice to master this skill. This laboratory will show you how to use these instruments to their best advantage.

Outline

EXERCISE 4.1

The Compound Microscope

Objectives

After completing this exercise, you should be able to

1. Determine total magnification for the lenses on your microscope.
2. Explain the difference between magnification and resolving power.
3. Identify the parts of the compound microscope and give the function of each part.
4. Describe the procedure you would follow to locate and focus on any specimen using the scanning, low-power, or high-power objective.
5. Define field of view and describe how the field of view changes with magnification.
6. Define depth of focus and explain how it changes with magnification and how to adjust for it when viewing specimens.

The compound light microscope has two sets of lenses: the eyepiece, which is near the eye, and the objective, which is near the specimen. The total magnification of the specimen is calculated by multiplying the magnification of each set of lenses:

total magnification =
magnification of objective × magnification of eyepiece

The eyepiece magnification is usually fixed, and ranges from 8 times (8×) to 15×, depending upon the microscope model. The objective magnification is changeable. Compound microscopes generally have three to five different objectives. Typical magnifications are 4× (scanning power), 10× (low power), 45× (high power), and 100× (oil immersion). The magnification is engraved on each objective.

Calculate the total magnification for the lens combinations on your microscope:

Scanning power: eyepiece _____× times objective _____× = _____×

Low power: eyepiece _____× times objective _____× = _____×

High power: eyepiece _____× times objective _____× = _____×

Oil immersion: eyepiece _____× times objective _____× = _____×

Although theoretically we could put even higher powered lenses on microscopes to achieve greater magnifications, there is a practical limit imposed by the resolving power of the optical system. The **resolving power** of a microscope is its ability to distinguish two objects that are very close together as separate; it is what allows us to see detail.

When two objects cannot be distinguished as separate, the image looks blurry. Resolving power is thus a measure of clarity. Two microscopes may give the same magnification, yet the clarity of images provided by one may be better due to superior resolving power, which in turn is a function of the quality of the objective lens.

Activity A: The Parts of the Compound Microscope

Although there are numerous variations in the features of different models of compound microscopes, they are all constructed on a common plan. Figure 4.1 shows a typical model.

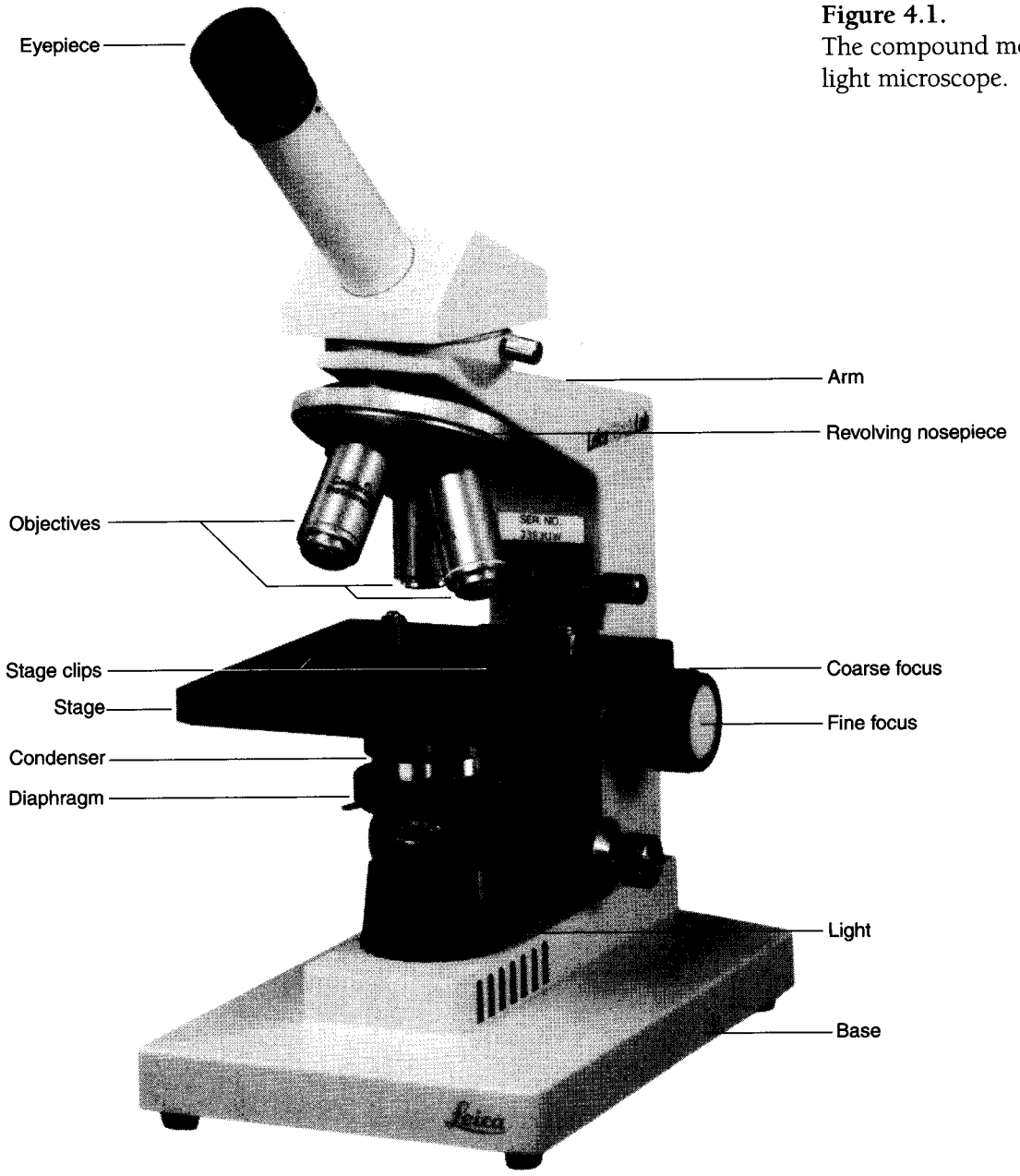

Figure 4.1.
The compound monocular light microscope.

Locate the following parts on your microscope. The descriptions of parts include the common variations found in different models of microscopes. In the space below each item, answer the questions about the microscope you will be using.

Eyepiece

The eyepiece, also called the ocular, is the topmost lens system, and serves to magnify the image of the specimen. Some models have one eyepiece (monocular). When using a monocular scope, it is recommended that you leave the other eye open (if you can master this technique) or else cover the other eye with one hand, rather than squinting with one eye tightly shut. Binocular (two-eyepiece) models are also available.

How many eyepieces does your microscope have?

Is it monocular or binocular?

On a binocular microscope, the eyepieces can be moved closer together or farther apart to match the distance between the user's pupils. There is sometimes also a diopter ring on one eyepiece, which allows you to adjust the focus for that eye before focusing with both eyes.

Objectives

The objectives magnify and resolve the image of the specimen. They are set in a revolving nosepiece so that the user can readily change lenses. Most microscopes have a ring above the lenses. Use this to turn the nosepiece rather than grabbing the lenses themselves. You should feel the lens click into place when it is engaged. This is the **working position.**

What are the magnifications of the objective lenses on your microscope?

Stage

The slide holding the specimen is placed on the stage and secured with clips. There is a hole in the center of the stage to allow light to pass through. On most microscopes, you move the slide around the stage manually in order to locate areas of interest on the specimen. Other models, however, have a movable stage. Once the slide is clipped in place, you rotate knobs to move the slide from side to side or toward or away from yourself.

Does your microscope have a movable stage?

Light

The specimen is illuminated by substage lighting. If the light source is built in, the light is focused into a beam by a **condenser** located just below the stage. Other models use an external light source; you adjust a mirror to direct the light upward through the stage. A **diaphragm** is used to control

the aperture (opening), or width of the beam of light that reaches the specimen. Some microscopes allow the user to vary the aperture continually by using a lever. Other models have a wheel with fixed apertures of various sizes; you turn the wheel to select the appropriate size.

Describe the illumination system for your microscope:

Focus Knobs

Once the slide containing the specimen is mounted on the stage, you look through the eyepiece and adjust the focus using first the coarse-focus knob (to bring the specimen into view) and then the fine-focus knob (to sharpen the image). On most microscopes, turning a focus knob toward you raises the objective, and turning it away from you lowers the objective. On some microscopes, however, the stage, rather than the objectives, moves up and down.

Describe how the focus knobs on your microscope work:

Support Structure

The body of the microscope is constructed to protect the lenses and the focusing system and to provide a sturdy, vibration-free support stand.

You should never attempt to remove the lenses or any other part of the microscope.

When working with a microscope, observe the following rules:

1. Carry the microscope with two hands. Hold the arm with one hand and use your other hand to support the base.
2. Keep all parts of the microscope dry. If you spill anything on the stage, wipe it up immediately.
3. If the lenses need to be cleaned, always use lens paper. Never use paper towels, tissues, or cleaning tissues. They will scratch the lenses.
4. When you are finished using the microscope, remove the slide and turn the lowest power objective to the working position.

Activity B: Using the Compound Microscope

Before you begin using the microscope to examine unfamiliar creatures, you will first do an exercise using a recognizable object, the letter "e," to help you learn to use the microscope effectively. The keys to successful microscope work are locating the specimen, focusing on the specimen, and adjusting the light for the best contrast and resolution.

If you wear glasses, you should remove them to look through the microscope unless they correct an astigmatism.

Procedure

1. Turn on the light source and make sure the lowest power (scanning power, if available) objective is in the working position.
2. Place the "e" slide on the stage, centering the "e" over the hole in the stage. Secure the slide with the clips.
3. Look at the slide on the stage from the side (*not* through the eyepiece) and turn the coarse-focus knob until the objective is as close to the stage as it will go. (Most microscopes have a built-in stop to prevent you from crashing the lens into the stage, but be careful in case yours doesn't.)
4. If your microscope is binocular, adjust the distance between the eyepieces by pushing them closer together or moving them farther apart until you can comfortably see one image. If there are markings on the microscope, record the setting so you can preset it in the future:

5. Look through the eyepiece(s) and slowly move the stage and objective farther apart by turning the coarse-focus knob until you have the "e" or some part of it in focus. Move the slide to center the "e" in your **field of view** (the area of the image that you see).

You may see a black line in the field of view. This is a pointer. It allows you to indicate a structure that you want someone else to view.

6. If you are using a monocular microscope or a binocular microscope that doesn't have a focusing ring for one eyepiece, use the fine-focus knob to bring the image into sharp focus.

   If you are using a binocular scope that has a focusing ring, first look only through the eyepiece that doesn't have the focusing ring. If the focusing ring is on the left eyepiece, look through the right eyepiece and cover the left eyepiece. Focus the "e" for that eye using the fine-focus knob. Then look through the eyepiece with the focusing ring, keeping the other eye covered. Adjust the focusing ring until the image is sharp. Finally, view the image with both eyes.

   How is the image of the "e" oriented compared to the image of the "e" on the slide itself?

   Look through the eyepiece and move the slide to your right.

What happens to the "e"?

What happens when you move the slide toward you?

7. Open the diaphragm all the way (or set the disk to the largest aperture), and then slowly close it (or choose smaller and smaller apertures in the disk). You can see that the amount of light affects the image you receive. In general, you should use a smaller opening for scanning and low-power objectives and a larger opening for high-power and oil-immersion objectives. However, the type of specimen also affects how much light is needed, so it is best for you to try different settings. Begin with the largest aperture and reduce the aperture until the image has good contrast, but you are still able to see details.
8. Bring the low-power objective into the working position. If your microscope is parfocal, you should only need to adjust the focus a little using the fine-focus knob. **Parfocal** means that when an image is focused with one objective, it will also be in focus in the other objectives. Center the "e" in your field of view.

   Can you still see the entire "e"?

9. Now turn the high-power objective to the working position, being careful not to bang the objective on the stage clips as you turn it.

This objective leaves very little space between the slide and the objective. You should use only the fine-focus knob when you are on high power.

Describe what you see at this magnification.

How does the field of view at low power compare to the field of view at high power?

Steps 10–15 will give you an idea of the relative fields of view that you see with your microscope.

10. Put the scanning (or lowest power) objective in the working position.
11. Turn the coarse-focus knob so that it is as close to the stage as it will go.

12. Place a transparent ruler across the stage and focus on the edge that has millimeter markings.

If you can't bring the markings into focus, put a clean glass slide under the ruler to raise it closer to the lens.

13. Move the ruler so that one of the markings is at the very edge of your field of view (see Figure 4.2). Each marked interval on the ruler is 1 mm. Estimate the diameter of the field of view at this magnification.

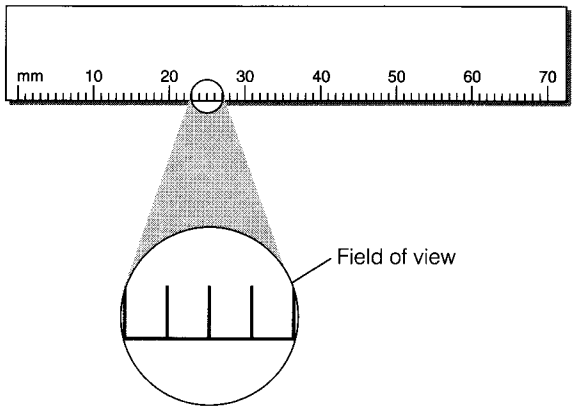

Figure 4.2.
Alignment of ruler to measure the field of view.

Magnification: _____

Diameter of field of view: _____

14. Change to the next higher objective and estimate the diameter of the field of view.

Magnification: _____

Diameter of field of view: _____

If your microscope only has low-power and high-power objectives, omit step 15.

15. Measure the field of view again at the next higher objective.

Magnification: _____

Diameter of field of view: _____

Obviously, microscopic images are too small to be measured with a ruler. Instead of expressing measurements in millimeters, they are usually expressed as micrometers (μm). 1 μm = 1×10^{-6} m. Since 1 mm = 1×10^{-3} m, 1 mm = 1×10^{3} μm. For example, to convert 5 mm to μm:

$$5 \text{ mm } (1 \times 10^{3} \text{ μm/mm}) = 5 \times 10^{3} \text{ μm} = 5000 \text{ μm}$$

If you are not familiar with these units or working with scientific notation, see Appendix A.

Convert the field of view measurements you recorded above to micrometers:

Magnification: _____

Diameter of field of view: _____

Magnification: _____

Diameter of field of view: _____

Magnification: _____

Diameter of field of view: _____

Why is this technique for measuring the field of view not useful for an oil immersion lens?

Activity C: Depth of Focus

Although a specimen mounted on a slide appears to be completely two-dimensional, there is actually 3 to 10 µm of depth. That might not sound like a lot, but keep in mind that the specimens are magnified up to 1000×. At higher magnifications, the lens will not focus on the entire specimen at once (Figure 4.3). It is therefore useful to focus up and down through the entire depth of a specimen using the fine-focus knob, especially when you are using high power. The thickness of the specimen that can be seen in focus at any time is called the **depth of focus.** You will investigate depth of focus in the following procedure.

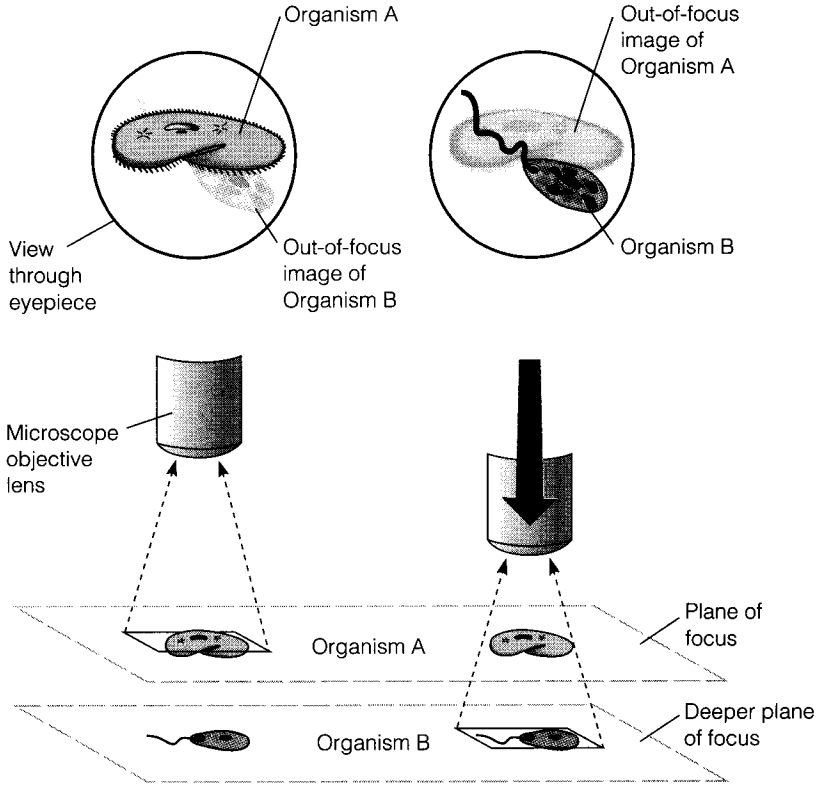

Figure 4.3.
Depth of focus. When a specimen is thick, only part of it can be in focus at a time.

You will also learn the technique for making a **wet mount**, which simply means that you place your specimen in a drop of water (or another liquid) on a slide and add a coverslip. The coverslip flattens the specimen, giving the slide a uniform depth for easier viewing, and protects the lens from getting wet.

Procedure

1. Get a clean microscope slide. With forceps, pull a *tiny* amount of cotton off of a cotton ball and put it on the middle of the slide.
2. Put a drop or two of *Paramecium* culture onto the cotton. (Hint: get a drop from the bottom of the container.)
3. Add a coverslip, using a dissecting needle (as shown in Figure 4.4) to lower the coverslip onto the slide. The trick is to do it slowly enough to keep any air bubbles from being trapped under the coverslip.

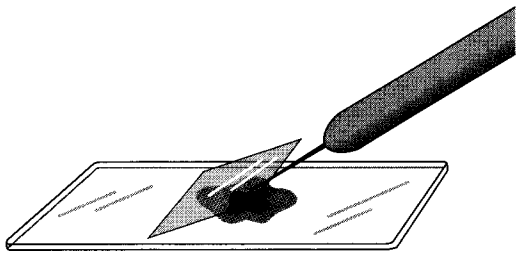

Figure 4.4.
Making a wet mount.

4. Put the slide on the stage of the microscope and focus using scanning power. Of the cotton threads that appear in your field of view, how many are in focus?

5. Try different apertures with the scanning power objective. Which aperture is best for this objective?

6. Change the objective to low power. Remember, if your microscope is parfocal you should only have to adjust the fine focus a little. How many of the cotton threads are in focus now?

   Try different apertures with the low-power objective. Which aperture is best for this objective?

7. Change the objective to high power. You may need to adjust the fine focus slightly, but don't touch the coarse focus! Now how much of the cotton is in focus?

   Use the fine-focus knob to focus all the way down to the bottom thread, then back up.

8. Try different apertures with the high-power objective. Which aperture is best for this objective?

9. Locate a *Paramecium* and watch it swim among the threads.

EXERCISE 4.2

Observing Cells

Objectives

After completing this exercise, you should be able to

1. Explain what stains are and why they are often used with biological materials.
2. Draw a cheek cell and identify its nucleus and plasma membrane.
3. Draw an *Elodea* cell and identify the chloroplasts and cell wall.

Activity A: Animal Cells

Animal cells contain numerous organelles—such as Golgi bodies, endoplasmic reticulum, lysosomes, microbodies, and mitochondria—that are too small to be seen with the compound light microscope. The only animal organelle that is reliably visible by compound light microscopy is the nucleus, which may be 4–6 μm in diameter. You can also tell where the plasma membrane is (though you can't see it, since it's less than 0.01 μm thick) because it forms a boundary around each cell.

Most animal cells are relatively colorless and appear transparent under the light microscope. We can tag certain structures by attaching pigment molecules to them in a procedure called **staining.** In this exercise you will use the stain methylene blue, which dyes the acidic molecules of the nucleus (the nucleic acids) blue. It also imparts a light blue tint to the rest of the cell.

Procedure

1. Gently scrape some cheek cells from the inside of your cheek with a clean, flat toothpick.

Discard your toothpick immediately after use in the container provided.

2. Spread the scrapings in the middle of a clean slide. Wait until the slide dries before proceeding.

3. Set half of a small petri dish inside half of a large petri dish. Place the slide on top of the small petri dish (see Figure 4.5).

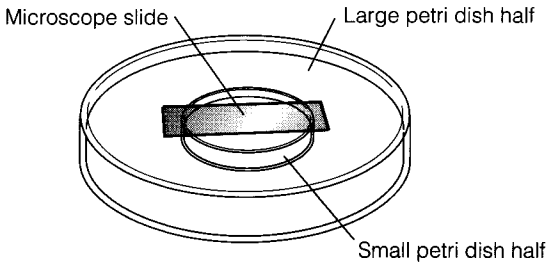

Figure 4.5.
Arrangement of petri dishes for staining cheek cells. The small petri dish serves as a platform for the slide, and the large petri dish will catch the excess water that washes off the slide.

4. Put several drops of methylene blue on the cheek scrapings. Wait 2 minutes for the stain to take effect.
5. *Gently* rinse the methylene blue off the slide with water from a squirt bottle. (If you rinse too vigorously, you may wash the cells off, too.)
6. Use a paper towel to blot dry the bottom of the slide. Do not wipe the top!
7. Add a small drop of water to the cheek scrapings; then add a coverslip.
8. Examine your cheek cells under the compound microscope.

Since the cells are still relatively transparent, adjust the aperture of the diaphragm to obtain maximum contrast.

9. Sketch the cheek cells in the margin of your lab manual and label the plasma membrane and nucleus.

If your microscope is equipped with an oil immersion lens, complete steps 10–14.

10. Be sure your slide is focused and centered at high power.
11. Move the high-power objective out of the way so that no objective is directly over the slide.
12. Put a small drop of immersion oil on the coverslip in the area you wish to view.
13. Turn the ring above the objectives so that the oil immersion lens is in the working position. When it is in place, the lens will actually be in the oil drop—this is why it's called oil immersion. View the specimen. You will need to open the diaphragm as wide as possible to get enough light. Sketch a cell in the margin of your lab manual.

    What has happened to the size of your field of view?

14. When you are finished, wipe the oil immersion lens with lens paper to remove the oil.

Your cheek cells are a type of tissue called **squamous epithelium,** the same kind of tissue that makes up your skin and other internal and external linings of your body. The cells are loosely joined together in sheets (see Figure 4.6).

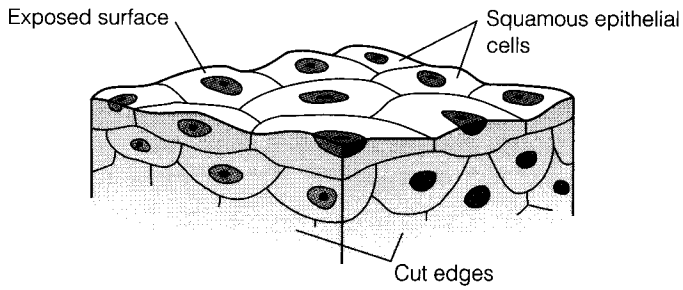

Figure 4.6.
Squamous epithelium.

The specimen you prepared showed cells lying flat on the slide, and you looked down on top of them through the microscope. Most tissues, however, don't peel off in sheets the way squamous epithelium does. For example, if you want to examine the skin tissues that lie beneath the squamous epithelium, you have to make a very thin slice from a block of tissue so that light can pass through it (see Figure 4.7). The tissue is first fixed, or killed, and then sliced and stained. When the specimen is mounted on a slide, a coverslip is fixed in place permanently. This type of slide is called a prepared slide, in contrast to the wet mounts you make yourself. You will frequently use prepared slides in this course.

Procedure

View a prepared slide of skin. How can you identify the squamous epithelium?

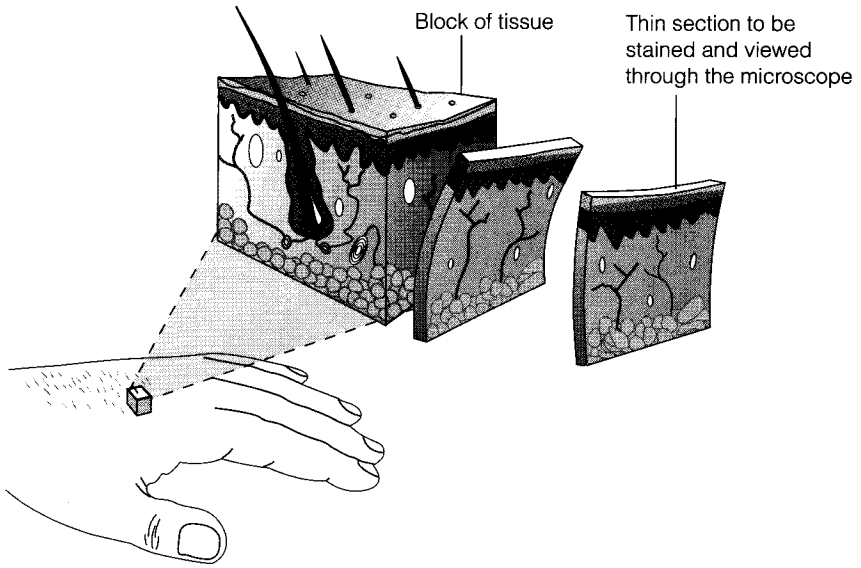

Figure 4.7.
Preparation of a thin section of tissue. After a block of tissue is removed from a larger specimen, it is sliced thinly enough to allow light to pass through it.

Activity B: Plant Cells

In addition to the nucleus, chloroplasts and the cell wall are visible when you look at a plant cell under the light microscope. Chloroplasts are the large (~8 μm), green, football-shaped organelles responsible for photosynthesis. The cell wall is composed of cellulose fibrils that provide strength and some rigidity. (As is the case with the plasma membrane, you cannot actually see the cell wall, but can infer its position.) There is a plasma membrane pressed up against the inside of the cell wall. Mature plant cells also have a large central vacuole, which is filled with water and water-soluble substances and may occupy 90% of the cell volume.

As an example of plant cells, you will examine *Elodea,* an aquatic plant commonly used in aquaria. The leaves are only a few cell layers thick, so a wet mount of a whole leaf allows enough light to pass through to examine the cells with light microscopy.

Procedure

1. Place one *Elodea* leaf on a slide in a drop of water.
2. Add a coverslip as shown in Figure 4.4. Try to avoid trapping air bubbles on the leaf surface.
3. Focus on the *Elodea* cells at scanning power, then at low power, and finally at high power.
4. With the high-power objective in place, move the fine-focus knob slightly up and down to see the entire thickness of a cell.

Sketch a few *Elodea* cells in the margin of your lab manual. Label the cell wall and chloroplasts. (You may not be able to see the nucleus.) In some cells you should be able to see cytoplasmic streaming as the chloroplasts move around the cell on microfilament tracks.

Try to visualize the *Elodea* cell in three dimensions. The cell is shaped like a shoebox, and inside the cell wall is the plasma membrane. A thin layer of cytoplasm lines the cell, but the largest volume is occupied by the central vacuole, which is interior to the cytoplasm. As Figure 4.8 shows, you are looking down on the top of the cell, so your view of the vacuole is blocked by the cytoplasm.

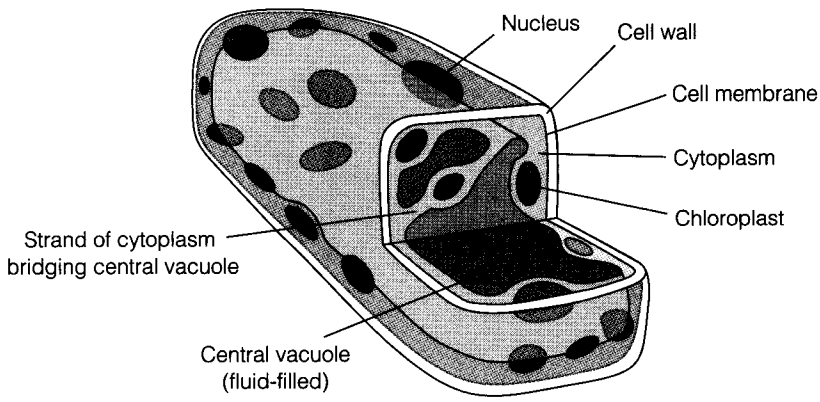

Figure 4.8.
Three-dimensional view of *Elodea* cell.

EXERCISE 4.3

The Stereoscopic Dissecting Microscope

Objectives

After completing this exercise, you should be able to

1. Explain how a dissecting scope differs from the compound microscope.
2. Identify the parts of the dissecting scope and give the function of each part.
3. Describe how to locate and focus on a specimen using the dissecting scope.
4. Identify situations in which a dissecting scope would be more useful than a compound light microscope.

In order to be viewed with the compound light microscope, specimens must be thin enough for light to pass through them. They must be mounted on glass slides; usually they are covered by a coverslip, so little manipulation of the specimen is possible while it is being viewed. In addition, the minimum magnification is typically 40×.

The stereoscopic dissecting microscope, usually referred to as a dissecting scope, provides a lower range of magnifications, usually between 5× and 50×, and a large working distance for manipulation of specimens. This type of microscope is also a compound microscope, since there are two lens systems, but in this case the objective lenses are housed inside the scope and are changed using a knob or dial. The number and magnifications of objective lenses vary considerably from one model of microscope to another. Some have only two settings, low power and high power, while others can be varied continuously like a zoom lens.

All dissecting scopes have two eyepieces. In contrast to a binocular compound microscope, in which you see the same image with both eyes, in the dissecting scope each eye sees through an independent system—there are actually two objectives. The result is that you see depth the way you do with unaided eyes.

Activity A: The Parts of the Dissecting Microscope

Locate the following parts on your dissecting scope (see Figure 4.9).

Eyepieces (oculars)

These lenses magnify the specimen. Notice that the distance between the eyepieces can be adjusted to fit the distance between your eyes. Often one eyepiece has a diopter ring on it, which allows you to adjust the focus of the two eyepieces independently.

What is the eyepiece magnification on your dissecting scope?

Stage

Usually the specimen may be placed directly on the stage to be examined. A slide is required only for some types of specimens (for instance, if the specimen must be viewed in a drop of water). A hole in the center of the stage allows light to be transmitted from beneath the stage.

Magnification Changer

This dial or knob changes the objective lenses inside the microscope. It is marked to indicate the magnifications possible for the objectives. There may be a setting that is less than 1 (for example, 0.7). If the eyepiece is 10×, this objective would give a total magnification of 7×.

What are the objective magnifications on your dissecting scope?

What are the total magnifications for your dissecting scope?

Focus Knob

Note that there is only one type of focus knob on this kind of microscope.

Light Sources

Two types of lighting are used with dissecting scopes: transmitted light, which comes from beneath the stage, and reflected light, which shines down on the specimen from above. Transmitted light is used when the specimen is thin and transparent. Reflected light is used for specimens that are opaque. It enhances the three-dimensional quality of the image.

Describe the illumination system in your microscope.

Activity B: Using the Dissecting Microscope

Familiarize yourself with the operation of the dissecting scope by using the "e" slide again.

Procedure

1. Position the "e" slide on the stage so that the "e" is directly over the hole in the center of the stage.
2. Set the objective magnification at its lowest power.
3. Turn on the transmitted (substage) light.
4. Focus on the "e." (You may need to complete steps 5 and 6 to get the best image.)
5. While looking through the eyepieces, move them together or apart until you see a single image. If there are markings on the microscope, record the setting for your intereye distance for future reference:

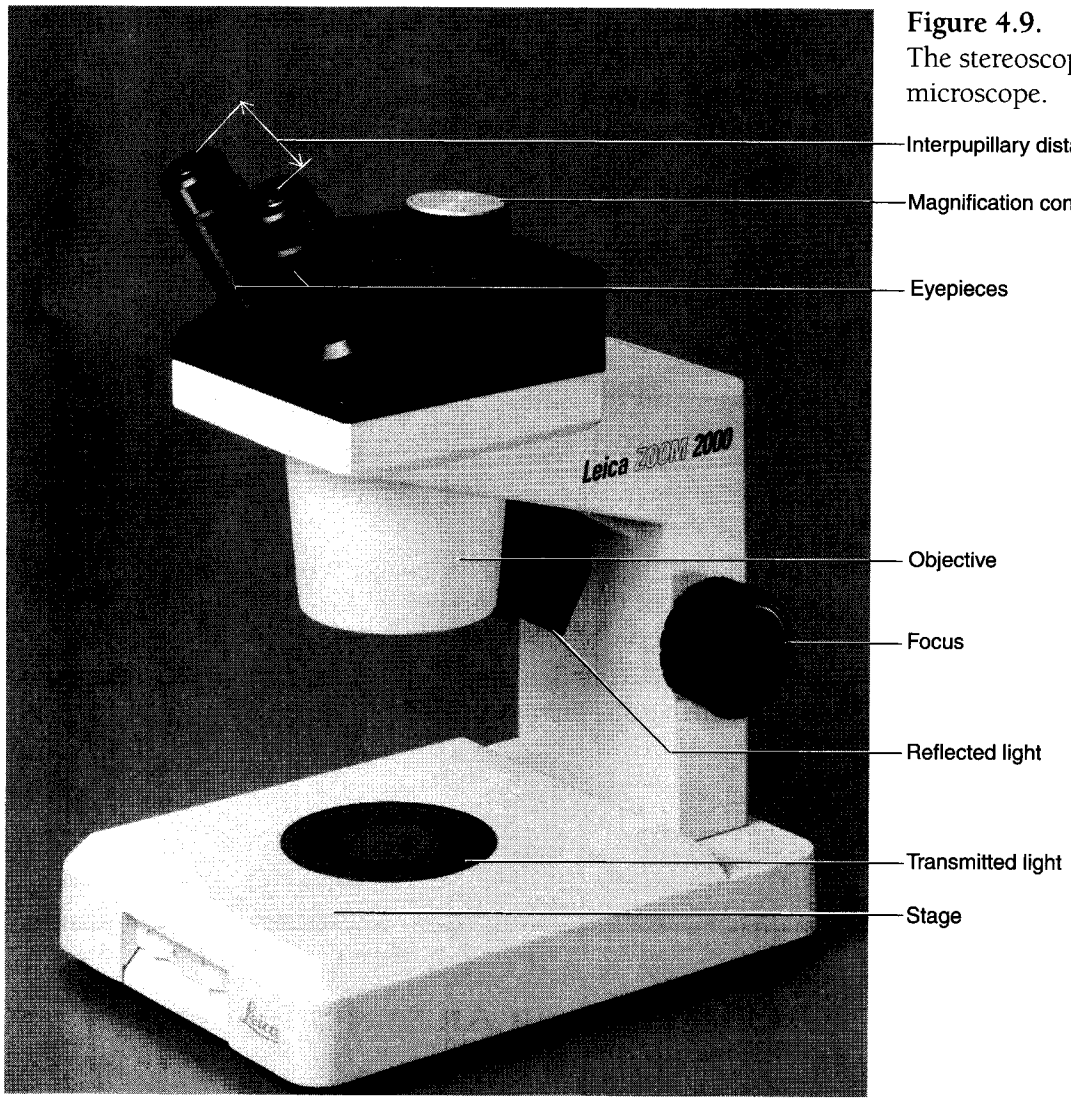

Figure 4.9.
The stereoscopic (dissecting) microscope.

6. With one eye, look through the eyepiece that doesn't have the focusing (diopter) ring. Cover the other eye. Use the focus knob to bring the image into sharp focus. Then look through the eyepiece that has the focusing ring and cover the other eye. Turn the ring until you get the sharpest focus for that eye. Then look with both eyes. The image should be in focus.

   How is the image of the "e" oriented compared to the image of the "e" on the slide itself?

   Look through the eyepiece and move the slide to your right.

   What happens to the "e"?

   What happens when you move the slide toward you?

7. Turn the magnification knob so that you see the "e" through the entire range of magnifications available.
8. Turn off the transmitted light and turn on the reflected light. How does this change the image you see?

   Which light source is better for this slide?

9. Next, get a photograph that has been cut out of a newspaper and place it on the stage.
10. Using reflected light, focus on the image. When you magnify the picture, you can see that it is composed of dots. The individual dots are not resolved by the unaided eye.
11. Turn off the reflected light and turn on the transmitted light. How does this change the image you see?

EXERCISE 4.4

Observing an Animal Specimen

Objective

After completing this exercise, you should be able to

1. Determine the best illumination and magnification to use for viewing a specimen on the dissecting scope.

As you have seen, the dissecting scope is the best choice if you are working with large specimens requiring relatively little magnification. In this exercise, you will examine a hydra, an animal related to sea anemones and corals. The hydra is commonly found in ponds and streams and is a favorite laboratory organism for introductory biology.

Procedure

1. Get a prepared slide of a hydra and place it on the stage of the dissecting scope.
2. Use a magnification that allows you to see the entire hydra and focus on it.

   Which type of light gives you the best image?

3. Sketch the hydra in the margin of your lab manual.
4. Get a dish containing living hydra. Place it on the stage and focus on one hydra.

   Which type of light gives you the best image?

   Describe how the image of the living hydra is different from the image of the prepared slide.

5. Dip a piece of thread in the juice from liver. While you are looking at the hydra under the dissecting scope, place the thread in the dish near the hydra. Describe the animal's behavior.

Your instructor will provide other specimens for viewing under the dissecting scope.

Questions for Review

1. If the eyepiece on a microscope has a magnification of 10×, what is the total magnification with a 10× objective?

   What is the total magnification with a 45× objective?

2. A microscope gives a total magnification of 1500×, but the image is too blurry to be useful. What might be the problem with this microscope?

3. Why is it important to center a specimen on low power before attempting to focus on it at high power?

4. A student focuses on a specimen at low power and carefully centers it before changing to high power. At high power, however, he doesn't see the part of the specimen he was interested in. What might be the problem?

5. Inspired by her biology lab, a student decides to make a closer study of the food she eats. She uses a razor blade to make a very thin section from a raw potato and mounts it in a drop of water on a slide. To her disappointment, she can barely make out the cells under the microscope. What might she do to improve her results?

6. Compare the image obtained using a compound light microscope with the image obtained using a stereoscopic dissecting microscope.