Figure 3.2 Microscopes and Magnification.

Units of Measurement

- Microorganisms are measured in **micrometers** (μm) and **nanometers** (nm)
  - 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

Figure 1.2b Anton van Leeuwenhoek's microscopic observations.
Light Microscopy

- 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 (DIC) microscopy
  - Fluorescence microscopy
  - Confocal microscopy

![Diagram of a compound light microscope with labeled parts](image)
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

---

Figure 3.1b  The compound light microscope.

- Ocular lens
- Line of vision
- Path of light
- Prism
- Body tube
- Objective lenses
- Specimen
- Condenser lenses
- Illuminator
- Base with source of illumination

(b) The path of light (bottom to top)
Compound Light Microscopy

- **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 at least 0.4 nm apart
- Shorter wavelengths of light provide greater resolution

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Compound Light Microscopy

- The **refractive index** is a measure of the light-bending ability of a medium
- Light may refract after passing through a specimen to an extent that it does not pass through the objective lens
- Immersion oil is used to keep light from refracting
Compound Light Microscopy

- **Brightfield illumination**
  - Dark objects are visible against a bright background
  - Light reflected off the specimen does not enter the objective lens
Figure 3.4a Brightfield, darkfield, and phase-contrast microscopy.

(a) Brightfield. (Top) The path of light in brightfield microscopy, the type of illumination produced by regular compound light microscopes. (Bottom) Brightfield illumination shows internal structures and the outline of the transparent pellicle (external covering).

Darkfield Microscopy

- Light objects are visible against a dark background
- Opaque disk placed in condenser
- Only light reflected off the specimen enters the objective lens
Phase-Contrast Microscopy

- Allows examination of living organisms and internal cell structures
- Brings together two sets of light rays, direct rays, and diffracted rays to form an image
Differential Interference Contrast (DIC) Microscopy

- Similar to phase-contrast
- Uses two light beams and prisms to split light beams, giving more contrast and color to the specimen
Fluorescence Microscopy

- Uses UV (short wavelength) light
- Fluorescent substances absorb UV light and emit longer wavelength (visible) light
- Cells may be stained with fluorescent dyes (fluorochromes) if they do not naturally fluoresce
Confocal Microscopy

- Cells are stained with fluorochrome dyes
- Short-wavelength (blue) light is used to excite a single plane of a specimen
- Each plane in a specimen is illuminated and a three-dimensional image is constructed with a computer
Two-Photon Microscopy

- Cells are stained with fluorochrome dyes
- Two photons of long-wavelength (red) light are used to excite the dyes
- Can study living cells up to 1 mm deep
Scanning Acoustic Microscopy

- Measures sound waves that are reflected back from a specimen
- Used to study cells attached to surfaces
- Resolution of 1 µm
Electron Microscopy

- Uses electrons instead of light
- The shorter wavelength of electrons gives greater resolution
- Used for images too small to be seen with light microscopes, such as viruses
Transmission Electron Microscopy

- A beam of electrons passes through ultrathin sections of a specimen, then through an electromagnetic lens, then focused on a projector lens
- Specimens may be stained with heavy-metal salts for contrast

Figure 3.10a Transmission and scanning electron microscopy.

(a) Transmission. (Left) In a transmission electron microscope, electrons pass through the specimen and are scattered. Magnetic lenses focus the image onto a fluorescent screen or photographic plate. (Right) This colorized transmission electron micrograph (TEM) shows a thin slice of Paramecium. In this type of microscopy, the internal structures present in the slice can be seen.
Transmission Electron Microscopy

- Magnifies objects 10,000 to 100,000×; resolution of 10 pm

Scanning Electron Microscopy

- An electron gun produces a beam of electrons that scans the surface of an entire specimen
- Secondary electrons emitted from the specimen produce a three-dimensional image
Figure 3.10b  Transmission and scanning electron microscopy.

(b) Scanning. (Left) In a scanning electron microscope, primary electrons sweep across the specimen and knock electrons from its surface. These secondary electrons are picked up by a collector, amplified, and transmitted onto a viewing screen or photographic plate. (Right) In this colorized scanning electron micrograph (SEM), the surface structures of *Paramecium* can be seen. Note the three-dimensional appearance of this cell, in contrast to the two-dimensional appearance of the transmission electron micrograph in part (a).

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**Scanning Electron Microscopy**

- Magnifies objects 1000 to 10,000×; resolution of 10 nm
Scanning Tunneling Microscopy

- Uses a tungsten probe to scan a specimen and reveal details of its surface
- Resolution of 1/100 of an atom

Figure 3.11a Scanned-probe microscopy.
Atomic Force Microscopy

- Uses a metal-and-diamond probe placed onto a specimen
- Produces three-dimensional images
Preparation Smears for Staining

- **Staining**: coloring microorganisms with a dye that emphasizes certain structures
- **Smear**: a thin film of a material containing microorganisms spread over a slide
- Microorganisms are fixed (attached) to the slide, which kills the microorganisms

Preparation Smears for Staining

- Live and/or unstained specimens have little contrast with the surrounding medium. Live specimens are used to study cell behavior.
Preparing Smears for Staining

- Stains consist of a positive and negative ion, one of which is colored (chromophore)
- 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
- Highlights the entire microorganism to visualize cell shapes and structures
- 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 have thick peptidoglycan cell walls
  - Gram-negative bacteria have thin peptidoglycan cell walls and a layer of lipopolysaccharides
Figure 3.12a  Gram staining.

Application of crystal violet (purple dye)

Application of iodine (mordant)

Alcohol wash (decolorization)

Application of safranin (counterstain)

KEY
- Crystal violet
- Iodine
- Alcohol
- Safranin

Gram-positive
Gram-negative

Rod
(gram-negative)

Coccus
(gram-positive)

Figure 3.12b  Gram staining.
Acid-Fast Stain

- Binds only to bacteria that have a waxy material in their cell walls, which is not decolorized by acid-alcohol
- Used for the identification of
  - *Mycobacterium*
  - *Nocardia*

<table>
<thead>
<tr>
<th></th>
<th>Color of Acid-Fast</th>
<th>Color of Non-Acid-Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Stain: Carbolfuchsin</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>Decolorizing Agent: Acid-alcohol</td>
<td>Red</td>
<td>Colorless</td>
</tr>
<tr>
<td>Counterstain: Methylene Blue</td>
<td>Red</td>
<td>Blue</td>
</tr>
</tbody>
</table>
Special Stains

- Used to distinguish parts of microorganisms
  - Capsule stain
  - Endospore stain
  - Flagella stain
Negative Staining for Capsules

- **Capsules** are a gelatinous covering that do not accept most dyes
- Suspension of India ink or nigrosin contrasts the background with the capsule, which appears as a halo around the cell

Figure 3.14a Special staining.

(a) Negative staining

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Endospore Staining

- **Endospores** are resistant, dormant structures inside some cells that cannot be stained by ordinary methods
- Primary stain: malachite green, usually with heat
- Decolorize cells: water
- Counterstain: safranin
- Spores appear green within red or pink cells
Flagella Staining

- **Flagella** are structures of locomotion
- Uses a mordant and carbolfuchsins

![Flagellum](image)

*Figure 3.14c Special staining.*