Fixation and Staining of Specimens

Fixation

- The stained cells seen in a microscope should resemble living cells as closely as possible.
- **Fixation** is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position.
- It inactivates enzymes that might disrupt cell morphology and toughens cell structures so that they do not change during staining and observation.
- A microorganism usually is killed and attached firmly to the microscope slide during fixation.
- There are two fundamentally different types of fixation.
  - Bacteriologists heat-fix bacterial smears by gently flame heating an air-dried film of bacteria. This adequately preserves overall morphology but not structures within cells.
  - Chemical fixation must be used to protect fine cellular substructure and the morphology of larger, more delicate microorganisms.
- Chemical fixatives penetrate cells and react with cellular components, usually proteins and lipids, to render them inactive, insoluble, and immobile.
- Common fixative mixtures contain such components as ethanol, acetic acid, mercuric chloride, formaldehyde, and glutaraldehyde.

Dyes and Simple Staining

- The many types of dyes used to stain microorganisms have two features in common.
  - They have **chromophore groups,** groups with conjugated double bonds that give the dye its color.
  - They can bind with cells by ionic, covalent, or hydrophobic bonding.
- For example, a positively charged dye binds to negatively charged structures on the cell.
- Ionizable dyes may be divided into two general classes based on the nature of their charged group.
- **Basic dyes**—methylene blue, basic fuchsin, crystal violet, safranin, malachite green—have positively charged groups (usually some form of pentavalent nitrogen) and are generally sold as chloride salts.
- Basic dyes bind to negatively charged molecules like nucleic acids and many proteins. Because the surfaces of bacterial cells also are negatively charged, basic dyes are most often used in bacteriology.
- **Acid dyes**—eosin, rose bengal, and acid fuchsin—possess negatively charged groups such as carboxyls (—COOH) and phenolic hydroxyls (—OH).
- Acid dyes, because of their negative charge, bind to positively charged cell structures.

- The pH may alter staining effectiveness since the nature and degree of the charge on cell components change with pH.
- Thus anionic dyes stain best under acidic conditions when proteins and many other molecules carry a positive charge; basic dyes are most effective at higher pHs.
- Although ionic interactions are probably the most common means of attachment, dyes also bind through covalent bonds or because of their solubility characteristics.
- For instance, DNA can be stained by the Feulgen procedure in which Schiff's reagent is covalently attached to its deoxyribose sugars after hydrochloric acid treatment.
- Sudan III (Sudan Black) selectively stains lipids because it is lipid soluble but will not dissolve in aqueous portions of the cell.