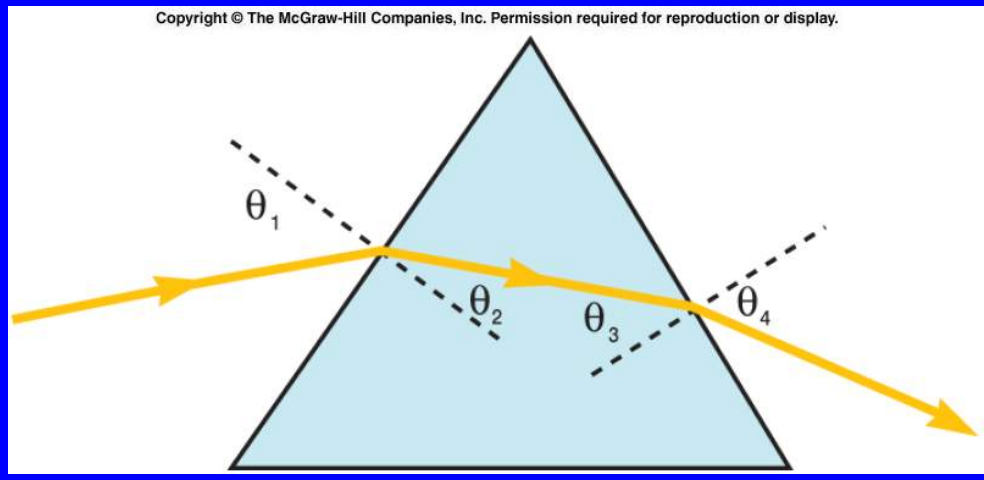
Chapter 2

The Study of Microbial Structure: Microscopy and Specimen Preparation

Lenses and the Bending of Light



- light is refracted (bent) when passing from one medium to another
- **refractive index**
  - a measure of how greatly a substance slows the velocity of light
- direction and magnitude of bending is determined by the refractive indexes of the two media forming the interface

Lenses

- focus light rays at a specific place called the **focal point**
- distance between center of lens and focal point is the **focal length**
- strength of lens related to focal length
  - short focal length ⇒more magnification

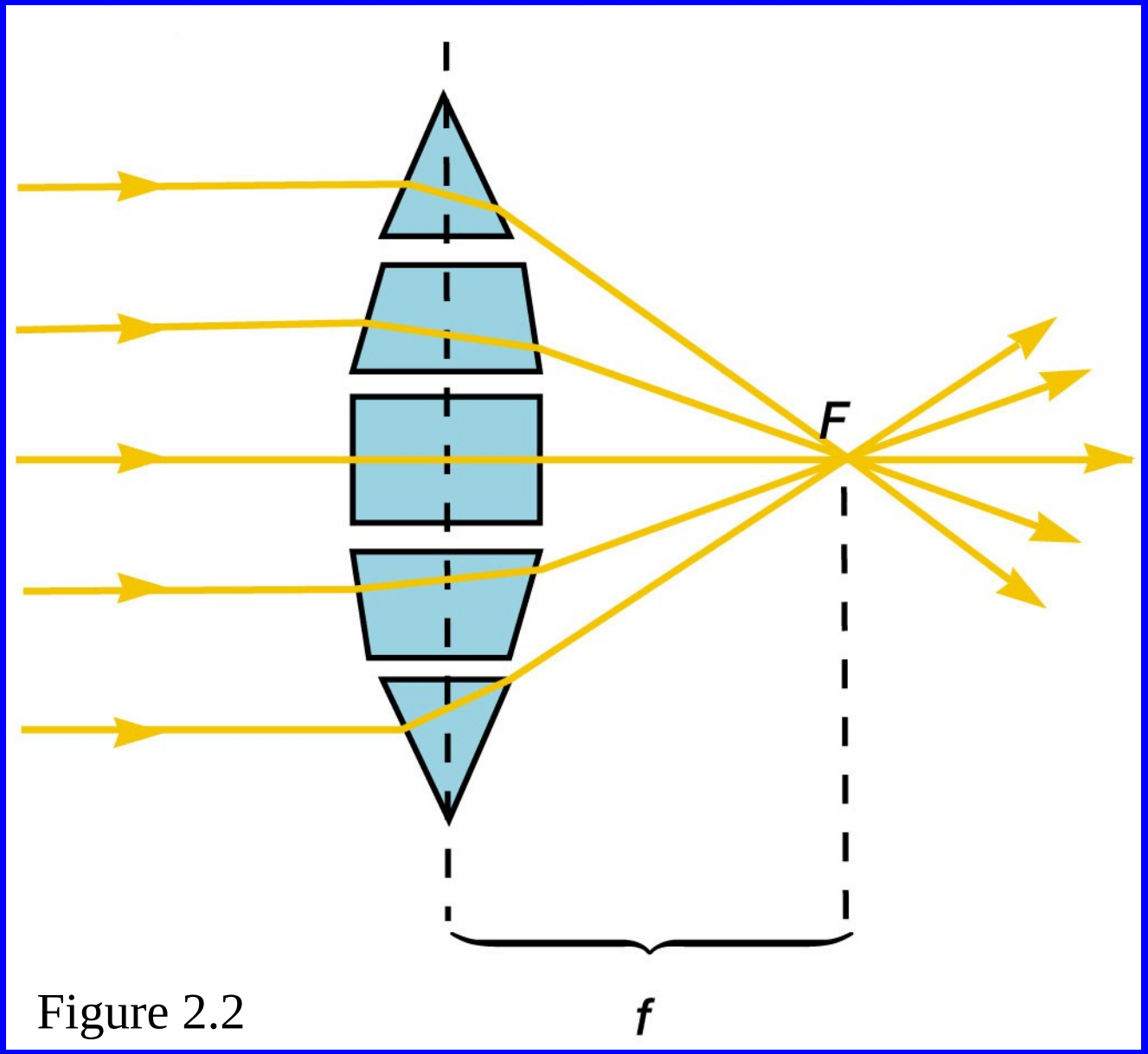


Figure 2.2

The Light Microscope

- many types
  - bright-field microscope
  - dark-field microscope
  - phase-contrast microscope
  - fluorescence microscopes
- are compound microscopes
  - image formed by action of ≥2 lenses

The Bright-Field Microscope

- produces a dark image against a brighter background
- has several objective lenses
  - **parfocal** microscopes remain in focus when objectives are changed
- **total magnification**
  - product of the magnifications of the ocular lens and the objective lens

Figure 2.3

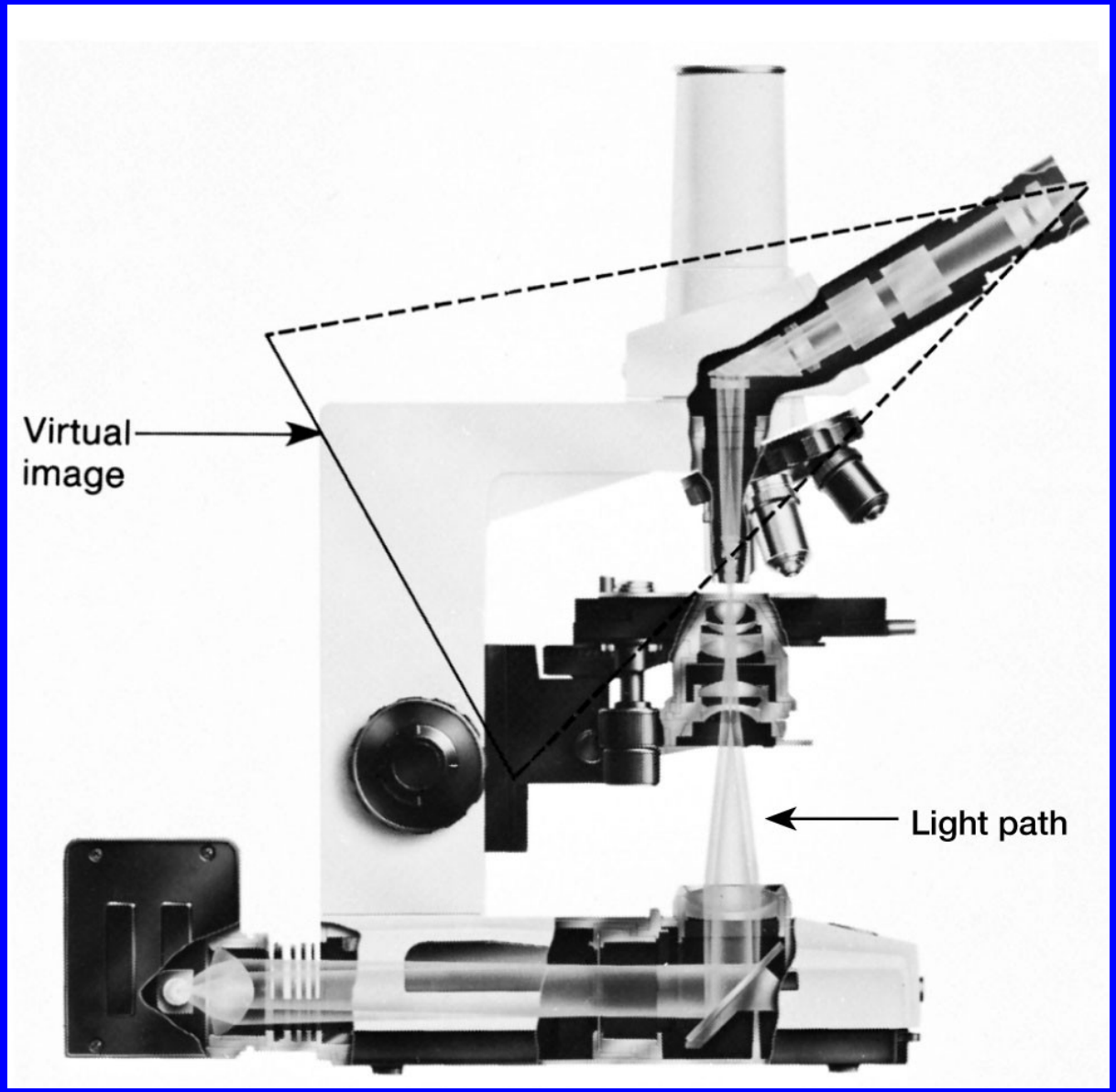


Figure 2.4

Microscope Resolution (*d*)

- **ability of a lens to separate or distinguish small objects that are close together**
  - $d = 0.5\lambda$/numerical aperture
  - NA = $n \sin \Theta$
- **wavelength of light used is major factor in resolution**

  shorter wavelength ⇒ greater resolution

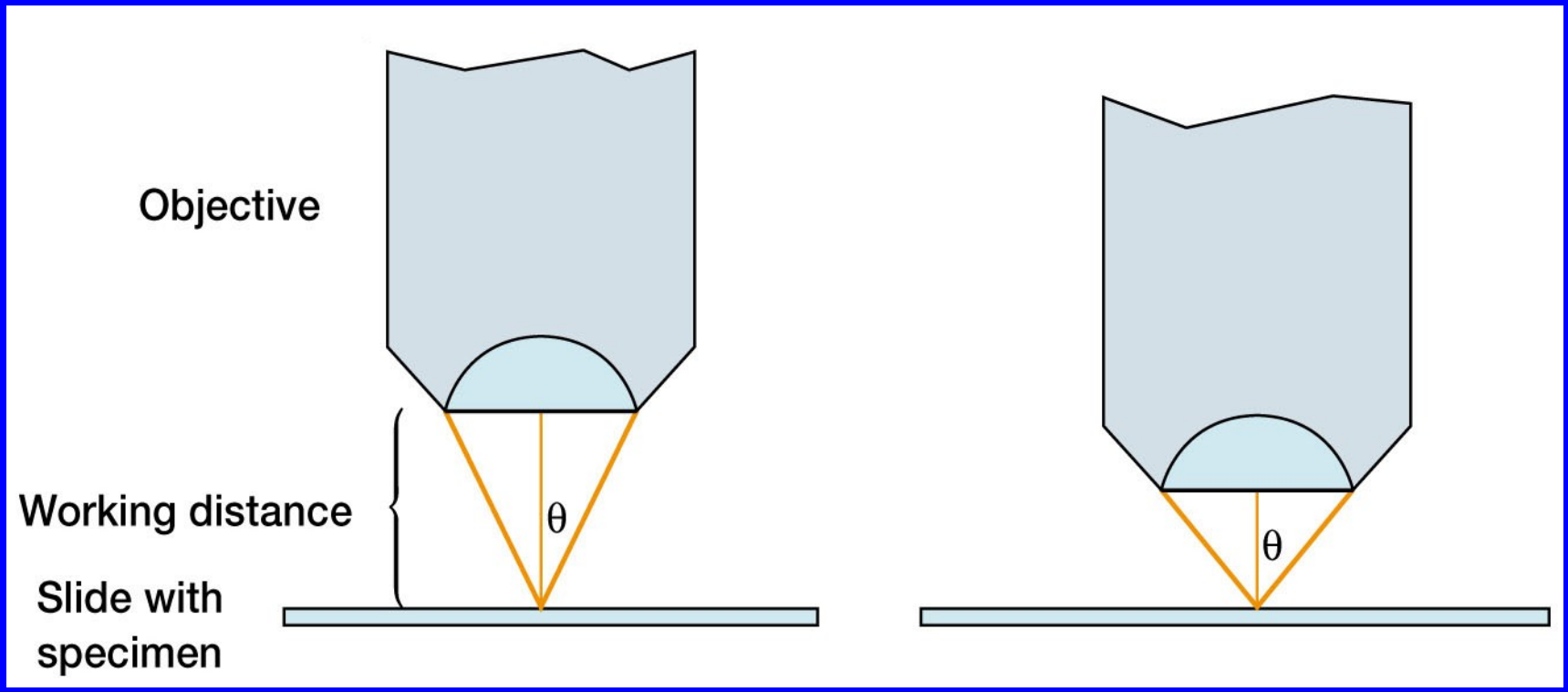


Figure 2.5

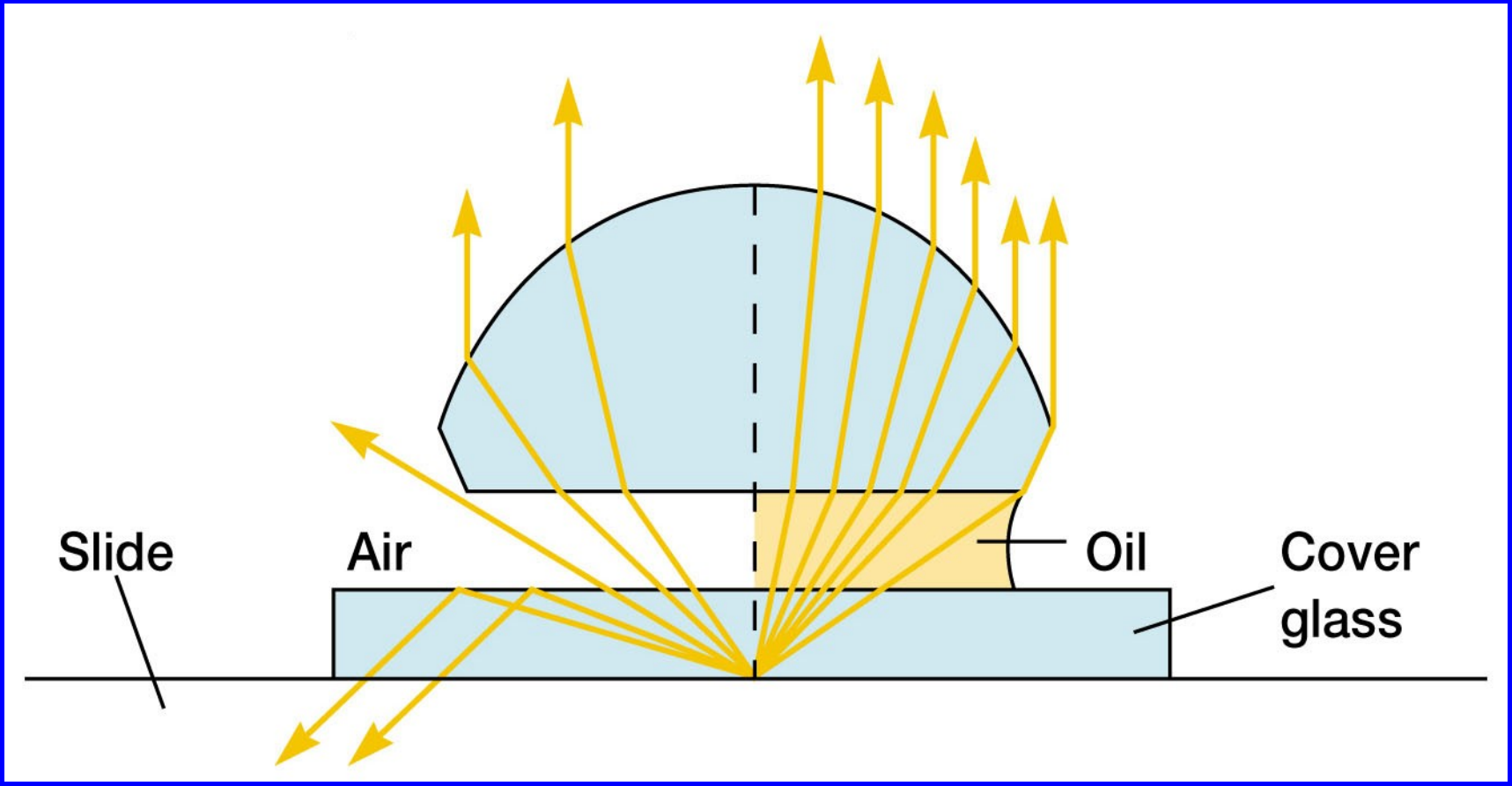


Figure 2.6

Table 2.2 The Properties of Microscope Objectives

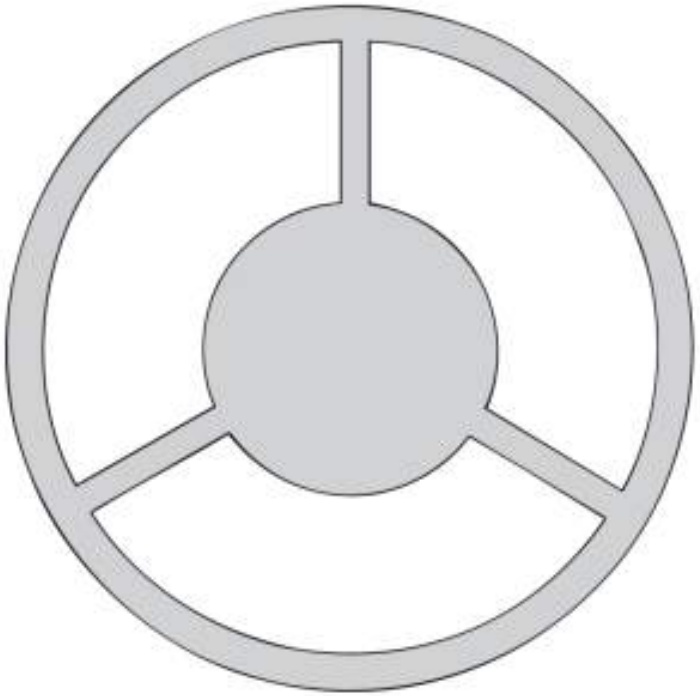
	Objective			
Property	**Scanning**	**Low Power**	**High Power**	**Oil Immersion**
Magnification	4×	10×	40–45×	90–100×
Numerical aperture	0.10	0.25	0.55–0.65	1.25–1.4
Approximate focal length (*f*)	40 mm	16 mm	4 mm	1.8–2.0 mm
Working distance	17–20 mm	4–8 mm	0.5–0.7 mm	0.1 mm
Approximate resolving power with light of 450 nm (blue light)	2.3 µm	0.9 µm	0.35 µm	0.18 µm

Mistake in equation on page 21 (5.30 nm is really 530 nm)

- working distance
  - — distance between the front surface of lens and surface of cover glass or specimen

The Dark-Field Microscope

- produces a bright image of the object against a dark background
- used to observe living, unstained preparations



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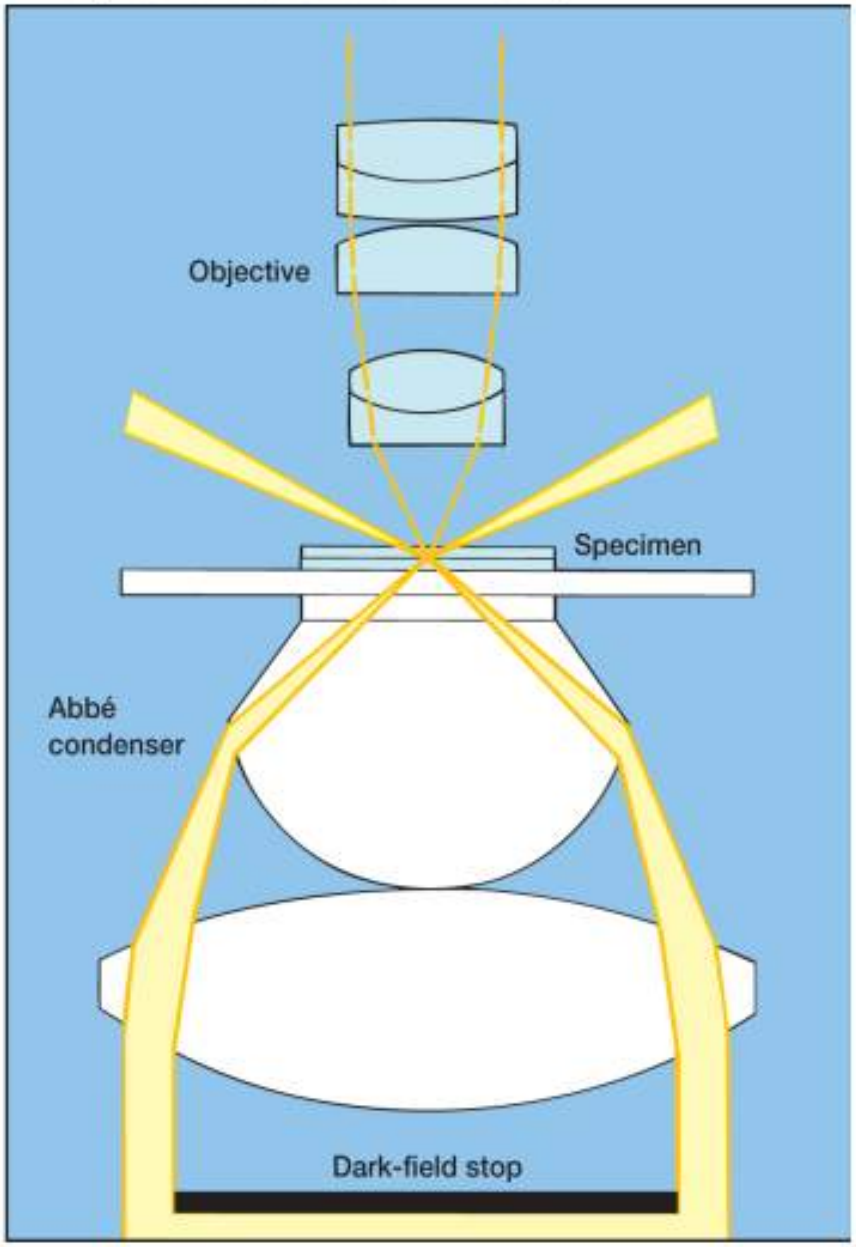
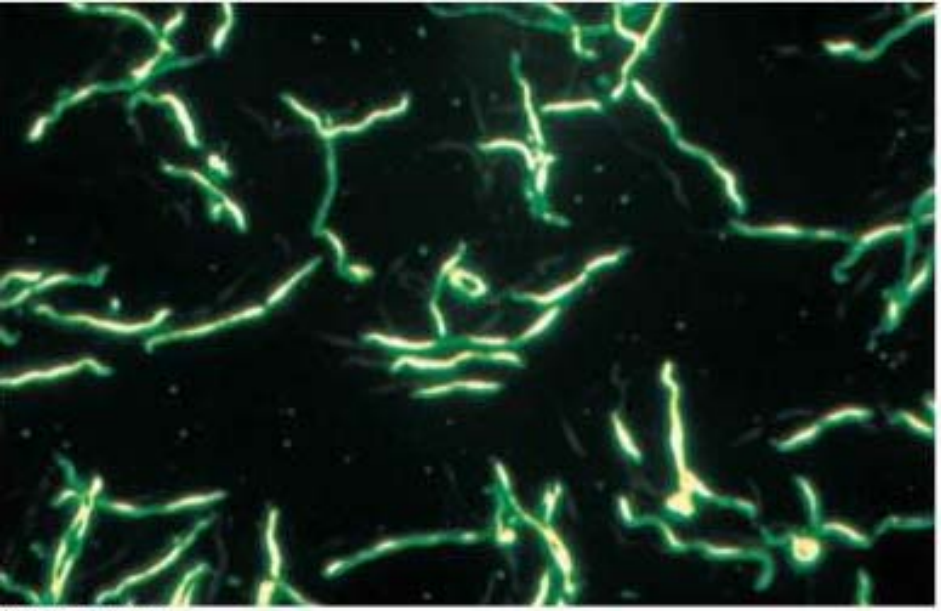
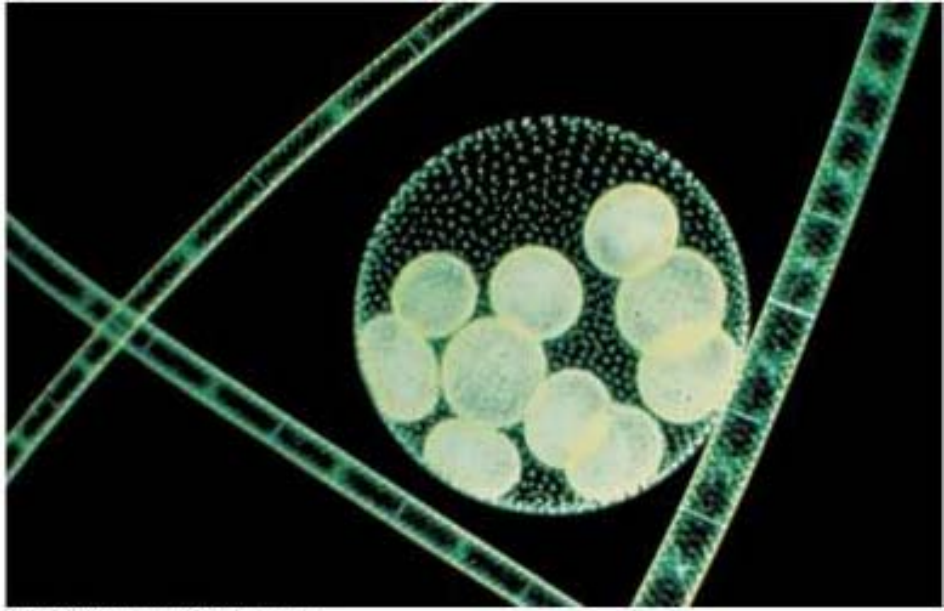


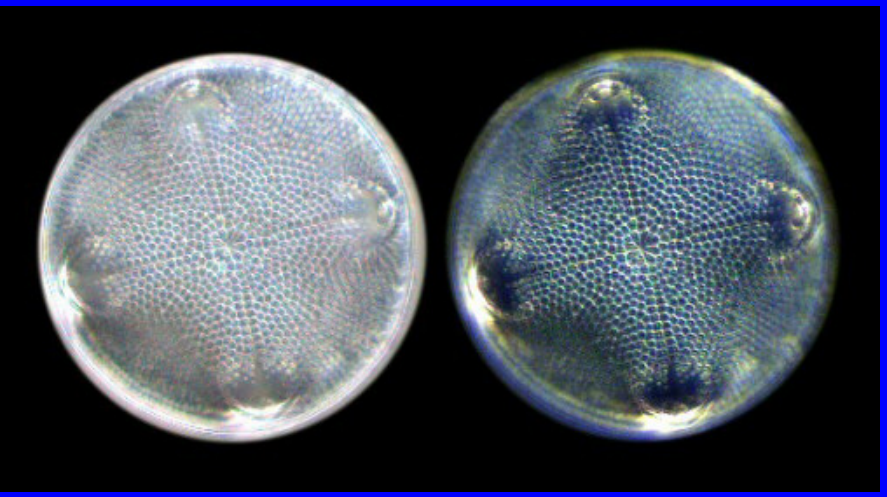
(b)



(a) *T. pallidum*



(b) *Volvox and Spirogyra*



The Phase-Contrast Microscope

- enhances the contrast between intracellular structures having slight differences in refractive index
- excellent way to observe living cells

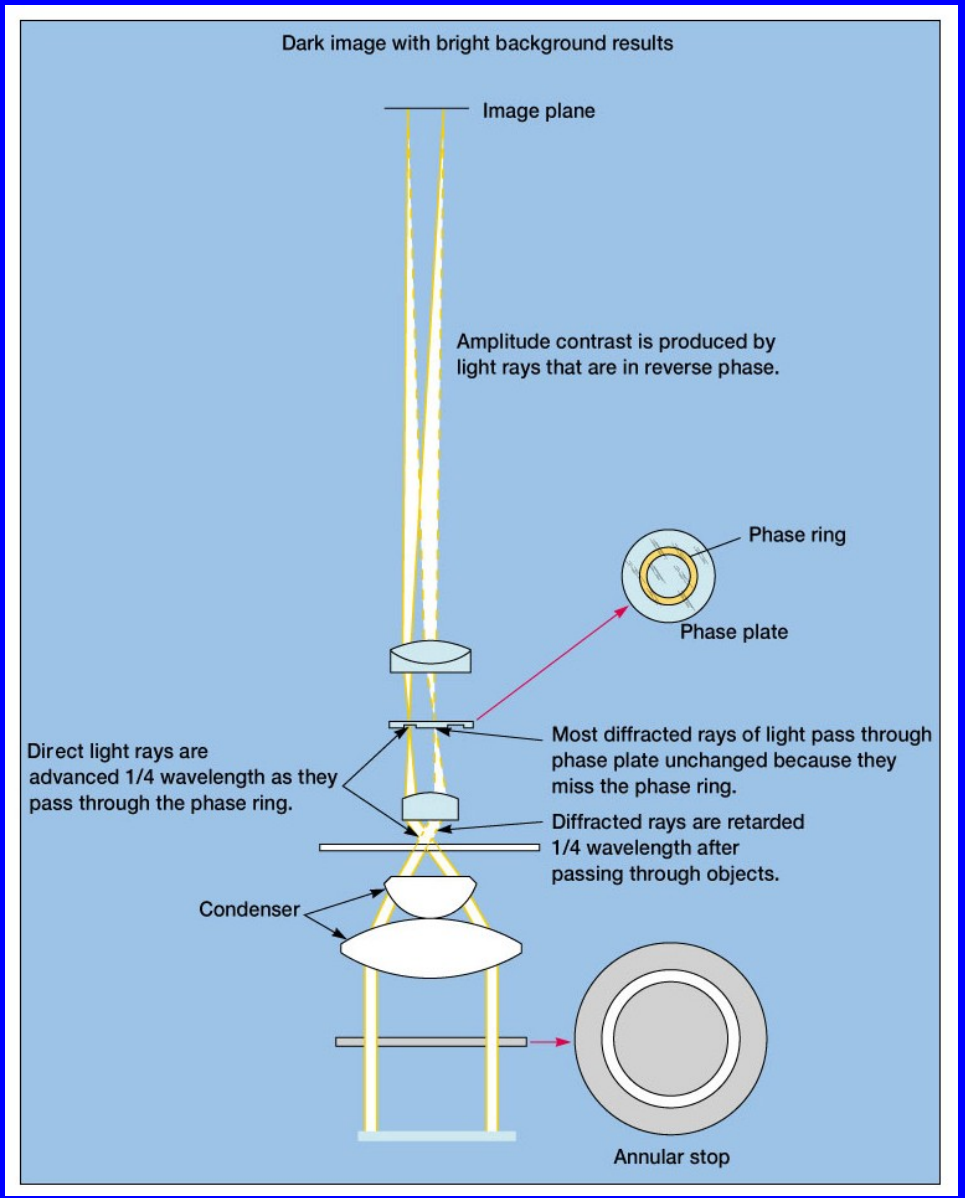


Figure 2.9

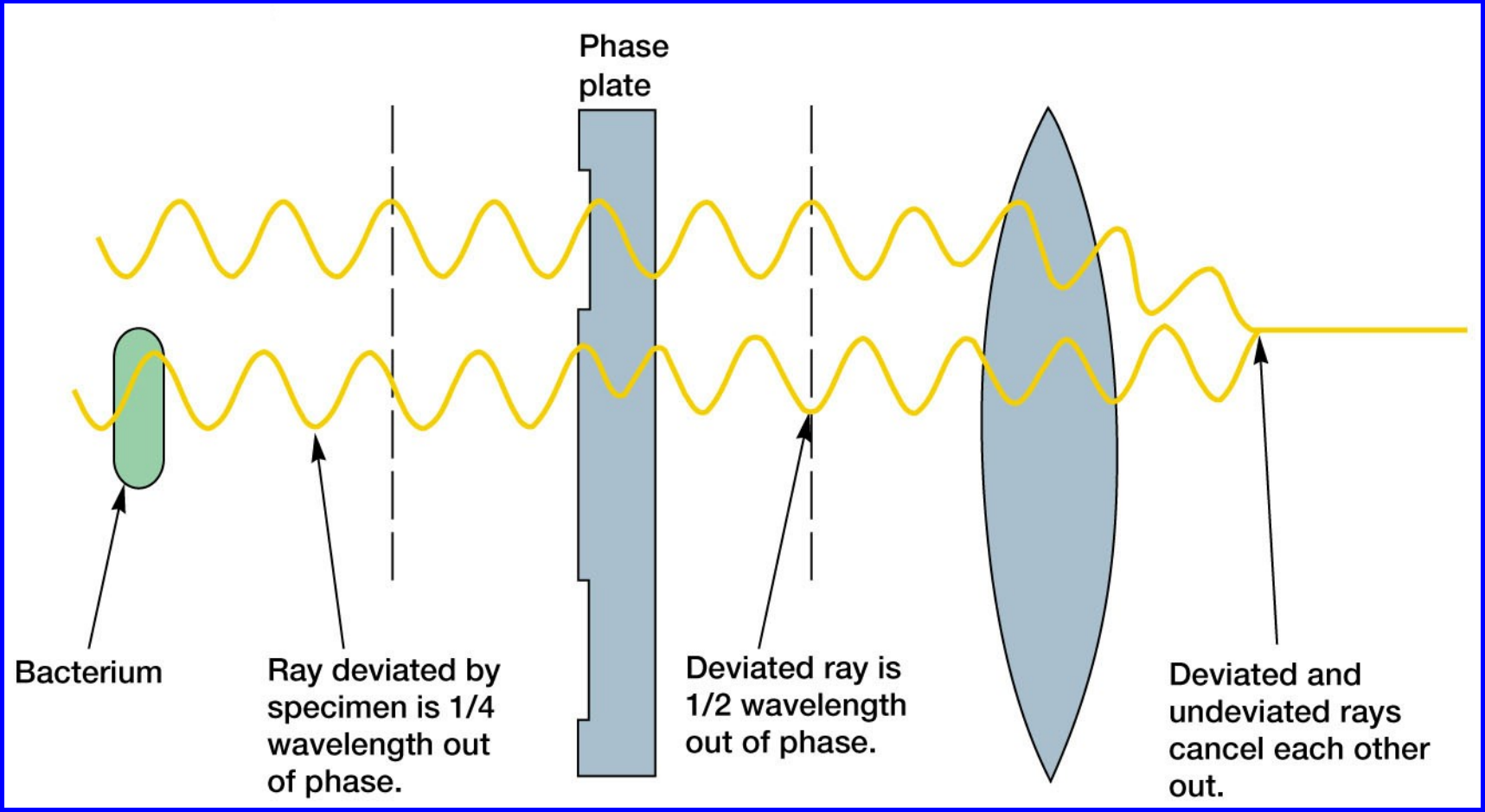
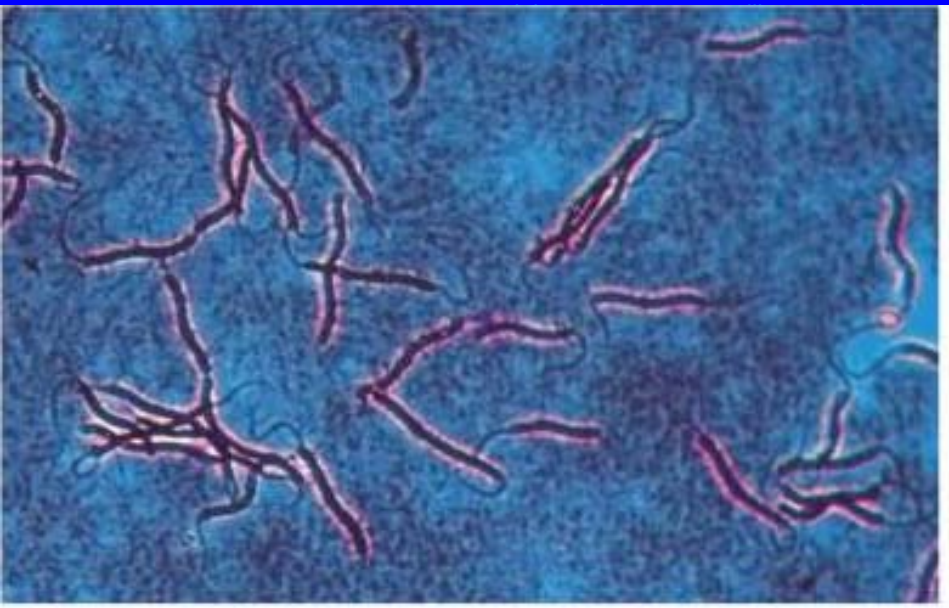
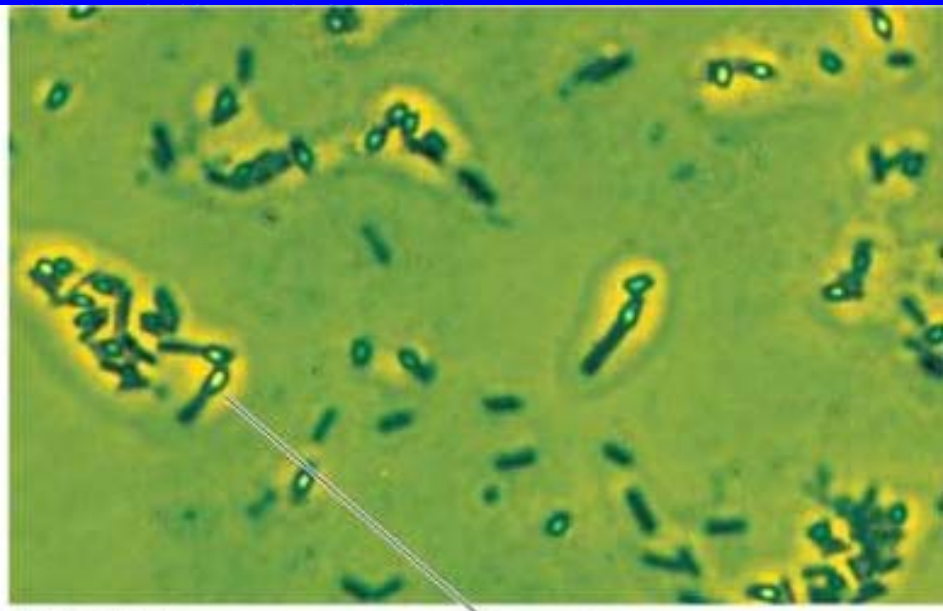


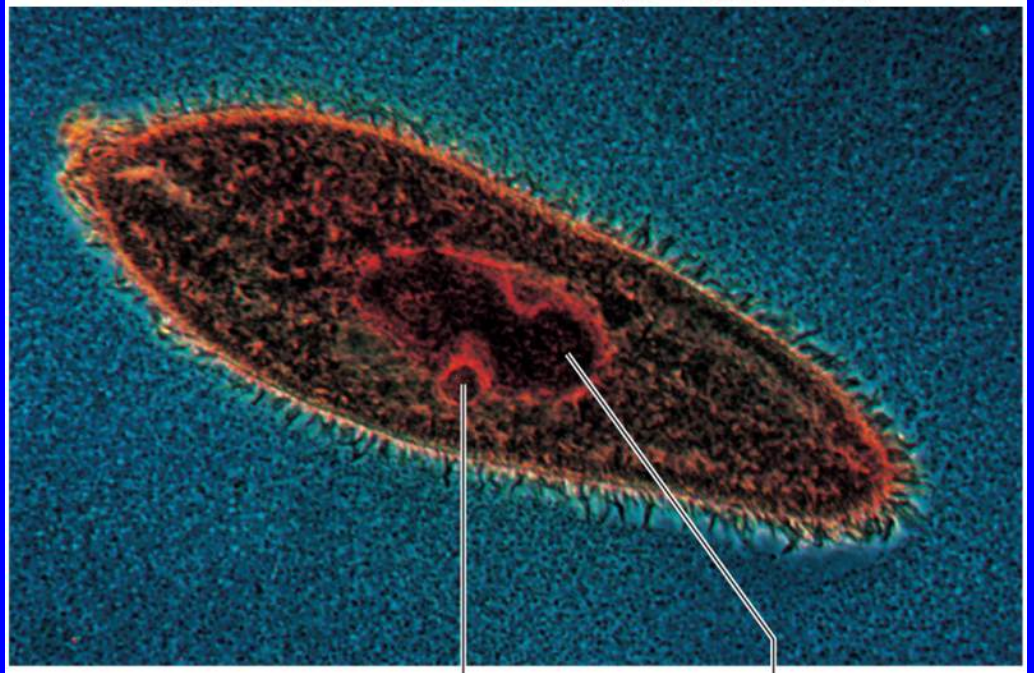
Figure 2.10



(c) *S. volutans*



(d) *C. botulinum*



(e) *Paramecium*

The Fluorescence Microscope

- exposes specimen to ultraviolet, violet, or blue light
- specimens usually stained with **fluorochromes**
- shows a bright image of the object resulting from the fluorescent light emitted by the specimen

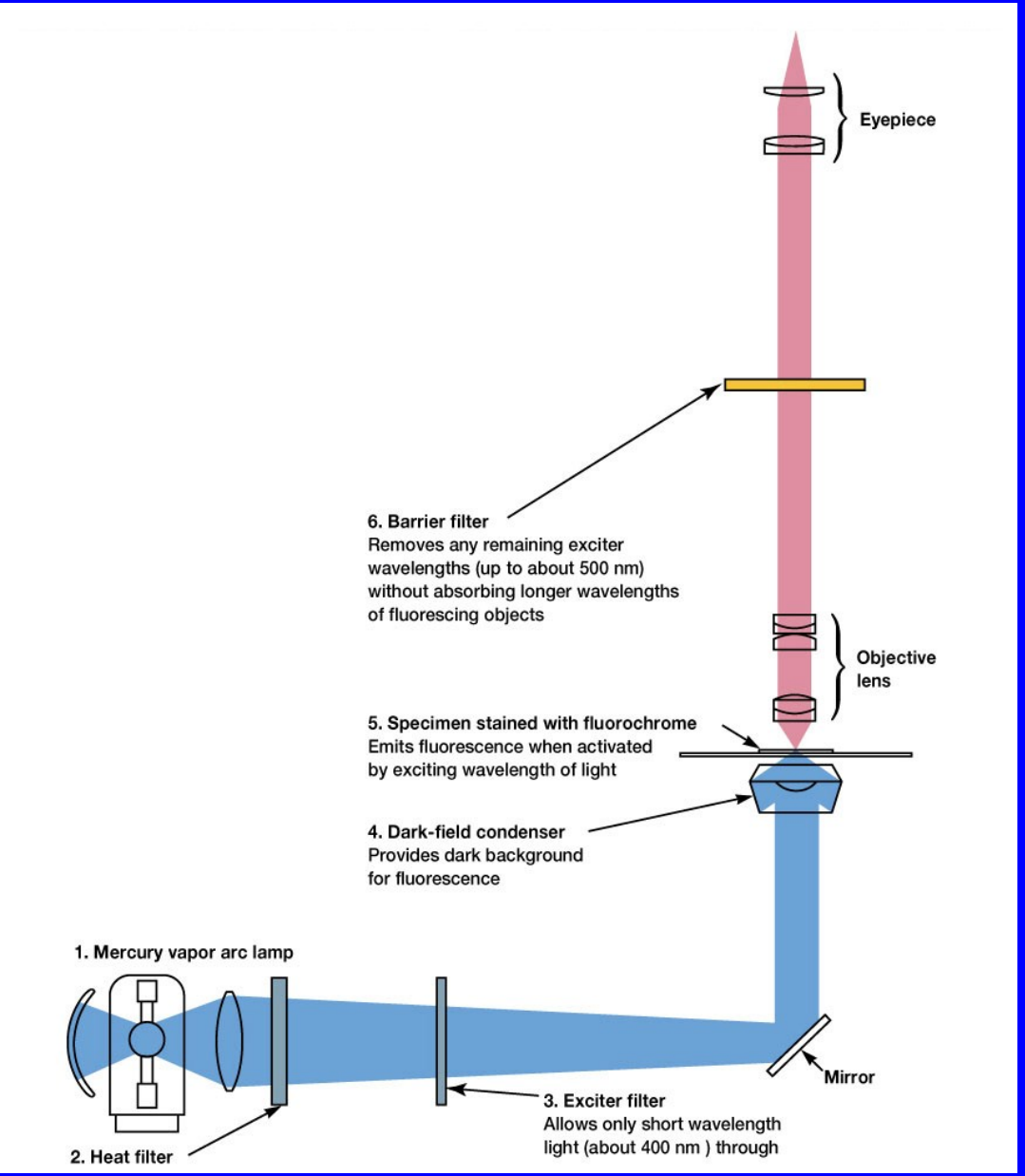
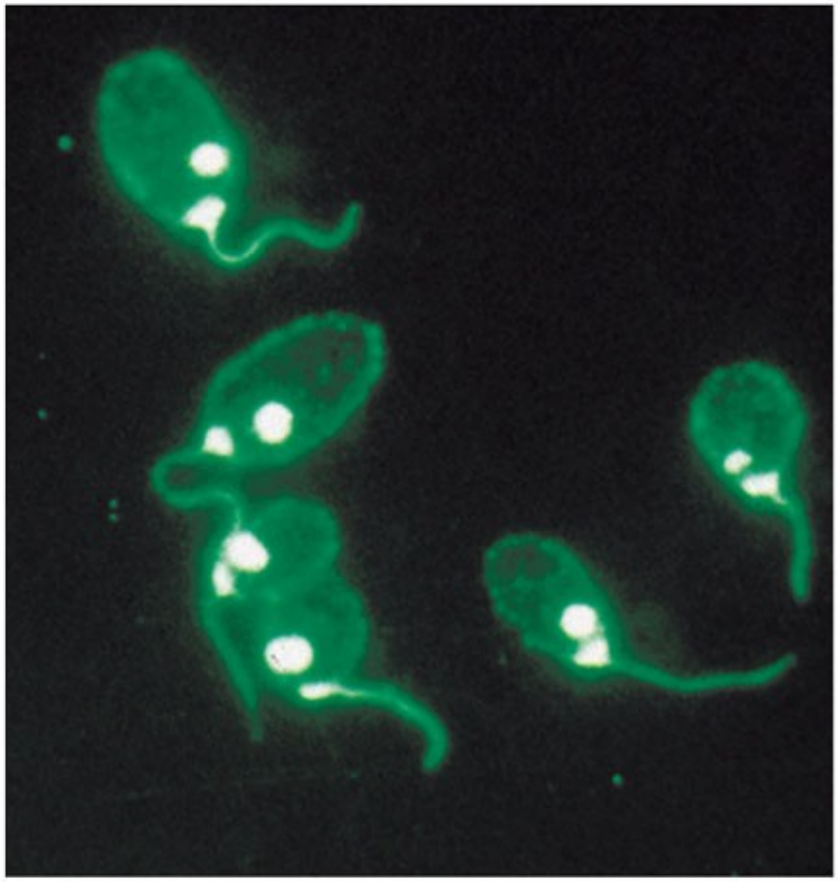


Figure 2.12



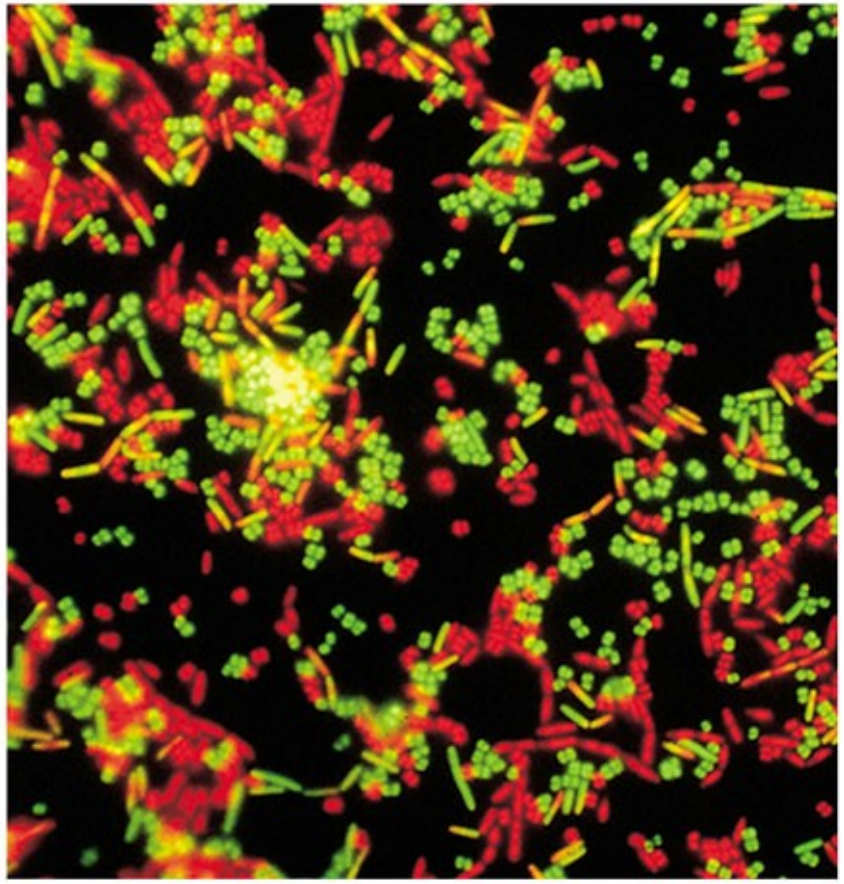


Figure 2.13c and d

Preparation and Staining of Specimens

- increases visibility of specimen
- accentuates specific morphological features
- preserves specimens

Fixation

- process by which internal and external structures are preserved and fixed in position
- process by which organism is killed and firmly attached to microscope slide
  - heat fixing
    - preserves overall morphology but not internal structures
  - chemical fixing
    - protects fine cellular substructure and morphology of larger, more delicate organisms

Dyes and Simple Staining

- dyes
  - make internal and external structures of cell more visible by increasing contrast with background
  - have two common features
    - chromophore groups
      - chemical groups with conjugated double bonds
      - give dye its color
    - ability to bind cells

Dyes and Simple Staining

- **simple staining**
  - **a single staining agent is used**
  - **basic dyes are frequently used**
    - **dyes with positive charges**
    - **e.g., crystal violet**

Differential Staining

- **divides microorganisms into groups based on their staining properties**
  - **e.g., Gram stain**
  - **e.g., acid-fast stain**

Gram staining

- most widely used differential staining procedure
- divides Bacteria into two groups based on differences in cell wall structure

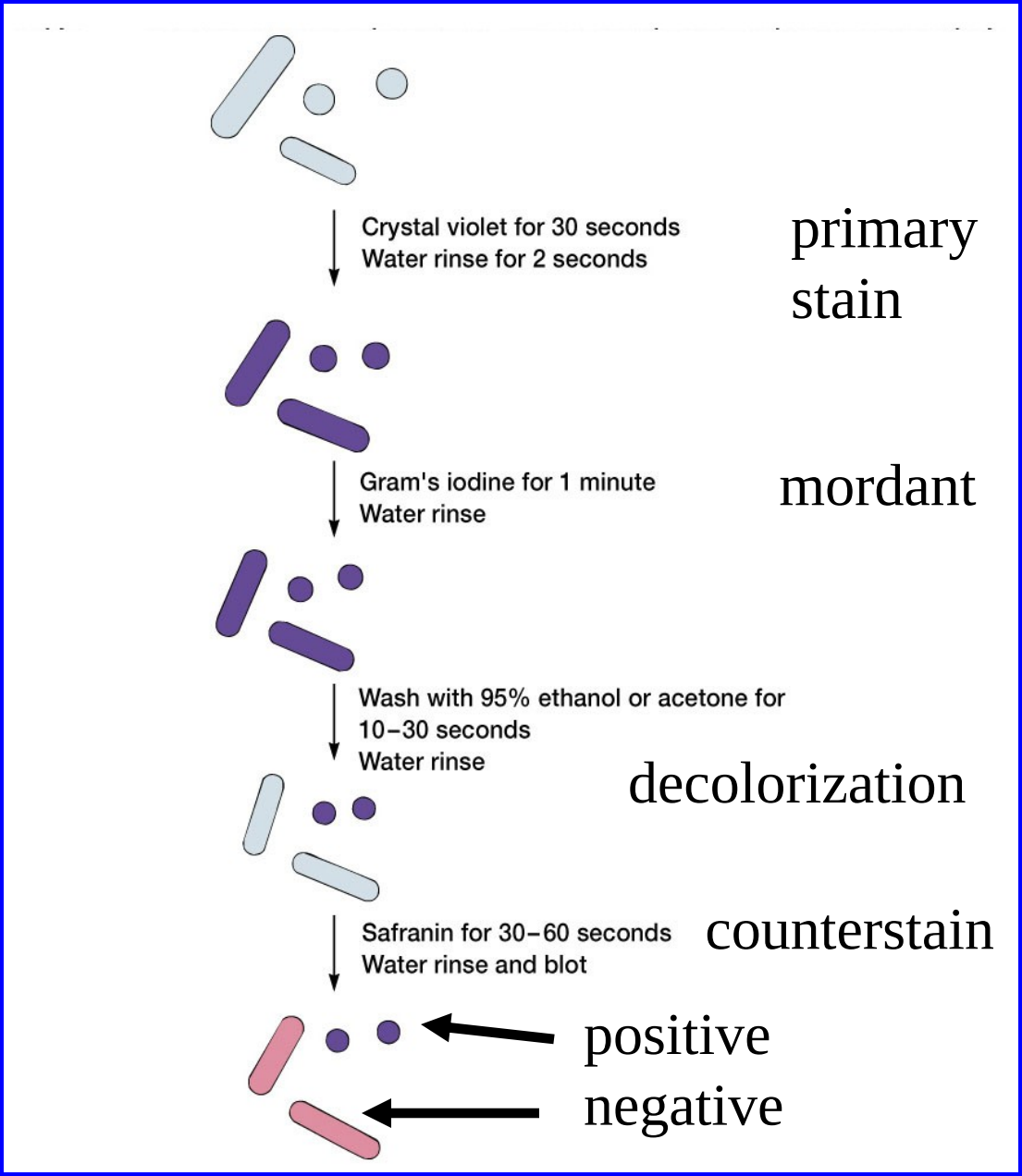


Figure 2.14

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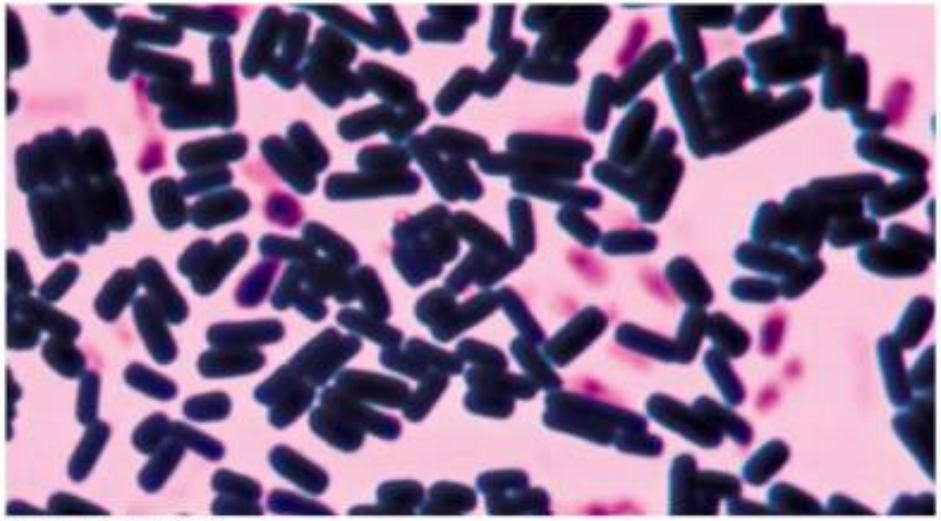
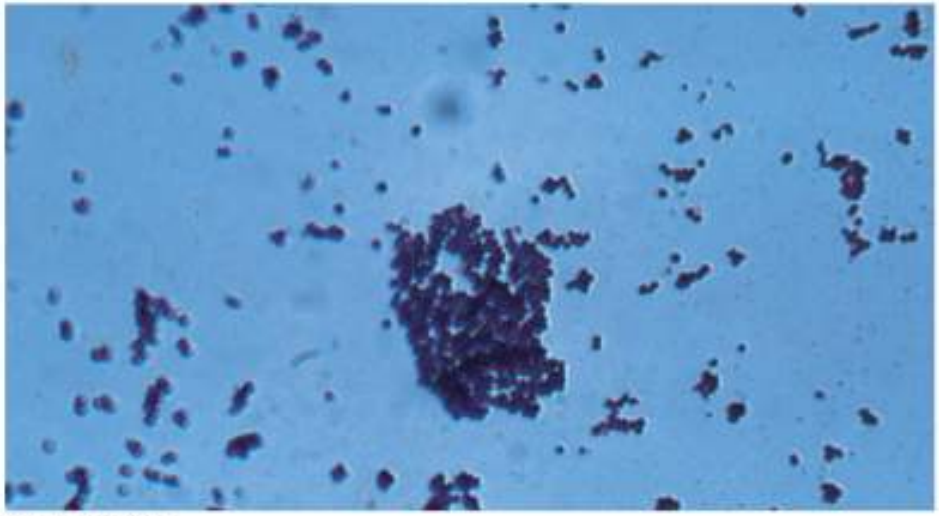
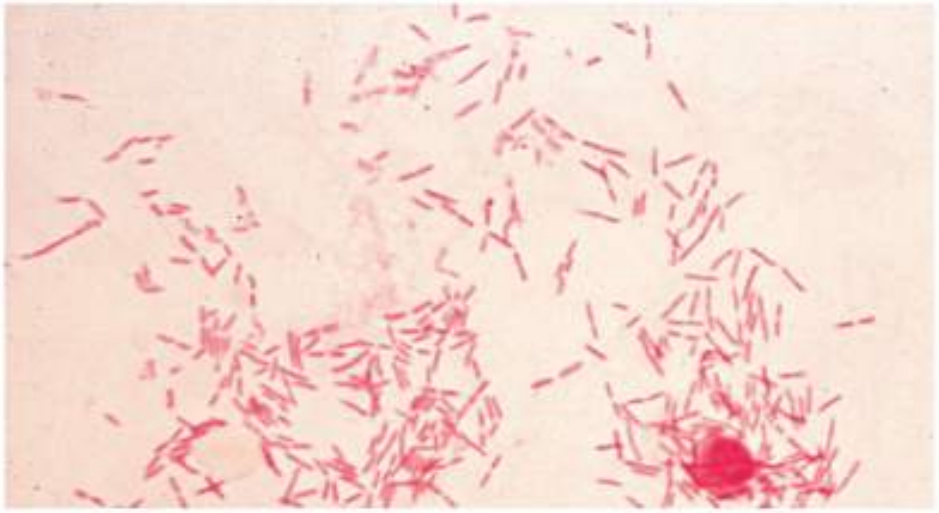


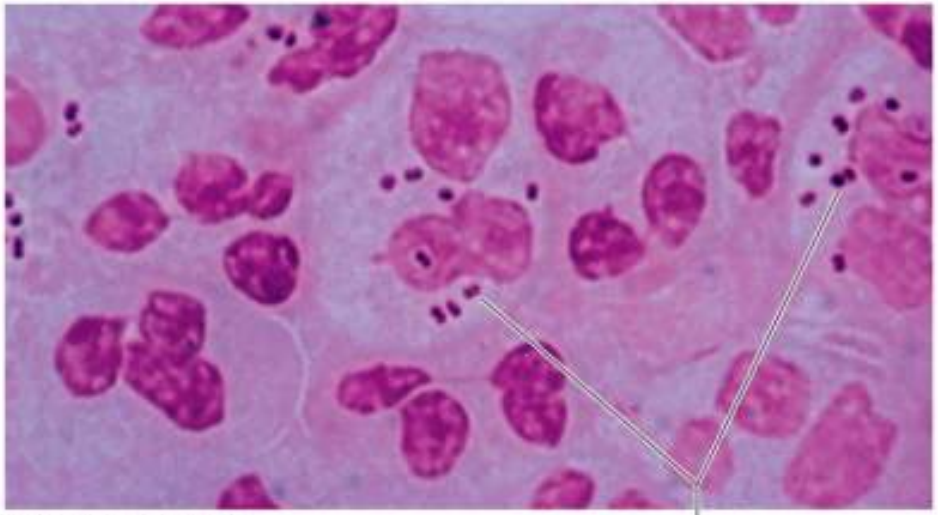
(a) *C. Perfringens*



(b) *S. aureus*



(c) *E. coli*



(d) *N. gonorrhoeae*

Acid-fast staining

- **particularly useful for staining members of the genus *Mycobacterium***
  - **e.g., *Mycobacterium tuberculosis* – causes tuberculosis**
  - **e.g., *Mycobacterium leprae* – causes leprosy**
  - **– high lipid content in cell walls is responsible for their staining characteristics**

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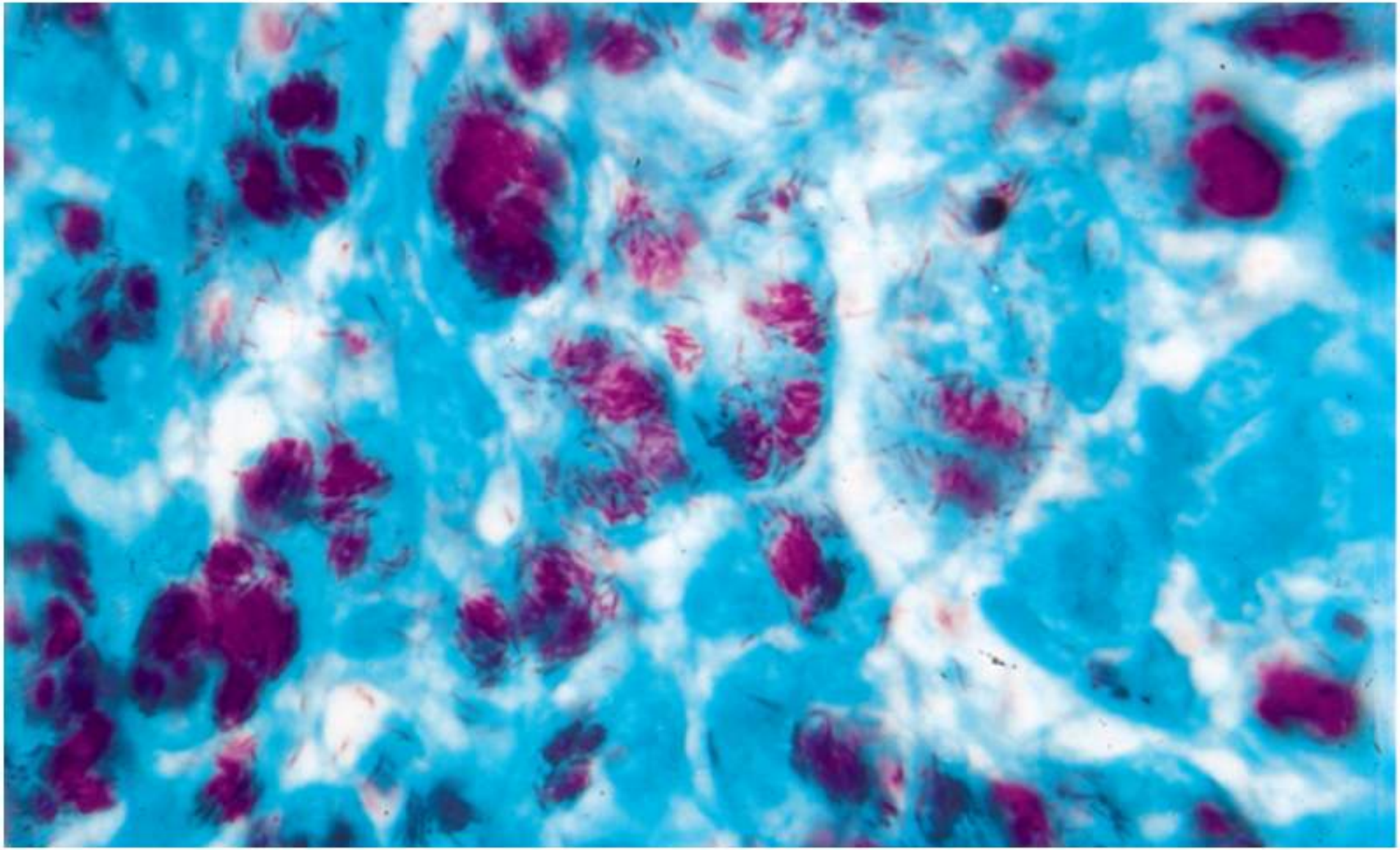
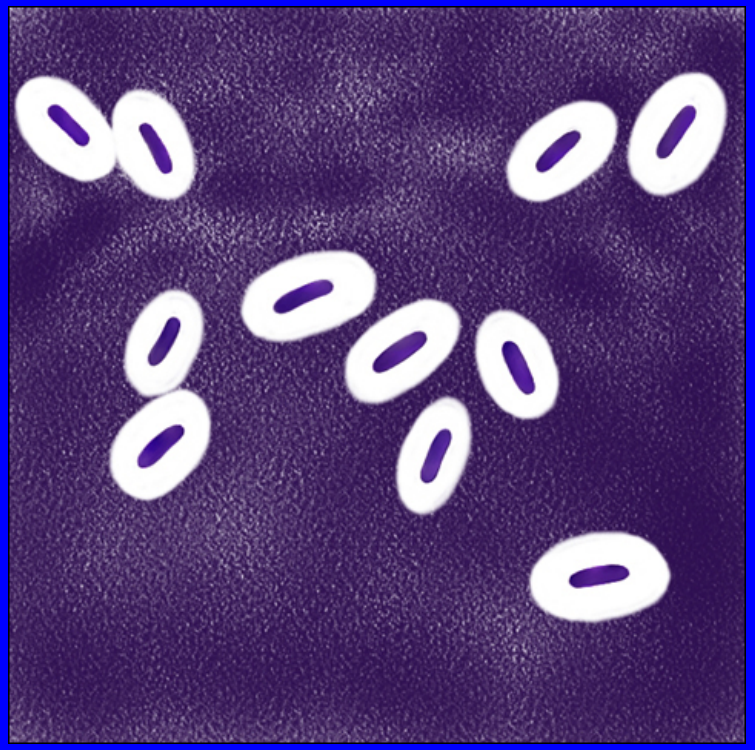


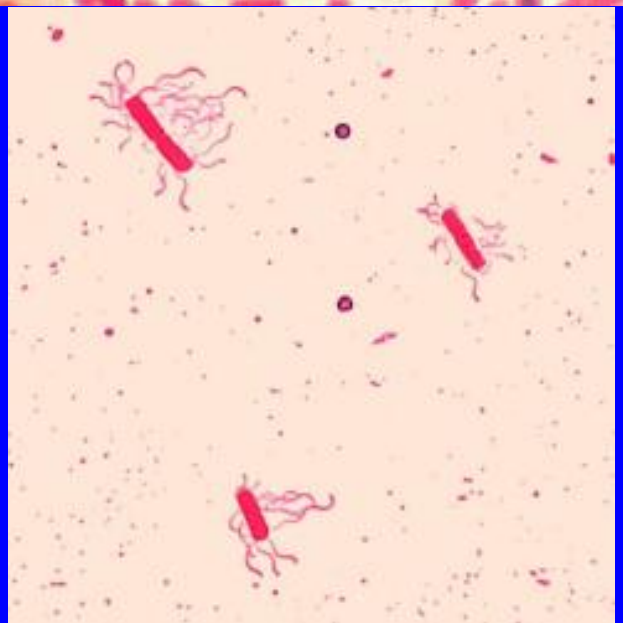
Staining Specific Structures

- **Negative staining**
  - **often used to visualize capsules surrounding bacteria**
  - **capsules are colorless against a stained background**



Staining Specific Structures

- **Spore staining**
  - double staining technique
  - bacterial endospore is one color and vegetative cell is a different color
- **Flagella staining**
  - mordant applied to increase thickness of flagella



Electron Microscopy

- beams of electrons are used to produce images
- wavelength of electron beam is much shorter than light, resulting in much higher resolution

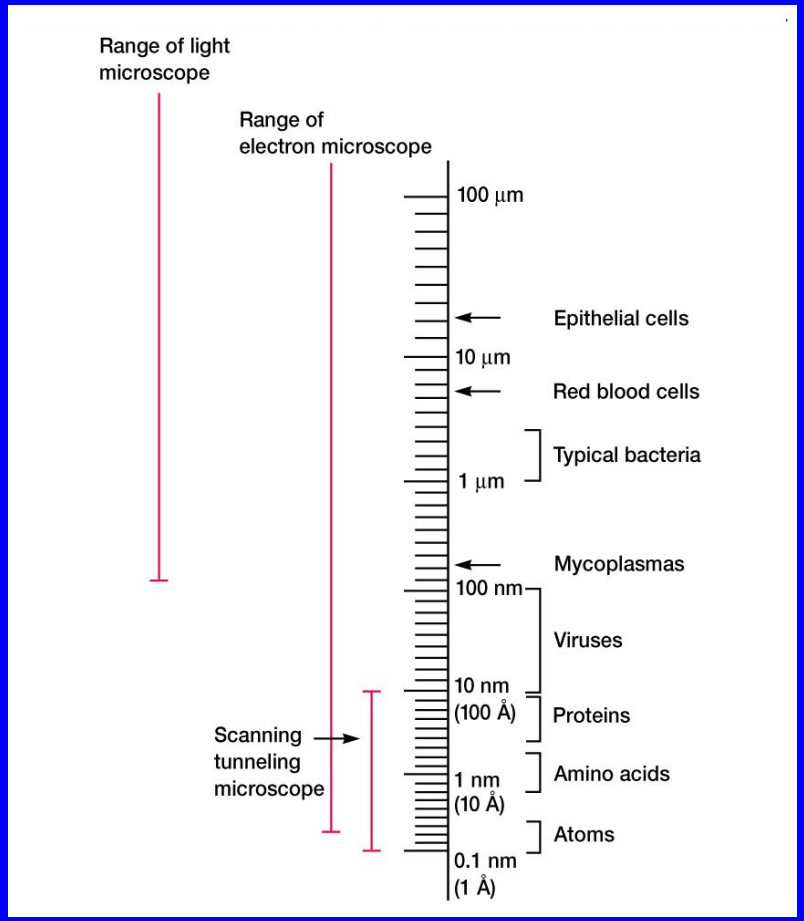
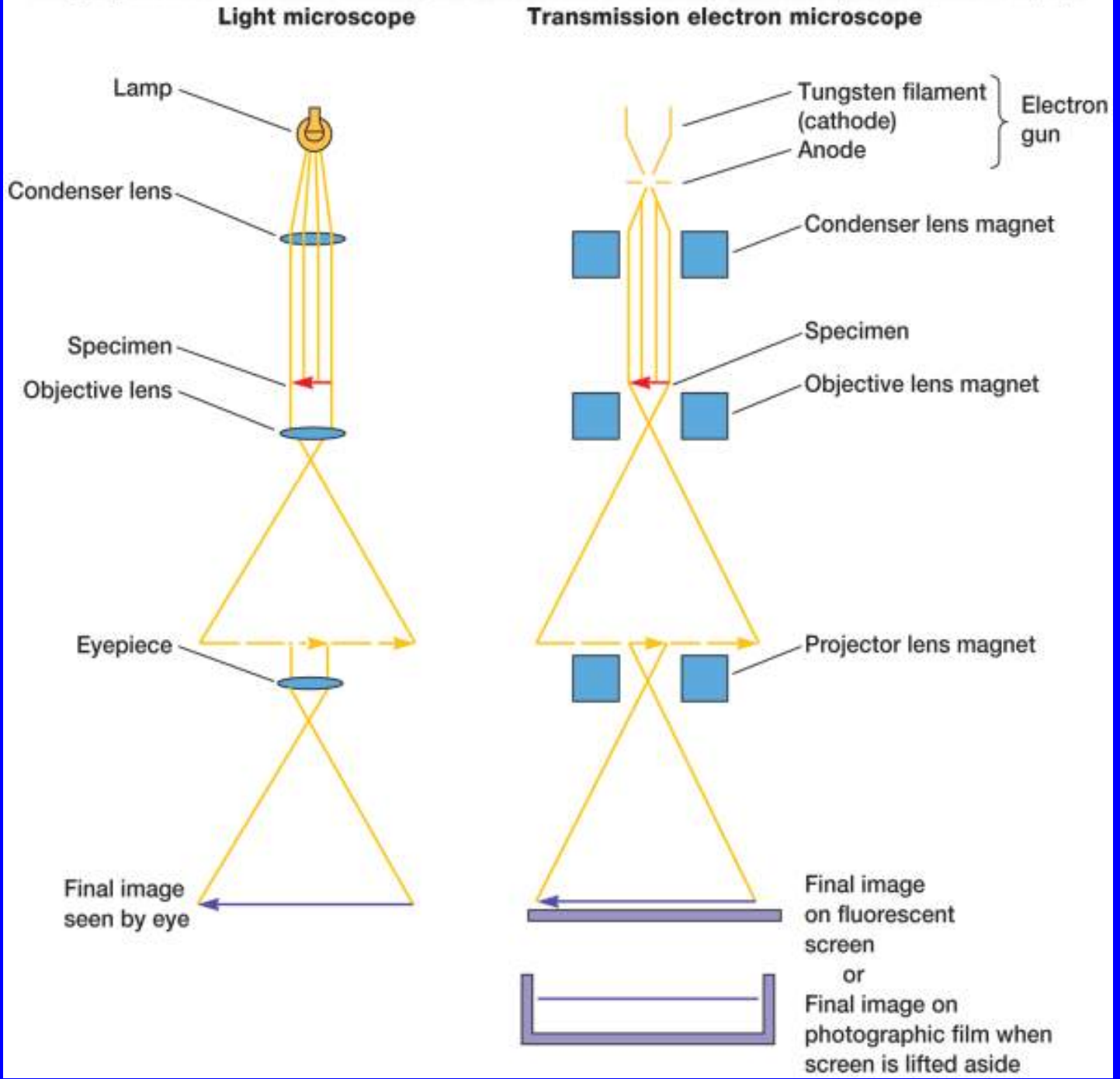


Figure 2.20

The Transmission Electron Microscope

- electrons scatter when they pass through thin sections of a specimen
- transmitted electrons (those that do not scatter) are used to produce image
- denser regions in specimen, scatter more electrons and appear darker



Specimen Preparation

- analogous to procedures used for light microscopy
- for transmission electron microscopy, specimens must be cut very thin
- specimens are chemically fixed and stained with electron dense material

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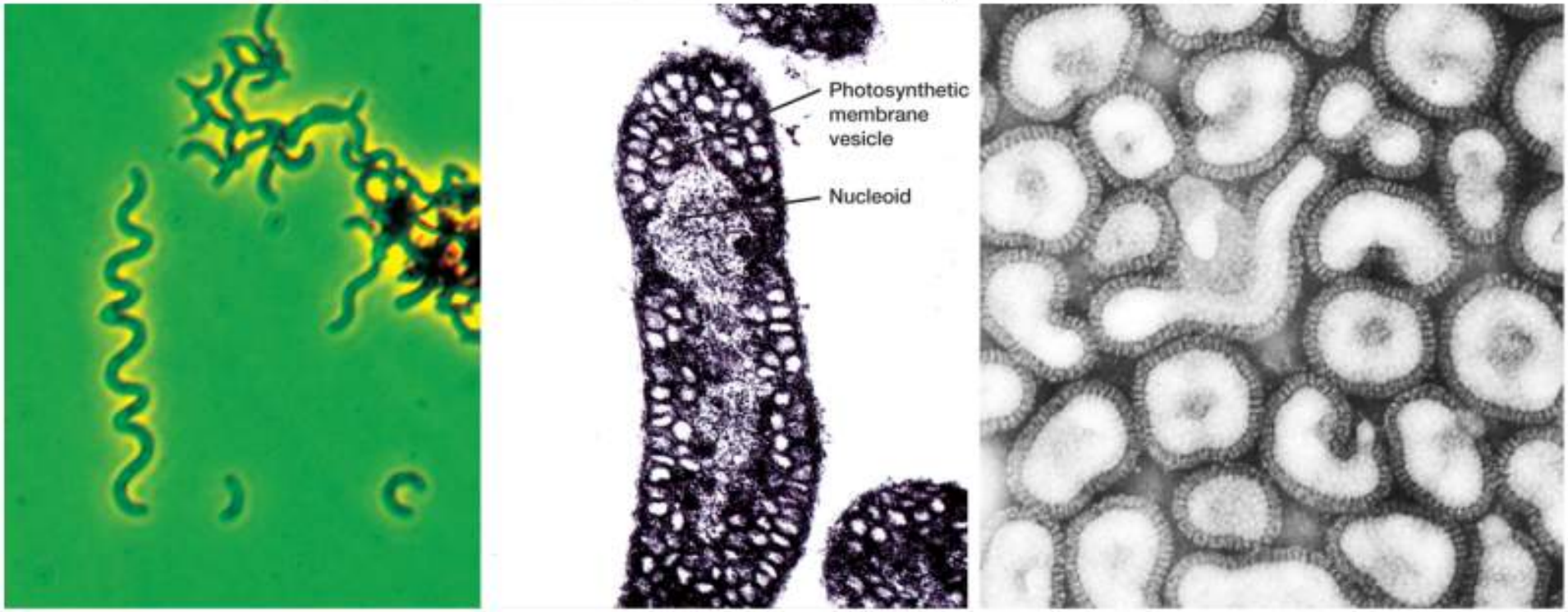
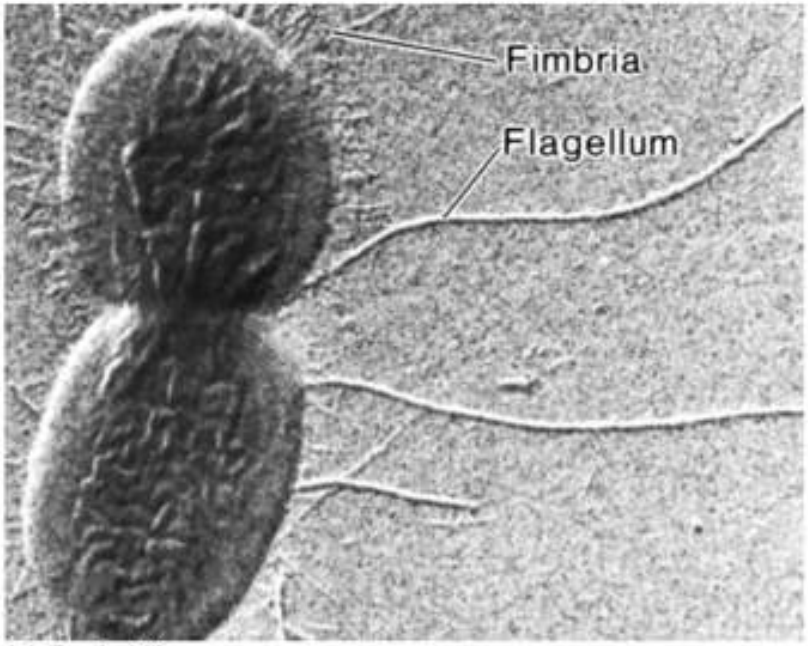
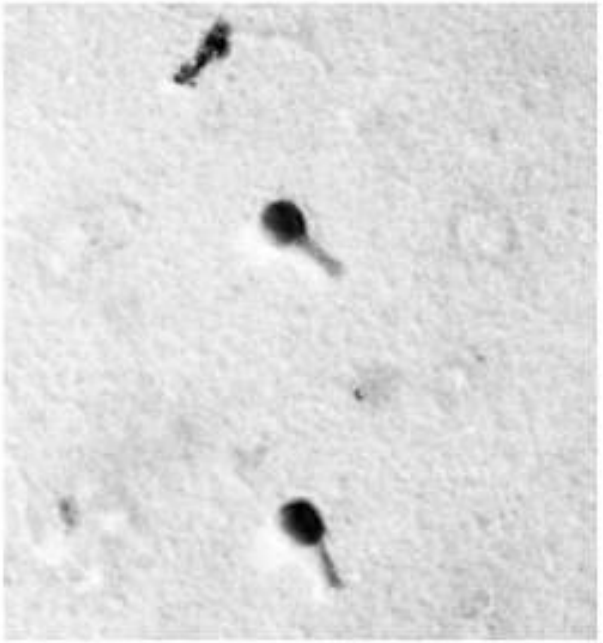


- **shadowing**
  - **coating specimen with a thin film of a heavy metal**

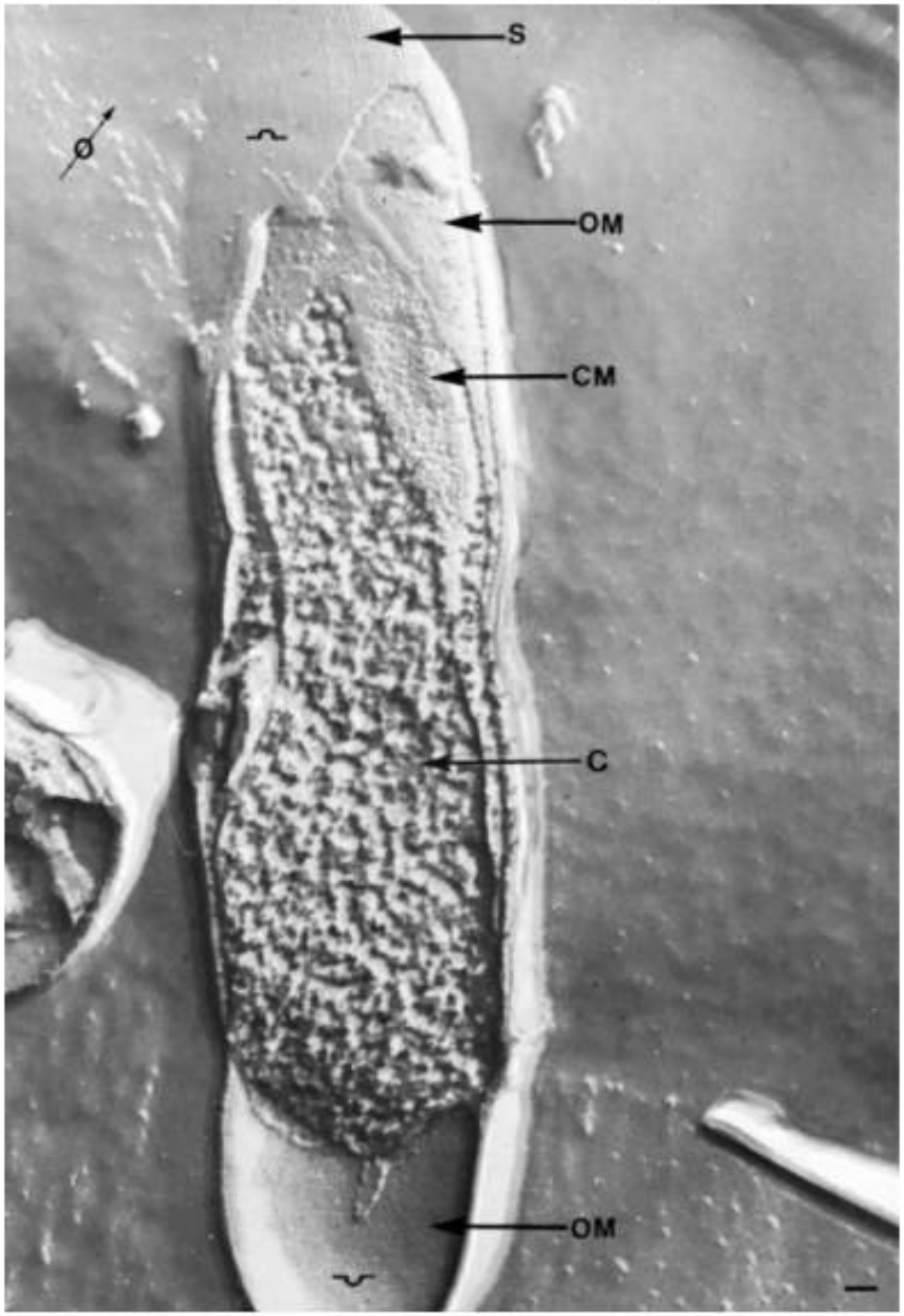


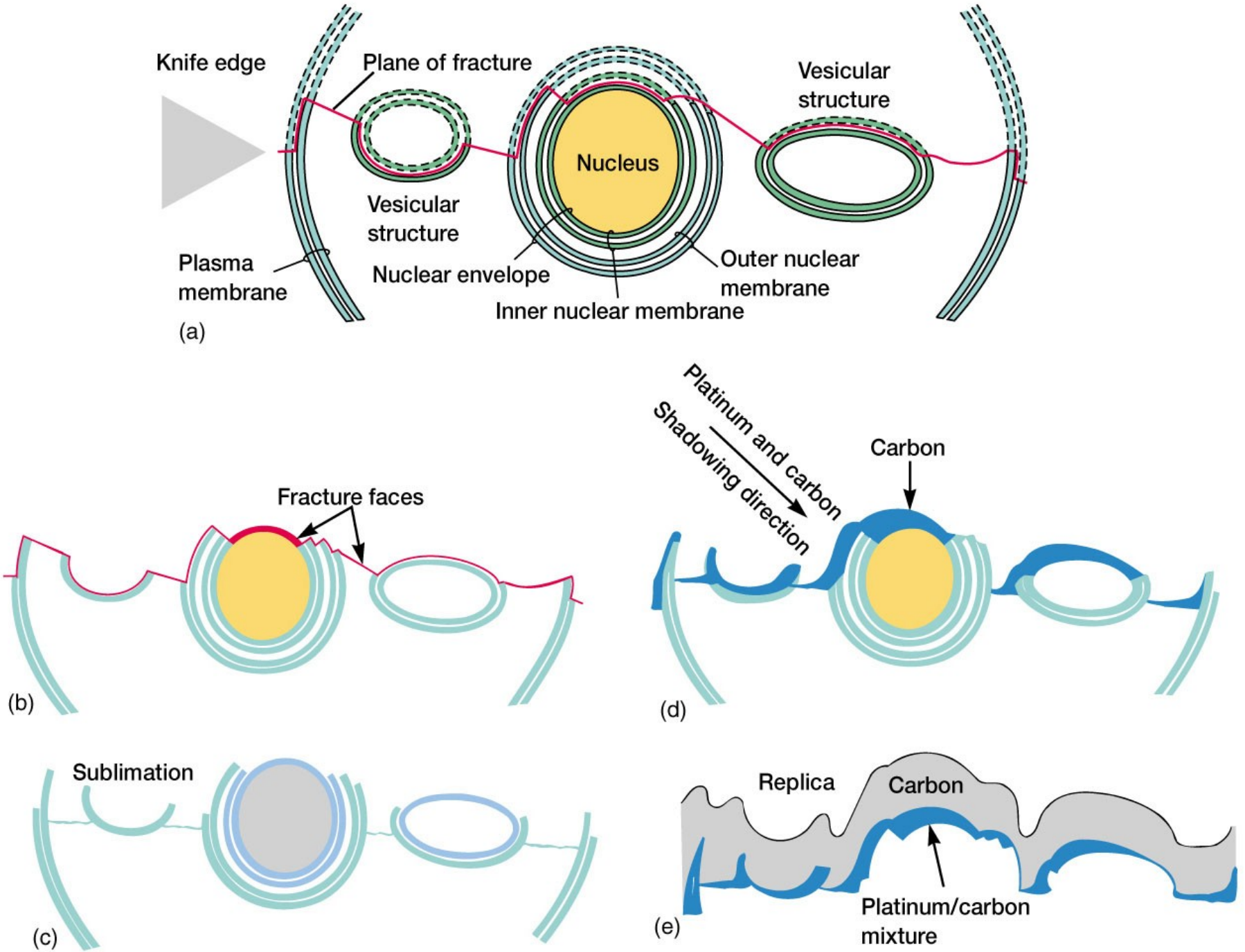
(a) *P. mirabilis*



(b) T4 coliphage

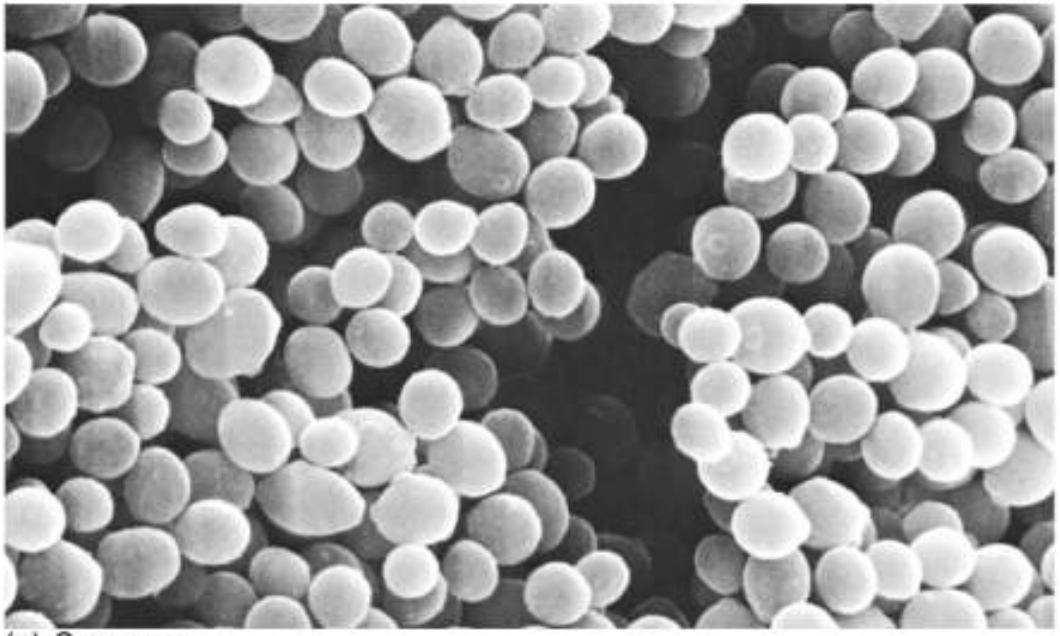
- **freeze-etching**
  - **freeze specimen then fracture along lines of greatest weakness (e.g., membranes)**



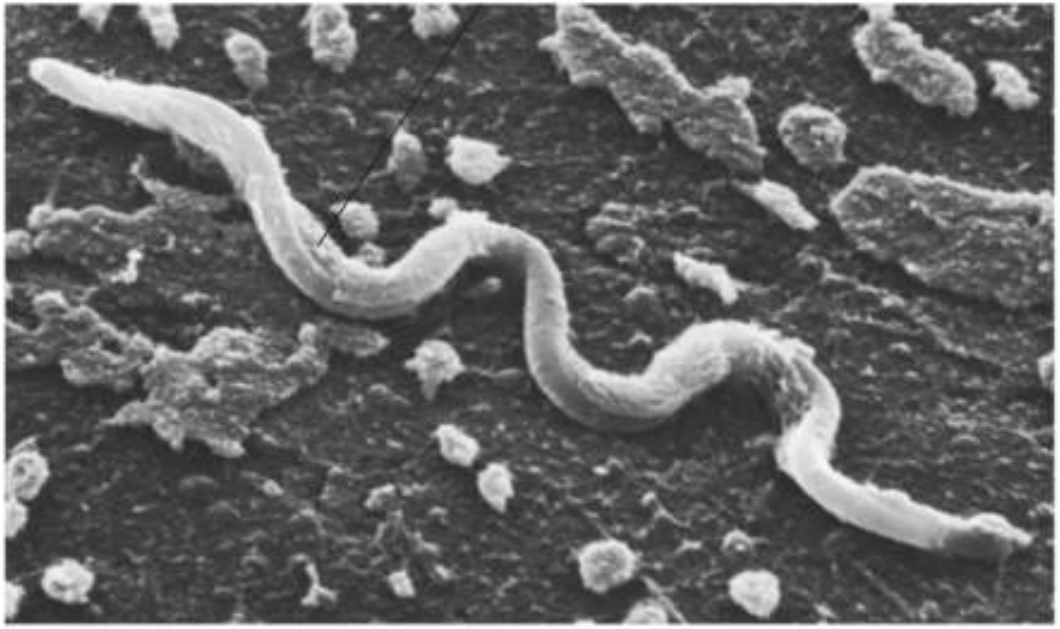

Knife edge
Plane of fracture
Vesicular structure
Nucleus
Vesicular structure
Plasma membrane
Nuclear envelope
Outer nuclear membrane
Inner nuclear membrane
(a)
Fracture faces
(b)
Sublimation
(c)
Platinum and carbon
Shadowing direction
Carbon
(d)
Replica
Carbon
Platinum/carbon mixture
(e)

The Scanning Electron Microscope

- uses electrons reflected from the surface of a specimen to create image
- produces a 3-dimensional image of specimen's surface features



(a) *S. aureus*



(b) *Cristispira*

Confocal Microscopy

- **confocal scanning laser microscope**
- **laser beam used to illuminate spots on specimen**
- **computer compiles images created from each point to generate a 3-dimensional image**

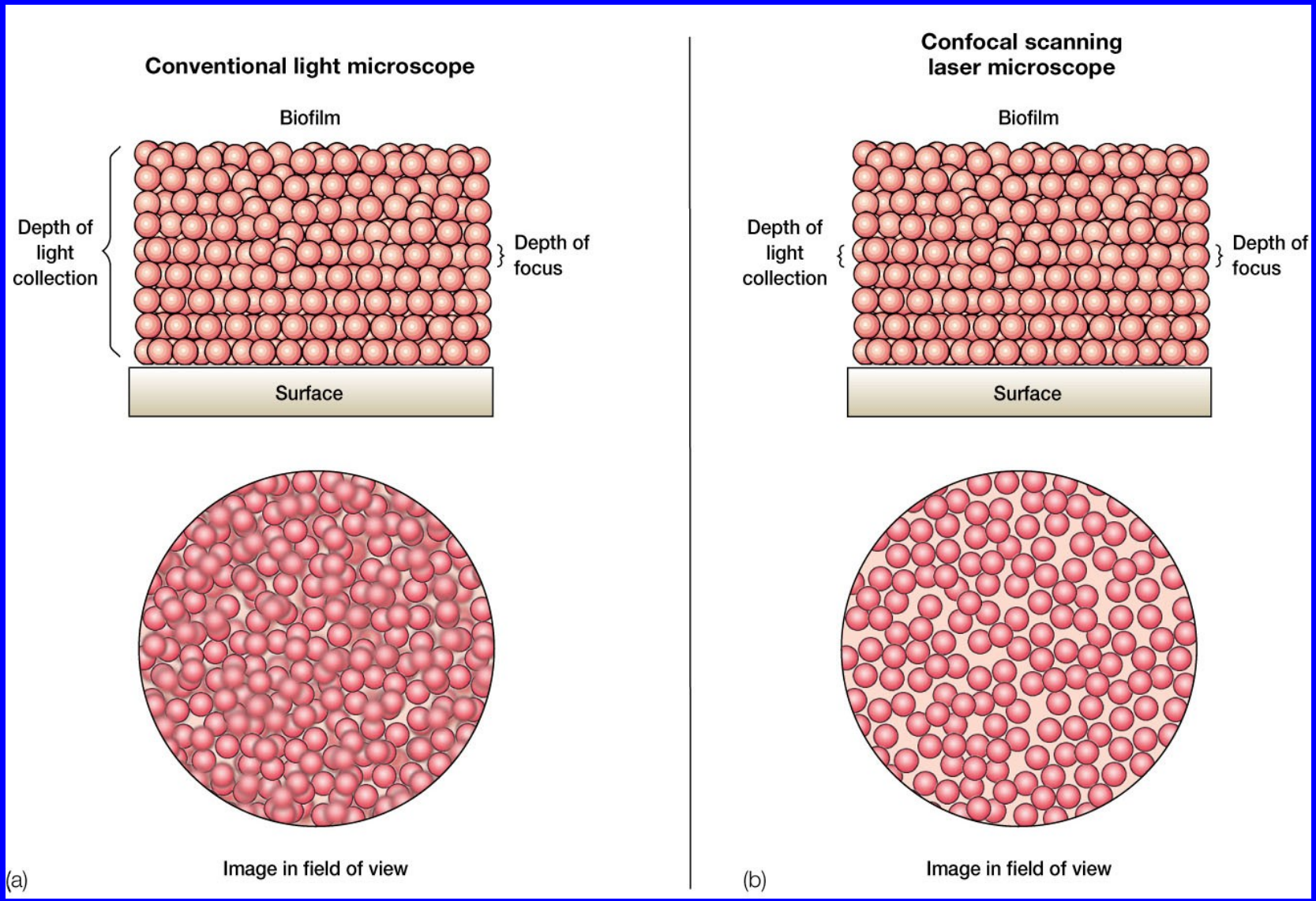


Figure 2.29

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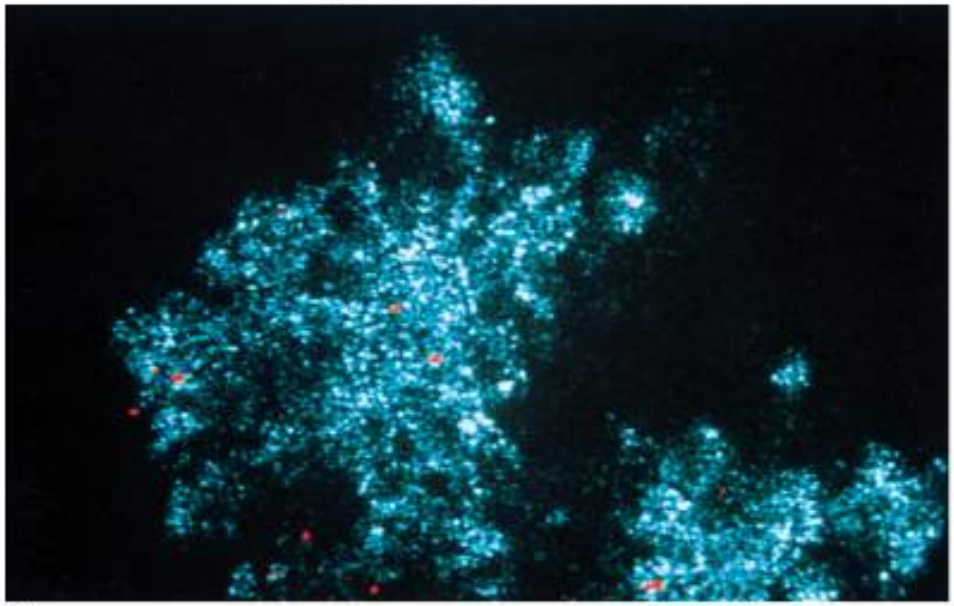

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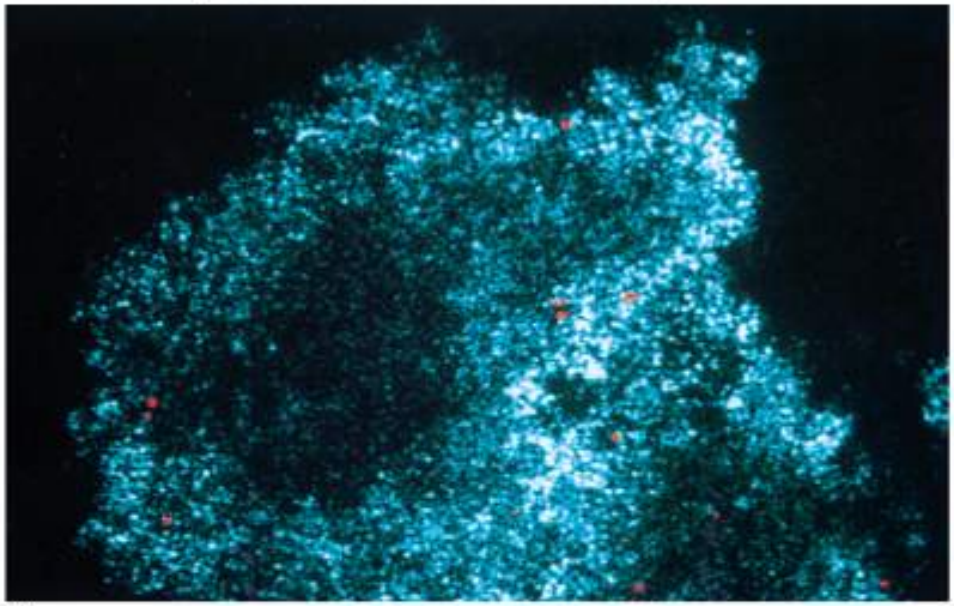
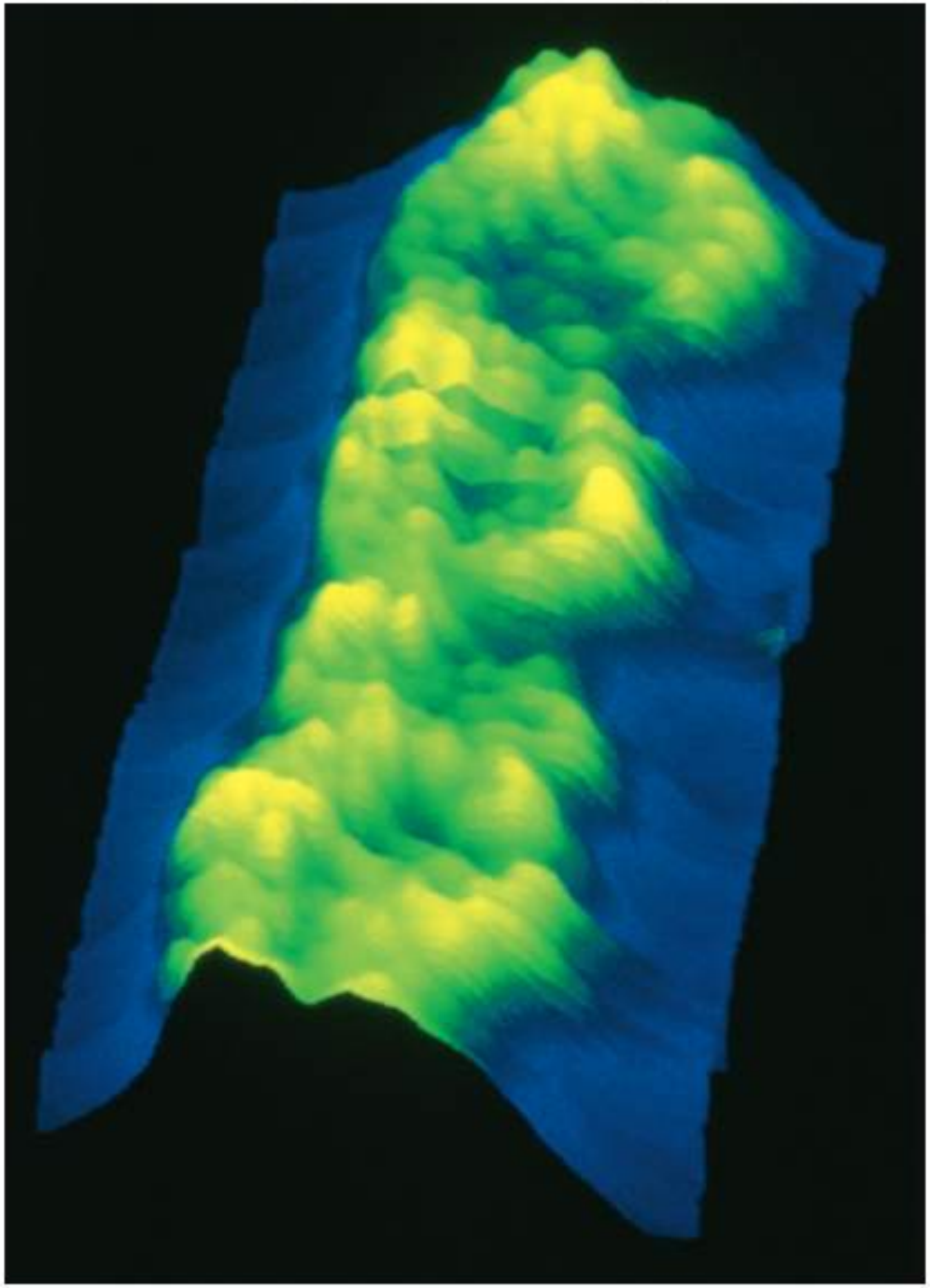


(b)

Scanning Probe Microscopy

- **scanning tunneling microscope**
  - **steady current (tunneling current) maintained between microscope probe and specimen**
  - **up and down movement of probe as it maintains current is detected and used to create image of surface of specimen**

DNA



Scanning Probe Microscopy

- **atomic force microscope**
  - **sharp probe moves over surface of specimen at constant distance**
  - **up and down movement of probe as it maintains constant distance is detected and used to create image**