Units of Measurement
- 1 μm = 10^{-6} m = 10^{-3} mm
- 1 nm = 10^{-9} m = 10^{-6} mm
- 1000 nm = 1 μm
- 0.001 μm = 1 nm

Microscopy: The Instruments
- A simple microscope has only one lens

Light Microscopy
- Use of any kind of microscope that uses visible light to observe specimens
- Types of **light microscopy**
  - Compound light microscopy
  - Darkfield microscopy
  - Phase-contrast microscopy
  - Differential interference contrast microscopy
  - Fluorescence microscopy
  - Confocal microscopy

Compound Light Microscopy
- In a **compound microscope**, the image from the objective lens is magnified again by the ocular lens
- **Total magnification** = objective lens × ocular lens
- **Resolution** is the ability of the lenses to distinguish two points
- A microscope with a resolving power of 0.4 nm can distinguish between two points ≥ 0.4 nm
- Shorter wavelengths of light provide greater resolution
- The **refractive index** is a measure of the light-bending ability of a medium
- The light may bend in air so much that it misses the small high-magnification lens
- Immersion oil is used to keep light from bending

Brightfield Illumination
- Dark objects are visible against a bright background
- Light reflected off the specimen does not enter the objective lens

Darkfield Illumination
- Light objects are visible against a dark background
- Light reflected off the specimen enters the objective lens

Phase-Contrast Microscopy
- Accentuates diffraction of the light that passes through a specimen

Differential Interference Contrast Microscopy
- Accentuates diffraction of the light that passes through a specimen; uses two beams of light

Fluorescence Microscopy
- Uses UV light
- Fluorescent substances absorb UV light and emit visible light
- Cells may be stained with fluorescent dyes (fluorochromes)

Confocal Microscopy
- Cells stained with fluorochrome dyes
- Short wavelength (blue) light used to excite the dyes
- The light illuminates each plane in a specimen to produce a three-dimensional image
  - Up to 100 μm deep

Two-Photon Microscopy
- Cells stained with fluorochrome dyes
- Two photons of long- wavelength (red) light used to excite the dyes
- Used to study cells attached to a surface
  - Up to 1 mm deep

Scanning Acoustic Microscopy (SAM)
- Measures sound waves that are reflected back from an object
- Used to study cells attached to a surface
- Resolution 1 μm

Electron Microscopy
- Uses electrons instead of light
- The shorter wavelength of electrons gives greater resolution

Transmission Electron Microscopy (TEM)
- Ultrathin sections of specimens
- Light passes through specimen, then an electromagnetic lens, to a screen or film
- Specimens may be stained with heavy metal salts
- 10,000–100,000×; resolution 2.5 nm

Scanning Electron Microscopy (SEM)
- An electron gun produces a beam of electrons that scans the surface of a whole specimen
- Secondary electrons emitted from the specimen produce the image
- 1,000–10,000×; resolution 20 nm

Scanned-Probe Microscopy
- **Scanning tunneling microscopy (STM)** uses a metal probe to scan a specimen
- Resolution 1/100 of an atom
- **Atomic force microscopy (AFM)** uses a metal- and-diamond probe inserted into the specimen.
- Produces three-dimensional images.

Stains and Smears
- **Staining**: Coloring the microbe with a dye that emphasizes certain structures
- **Smear**: A thin film of a solution of microbes on a slide
- A smear is usually **fixed** to attach the microbes to the slide and to kill the microbes

Preparing Smears for Staining
- Live or unstained cells have little contrast with the surrounding medium. Researchers do make discoveries about cell behavior by observing live specimens.
- Stains consist of a positive and negative ion
- In a **basic dye**, the chromophore is a cation
- In an **acidic dye**, the chromophore is an anion
- Staining the background instead of the cell is called **negative staining**

Simple Stains
- **Simple stain**: Use of a single basic dye
- A **mordant** may be used to hold the stain or coat the specimen to enlarge it

Differential Stains
- Used to distinguish between bacteria
  - Gram stain
  - Acid-fast stain

Gram Stain
- Classifies bacteria into gram-positive or gram-negative
  - Gram-positive bacteria tend to be killed by penicillin and detergents
  - Gram-negative bacteria are more resistant to antibiotics

Acid-Fast Stain
- Stained waxy cell wall is not decolorized by acid-alcohol

- *Mycobacterium*
- *Nocardia*

Special Stains
- Used to distinguish parts of cells
  - Capsule stain
  - Endospore stain
  - Flagella stain

Negative Staining for Capsules
- Cells stained
- Negative stain

Endospore Staining
- Primary stain: Malachite green, usually with heat
- Decolorize cells: Water
- Counterstain: Safranin

Flagella Staining
- Mordant on flagella
- Carbolfuchsin simple stain