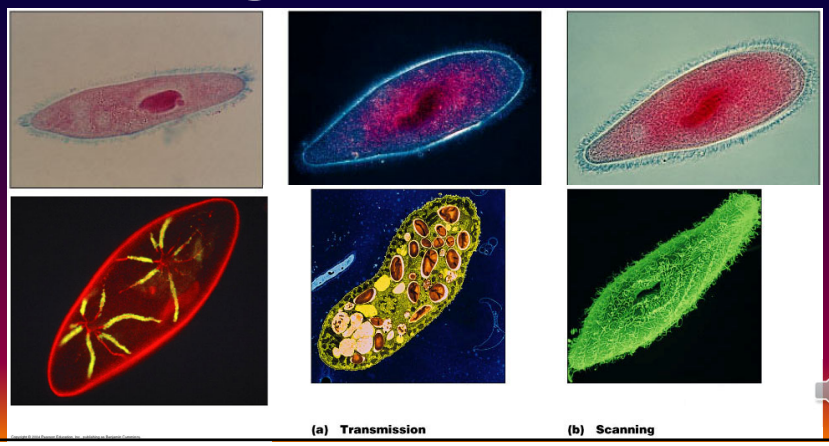
Chapter 3

Observing Microorganisms Through a Microscope



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Chapter 3:

Learning Objectives: Students should be able to…

1. Convert between **metric size units** of meters, centimeters, millimeters, microns, and nanometers.
2. Describe the basic differences between **Light Microscopy** (bright field, dark field, phase-contrast, and fluorescent) and **Electron Microscopy** (Transmission and Scanning EM).
3. Describe the method and utility of several different specimen **staining procedures**.

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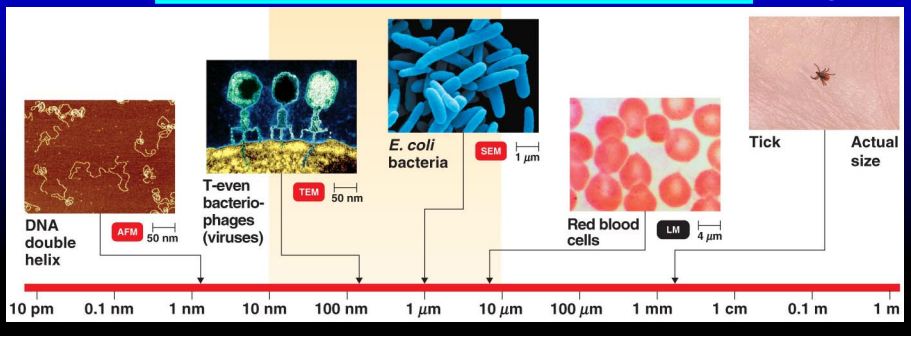
3.1) Units of Measurement

NANOMETERS & MICRONS:

- 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

Figure 3.2



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3.2) Microscopy: The Instruments

- A simple microscope has only one lens.

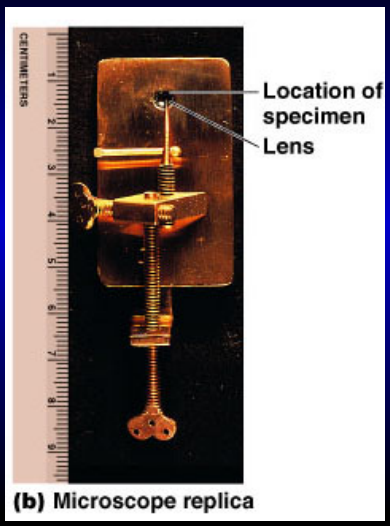

Figure 1.2b

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Microscopy: The Instruments

- In a compound microscope the image from the objective lens is magnified again by the ocular lens.
- Total magnification = objective lens (10, 40, 100X) × ocular lens (10X)

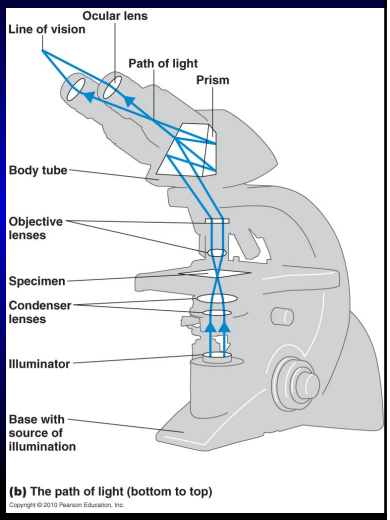

Figure 3.1b

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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 apart.
- Shorter wavelengths of light provide greater resolution
  - (eg: UV).

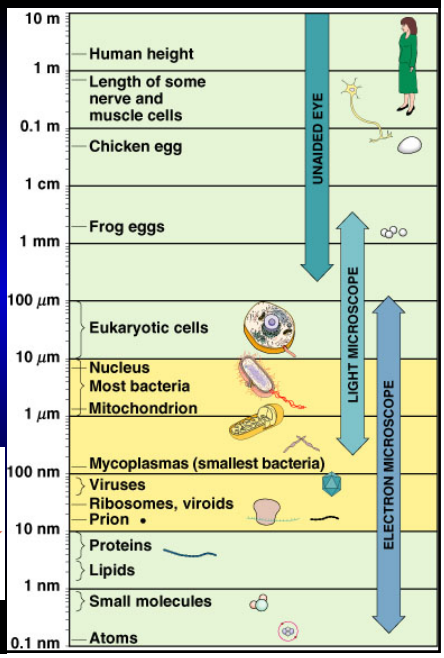

Figure 3.2

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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-magn'n lens.
- *Immersion oil is used to keep light from bending.*

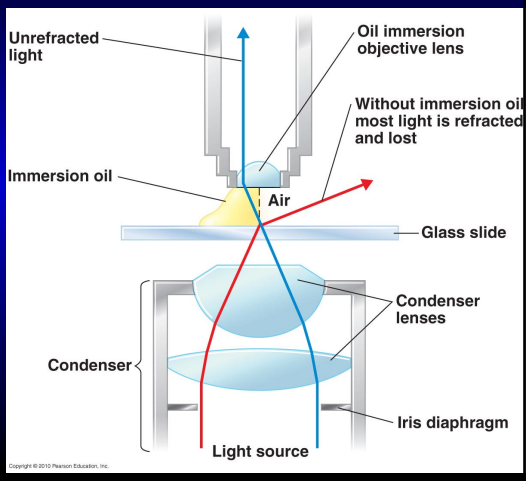

Figure 3.3

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A. Brightfield Illumination

- Dark objects are visible against a bright background.
  - *Light reflected off the specimen does not enter the objective lens.*
    - *Creates contrast / **positive image.***

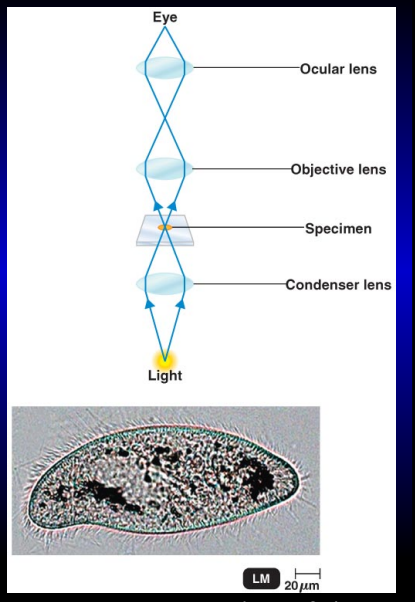

Figure 3.4a, b

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B. Darkfield Illumination

- Light objects are visible against a dark background.
  - *Light reflected off the specimen enters the objective lens.*
    - *Creates contrast / **negative image.***

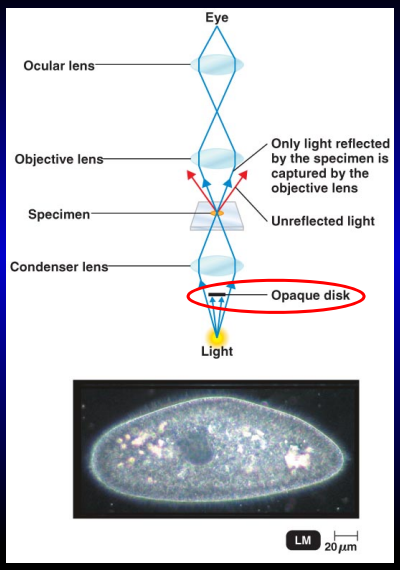

Figure 3.4a, b

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C. Phase-Contrast Microscopy

- Accentuates ***diffraction*** of the light that passes through a specimen.
  - Peaks and valleys of incoming light waves accentuate bright and dark regions of the specimen.

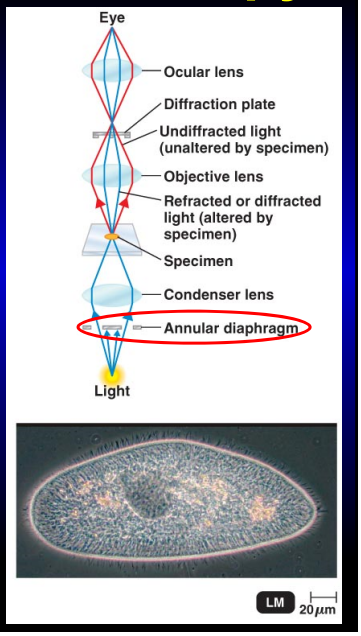

Figure 3.4c

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D. Fluorescence Microscopy

- Uses UV (to red) light.
- Fluorescent substances
  - absorb UV light (or other wavelengths) and
  - emit visible light (at a different wavelength).
- Cells may be stained with fluorescent dyes ("***fluorochromes***").

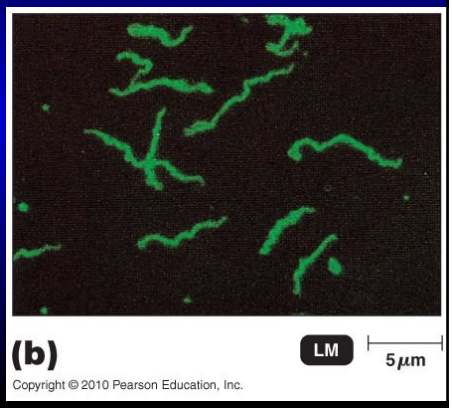

Figure 3.6b

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E. 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

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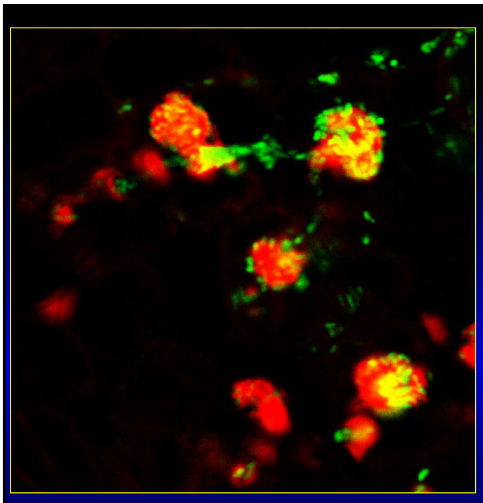
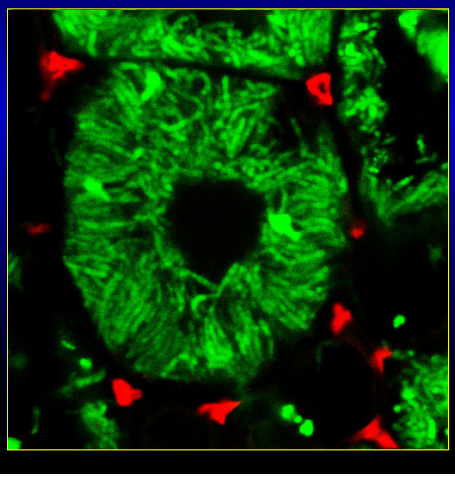


Transgenic, green GFP-expressing bacteria invading plant root tissues

- Symbiotic plant cell wall proteins labeled Red
- Allows visualization of LIVE biological processes!!



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3.3) Electron Microscopy

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

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F. Transmission Electron Microscopy (TEM)

- Ultrathin sections of specimens.
- **Electrons** pass 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**

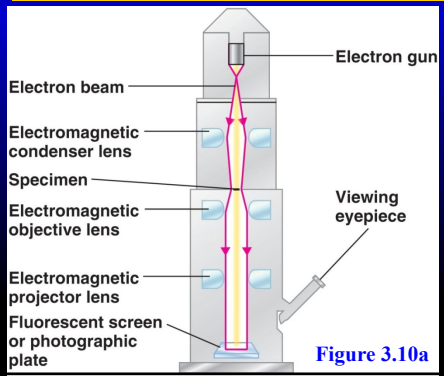
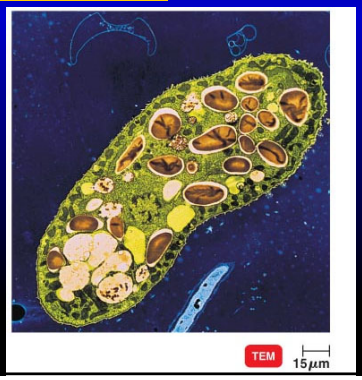


Figure 3.10a



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G. 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.
- **1000-10,000×; resolution = 20 nm**

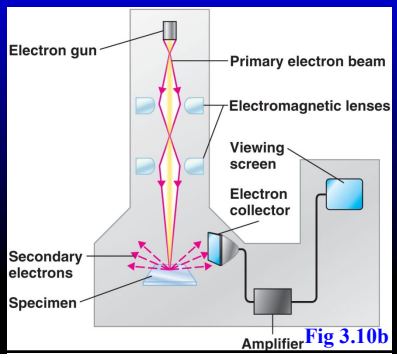

Fig 3.10b

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3.4) 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.

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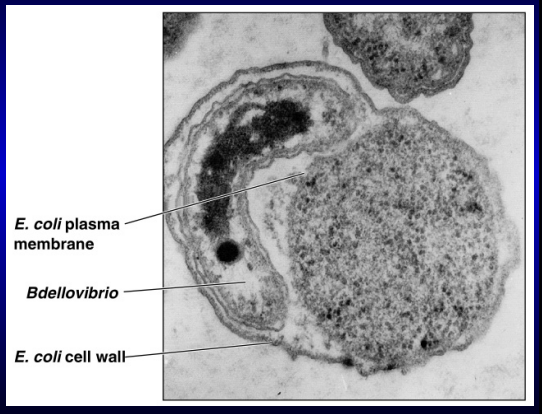
Preparing Smears for Staining

- Live or unstained cells have little contrast with the surrounding medium.
- However, researchers do make discoveries about cell behavior looking at live specimens.
  - Eg: ***Bdellovibrio***



https://youtu.be/-uZjo0ohjFw

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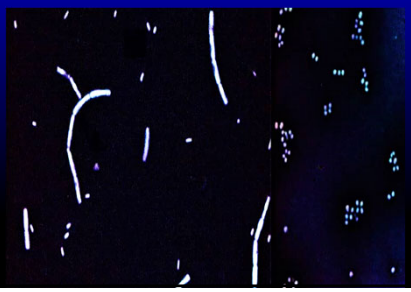
Preparing Smears for Staining

- ***Stains*** consist of a positive and negative ion.
  - In a **basic** dye, the ***chromophore*** (colored molecule) is a **cation**.
    - Carbol fuschin, methylene blue, crystal violet, safranin
  - In an **acidic** dye, the chromophore is an **anion**.
- Staining the background instead of the cell is called ***negative staining***.
  - Use acidic dyes:
    - India ink, nigrosin.



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A. 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.

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B. 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.

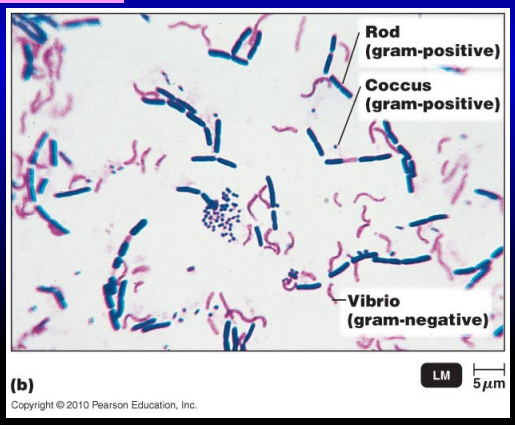

Figure 3.12b

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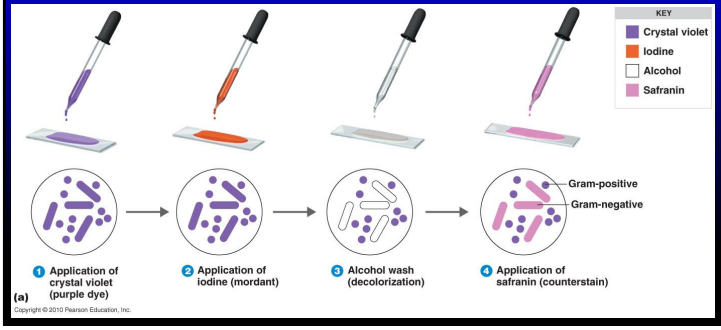
Gram Stain

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



http://highered.mheducation.com/olcweb/cgi/pluginpop.cgi?it=swf::530::530::/sites/dl/free/0073525502/930300/Gram_Stain.swf::Gram%20Stain

http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab6/images/gram_stain_11.swf

Figure 3.12a

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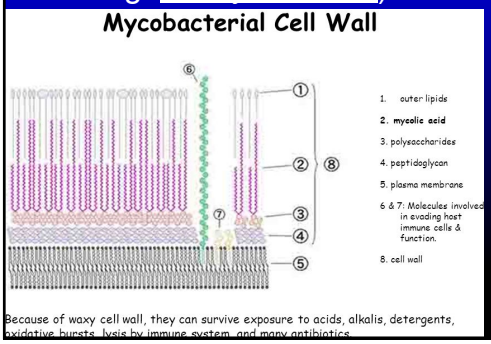
C. Differential Stains: Acid-Fast Stain

- Waxy cells that retain a basic stain in the presence of acid-alcohol are called ***acid-fast***. Lipid-rich walls.
  - ***Mycobacterium; Nocardia***
- Non–acid-fast cells lose the basic stain when rinsed with **acid-alcohol.** (heated and phenol solvent)
  - usually counterstained (with a different color basic stain; eg: methylene blue) to see them.



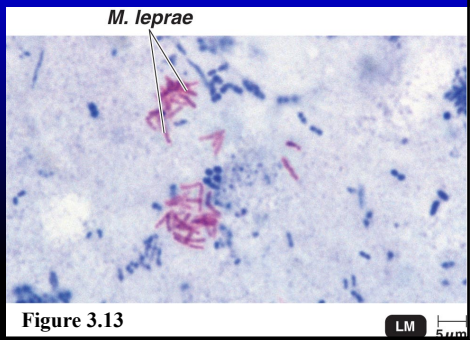


Figure 3.13

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D. Special Stains

- **Negative staining** is useful for capsules.
- Differential: **Endospore Stain** – Heat is required to drive a stain into endospores.
  - Phenol solvent sometimes added.

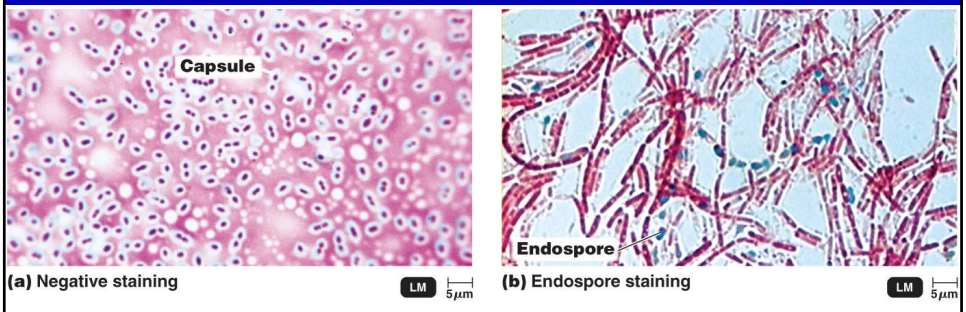

Figure 3.14

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