Lecture 9
27/01/2023
By Prof Manish Kumar

Observing Microorganisms

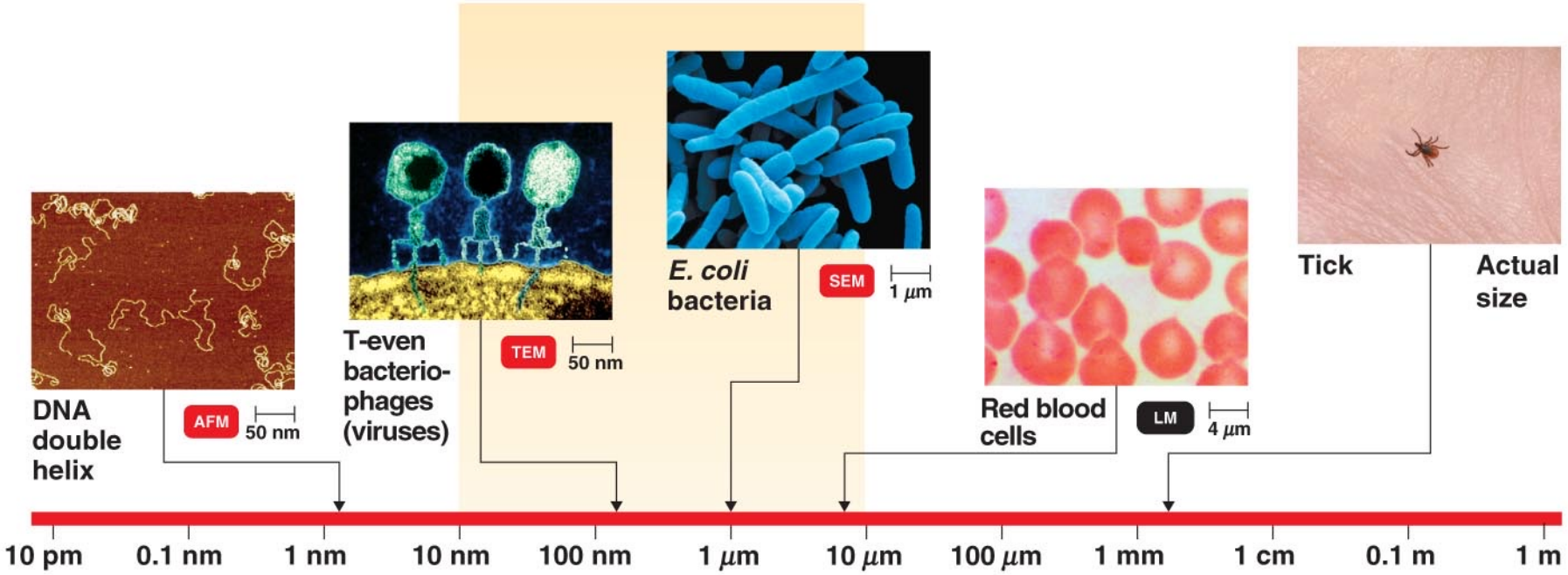


Figure 3.2

Units of Measurement

- 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

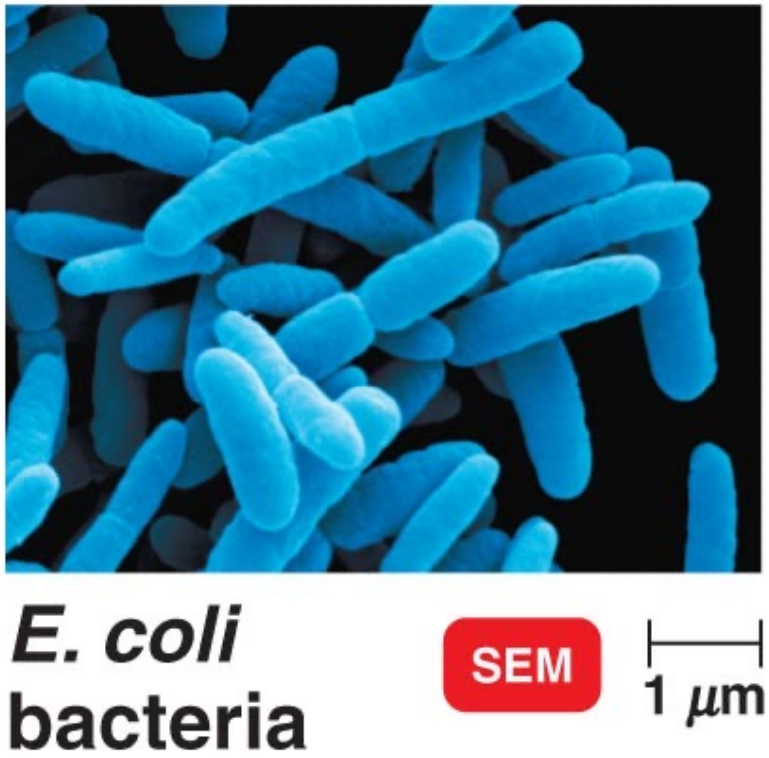
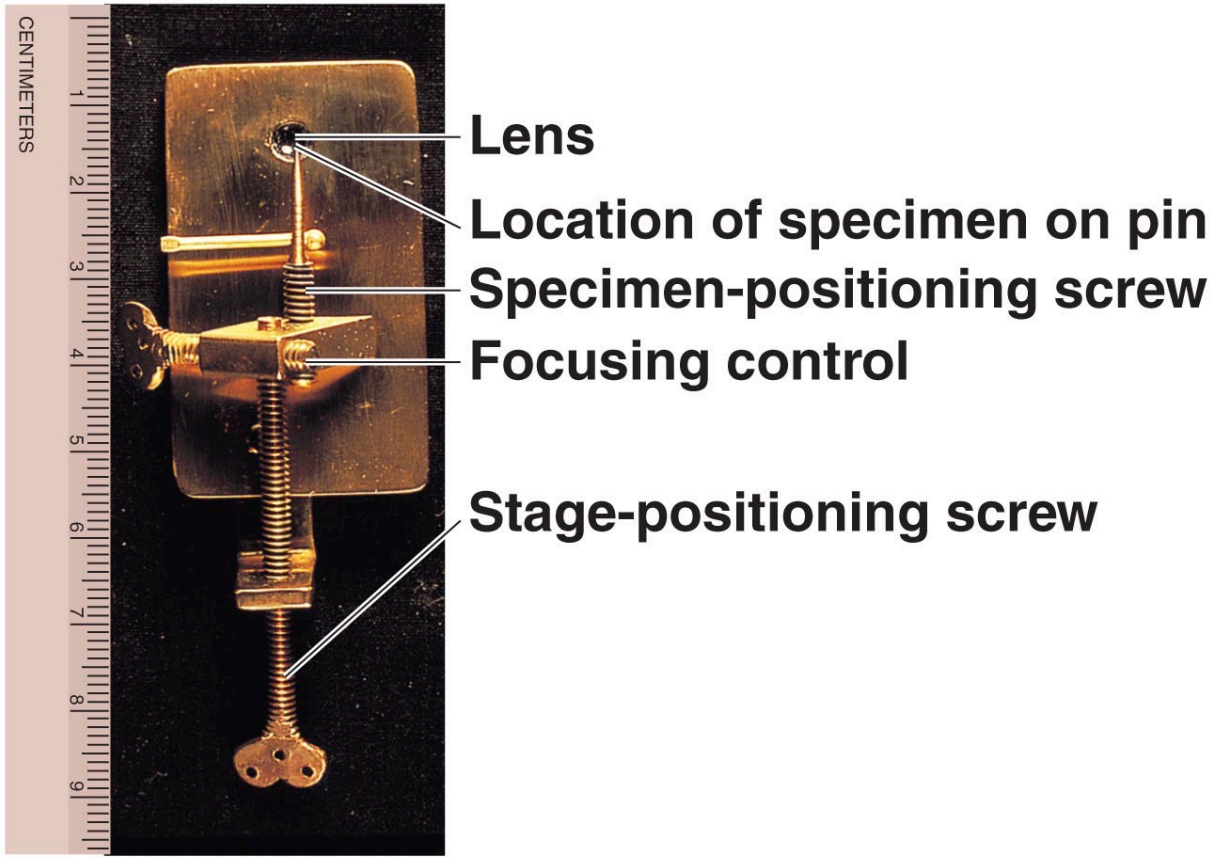


Figure 3.2

Microscopy: The Instruments

- A simple microscope has only one lens



(b) Microscope replica

Figure 1.2b

Light Microscopy

- Use of 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 microscopy
  - Fluorescence microscopy
  - Confocal microscopy

The Compound Light Microscope

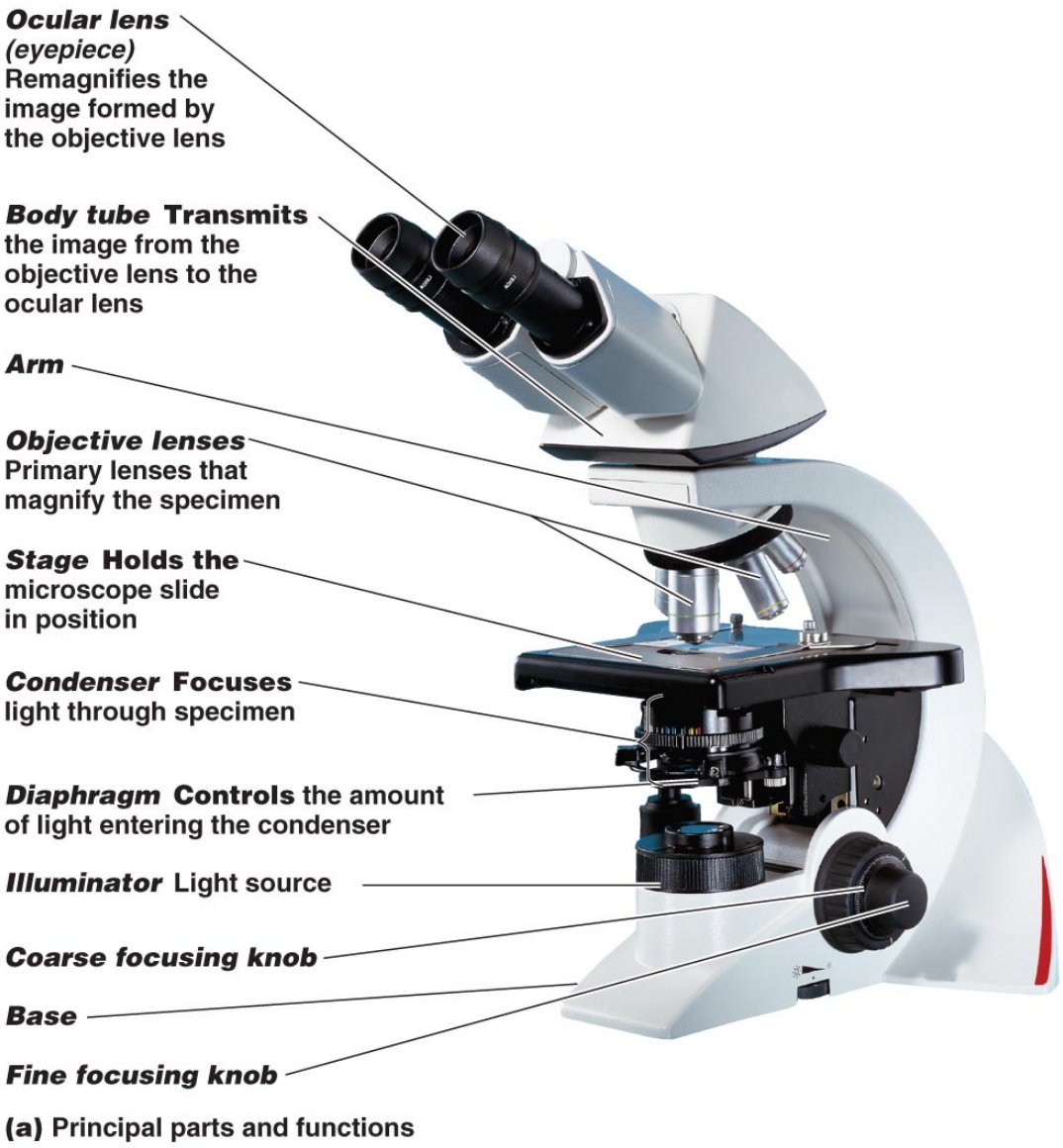
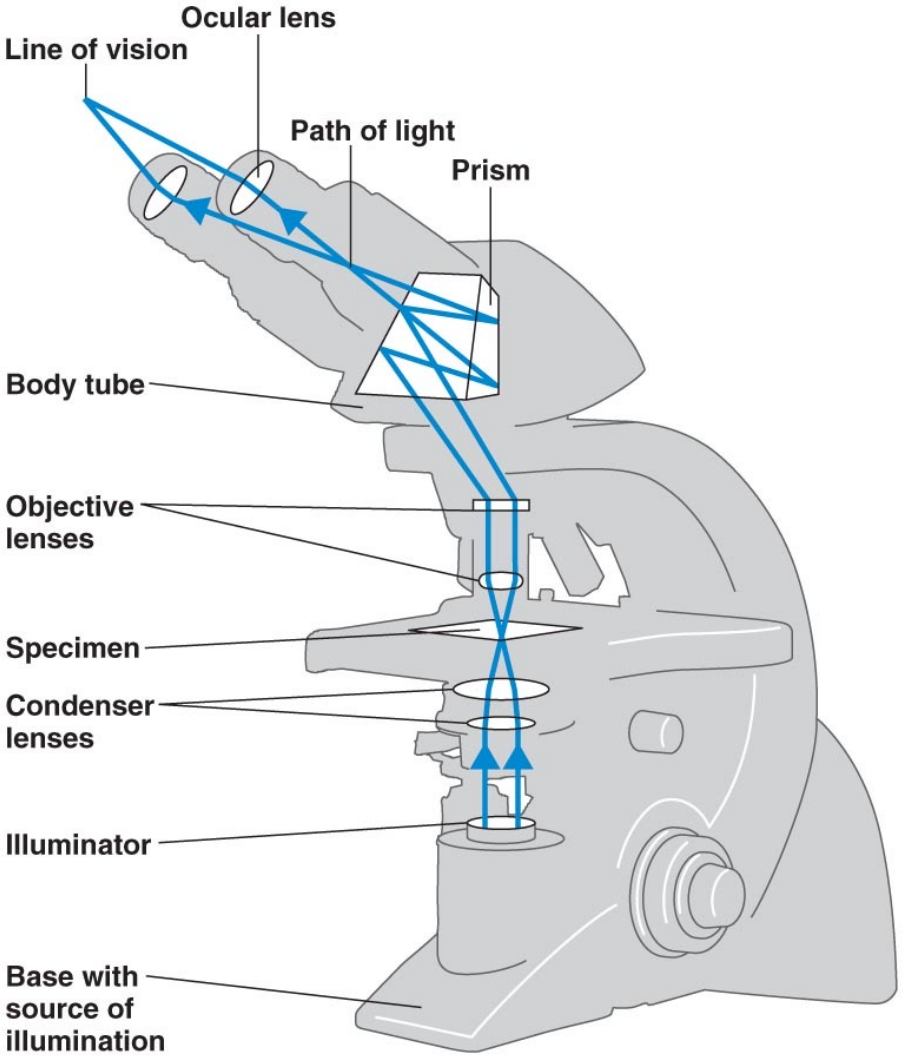


Figure 3.1a

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



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

Figure 3.1b

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 ≥ 0.4 nm
- Shorter wavelengths of light provide greater resolution

Compound Light Microscopy

- The **refractive index** is a measure of 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

Refraction in the Compound Microscope

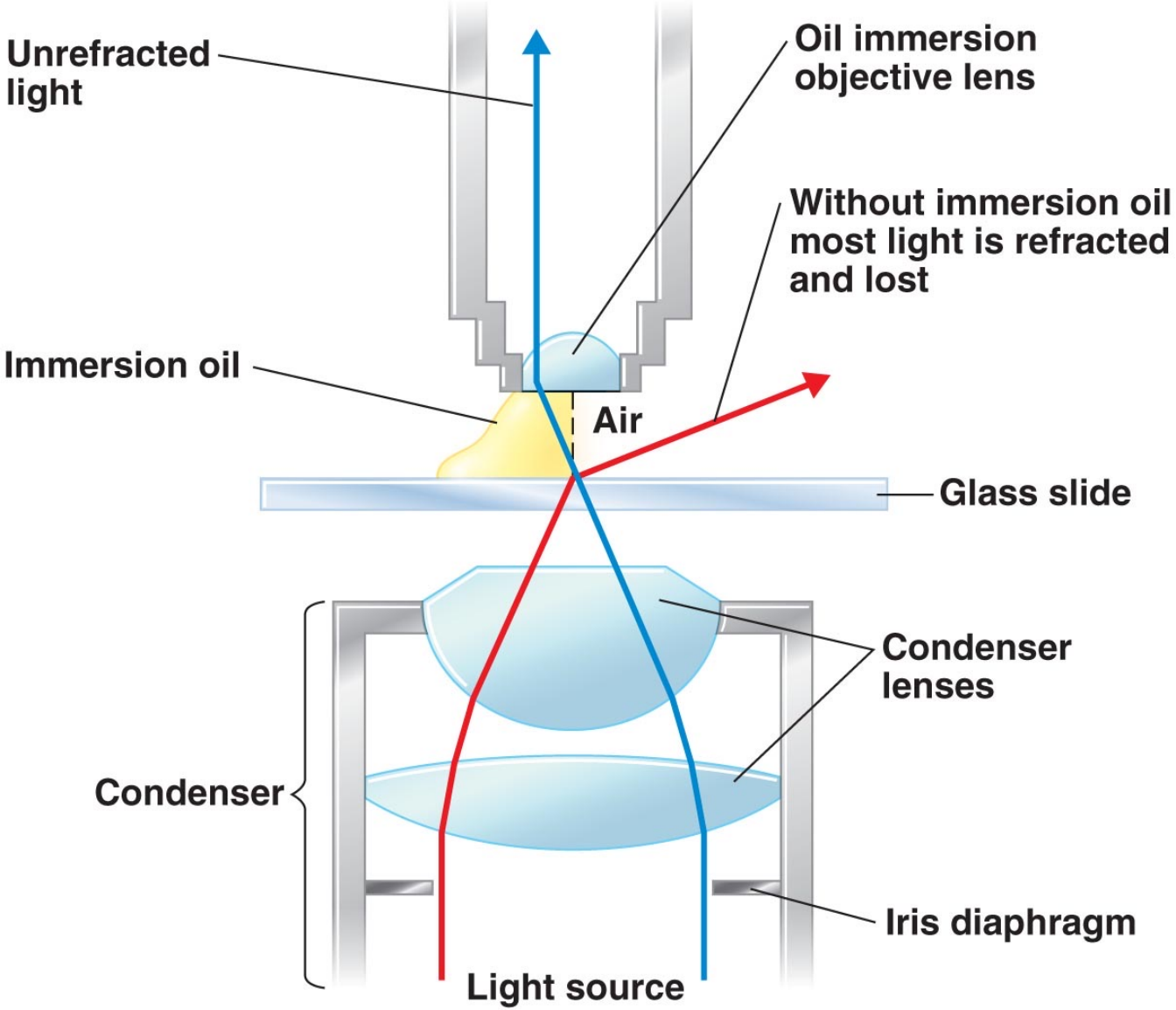
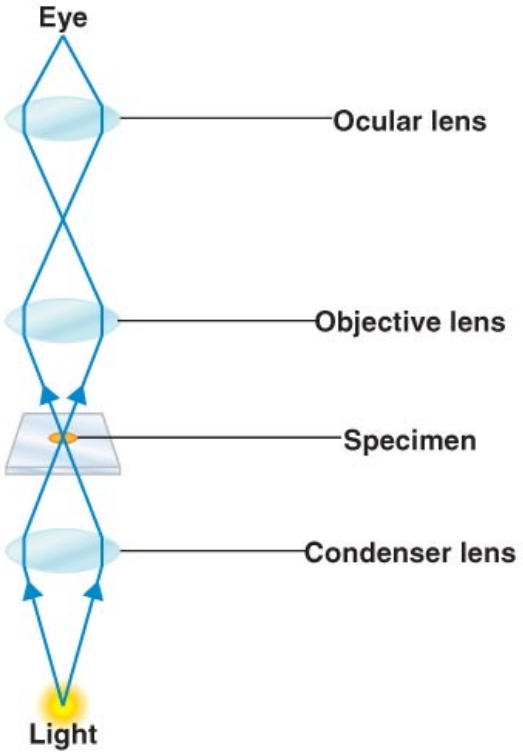


Figure 3.3

Brightfield Illumination

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



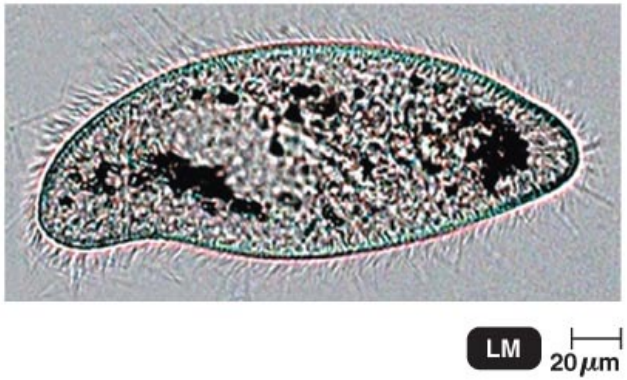
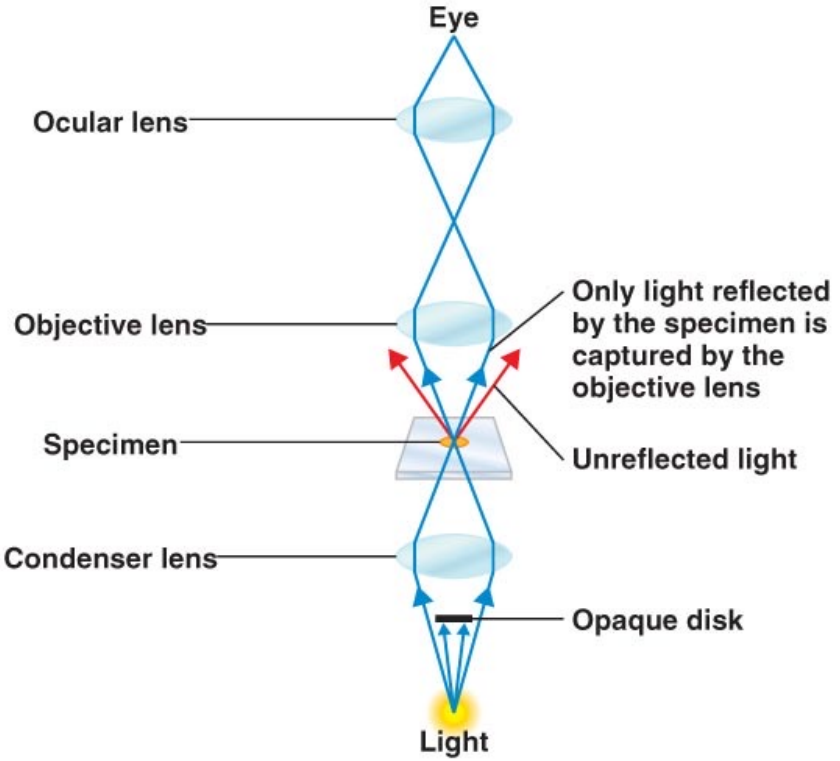


Figure 3.4a

Darkfield Illumination

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



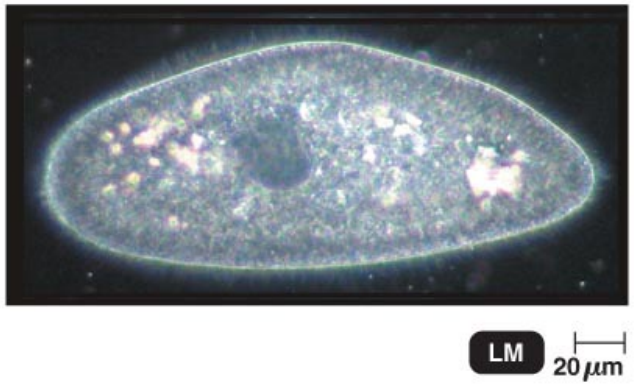


Figure 3.4b

Phase-Contrast Microscopy

- Accentuates (emphasize) diffraction of the light that passes through a specimen
- The wave nature of light rays.
- Light rays can be in phase (their crests and trough match) or out of phase
- Light rays (direct and diffracted/refracted) interact to produce reinforcement (relative brightness) or interference (relative darkness)

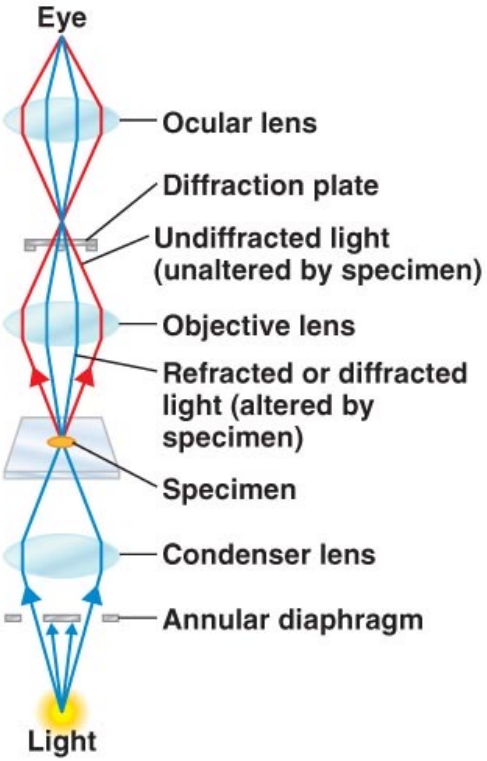


Figure 3.4c

Differential Interference Contrast Microscopy

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

- Employs polarizer in the condenser.

- The polorized light is passed through prism and generates two beams
- Image is brightly colored and appears 3-D

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)

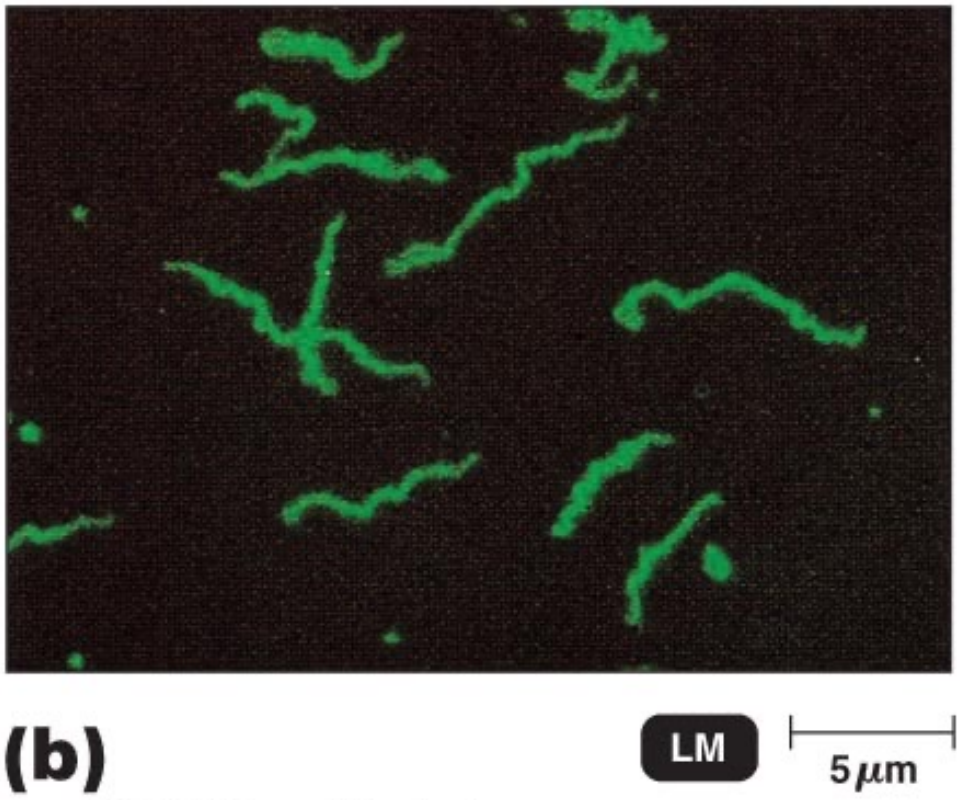


Figure 3.6b

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By Prof Manish Kumar

Confocal Microscopy

- Cells stained with fluorochrome dyes
- Short wavelength (blue) light used to excite the dyes
- The light illuminates each plane in a specimen to produce a three-dimensional image
  - Up to 100 μm deep

Figure 3.7

Scanning Acoustic Microscopy (SAM)

- Measures sound waves that are reflected back from an object
- Used to study cells attached to a surface (cancer cells, artery plaque)
- Resolution 1 μm

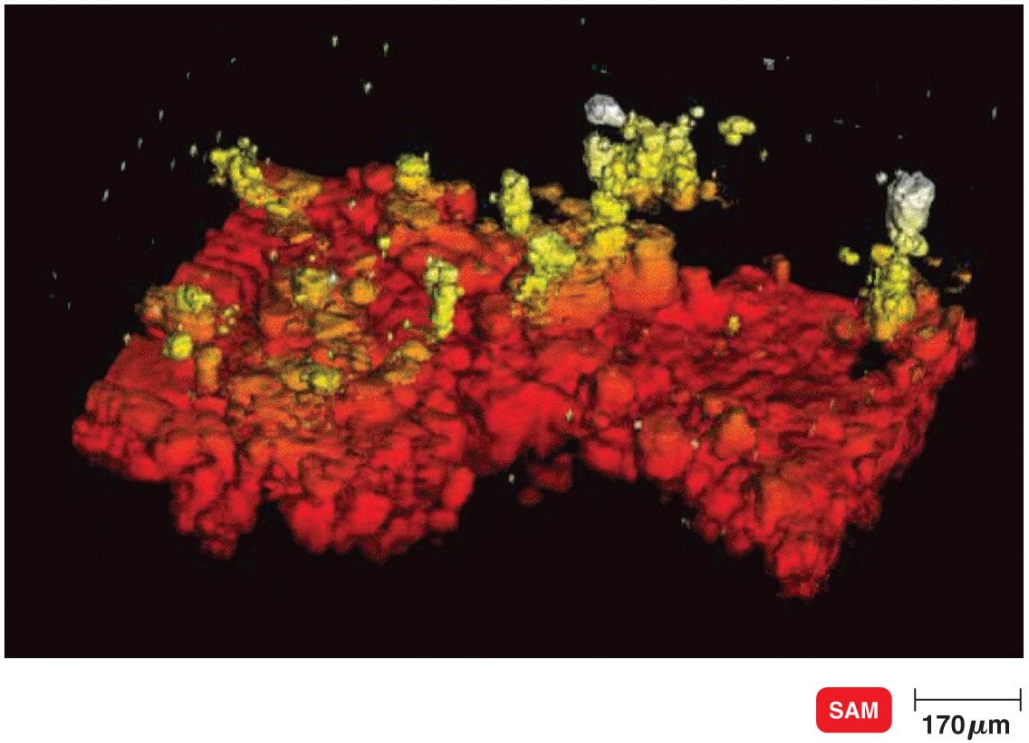

Figure 3.9

Electron Microscopy

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

Transmission Electron Microscopy (TEM)

- Ultra thin sections of specimens
- Specimens are placed on copper mesh grids
- Beam of electrons passes through specimen, then an electromagnetic lens, to a screen or film
- Specimens may be stained with heavy metal salts
- Magnify 10000 to 100000X.

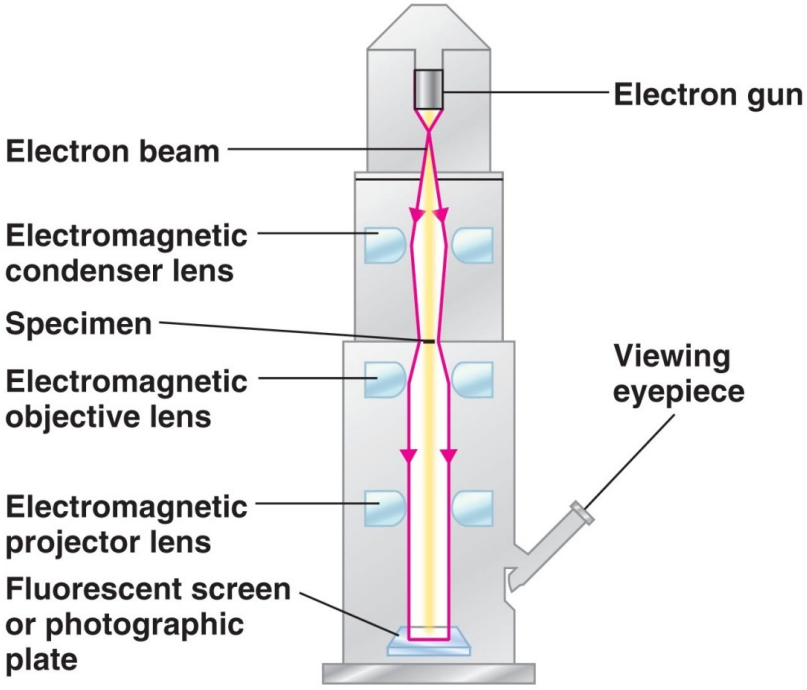

Figure 3.10a

Transmission Electron Microscopy (TEM)

- 10,000–100,000×; resolution 2.5 nm

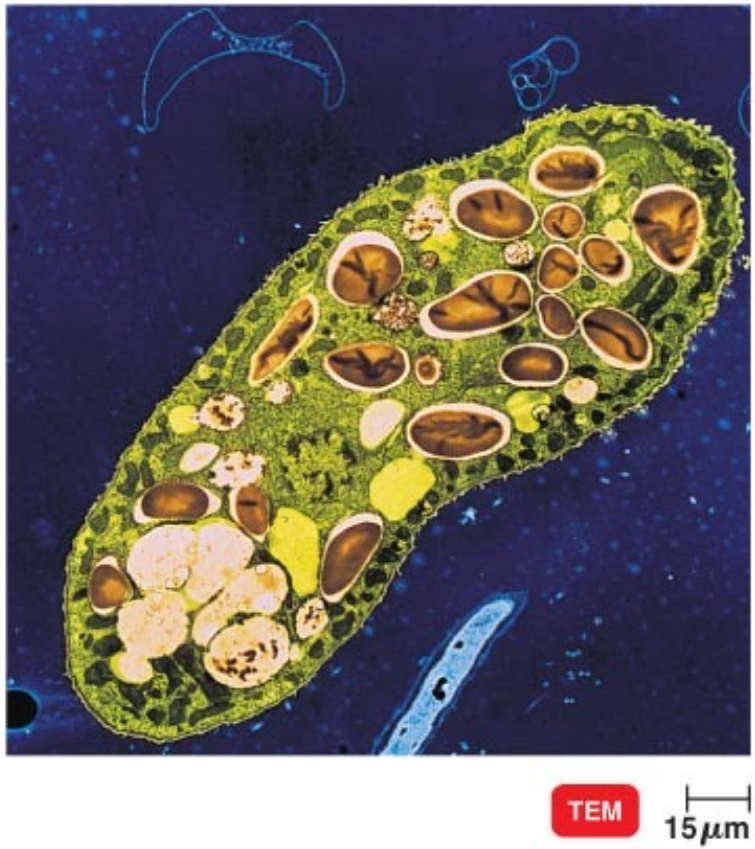

Figure 3.10a

Scanning Electron Microscopy (SEM)

- Overcomes problem of sectioning specimen
- 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

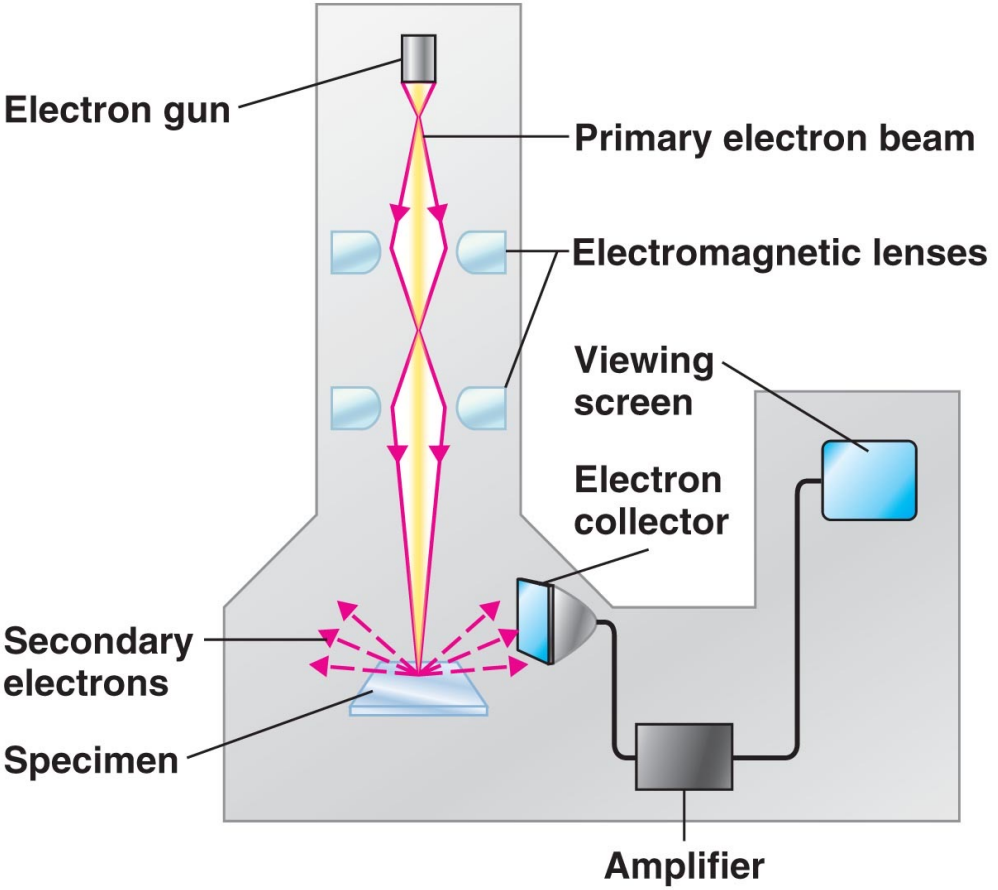

Figure 3.10b

Scanning Electron Microscopy (SEM)

- 1,000–10,000×; resolution 20 nm
- Useful for surface structures of intact cells and viruses.

Figure 3.10b

Scanned-Probe Microscopy

- **Scanning tunneling microscopy (STM)** uses a metal probe to scan a specimen
- Resolution 1/100 of an atom (thus greater than EM)

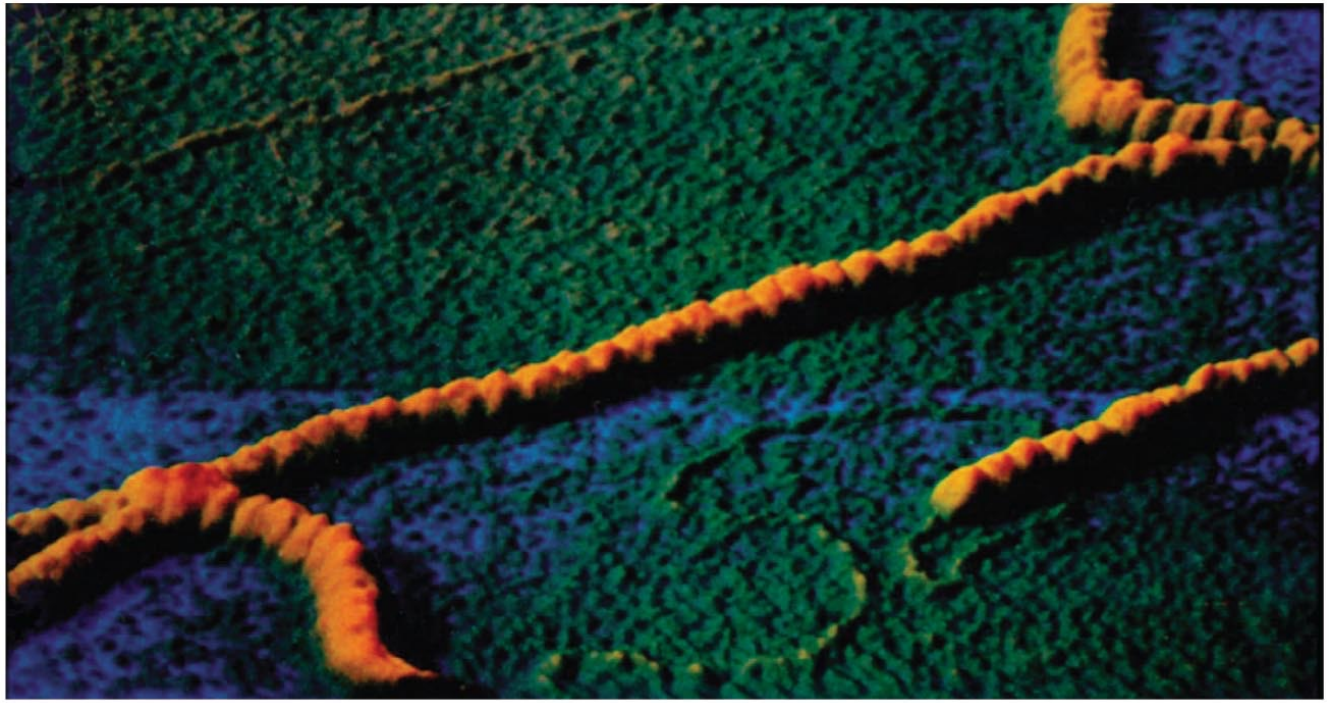

Figure 3.11a

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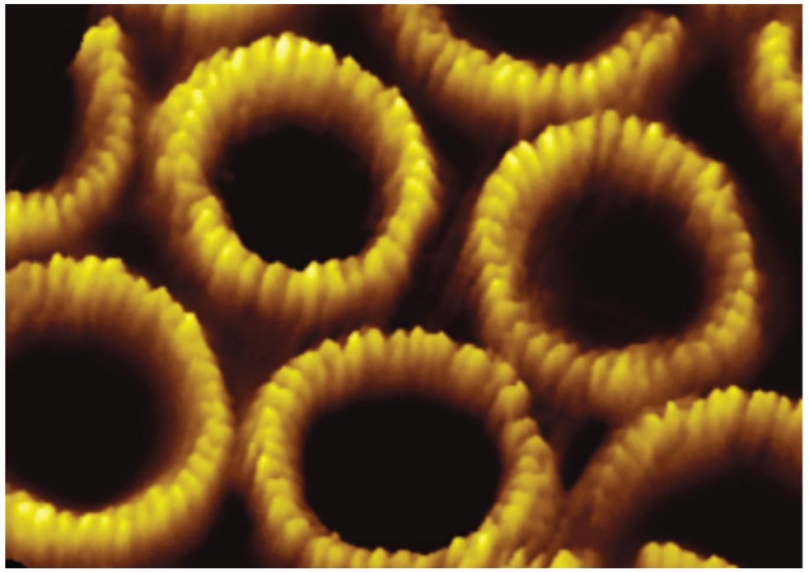
Scanned-Probe Microscopy

- **Atomic force microscopy (AFM)** uses a metal- and-diamond probe inserted (stylus) into the specimen.
- Produces three-dimensional images.



Perfringoglysin O toxin

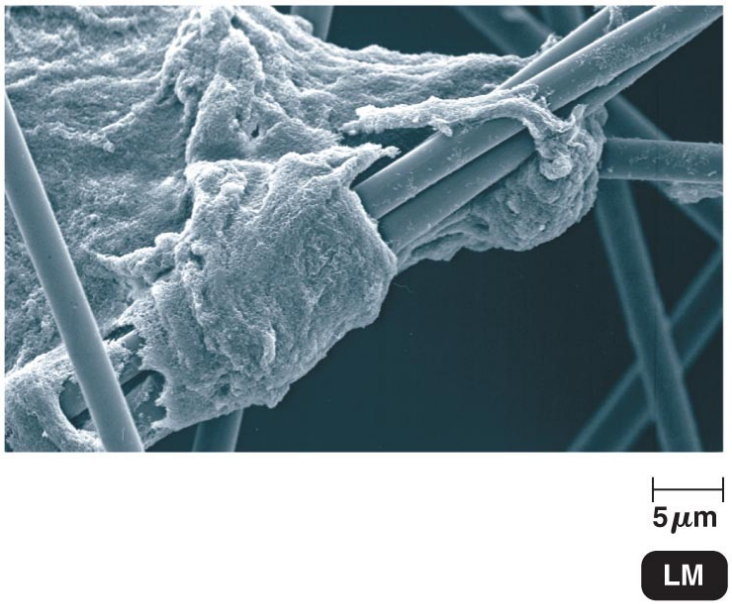
Figure 3.11b

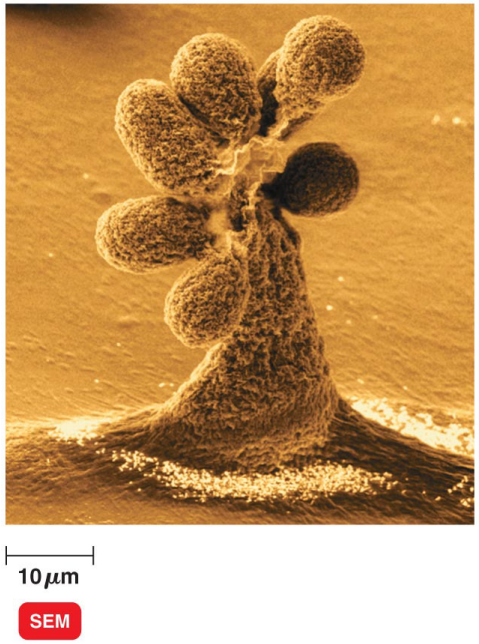
Preparing Smears for Staining

- **Staining**: Coloring the microbe with a dye that emphasizes certain structures
- **Smear**: A thin film of a solution of microbes on a slide
- 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. Researchers do make discoveries about cell behavior by observing live specimens.





Figures B and C

Preparing Smears for Staining

- Stains are salts composed of a positive and negative ion
- In a **basic dye (methylene blue, malachite green and safranin)**, the chromophore is a cation
- Bacteria are slightly negatively charged at pH7.
- In an **acidic dye**, the chromophore is an anion
- Acidic dye stain the background instead of the cell is called **negative staining**

Simple Stains

- **Simple stain**: Use of a single basic dye. Eg. Methylene blue, carbolfuchsin, crystal violet, and safranin.
- A **mordant** may be used to hold the stain or coat the specimen to thicken it for easier visibility

Differential Stains

- Used to distinguish between bacteria
  - Gram stain
  - Acid-fast stain

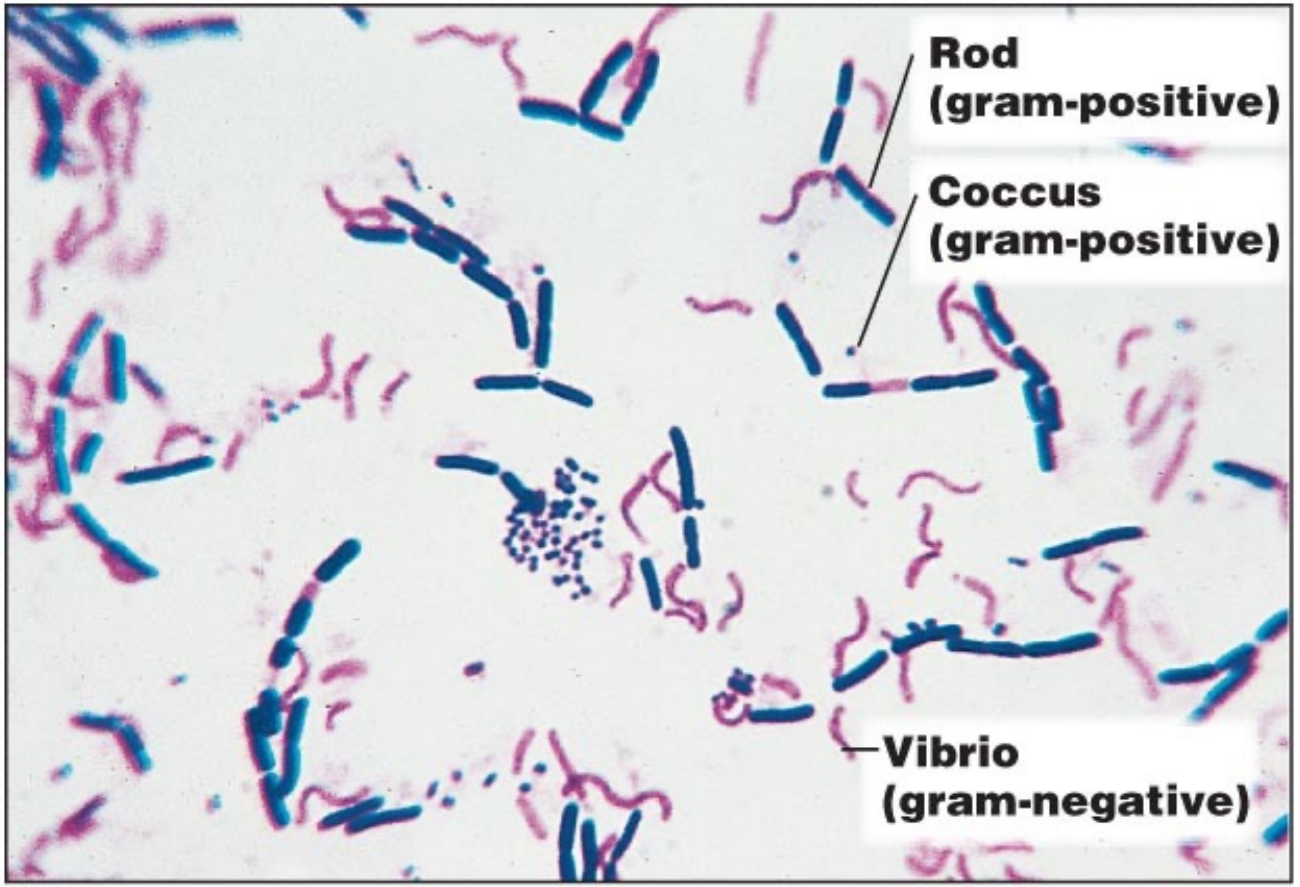
Gram Stain

- Classifies bacteria into gram-positive or gram-negative
  - Gram-positive bacteria tend to be killed by penicillin and detergents
  - Gram-negative bacteria are more resistant to antibiotics

Gram Stain

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

Micrograph of Gram-Stained Bacteria



(b)

Figure 3.12b

Acid-Fast Stain

- Stained waxy cell wall is not decolorized by acid-alcohol
- *Mycobacterium*
- *Nocardia*

Acid-Fast Stain

	Color of Acid-fast	Color of Non–Acid-fast
Primary stain: Carbolfuchsin	Red	Red
Decolorizing agent: Acid-alcohol	Red	Colorless
Counterstain: Methylene blue	Red	Blue

Acid-Fast Bacteria

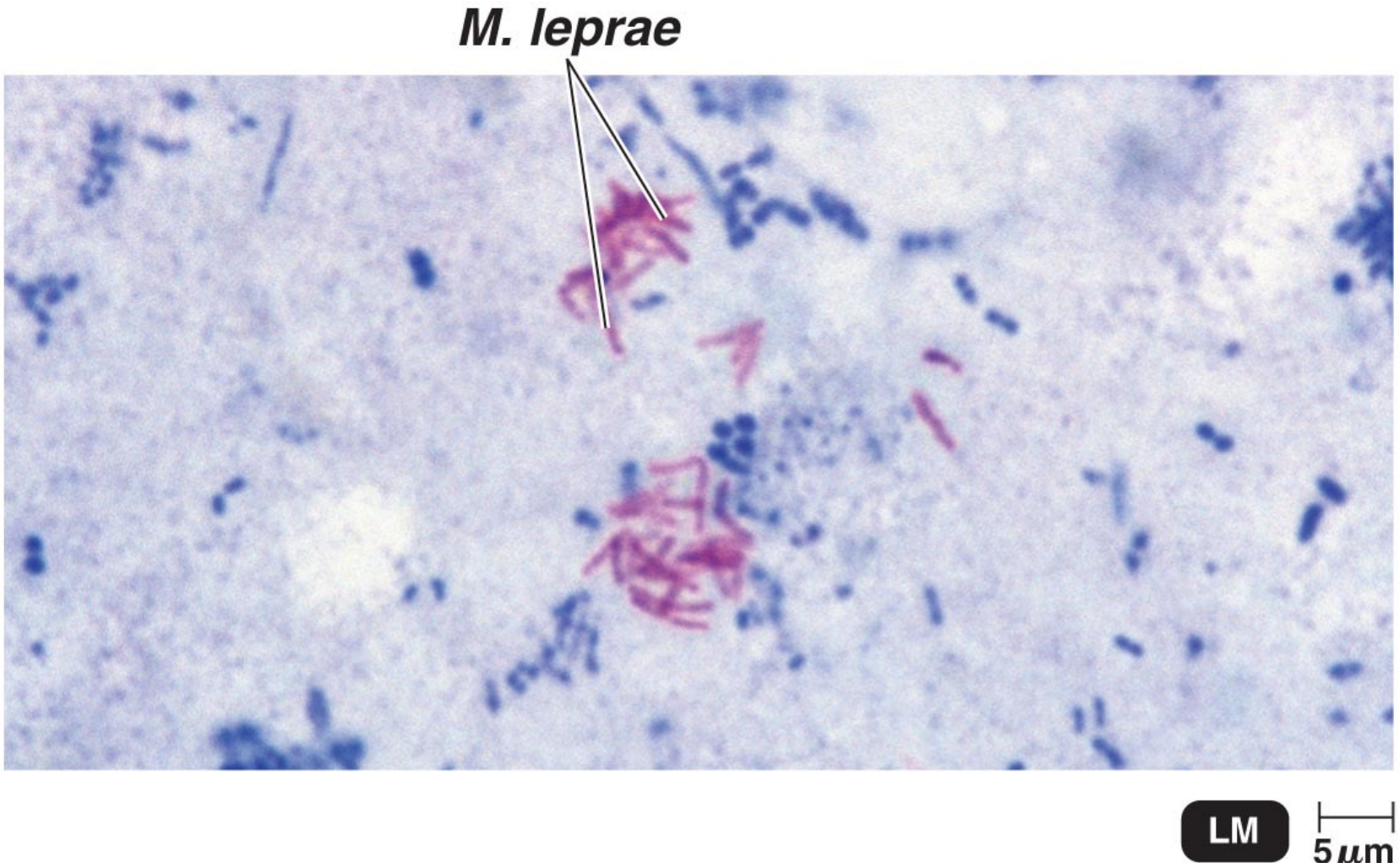


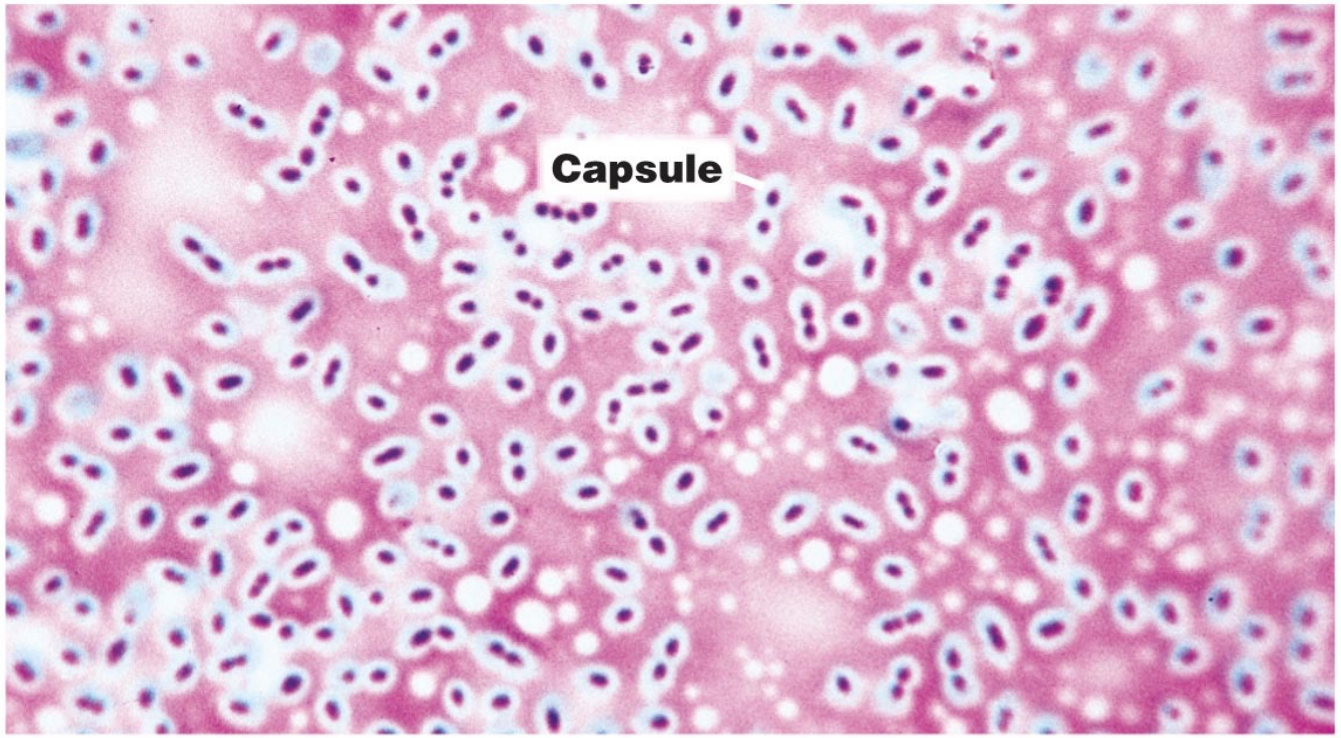
Figure 3.13

Special Stains

- Used to distinguish parts of cells
  - Capsule stain
  - Endospore stain
  - Flagella stain

Negative Staining for Capsules

- Cells stained
- Negative stain

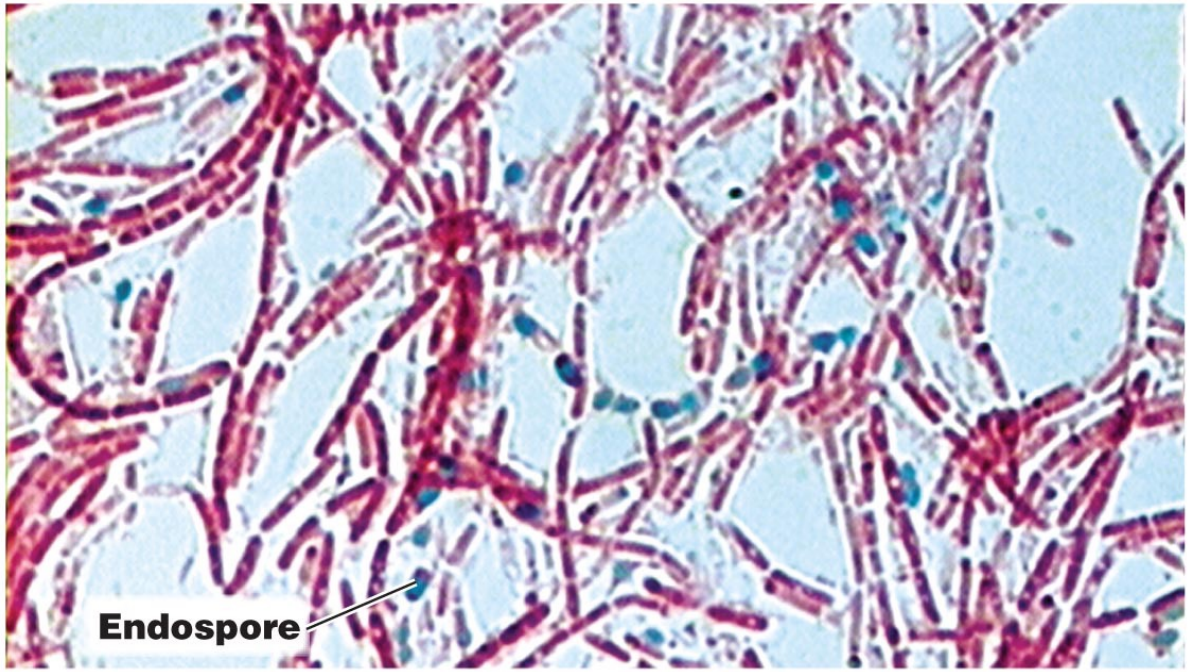


(a) Negative staining

Figure 3.14a

Endospore Staining

- Primary stain: Malachite green, usually with heat
- Decolorize cells: Water
- Counterstain: Safranin

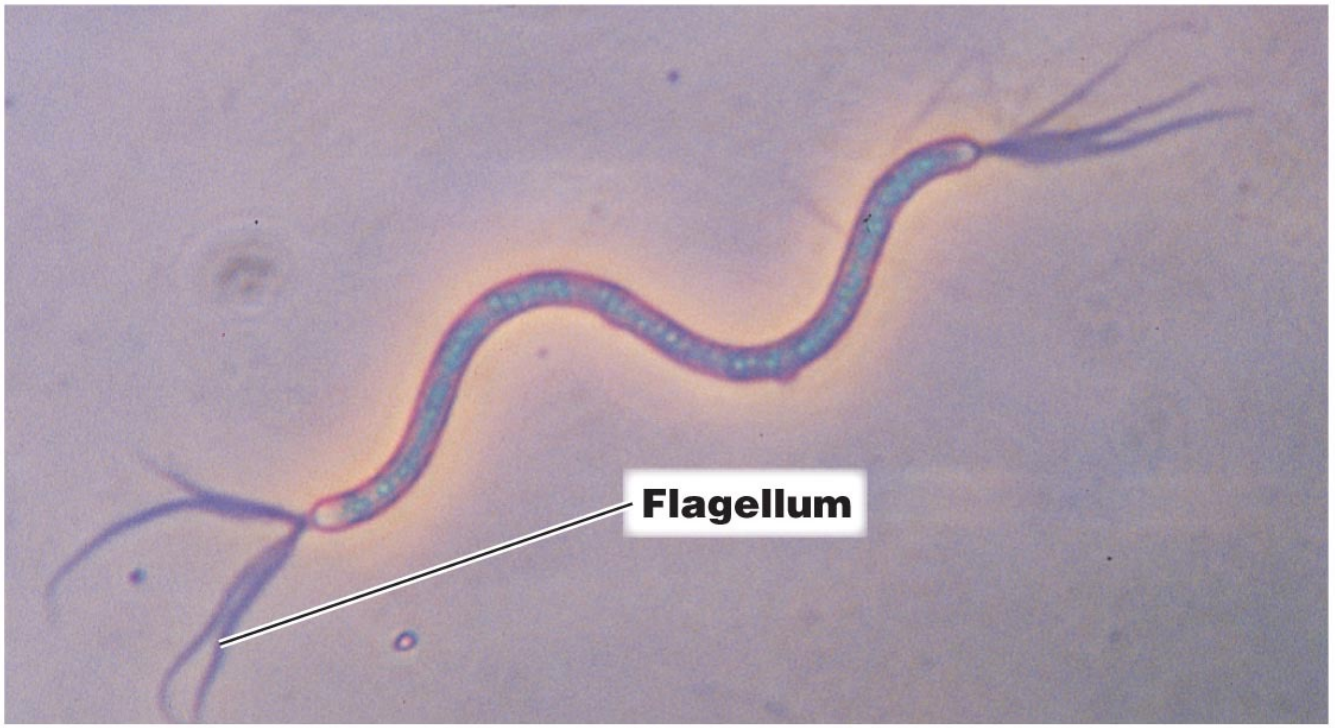


(b) Endospore staining

Figure 3.14b

Flagella Staining

- Mordant on flagella
- Carbolfuchsin simple stain



(c) Flagella staining

Figure 3.14c