

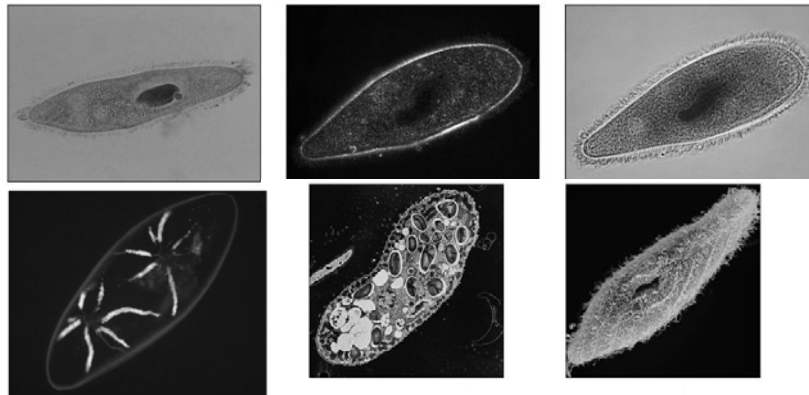
# Chapter 3:

## 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**.

# Chapter 3

## Observing Microorganisms Through a Microscope



(a) Transmission

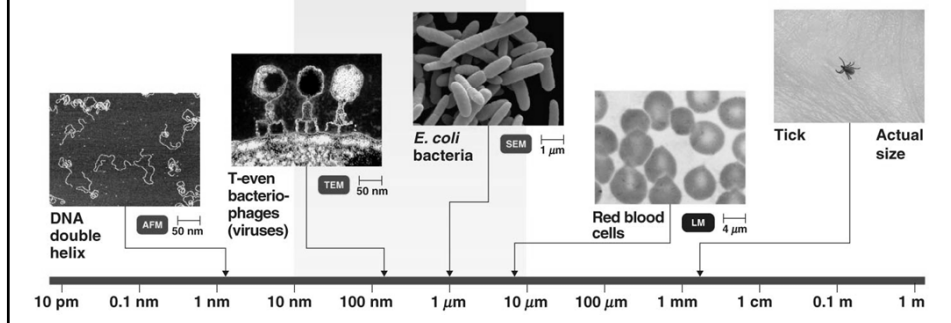
(b) Scanning

## 3.1) Units of Measurement

### NANOMETERS & MICRONS:

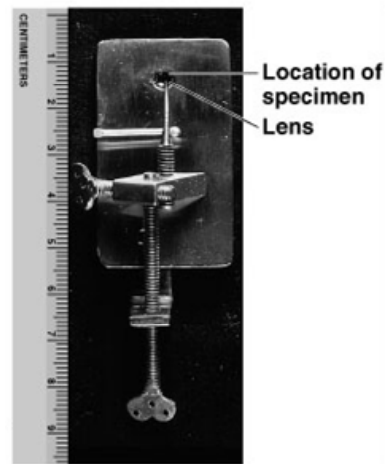
- $1 \mu\text{m} = 10^{-6} \text{ m} = 10^{-3} \text{ mm}$
- $1 \text{ nm} = 10^{-9} \text{ m} = 10^{-6} \text{ mm}$
- $1000 \text{ nm} = 1 \mu\text{m}$
- $0.001 \mu\text{m} = 1 \text{ nm}$

Figure 3.2



## 3.2) Microscopy: The Instruments

- A simple microscope has only one lens.

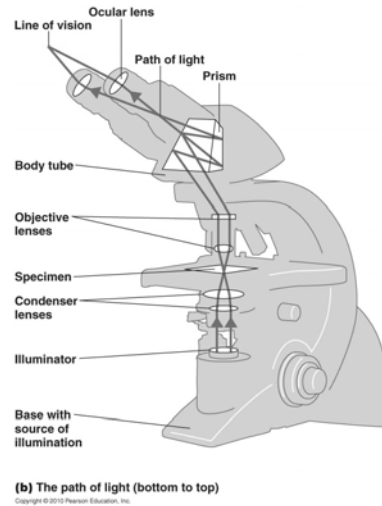


(b) Microscope replica

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 (10, 40, 100X) × ocular lens (10X)



(b) The path of light (bottom to top)  
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**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 apart.
- Shorter wavelengths of light provide greater resolution
  - (eg: UV).

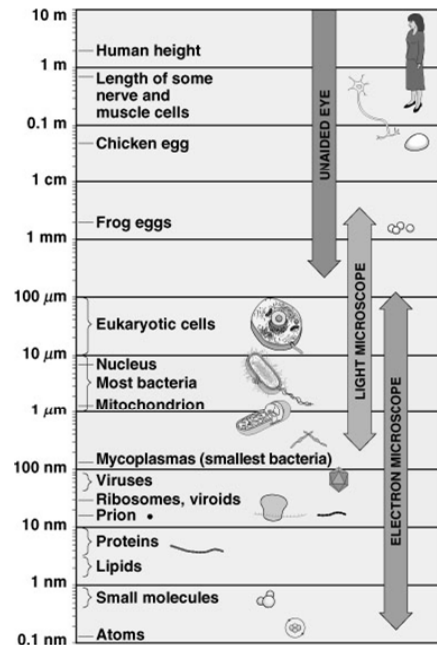


Figure 3.2

## 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.*

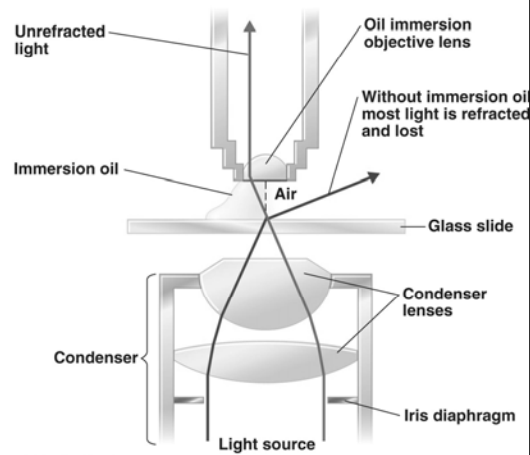
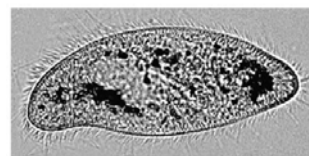
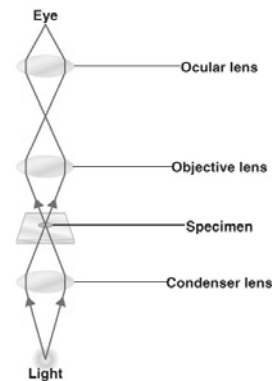


Figure 3.3

## A. Brightfield Illumination

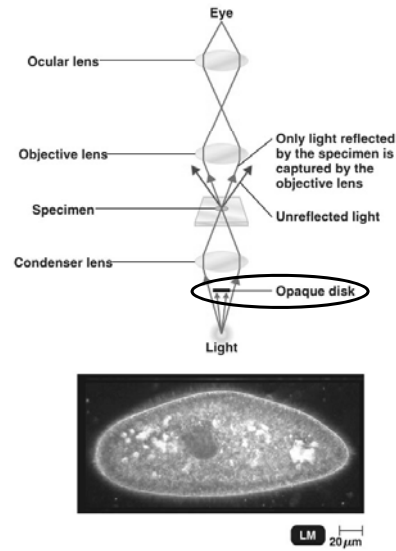
- Dark objects are visible against a bright background.
  - *Light reflected off the specimen does not enter the objective lens.*
    - *Creates contrast / positive image.*



LM 20 μm  
Figure 3.4a, b

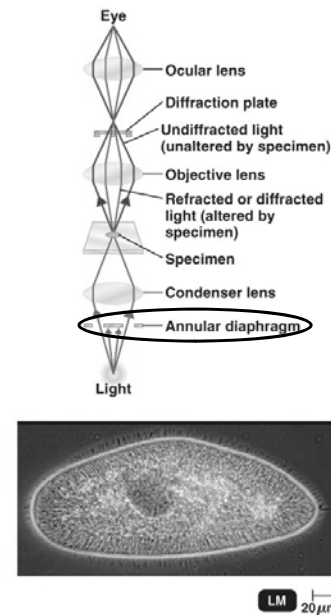
## B. Darkfield Illumination

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



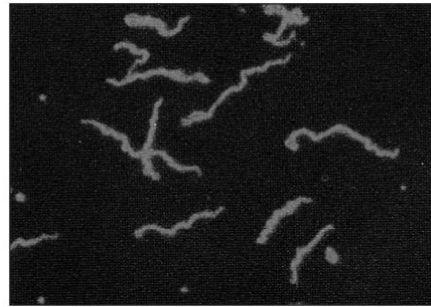
## C. Phase-Contrast Microscopy

- 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.



## 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***”).



(b)

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LM

5 μm

Figure 3.6b

## E. Confocal Microscopy

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

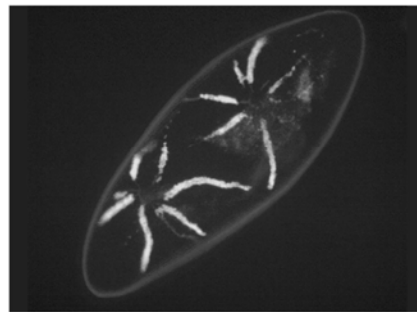
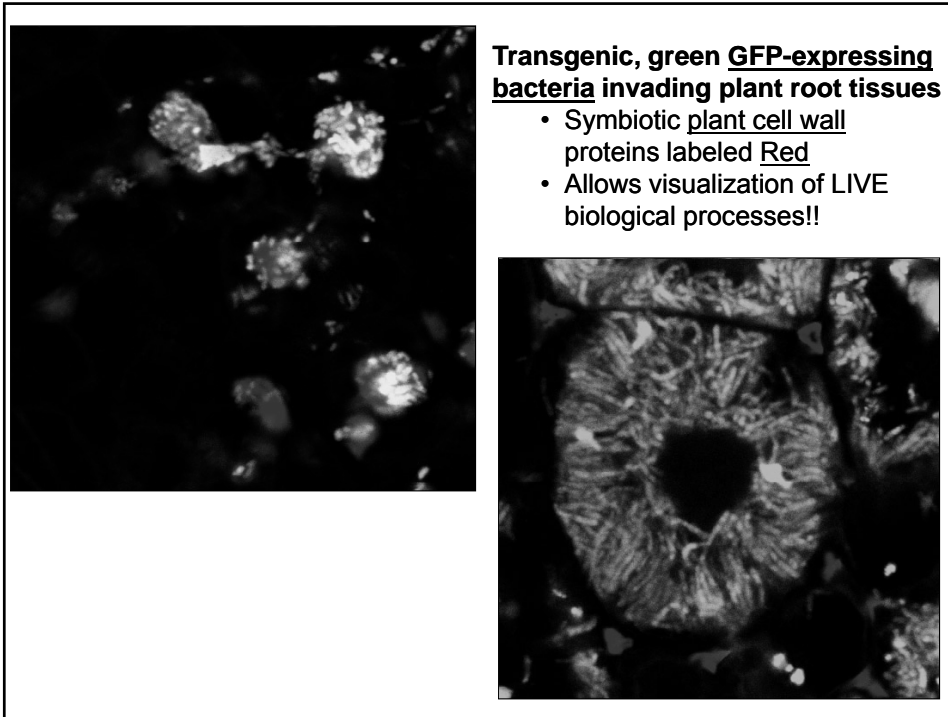


Figure 3.7

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CF

20 μm

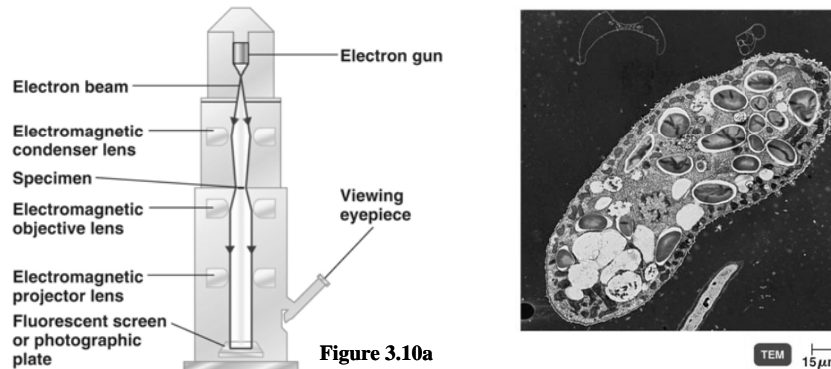


### 3.3) Electron Microscopy

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

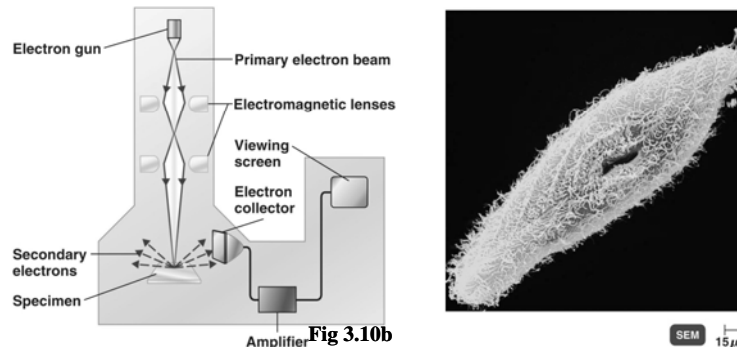
## F. Transmission Electron Microscopy (TEM)

- 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**



## 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**

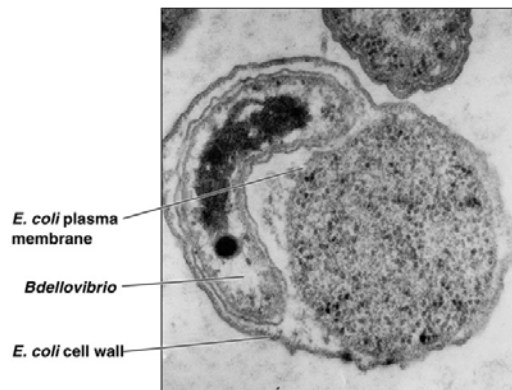


## 3.4) Preparation of Specimens for Light Microscopy

- 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.

## 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

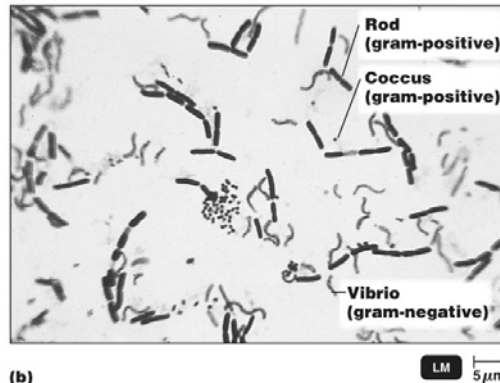
- **Stains** consist of a positive and negative ion.
  - In a **basic dye**, the **chromophore** (colored molecule) is a **cation**.
    - Carbol fuschin, methylene blue, crystal violet, safranin
  - In an **acidic dye**, the chromophore is an **anion**.
- Staining the background instead of the cell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

### 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.

## 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.

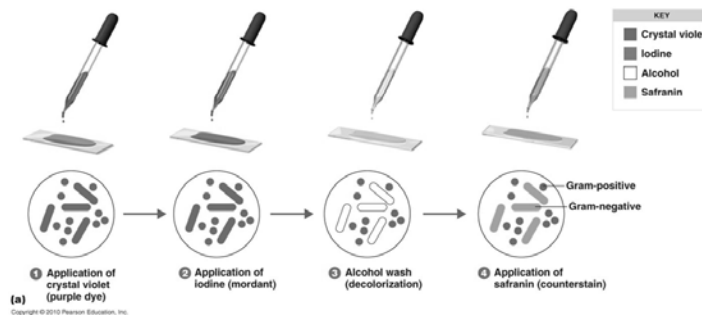


(b)  
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Figure 3.12b

## Gram Stain

	Color of Gram + cells	Color of Gram – cells
Primary stain: <u>C</u> ystal violet	Purple	Purple
Mordant: <u>I</u> odine	Purple	Purple
Decolorizing agent: <u>A</u> lcohol	Purple	Colorless
Counterstain: <u>S</u> afranin	Purple	Red/Pink



(a)  
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Figure 3.12a

## C. Differential Stains: Acid-Fast Stain

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

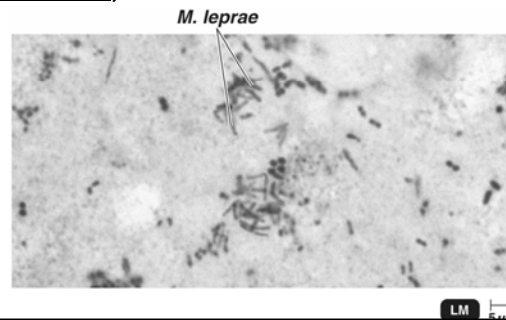
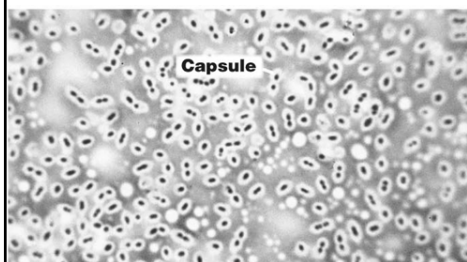


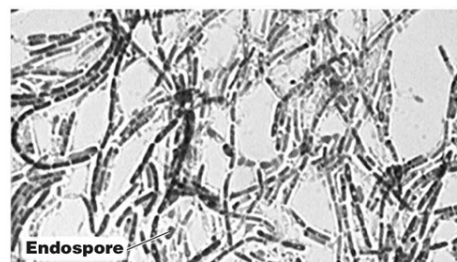
Figure 3.13

## 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.



(a) Negative staining



(b) Endospore staining

Figure 3.14