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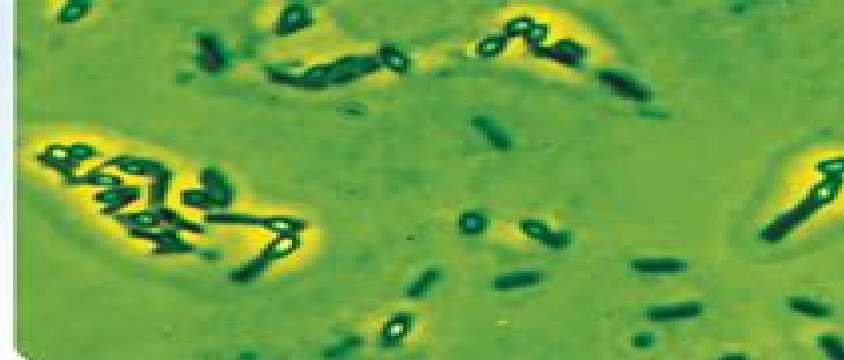
The Study of Microbial Structure: Microscopy and Specimen Preparation

PREVIEW

- Light microscopes use glass lenses to bend and focus light rays to produce enlarged images of small objects. The maximum resolution of a light microscope is about $0.2\ \mu\text{m}$.
- Many types of light microscopes have been developed, including bright-field, dark-field, phase-contrast, and fluorescence microscopes. Each yields a distinctive image.
- Bright-field microscopy requires the application of stains to microorganisms for easy viewing. Stains are also used to determine the nature of bacterial cell walls or to visualize specific prokaryotic structures such as flagella and capsules.
- The useful magnification of a light microscope is limited by its resolving power. The resolving power is limited by the wavelength of the illuminating beam.
- Electron microscopes use beams of electrons rather than light to achieve very high resolution (up to $0.5\ \text{nm}$) and magnification.
- New forms of microscopy are improving our ability to observe microorganisms and molecules. Two examples are the confocal scanning laser microscope and the scanning probe microscope.

Microbiology usually is concerned with organisms so small they cannot be seen distinctly with the unaided eye. Because of the nature of this discipline, the microscope is of crucial importance. Thus it is important to understand how the microscope works and the way in which specimens are prepared for examination.

In this chapter we begin with a detailed treatment of the standard bright-field microscope and then describe other common types of light microscopes. Next we discuss preparation and staining of specimens for examination with the light microscope. This is followed by a description of transmission and scanning electron microscopes, both of which are used extensively in cur-



Clostridium botulinum is a rod-shaped bacterium that forms endospores and releases botulinum toxin, the cause of botulinum food poisoning. In this phase-contrast micrograph, the endospores are the bright, oval objects located at the ends of the rods; some endospores have been released from the cells that formed them.

rent microbiological research. We close the chapter with a brief introduction to two newer forms of microscopy: confocal microscopy and scanning probe microscopy.

2.1 LENSES AND THE BENDING OF LIGHT

To understand how a light microscope operates, one must know something about the way in which lenses bend and focus light to form images. When a ray of light passes from one medium to another, **refraction** occurs—that is, the ray is bent at the interface. The **refractive index** is a measure of how greatly a substance slows the velocity of light; the direction and magnitude of bending is determined by the refractive indices of the two media forming the interface. When light passes from air into glass, a medium with a greater refractive index, it is slowed and bent toward the normal, a line perpendicular to the surface (Figure 2.1). As light leaves glass and returns to air, a medium with a lower refractive

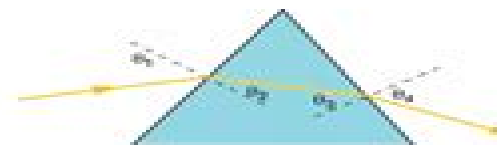


Figure 2.1 The Bending of Light by a Prism. Normals (lines perpendicular to the surface of the prism) are indicated by dashed lines. As light enters the glass, it is bent toward the first normal (angle θ_2 is less than θ_1). When light leaves the glass and returns to air, it is bent away from the second normal (θ_4 is greater than θ_3). As a result the prism bends light passing through it.

There are more animals living in the scum on the teeth in a man's mouth than there are men in a whole kingdom.

—Antony van Leeuwenhoek

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