**Chapter 4**

Microscopy, Staining and Classification

**General Principles of Microscopy**

Wavelength of radiation

Magnification

Resolution

* + Ability to see two objects as two distinct and separate objects.

Contrast

* + Differences in intensity between two objects, or between an object and background
  + Important in determining resolution
  + Staining increases contrast
  + Use of light that is in phase increases contrast

**Light Microscopy**

* Bright-field microscopes
* Simple
  + Contain a single magnifying lens
  + Similar to magnifying glass
  + Leeuwenhoek used simple microscope to observe microorganisms
  + Bright-field microscopes
* Compound
  + Use a series of lenses for magnification
  + Light rays pass through specimen and into objective lens (one of a series of objective lenses)
  + Oil immersion lens increases resolution because light does not refract
  + Have one or two ocular lenses
  + Total magnification = magnification of objective lens X magnification of ocular lens
  + Most have condenser lens to direct light through specimen
* Dark-field microscopes
  + Best for observing pale objects and external features
  + Only light rays scattered by specimen enter objective lens
  + Specimen appears light against dark background
  + Increases contrast and enables observation of more details
* Phase microscopes
  + Used to examine living organisms or specimens that would be damaged or altered by attaching them to slides or staining them
  + Treat one set of light rays differently from another set
  + Light rays in phase produce brighter image, while light rays out of phase produce darker image
  + Contrast is created because light waves are 1/2 wavelength out of phase
* Fluorescent microscopes
  + Direct UV light source at specimen; causes the specimen to radiate energy back as a longer, visible wavelength
  + UV light increases resolution and contrast
  + Some cells and molecules are naturally fluorescent, while others must be stained
  + Used in immunofluorescence to identify pathogens and to locate and make visible a variety of proteins
* Confocal microscopes
  + Use fluorescent dyes
  + Use UV lasers to illuminate fluorescent chemicals in a single plane that is no thicker than 1.0 mm
  + Resolution increased by up to 40% because emitted light passes through pinhole aperture
  + Computer constructs 3-D image from digitized images

**Electron Microscopy**

* Light microscopes cannot resolve structures closer than 200 nm because shortest wavelength of visible light is 400 nm
* Electrons have wavelengths of 0.01 nm to 0.001 nm, so electron microscopes have greater resolving power and greater magnification
* Magnifies objects 10,000X to 100,000X
* Gives detailed views of bacteria, viruses, internal cellular structures, molecules, and large atoms
* Two types
  + Transmission electron microscopes
  + Scanning electron microscopes

**Probe Microscopy**

* Uses minuscule, pointed, electronic probes to magnify more than 100,000,000 times
* Two types
  + Scanning tunneling microscopes
  + Atomic force microscopes

**Staining**

* Increases contrast and resolution by coloring specimens with stains/dyes
* Smear of microorganisms (thin film) air dried to slide and then fixed to surface by heat or chemical fixation
* Microbiological stains usually salts composed of cation and anion, with one colored (chromophore)
* Acidic dyes stain alkaline structures; more commonly, basic dyes stain acidic structures
* Simple stains
* Differential stains
  + Gram stain
  + Acid-fast stain
  + Endospore stain
* Special stains
  + Negative (capsule) stain
  + Flagellar stain
* Staining for Electron Microscopy
  + Chemicals containing heavy metals used for transmission electron microscopy
  + Stains may bind molecules in specimens or the background

**Classification & Identification of Microorganisms**

* Taxonomy consists of classification, nomenclature, and identification
* Enables scientists to organize large amounts of information about organisms and make predictions based on knowledge of similar organisms
* Linnaeus, Whittaker, and Taxonomic Categories
  + Linnaeus
    - Provided system that standardized the naming and classification of organisms based on characteristics in common
    - Grouped similar organisms that can successfully interbreed into categories called species
    - Used binomial nomenclature in his system
  + Whittaker
    - Linnaeus proposed only two kingdoms
    - Whittaker proposed a widely accepted taxonomic approach based on five kingdoms: Animalia, Plantae, Fungi, Protista, and Prokaryotae
  + Linnaeus’s goal was classifying and naming organisms as a means of cataloging them
  + More modern goal is understanding relationships among groups of organisms
  + Major goal of modern taxonomy is to reflect phylogenetic hierarchy
  + Greater emphasis on comparisons of organisms’ genetic material led to proposal to add domain
  + Domains
    - Carl Woese compared nucleotide sequences of rRNA subunits (changes occur rarely)
    - Proposal of three domains based on three basic types of cells as determined by ribosomal nucleotide sequences
    - Three Domains: Eukarya, Bacteria, and Archaea
    - Cells in the three domains also differ with respect to many other characteristics

**Taxonomic and Identifying Characteristics**

* + Physical characteristics
  + Biochemical tests
  + Serological tests
  + Phage typing
  + Analysis of nucleic acids