**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