
TORTORA • FUNKE • CASE

Microbiology

AN INTRODUCTION

10th EDITION

Chapter 3

Observing Microorganisms Through a Microscope

PowerPoint® Lecture Slide Presentation prepared by Christine L. Case Modified by Nick Kapp

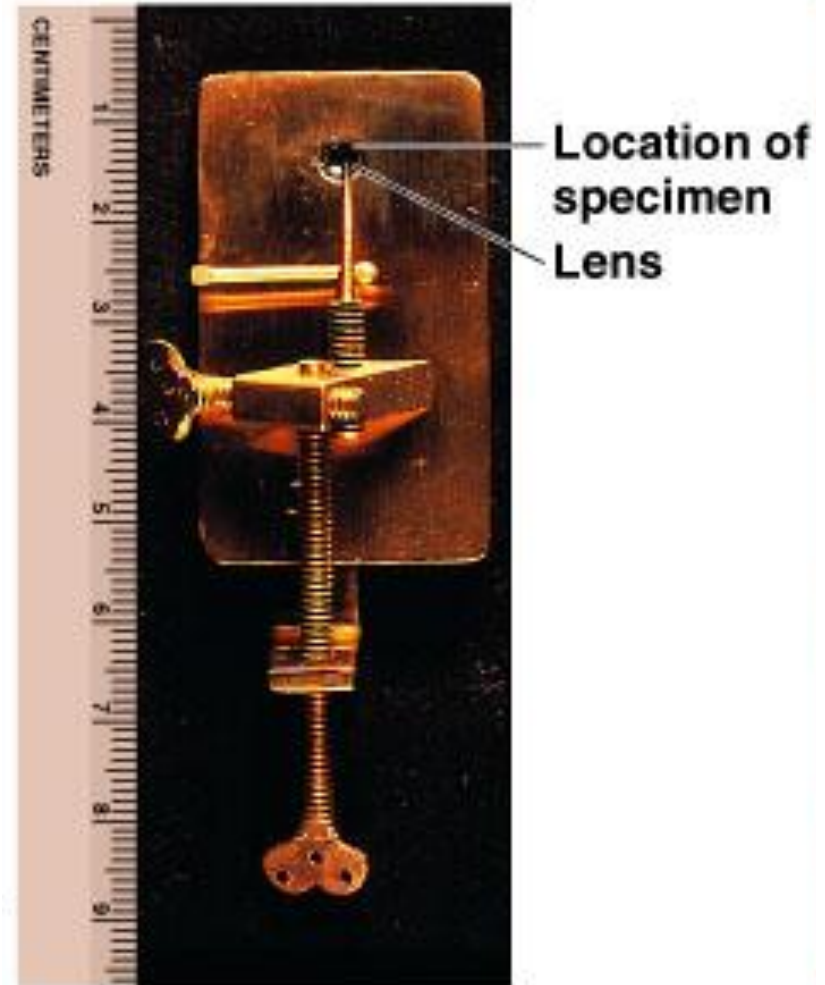
Copyright © Fresh for Today's classes

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

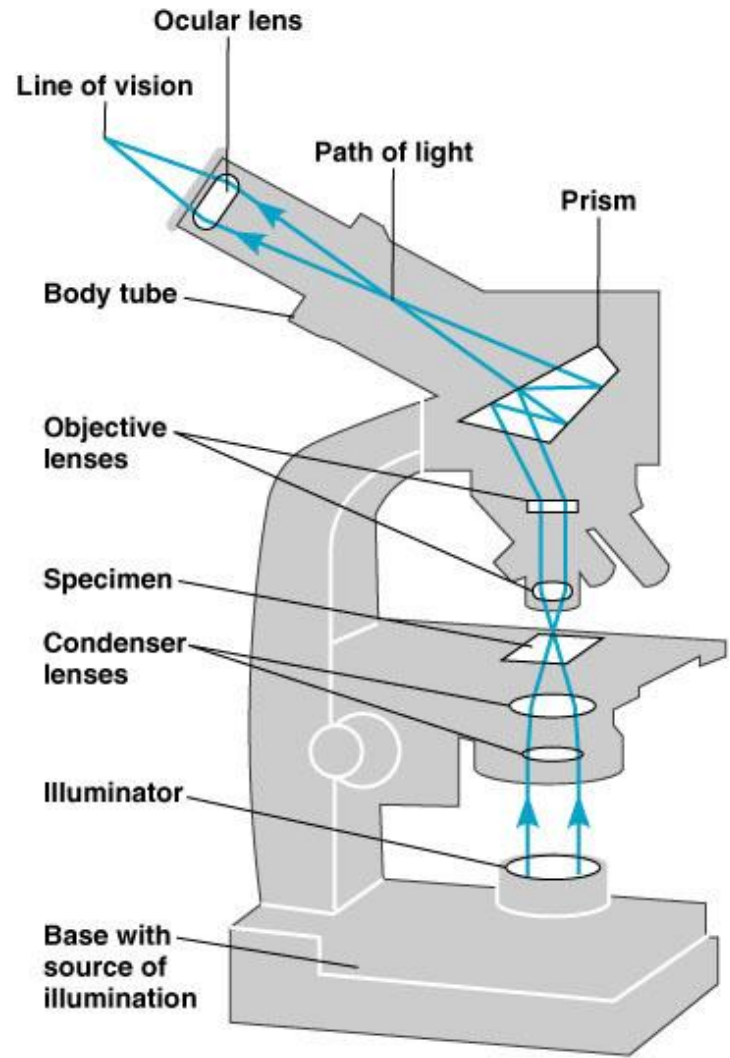
- A simple microscope has only one lens.



(b) Microscope replica

Microscopy: The Instruments

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



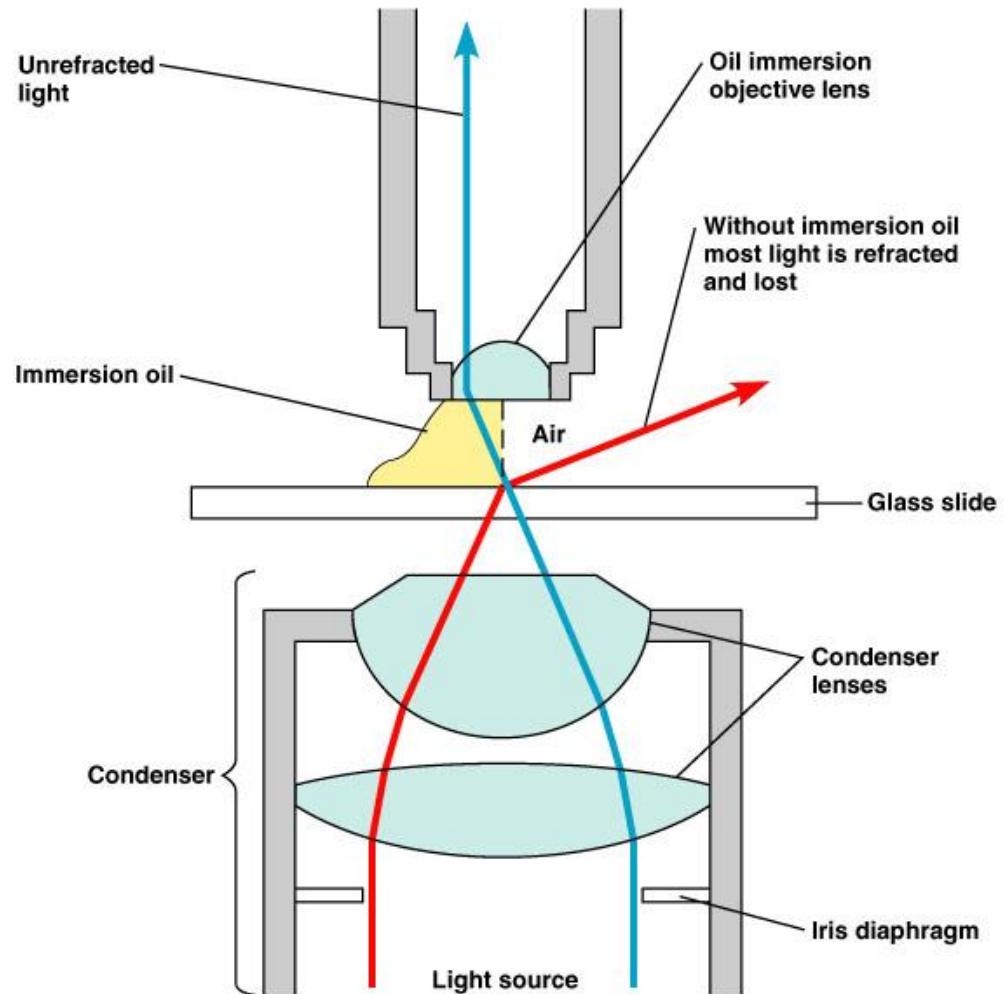
(b) The path of light (bottom to top)

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 / $2 \times$ numerical aperture (a property of the lens).

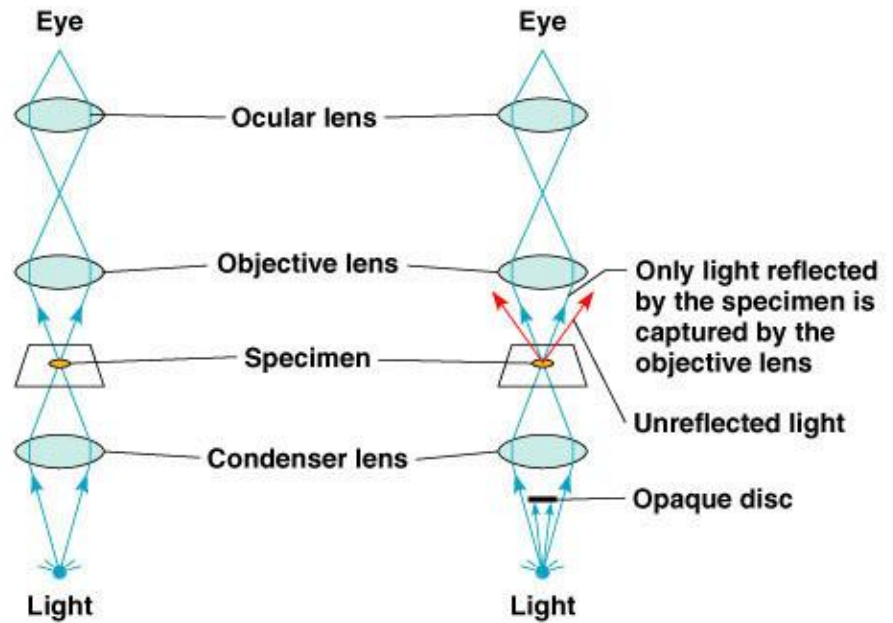
Microscopy: The Instruments

- 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.



Brightfield Illumination

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



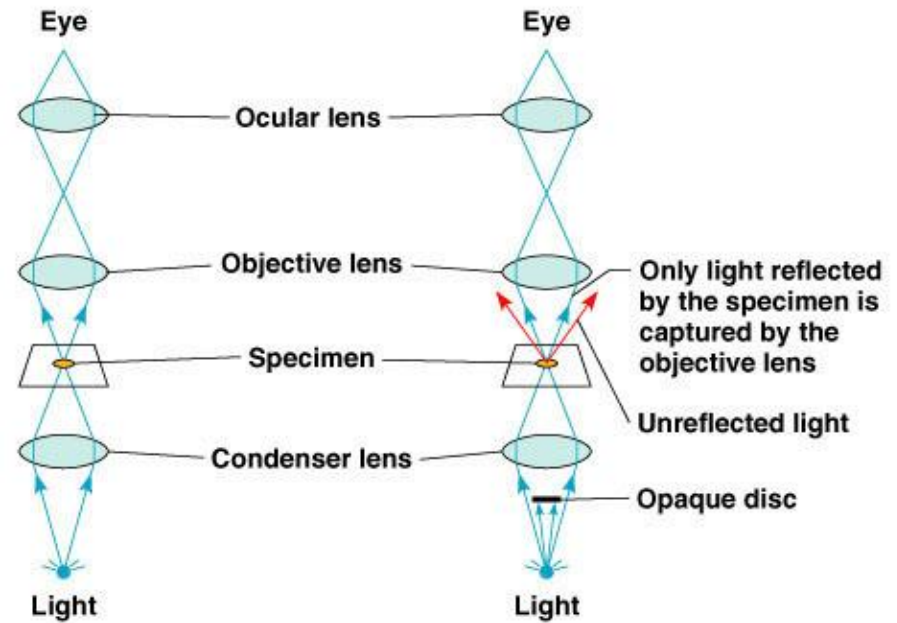
(a) Brightfield



(b) Darkfield

Darkfield Illumination

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



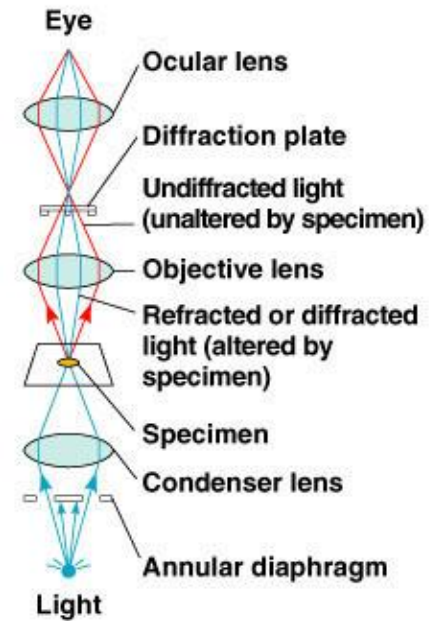
(a) Brightfield



(b) Darkfield

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



(c) Phase-contrast

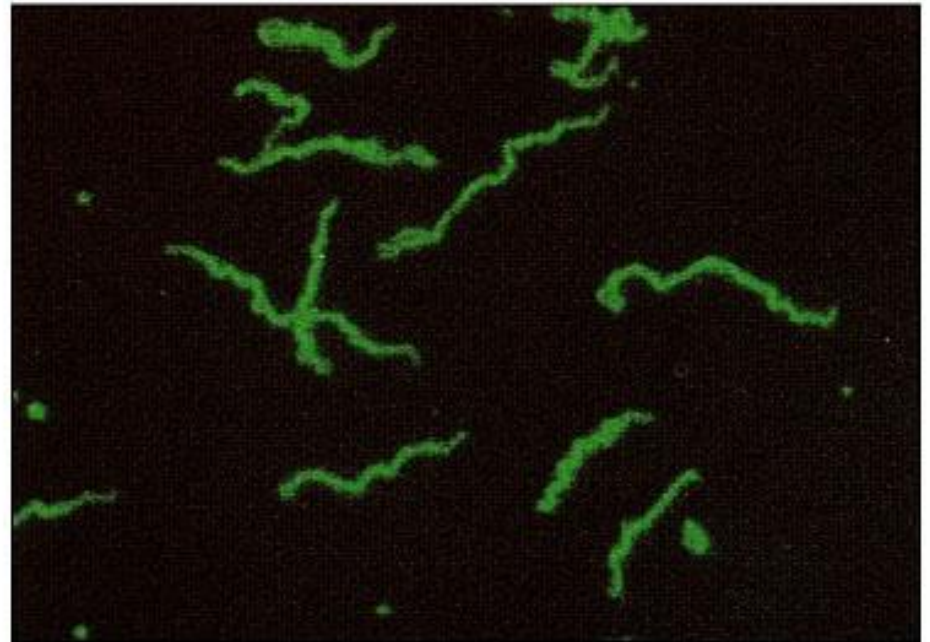
Differential Interference Contrast Microscopy

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



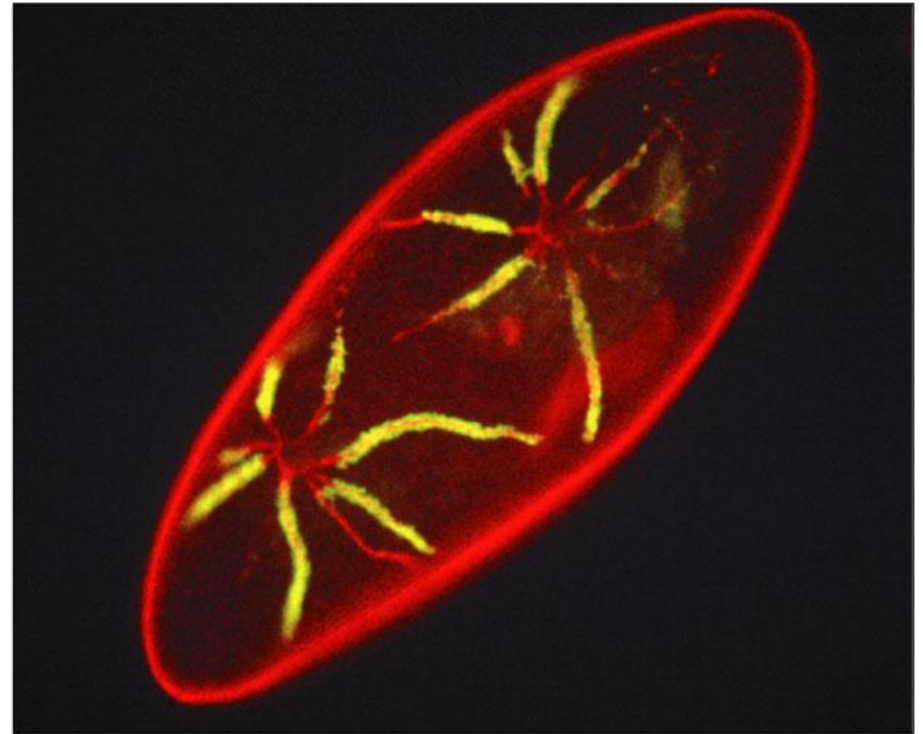
Fluorescence Microscopy

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



Confocal Microscopy

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

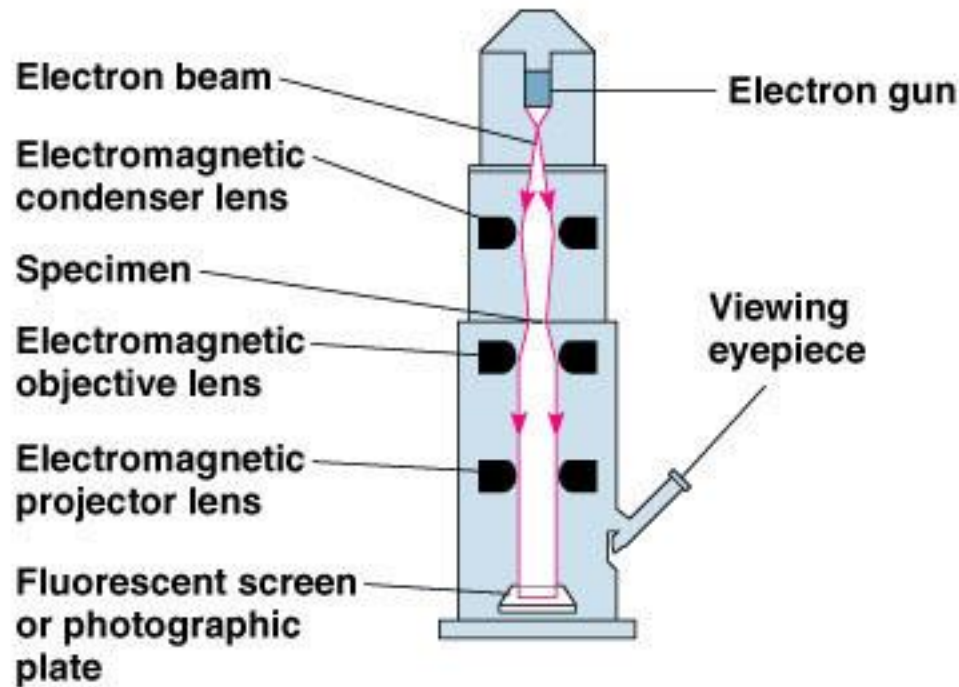


Electron Microscopy

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

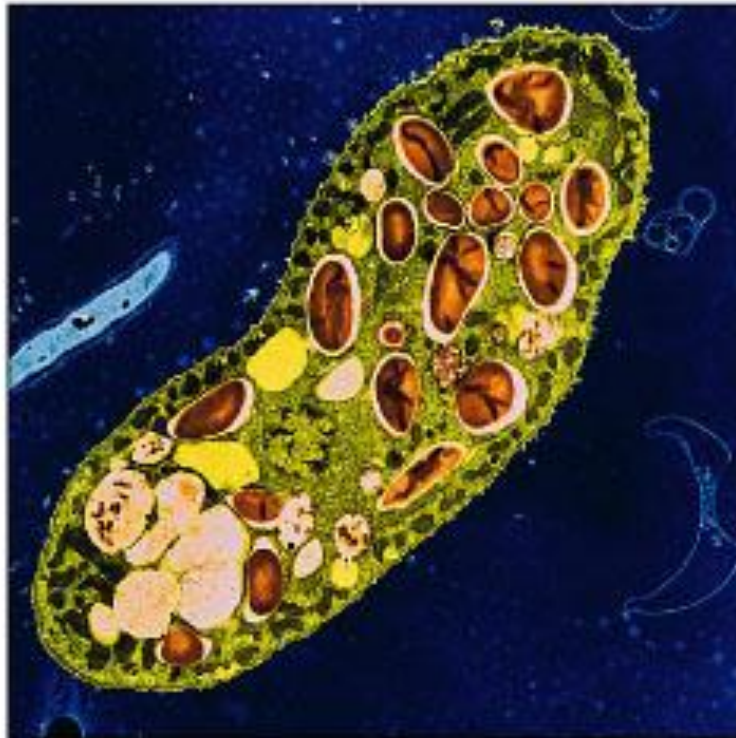
Transmission Electron Microscopy (TEM)

- 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.



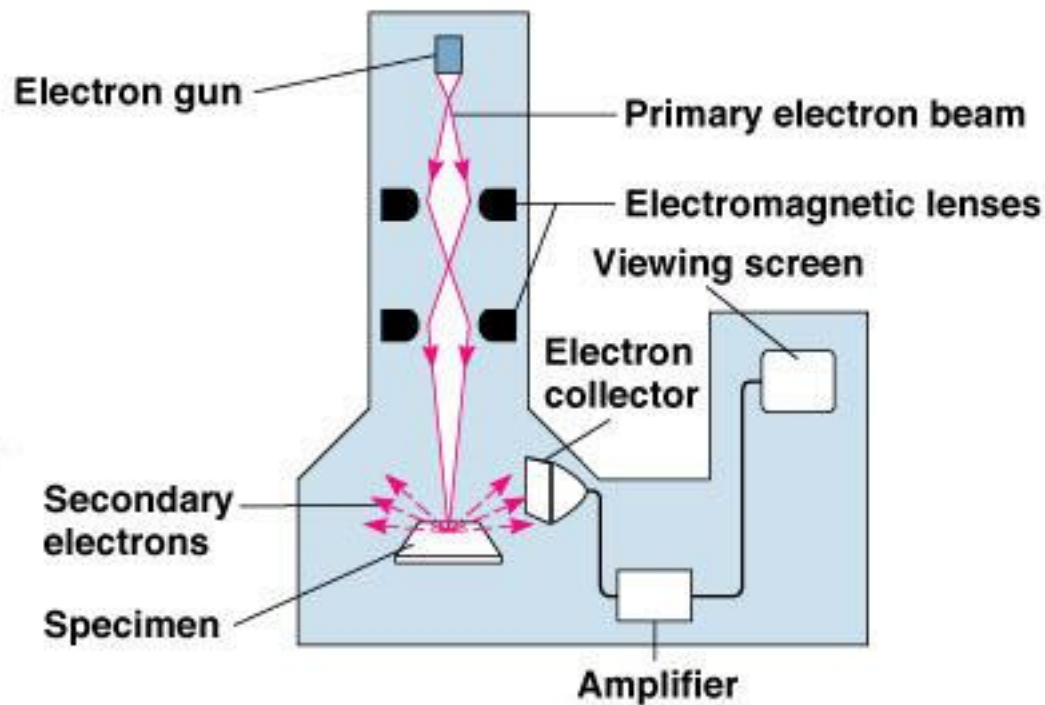
Transmission Electron Microscopy (TEM)

- 10,000-100,000 \times ; 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.



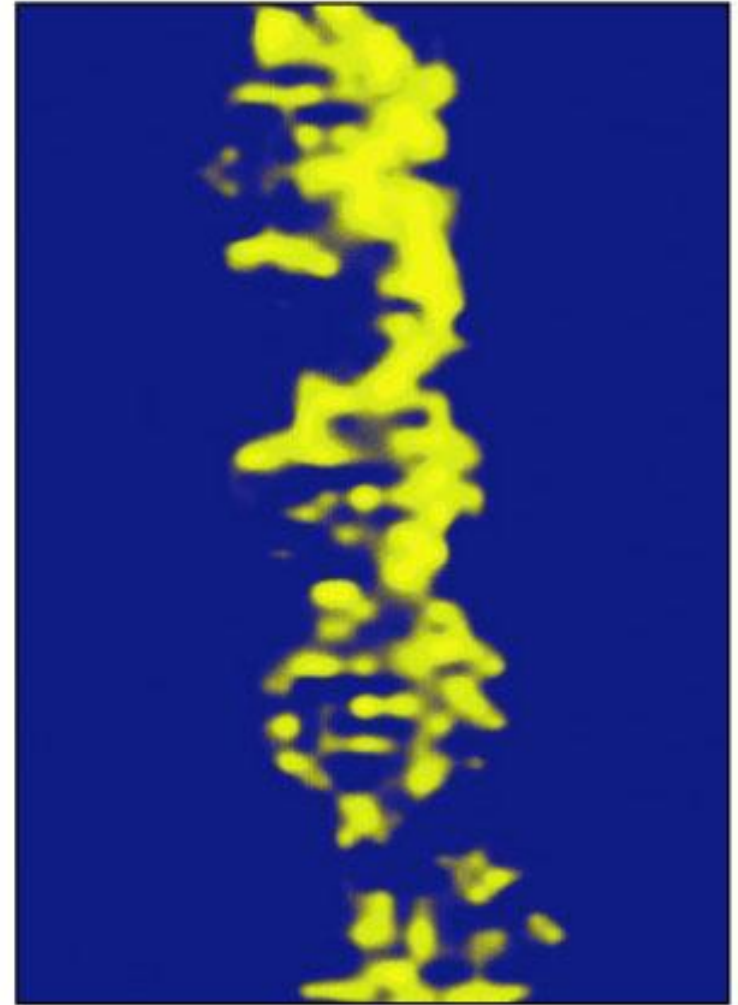
Scanning Electron Microscopy (SEM)

- 1000-10,000×; resolution 20 nm



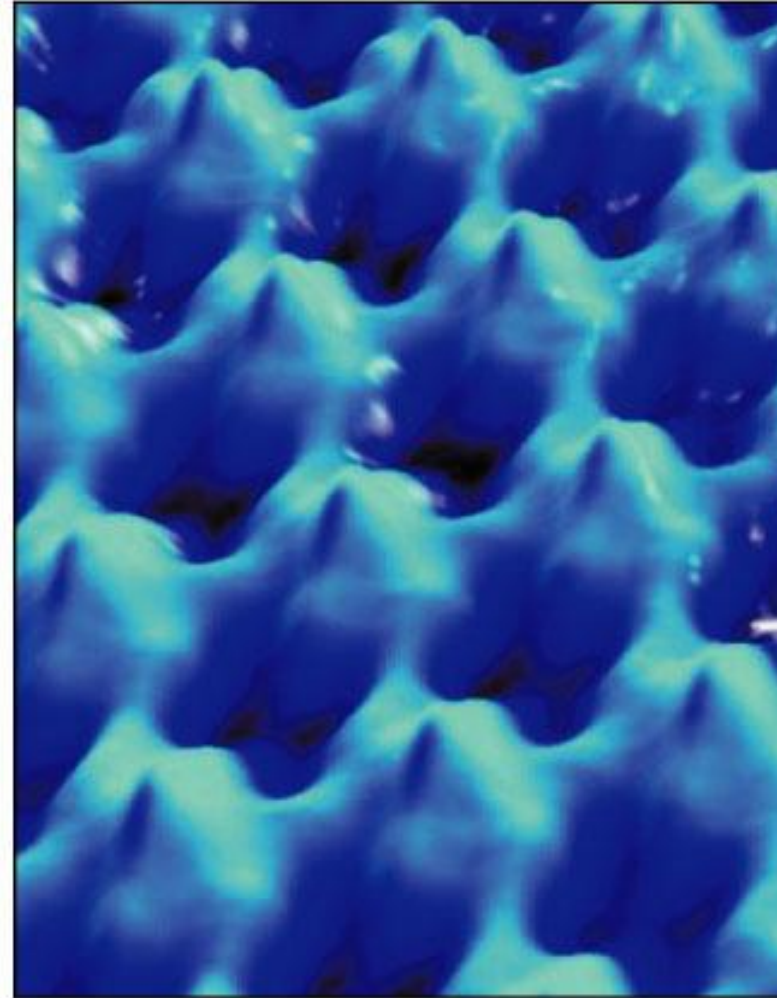
Scanning-Probe Microscopy

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



Scanning-Probe Microscopy

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

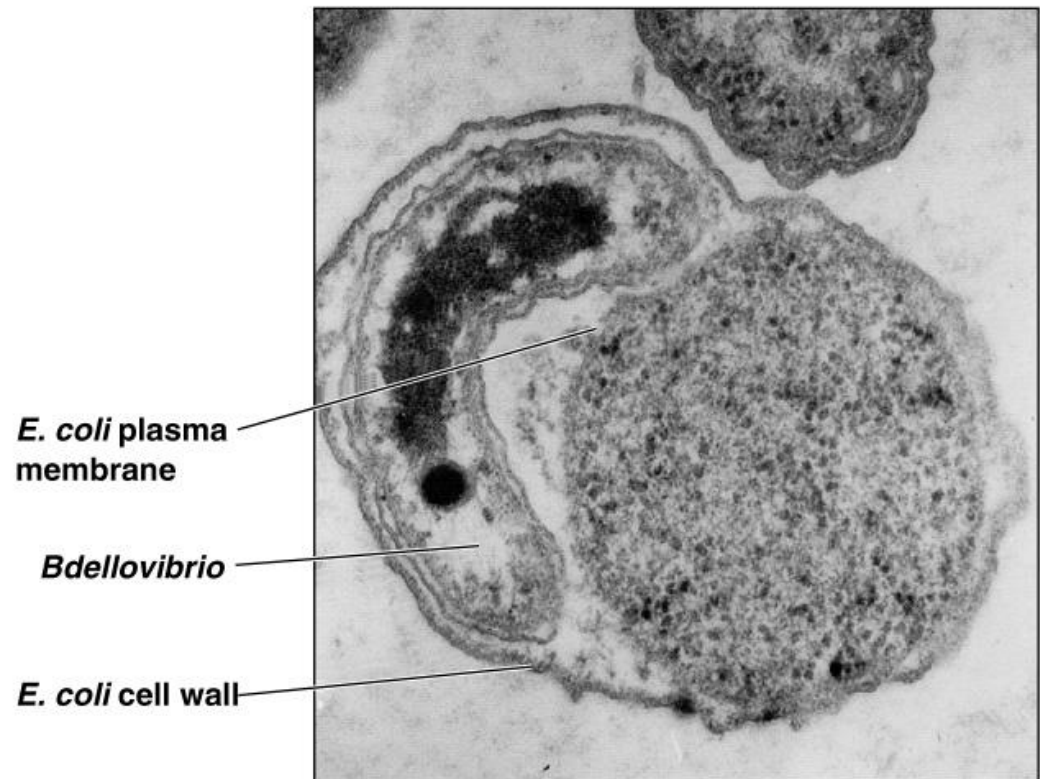


Preparation of Specimens for Light Microscopy

- 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.

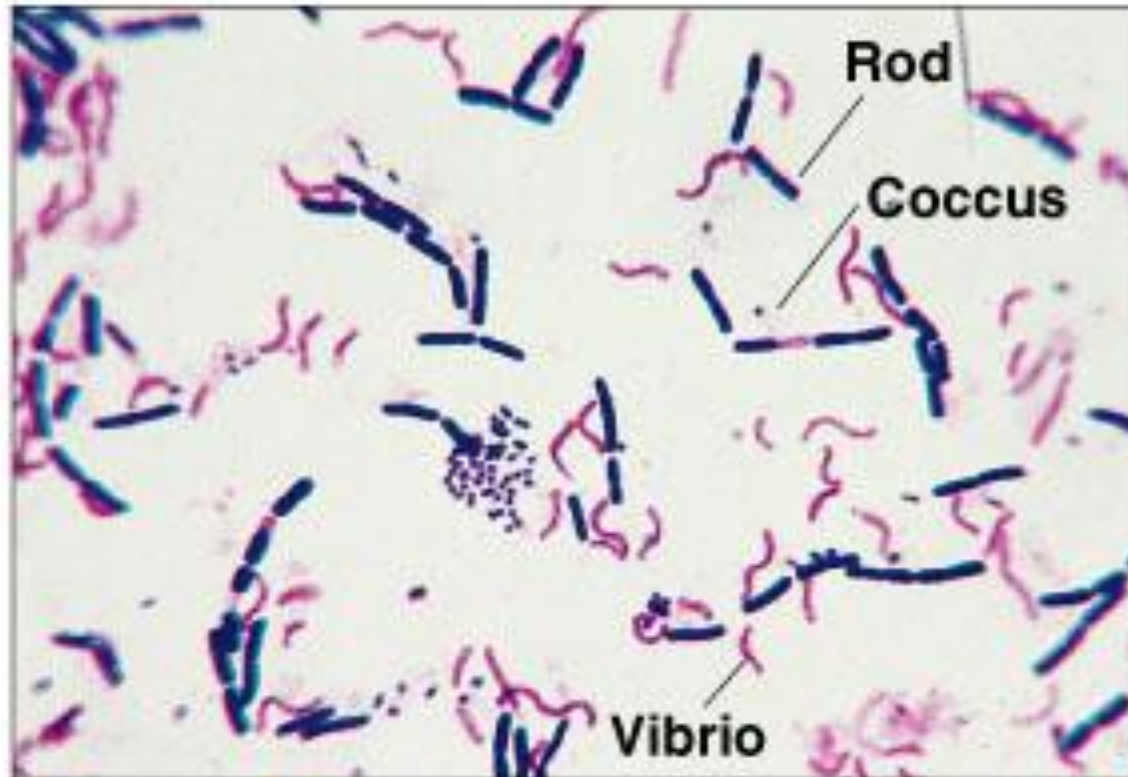
Differential Stains: Gram Stain

- 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

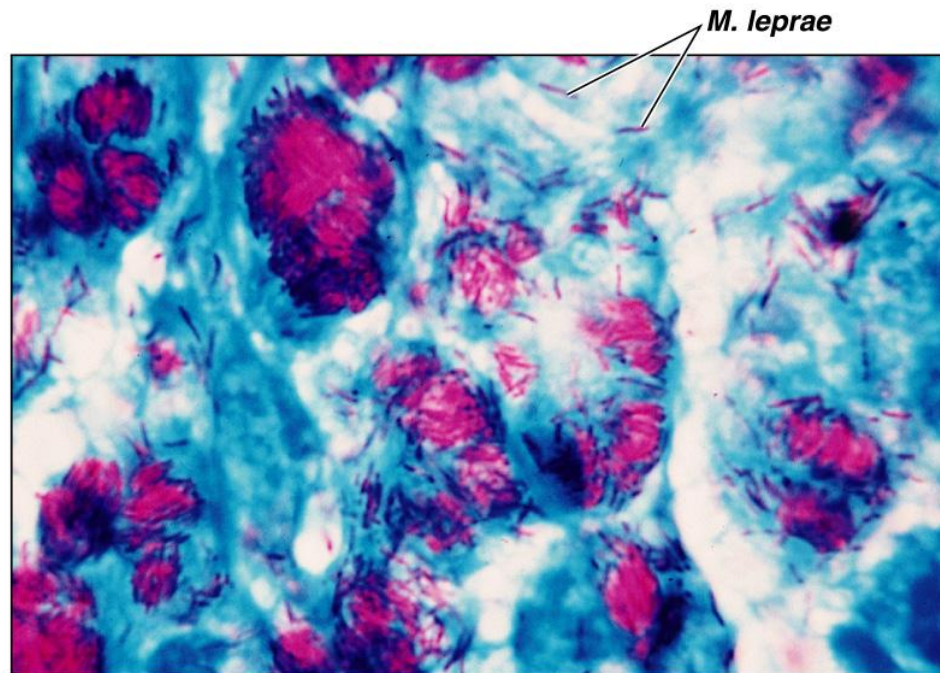
| | Color of Gram + cells | Color of Gram – cells |
|--|-----------------------|-----------------------|
| Primary stain: Crystal violet | Purple | Purple |
| Mordant: Iodine | Purple | Purple |
| Decolorizing agent: Alcohol-acetone | Purple | Colorless |
| Counterstain: Safranin | Purple | Red |

Differential Stains: Gram Stain



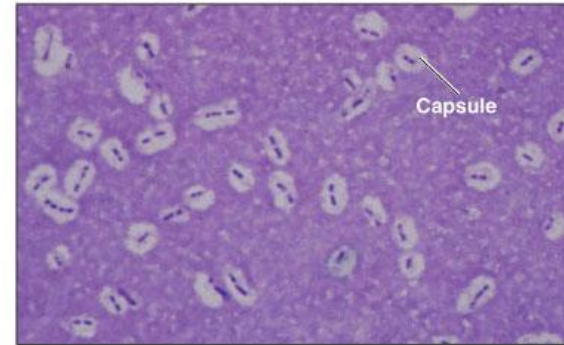
Differential Stains: Acid-Fast Stain

- 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.

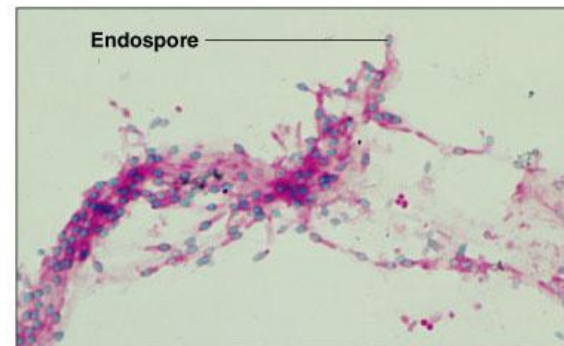


Special Stains

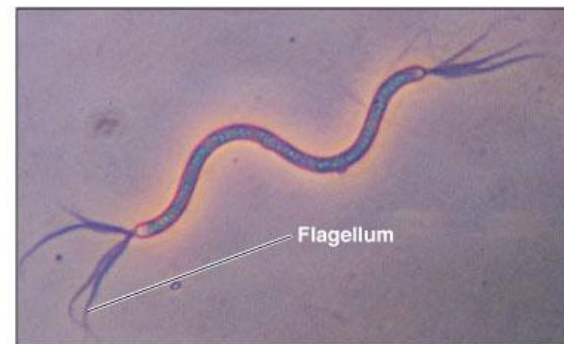
- Negative staining is useful for capsules.
- Heat is required to drive a stain into endospores.
- Flagella staining requires a mordant to make the flagella wide enough to see.



(a) Negative staining.



(b) Endospore staining.



(c) Flagella staining.

What should we know after this presentation?

Know the parts of the microscope

Power, resolution, magnification, 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