TORTORA • FUNKE • CASE

Microbiology

AN INTRODUCTION

10th EDITION

Chapter 3

Observing Microorganisms Through a Microscope

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Units of Measurement Table 3.1

- **1 µm micrometer** = 10^{-6} m = 10^{-3} mm
- **1 nm nanometer** = 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.

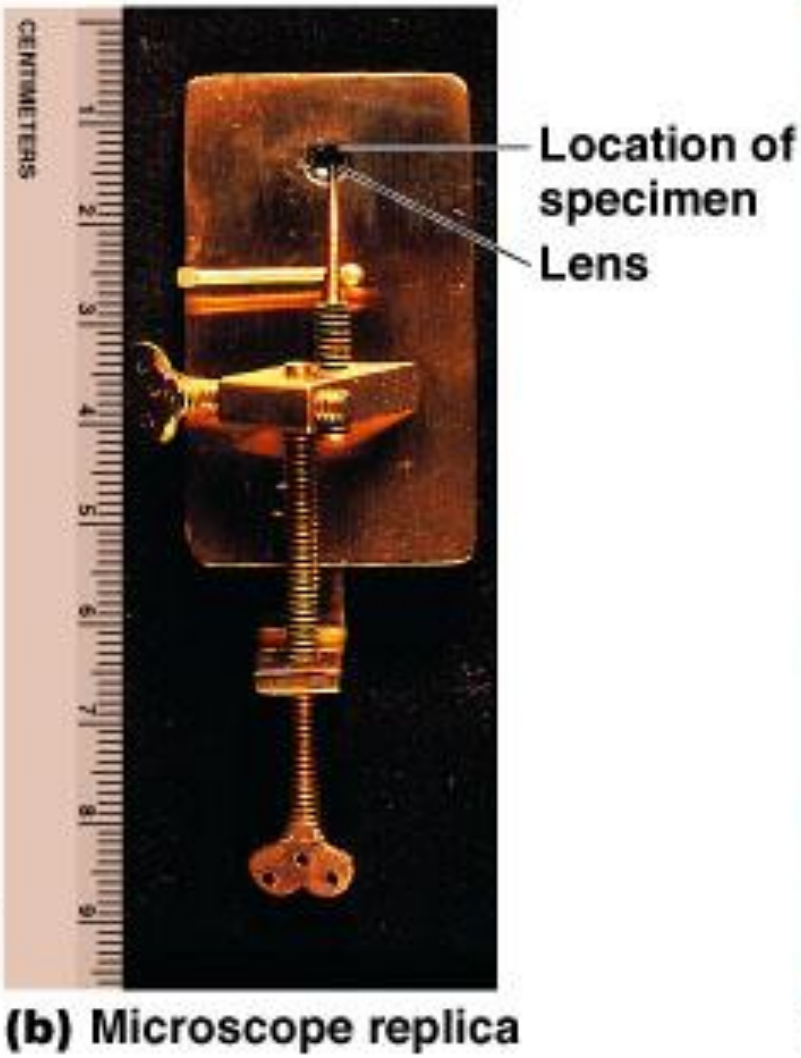
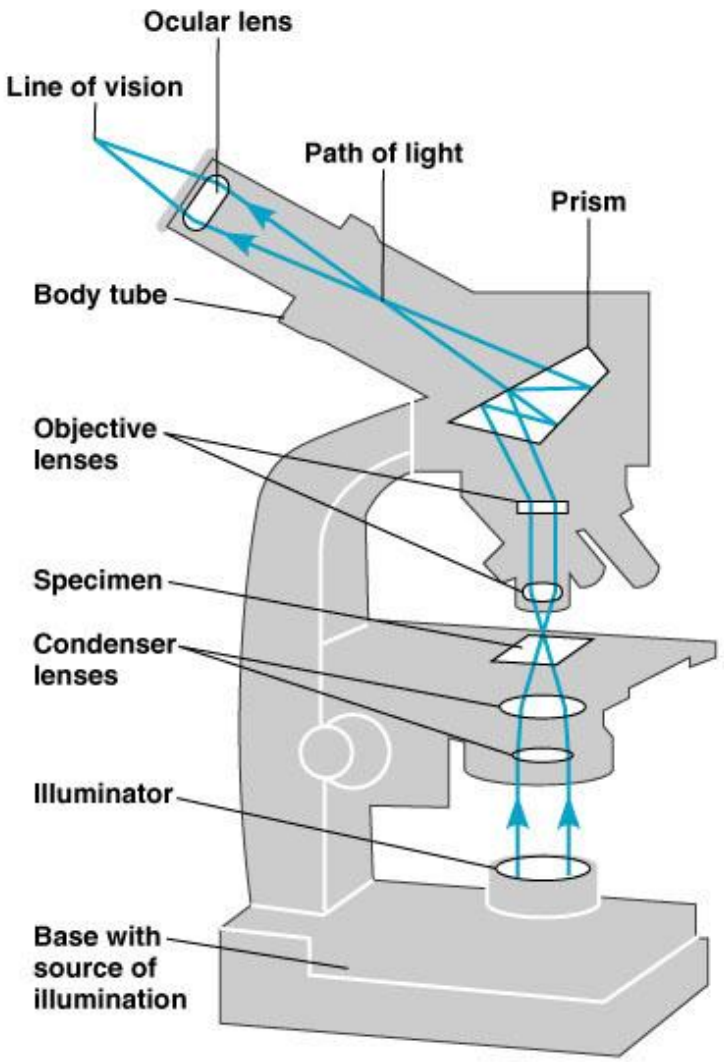


Figure 1.2b

Microscopy: The Instruments

- In a compound microscope the image from the objective lens is magnified again by the ocular lens.
- Total magnification = objective lens × ocular lens



(b) The path of light (bottom to top)

Figure 3.1b

Microscopy: The Instruments

- **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
- Resolving power=Wavelength of light used/2x numerical aperture(a property of the lens).

Microscopy: The Instruments

- Refractive index is 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.

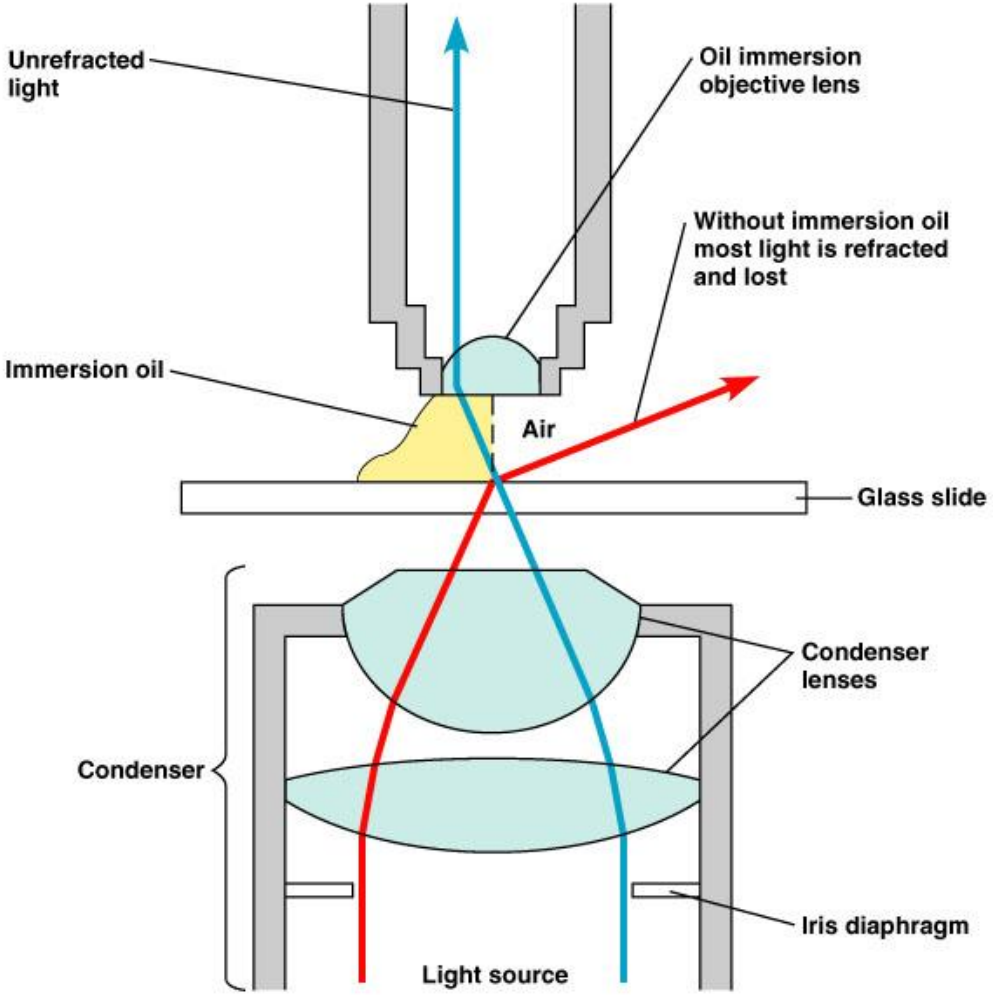


Figure 3.3

Brightfield Illumination

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

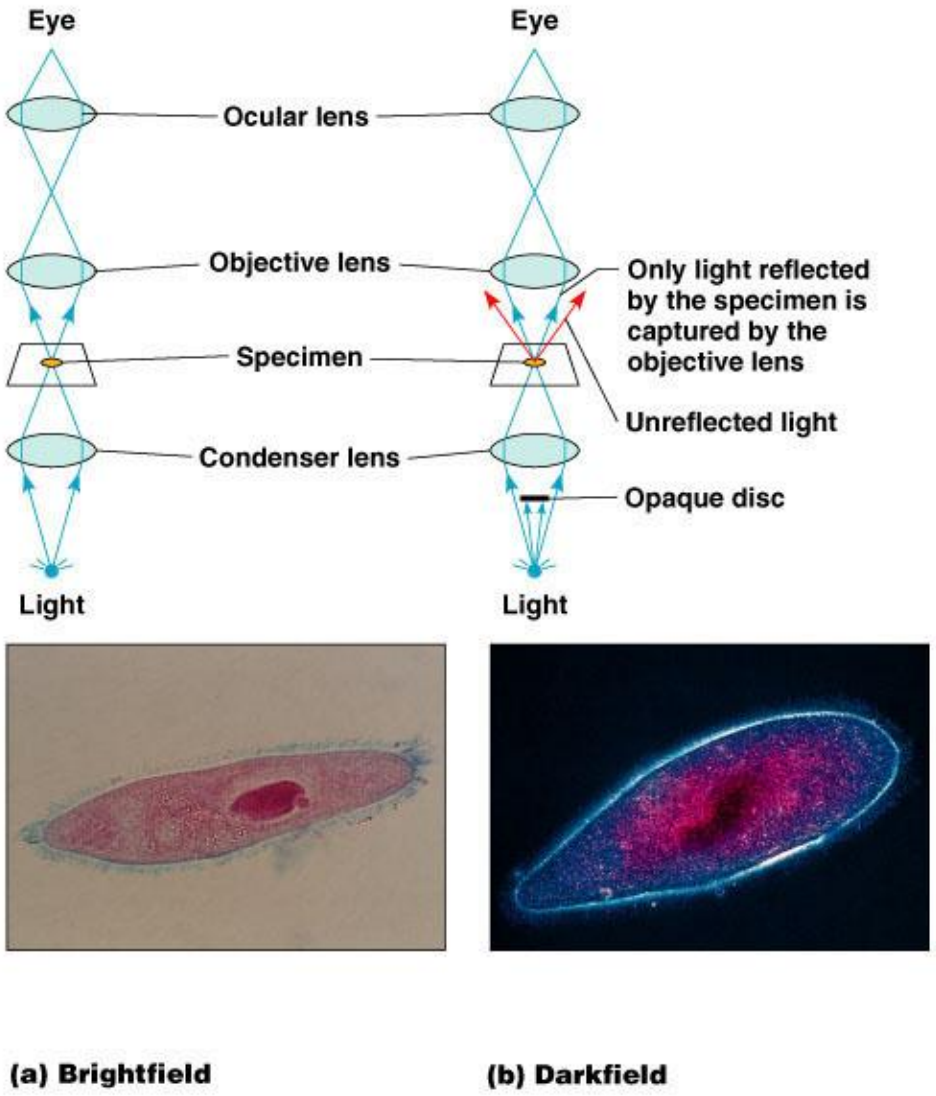


Figure 3.4a, b

Darkfield Illumination

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

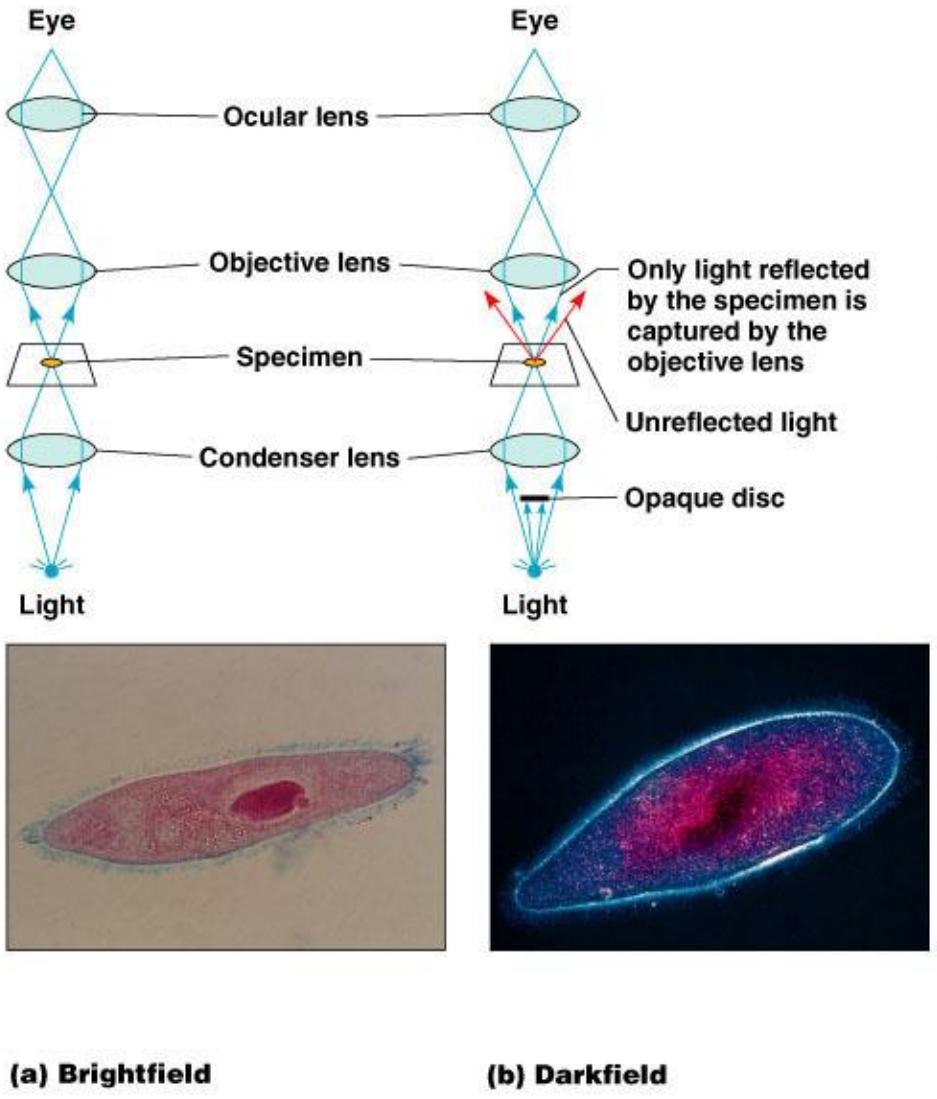


Figure 3.4a, b

Phase-Contrast Microscopy

- Accentuates diffraction of the light that passes through a specimen. Direct and reflected light rays are combined at the eye. Increasing contrast

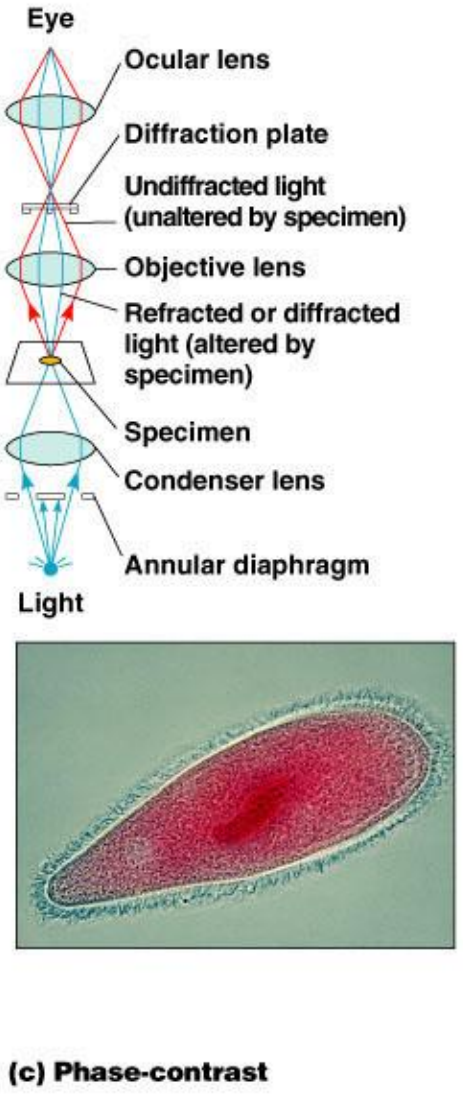


Figure 3.4c

Differential Interference Contrast Microscopy

- Accentuates diffraction of the light that passes through a specimen; uses two beams of light. Adding color

Figure 3.5

Fluorescence Microscopy

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

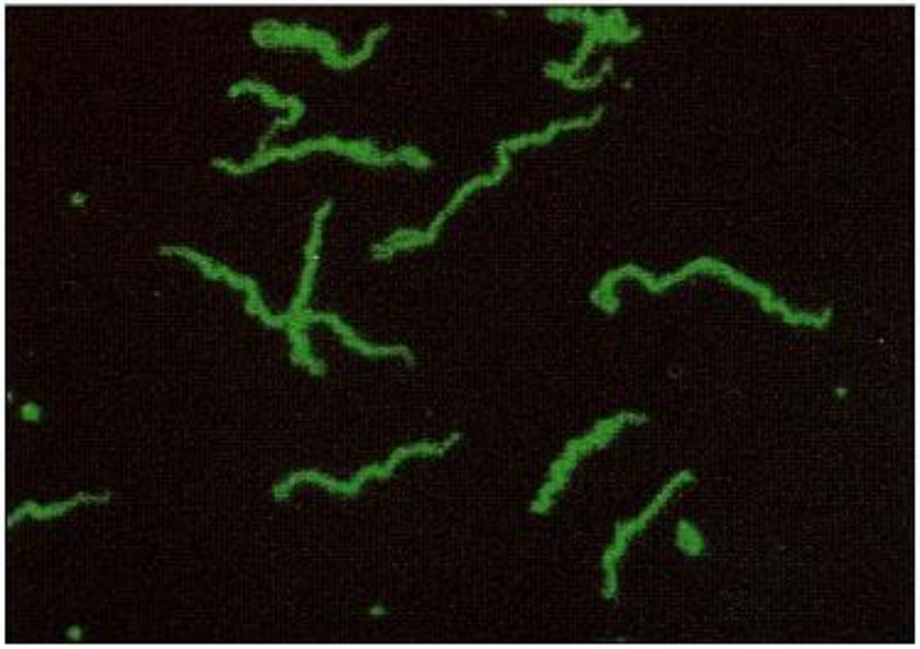


Figure 3.6b

Confocal Microscopy

- Uses fluorochromes and a laser light.
- The laser illuminates each plane in a specimen to produce a 3-D image.

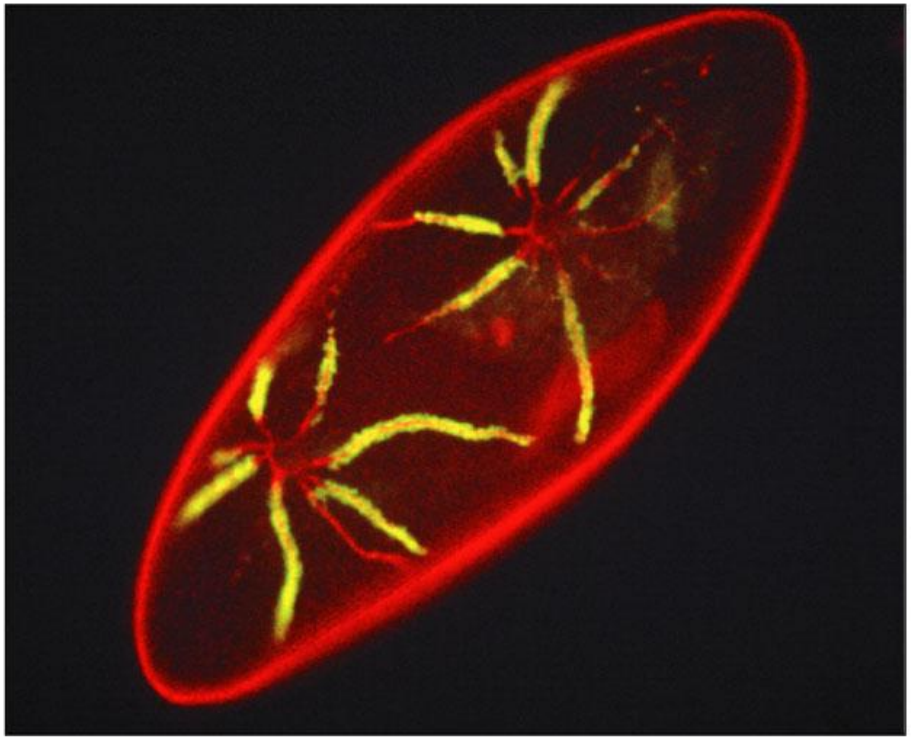


Figure 3.7

Electron Microscopy

- Uses electrons instead of light.
- The shorter wavelength of electrons gives greater resolution. Why?

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.

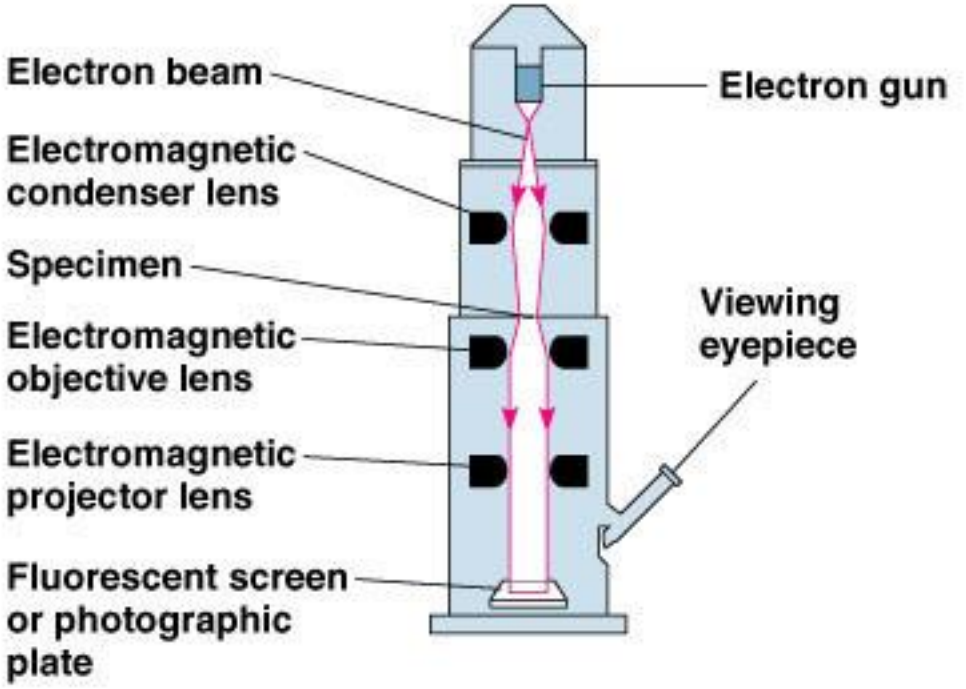


Figure 3.8a

Transmission Electron Microscopy (TEM)

- 10,000-100,000×; resolution 2.5 nm

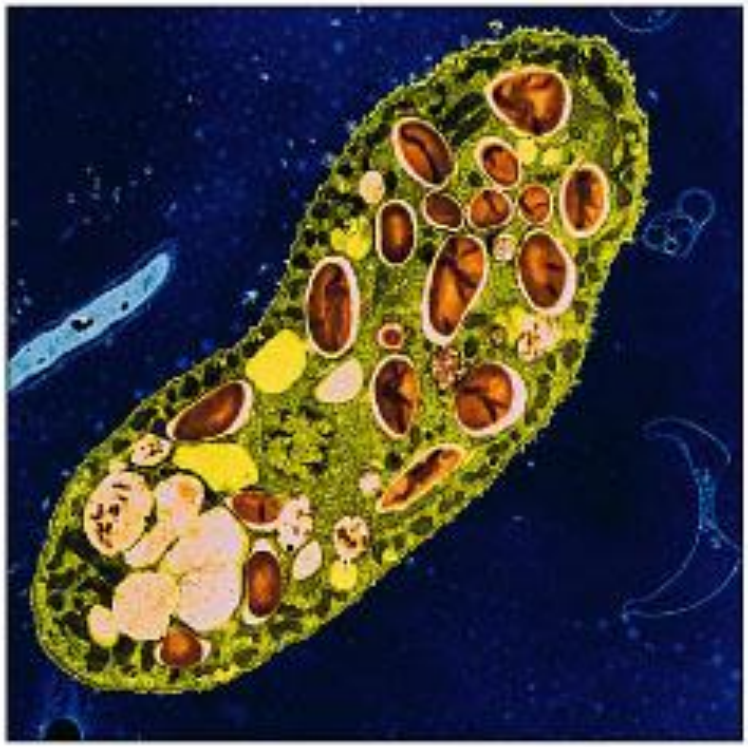


Figure 3.9

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.

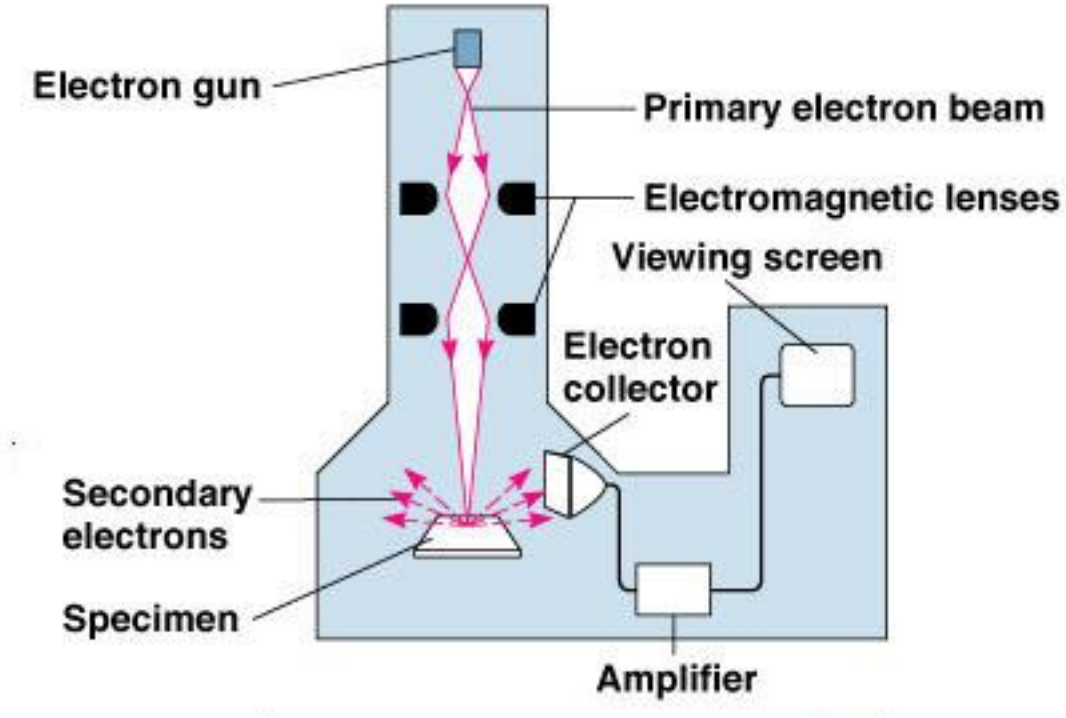


Figure 3.9b

Scanning Electron Microscopy (SEM)

- 1000-10,000×; resolution 20 nm

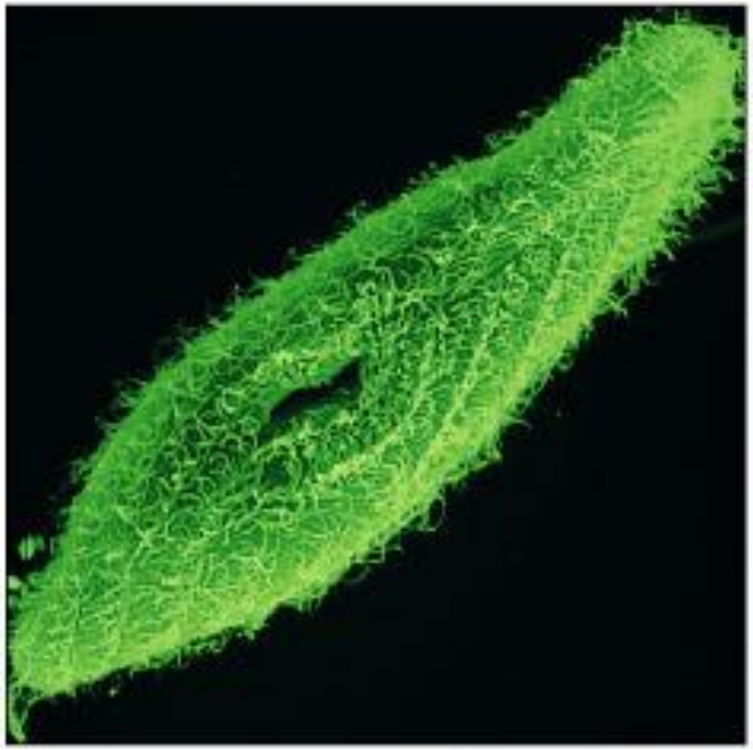


Figure 3.8b

Scanning-Probe Microscopy

- Scanning tunneling microscopy uses a metal probe to scan a specimen.
- Resolution 1/100 of an atom.

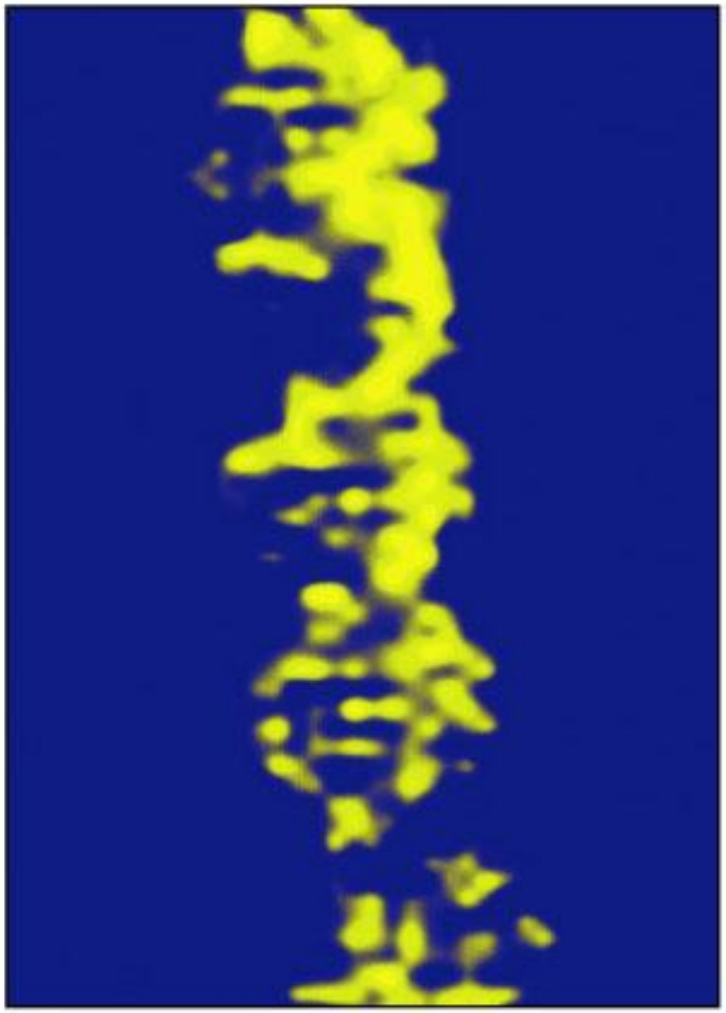


Figure 3.9a

Scanning-Probe Microscopy

- Atomic force microscopy uses a metal and diamond probe inserted into the specimen.
- Produces 3-D images.

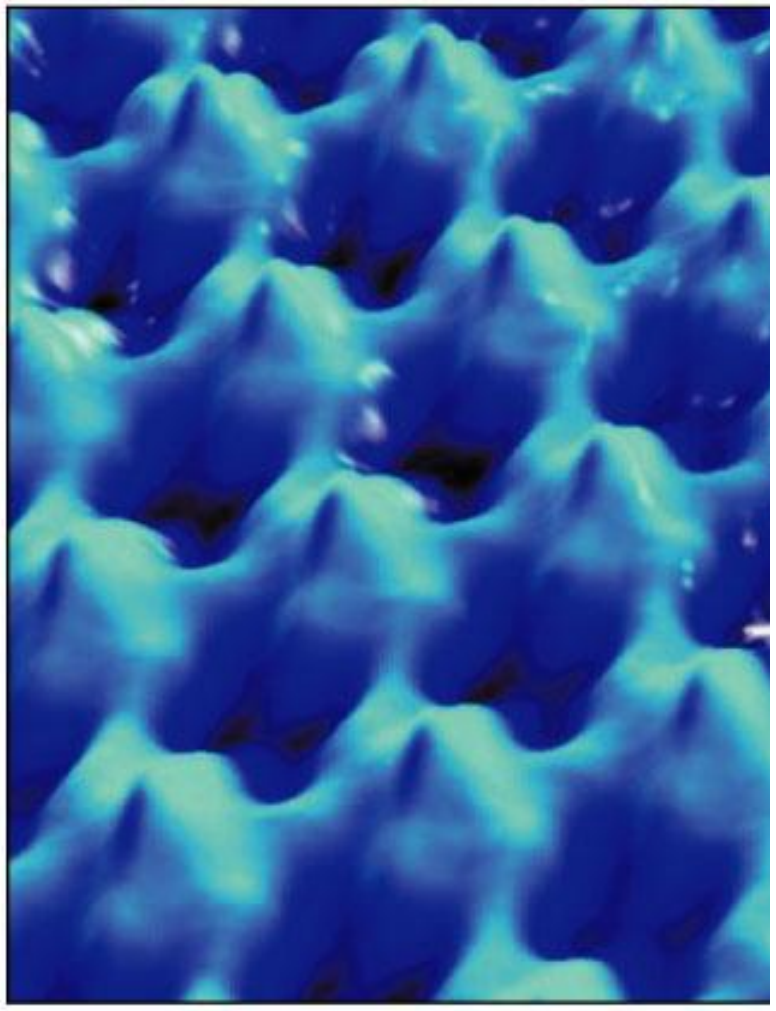


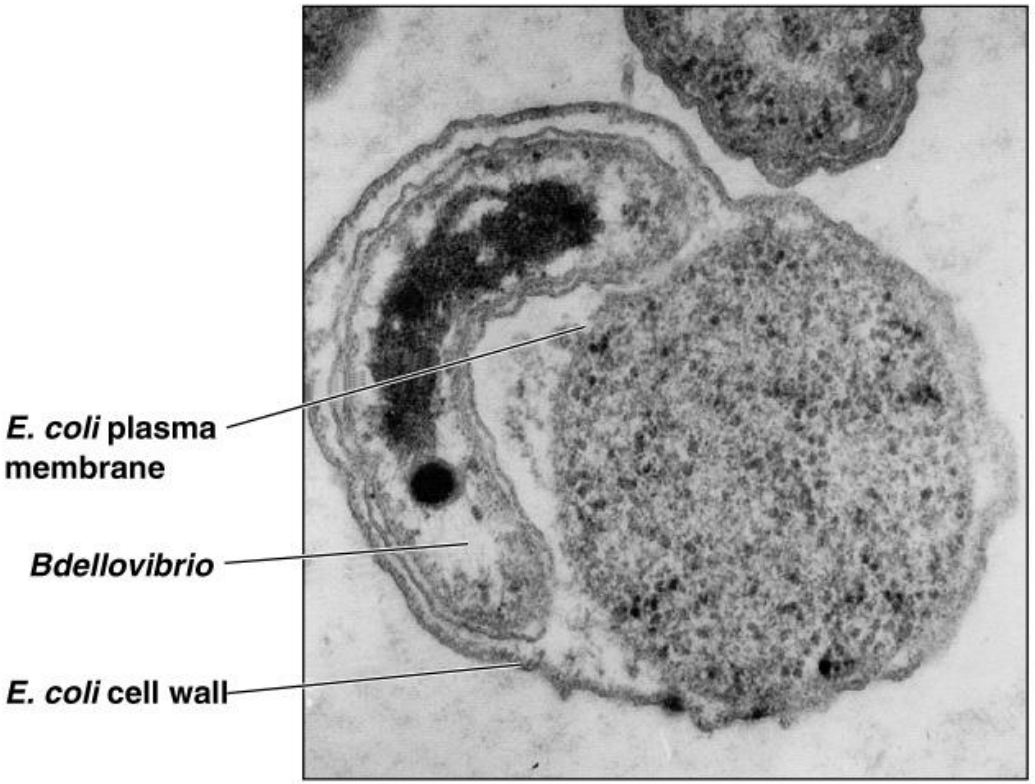
Figure 3.9b

Preparation of Specimens for Light Microscopy

- A thin film of a solution of microbes on a slide is a **smear.**
- 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*. However, researchers do make discoveries about cell behavior looking at live specimens.



Preparing Smears for Staining

- 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 (-).
- Bacteria are slightly negative at neutral pH
- Staining the background instead of the cell is called negative staining.

Simple Stains

- Use of a single basic dye is called a **simple stain**.
- A **mordant** may be used to hold the stain or coat the specimen to enlarge it.
- A mordant: substance, typically an inorganic oxide, that combines with a dye or stain and thereby fixes it in a material.

Differential Stains: Gram Stain

- The **Gram stain** classifies bacteria into gram-positive and gram-negative.
- **Gram-positive** bacteria tend to be killed by penicillin and detergents.
- **Gram-negative** bacteria are more resistant to antibiotics.

Differential Stains: Gram Stain

	Color of Gram + cells	Color of Gram – cells
Primary stain: Crystal violet	Purple	Purple
Mordant: Iodine	Purple	Purple
Decolorizing agent: Alcohol-acetone	Purple	Colorless
Counterstain: Safranin	Purple	Red

Differential Stains: Gram Stain

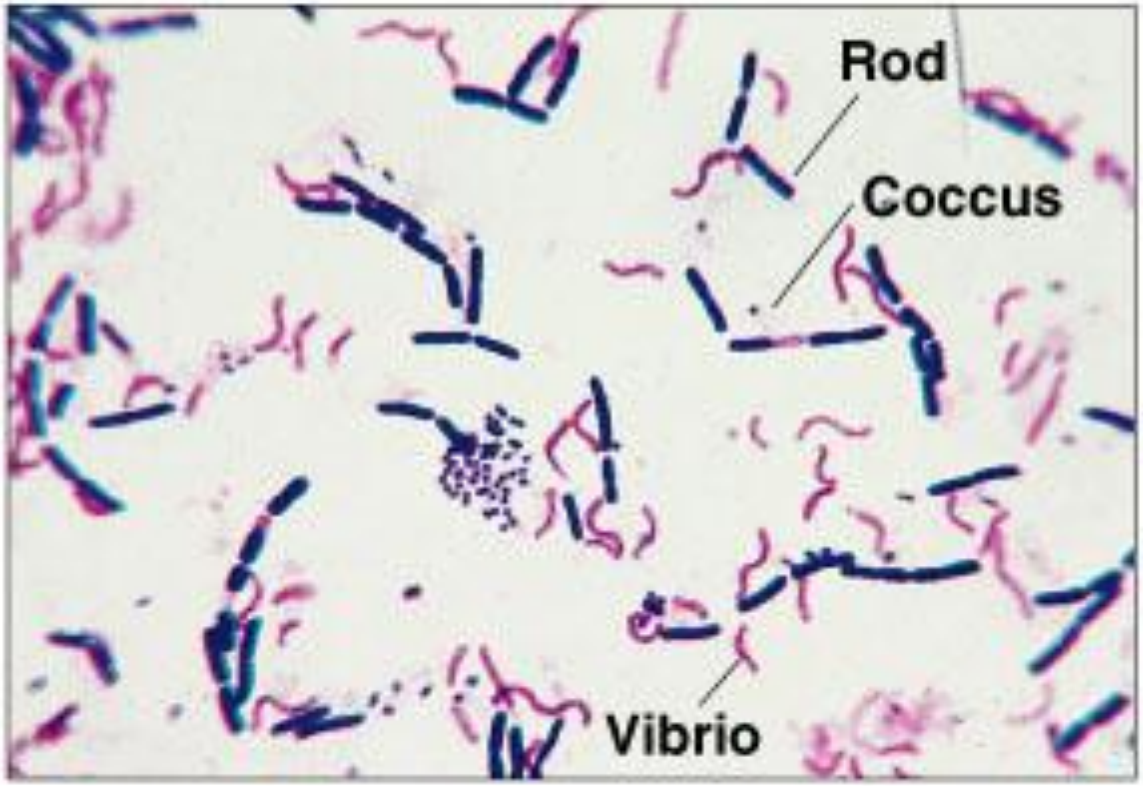


Figure 3.11b

Differential Stains: Acid-Fast Stain

- Cells that retain a basic stain in the presence of acid-alcohol are called acid-fast.
- Non–acid-fast cells lose the basic stain when rinsed with acid-alcohol, and are usually counterstained (with a different color basic stain) to see them.

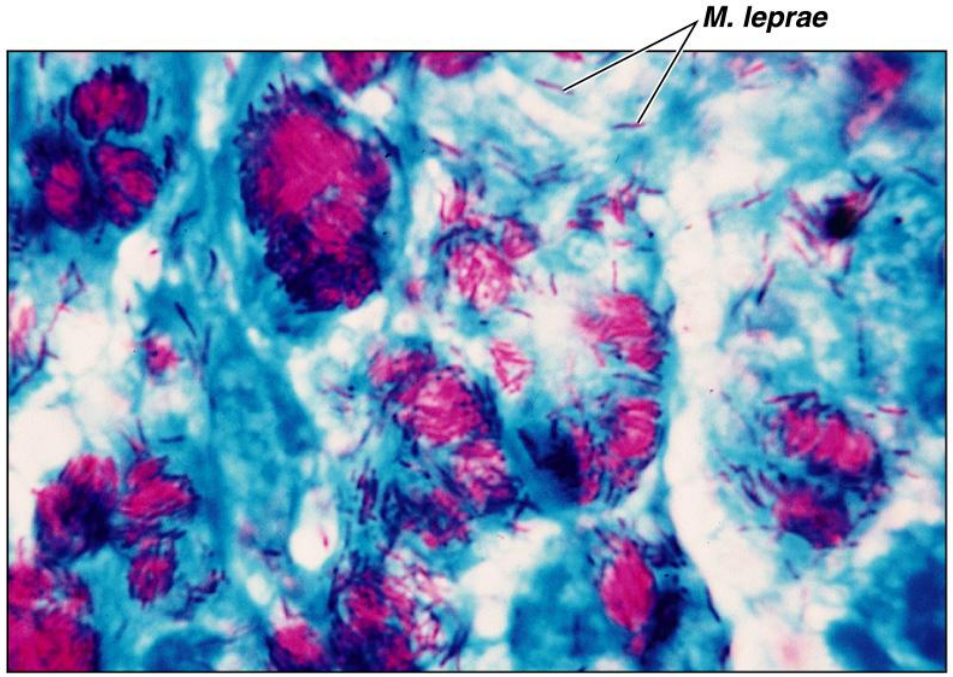
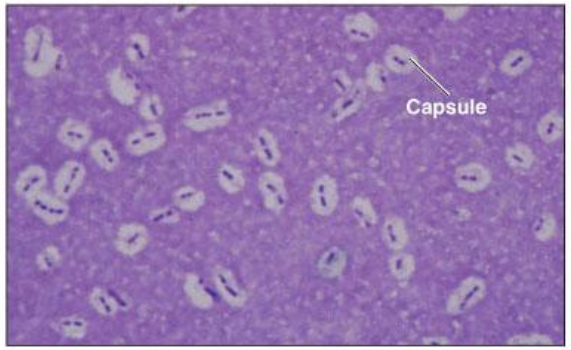


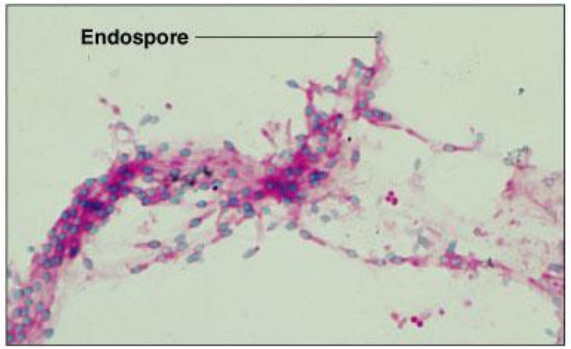
Figure 3.12

Special Stains

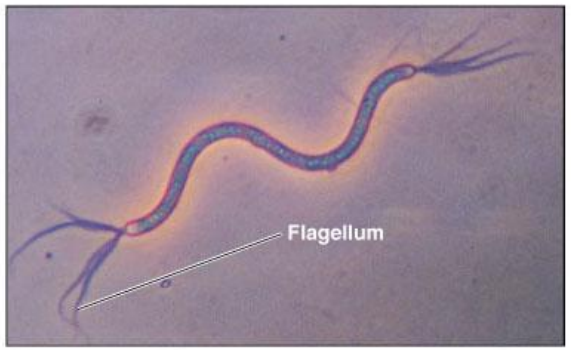
- Negative staining is useful for capsules.
- Heat is required to drive a stain into endospores.
- Flagella staining requires a mordant to make the flagella wide enough to see.



(a) Negative staining.



(b) Endospore staining.



(c) Flagella staining.

Figure 3.13a-c

What should we know after this presentation?

Know the parts of the microscope

Power, resolution, magnificaiton, focus

Know the types of light and electronic microscopes

- Power
- What they are good for observing

What are stains used for?

How do you do a gram stain