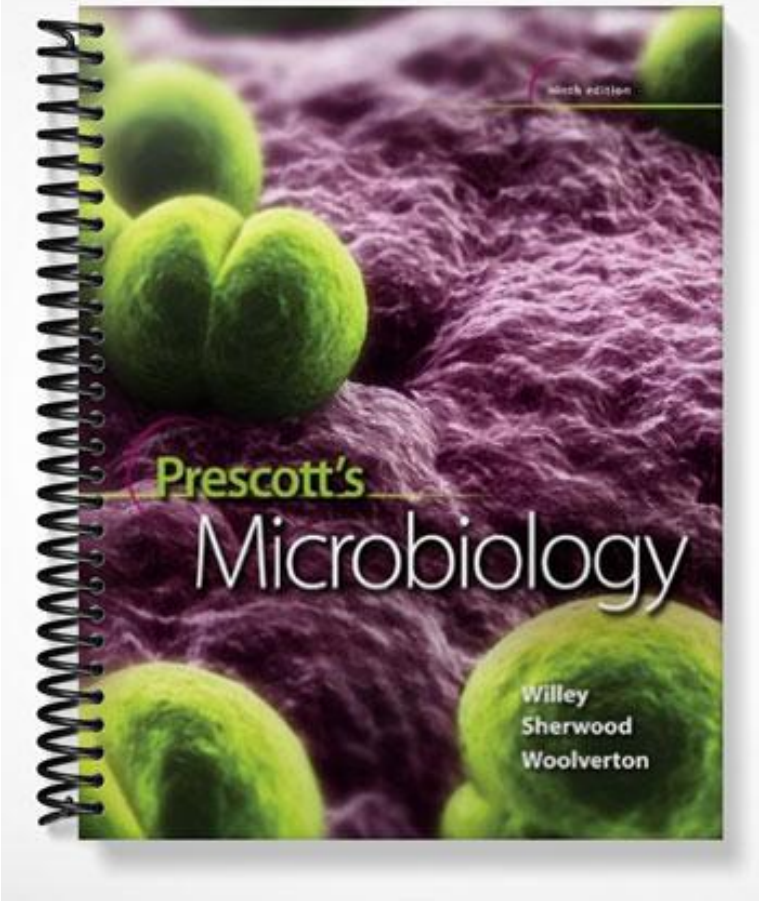
SOLUTIONS MANUAL



2 Microscopy

CHAPTER OVERVIEW

This chapter provides a relatively detailed description of the bright-field microscope and its use. Other common types of light microscopes are also described. Following this, various procedures for the preparation and staining of specimens are introduced. The chapter continues with a description of the two major types of electron microscopes and the procedures associated with their use. It concludes with descriptions of recent advances in microscopy: electron cryotomography and scanning probe microscopy.

LEARNING OUTCOMES

After reading this chapter students should be able to:

- relate the refractive indices of glass and air to the path light takes when it passes through a prism or convex lens
- correlate lens strength and focal length
- evaluate the parts of a light microscope in terms of their contributions to image production and use of the microscope
- predict the relative degree of resolution based on light wavelength and numerical aperture of the lens used to examine a specimen
- create a table that compares and contrasts the various types of light microscopes in terms of their uses, how images are created, and the quality of images produced
- recommend a fixation process to use when the microbe is a bacterium or archaeon and when the microbe is a protist
- plan a series of appropriate staining procedures to describe an unknown bacterium as fully as possible
- compare what happens to Gram-positive and Gram-negative bacterial cells at each step of the Gram-staining procedure
- create a concept map, illustration or table that compares transmission electron microscopes (TEM) to light microscopes
- decide when it would be best to examine a microbe by TEM, scanning electron microscopy (SEM), and electron cryotomography
- distinguish scanning tunneling from atomic force microscopes in terms of how they create images and their uses
- evaluate light microscopy, electron microscopy, and scanning probe microscopy in terms of their uses, resolution and the quality of the image created

GUIDELINES FOR ANSWERING THE MICRO INQUIRY QUESTIONS

Figure 2.2 *How would the focal length change if the lens shown here were thicker?*
As you increase the lens thickness, then the focal length would decrease, which in turn means the magnification would be greater. Note that this is true of the convex lenses used in microscopes, not for all lenses.

Figure 2.9 *What is the purpose of the annular stop in a phase-contrast microscope? Is it found in any other kinds of light microscopes?*
Using an annular stop and an objective phase plate, the microscope can be aligned to superimpose illuminating light rays passed through the annulus onto the objective phase ring to achieve phase-contrast illumination. Dark field microscopy uses a dark field stop similar to the annular ring to produce a hollow cone of light and differential interference contrast microscopy uses prisms to generate the two beams of light.

Figure 2.13 *How might the fluorescently labeled antibody used in figure 2.13b be used to diagnose strep throat?*
Fluorescent microscopy can be performed on a throat swab taken from the patient. Because the antibody is specific for the pathogen, an image should only be obtained with *S. pyogenes*. More commonly, the same antibody would be conjugated to an enzyme which gives a colorimetric reaction, thus a test can be run on the swab with a simple visual yes/no result. Rapid results such as these directly from clinical samples are much faster without the need for bacterial culture, and therefore allow rapid diagnosis for the patient.

Figure 2.16 *How does the light source differ between a confocal light microscope and other light microscopes?*
Confocal microscopes require a laser as source rather than just a bulb. Traditional light microscopes collect light from all areas of the sample to be viewed not just the plane of focus whereas the confocal microscope uses a laser beam for specimen illumination and any stray light is eliminated by the use of an aperture placed above the objective lens.

Figure 2.18 *Why is the decolorization step considered the most critical in the Gram-staining procedure?*
The difference between Gram-positive staining cells and Gram-negative staining cells is not absolute, but temporal. Thus, over-destaining (too long) causes all cells to appear Gram-negative (because with enough time, even Gram-positive cell walls will lose the crystal violet-iodine complex), and under-destaining (too short) causes all cells to falsely appear Gram-positive. So the destain step is technically critical. This is the step where the Gram-positive and Gram-negative cells are first visually different as well.

Figure 2.24 *Why are all electron micrographs black and white (although they are sometimes artificially colorized after printing)?*
Color in light comes from different wavelengths of the photons, while the electrons used do not have colors. The images are artificially colorized to make them easier to view (see Figure 2.27).

Figure 2.29 *Compare the magnification of this image with that shown in figures 2.24 (TEM) and 2.27 (SEM). Which has the highest magnification and which has the lowest?*
Scanning Tunneling Microscopy had the highest magnification of the image (x2,000,000) followed by Transmission Electron Microscopy (fig 2.24b, x 42,750), while the Scanning Electron Micrograph (fig 2.27, X 15,549) had the lowest magnification.

GUIDELINES FOR ANSWERING THE COMPARE, HYPOTHESIZE, INVENT QUESTIONS

1. *If you prepared a sample of a specimen for light microscopy, stained it with the Gram stain, and failed to see anything when you looked through your light microscope, list the things that you may have done incorrectly.*
This question should have students consider the many steps that have to be performed successfully with the Gram stain. There could be issues with: focusing, improper staining or detaining, insufficient heat fixation, etc.

2. *In a journal article, find an example of a light micrograph, a scanning or transmission electron micrograph, or a confocal image. Discuss why the figure was included in the article and why that particular type of microscopy was the method of choice for the research. What other figures would you like to see used in this study? Outline the steps that the investigators would take to obtain such photographs or figures.*
The student's understanding of why investigators choose particular systems and methods can be developed with this exercise. Discuss the theory behind each method and discuss why an understanding of the theory extends the interpretation of what is represented in the figure. Understand what aspects of a sample are revealed by each type of microscopy. The students can also develop critical thinking through experimental design.

3. *How are freeze etching and electron cryotomography similar? How do they differ? What is the advantage of electron cryotomography?*
Both methods are based on rapid deep-freezing to preserve cellular structures, thus avoiding fixation. While freeze etching makes an image along one surface of the specimen, cryotomography forms a 3D image of the specimen by reconstructing multiple views from a tilt series.

4. *STED microscopes were first developed about 10 years ago. They are modifications of confocal microscopes that use two highly focused beams of light rather than one as in typical confocal microscopy. The first beam uses a wavelength that excites the fluorophore with which a specimen is labeled. This creates a fluorescent spot where the fluorophore is located. The second beam is a hollow cone. It strikes the outermost edges of the fluorescent spot illuminated by the first beam. The wavelength used by the second beam returns the fluorophore to its ground state, thus only the center of the fluorescent spot is visible. The result is that the resolution of STED microscopes is greater and objects smaller than 200 nm can be resolved. In 2010 a STED microscope was used to observe the mechanism by which human immunodeficiency virus (HIV) particles are transferred from dendritic cells to T cells. These are important immune system cells and play critical roles in the development of HIV infection. Why was STED microscopy used rather than TEM or electron cryotomography?*
Read the original paper: Felts, R. L., et al. 2010. 3D visualization of HIV transfer at the virological synapse between dendritic cells and T cells. *Proc. Nat. Acad. Sci., USA*. 107:13336.
http://www.pnas.org/content/107/30/13336.full.pdf+html

A 3D image is needed, thus TEM would not be appropriate. While electron cryotomography can generate a 3D image, STED allows fluorescent tagging of specific viral or cellular proteins in the image, thus marking specific structures and allowing much greater detail in visualizing the viral-cellular interactions.