

# Study Outline Chapter 03

## Units of Measurement (p. 56)

- The standard unit of length is the meter (m).
- Microorganisms are measured in micrometers,  $\mu\text{m}$  (10<sup>-6</sup> m) and nanometers, nm (10<sup>-9</sup> m).

## Microscopy: The Instruments (pp. 57-67)

### Compound Light Microscopy (pp. 57-58)

- The most common microscope used in microbiology is the compound light microscope (LM), which has two sets of lenses: ocular and objective.
- The total magnification of an object is calculated by multiplying the magnification of the objective lens by the magnification of the ocular lens.
- The compound light microscope uses visible light.
- The maximum resolution, or resolving power (the ability to distinguish between two points) of a compound light microscope is 0.2  $\mu\text{m}$ ; maximum magnification is 2000 $\times$ .
- Specimens are stained to increase the difference between the refractive indexes of the specimen and the medium.
- Immersion oil is used with the oil immersion lens to reduce light loss between the slide and the lens.
- Brightfield illumination is used for stained smears.
- Unstained cells are more productively observed using darkfield, phase-contrast, or DIC microscopy. These types of microscopy use modified compound microscopes.

### Darkfield Microscopy (p. 59)

- The darkfield microscope shows a light silhouette of an organism against a dark background.
- It is most useful for detecting the presence of extremely small organisms.

### Phase-Contrast Microscopy (pp. 59-62)

- A phase-contrast microscope brings direct and reflected or diffracted light rays together (in phase) to form an image of the specimen on the ocular lens.
- It allows the detailed observation of living organisms.

### Differential Interference

### Contrast (DIC) Microscopy (p. 62)

- The DIC microscope provides a colored, three-dimensional image of the object being observed.
- It allows the detailed observations of living cells.

### Fluorescence Microscopy (p. 62)

- In fluorescence microscopy, specimens are first stained with fluorochromes and then viewed through a compound microscope by using an ultraviolet (or near-ultraviolet) light source.
- The microorganisms appear as bright objects against a dark background.

- Fluorescence microscopy is used primarily in a diagnostic procedure called fluorescent-antibody (FA) technique, or immunofluorescence.

#### Confocal Microscopy (p. 63)

- In confocal microscopy, a specimen is stained with a fluorescent dye and illuminated one plane at a time.
- Using a computer to process the images, two-dimensional and three-dimensional images of cells can be produced.

#### Electron Microscopy (pp. 63-64)

- A beam of electrons, instead of light, is used with an electron microscope.
- Electromagnets, instead of glass lenses, control focus, illumination, and magnification.
- Thin sections of organisms can be seen in an electron micrograph produced using a transmission electron microscope (TEM). Magnification: 10,000-100,000<sup>3</sup>. Resolving power: 2.5 nm.
- Three-dimensional views of the surfaces of whole microorganisms can be obtained with a scanning electron microscope (SEM). Magnification: 1000-10,000<sup>3</sup>. Resolving power: 20 nm.

#### Scanning Tunneling and Atomic Force Microscopy (pp. 65-67)

- Scanning tunneling microscopy (STM) and atomic force microscopy (AFM) produce three-dimensional images of the surface of a molecule.

### **Preparation of Specimens for Light Microscopy (p. 68)**

#### Preparing Smears for Staining (p. 68)

- Staining means coloring a microorganism with a dye to make some structures more visible.
- Fixing uses heat or alcohol to kill and attach micro-organisms to a slide.
- A smear is a thin film of material used for microscopic examination.
- Bacteria are negatively charged, and the colored positive ion of a basic dye will stain bacterial cells.
- The colored negative ion of an acidic dye will stain the background of a bacterial smear; a negative stain is produced.

#### Simple Stains (p. 68)

- A simple stain is an aqueous or alcohol solution of a single basic dye.
- It is used to make cellular shapes and arrangements visible.
- A mordant may be used to improve bonding between the stain and the specimen.

#### Differential Stains (pp. 68-70)

- Differential stains, such as the Gram stain and acid-fast stain, differentiate bacteria according to their reactions to the stains.
- The Gram stain procedure uses a purple stain (crystal violet), iodine as a mordant, an alcohol decolorizer, and a red counterstain.
- Gram-positive bacteria retain the purple stain after the decolorization step; gram-negative bacteria do not and thus appear pink from the counterstain.
- Acid-fast microbes, such as members of the genera *Mycobacterium* and *Nocardia*, retain carbolfuchsin after acid-alcohol decolorization and appear red;

non-acid-fast microbes take up the methylene blue counterstain and appear blue.

#### Special Stains (pp. 70-72)

- The endospore stain and flagella stain are special stains that color and isolate only certain parts of bacteria.
- Negative staining is used to make microbial capsules visible.