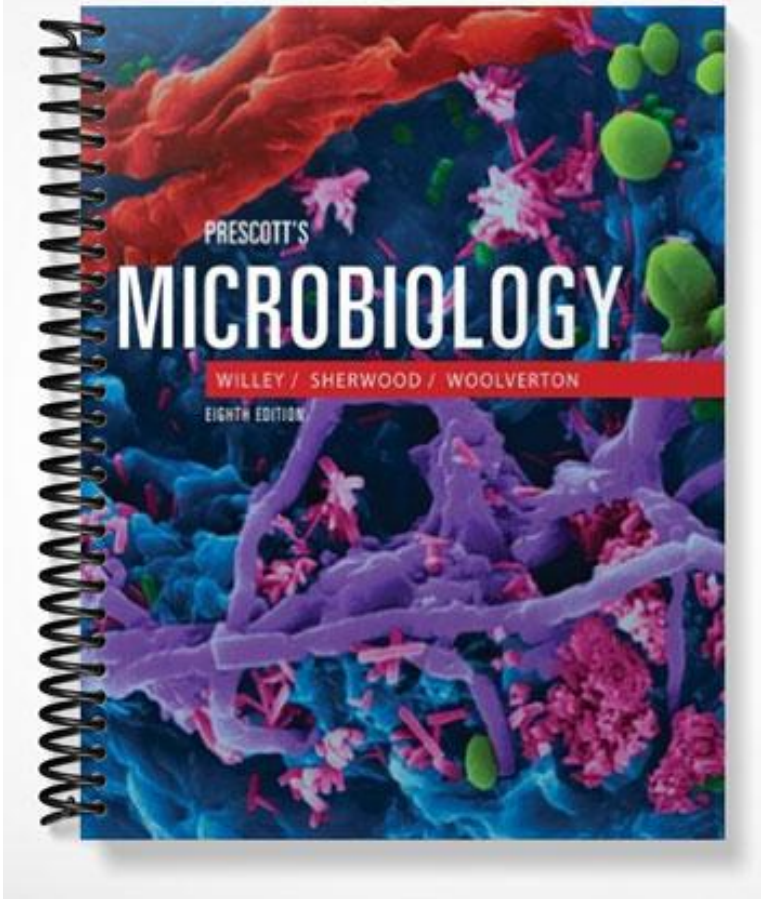
SOLUTIONS MANUAL



Chapter 2 - The Study of Microbial Structure: Microscopy and Specimen Preparation

CHAPTER OVERVIEW

This chapter provides a relatively detailed description of the bright-field microscope and its use. Other common types of light microscopes (the bright-field microscopy, the dark-field microscopy, the phase contrast microscopy, DIC microscopy, and fluorescence microscopy) 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 (TEM and SEM) and the procedures associated with their use. It concludes with descriptions of recent advances in microscopy: electron cryotomography, confocal microscopy and scanning probe microscopy.

CHAPTER OBJECTIVES

After reading this chapter, the student should be able to:

- describe how lenses bend light rays to produce enlarged images of small objects
- describe the various parts of the light microscope and how each part contributes to the functioning of the microscope
- describe the preparation and simple staining of specimens for observation with the light microscope
- describe the Gram-staining procedure and how it is used to categorize bacteria
- describe the basis for the various staining procedures used to visualize specific structures associated with microorganisms
- compare the operation of the transmission and scanning electron microscopes with each other and with light microscopes
- describe dark-field microscopy, phase-contrast microscopy, differential interference contrast microscopy, confocal microscopy, electron cryotomography, and scanning probe microscopy
- compare and contrast light microscopes, electron microscopes, confocal microscopes, and scanning probe microscopes in terms of their resolution, the types of specimens that can be examined, and the images produced.

ANSWERS TO MICRO INQUIRY QUESTIONS

Figure 2.2 The thicker the lens the shorter the focal length.

Figure 2.9 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 2.16 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 The decolorization step in gram staining is the most critical step because the presence of alcohol for too long a period will strip gram positive cells of crystal violet giving a false gram negative reactions and too little decolorization will give a false gram positive reaction for gram negative cells.

Figure 2.24 Electron micrographs are black and white because an electron beam is used as the radiation source instead of visible light.

Figure 2.29 Scanning Tunneling Microscopy had the highest magnification of the image (x2,000,000) followed by Transmission electron Microscopy (fig 2.24, x 42,750) and the Scanning Electron Micrograph (fig 2.24, X 15,549) had the lowest magnification.

ANSWER GUIDELINES FOR CRITICAL THINKING QUESTIONS

1 **Light microscopy of a gram stain sample**. This question encourages the student to scroll through all the steps of the Gram stain necessary to successfully visualize a specimen. Many manipulations are involved and all should be carefully performed to visualize the cell types.

2 **Microscopic picture in Journal Article**. 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.

3 **Electron cryomicroscopy**. This technique allows 3-D structure determination so the student needs to think of instances where structure impacts a system or mechanism: enzyme-substrate interactions, antigen-antibody interactions, virus-host cell receptor interactions, pathogen-drug interactions, are all possible examples.