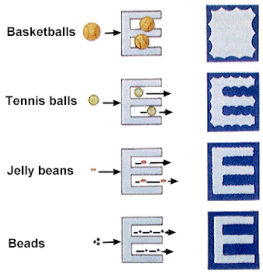
Chapter 3

Microscopy and Staining

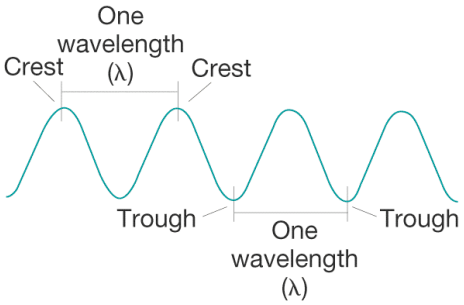
Measuring microorganisms

- 1 meter (m) 10^0m
- 1 centimeter (cm) 10^{-2}m
- 1 millimeter (mm) 10^{-3}m
- 1 micrometer (μm) 10^{-6}m
- 1 nanometer (nm) 10^{-9}m
- 1 angstrom (A) 10^{-10}m

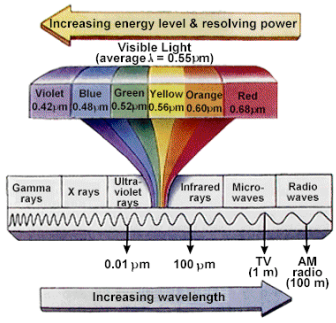
Resolution



Wavelength

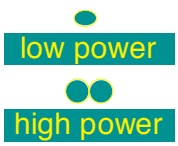


Wavelength Spectrum



Resolving Power

- The ability to determine the relationship between two objects.
- Are they two bacteria or just
- one bacterium?



Contrast

✓Visible shades in an organism

- ☞Bacteria and other microbes are usually transparent

✒Staining increases contrast

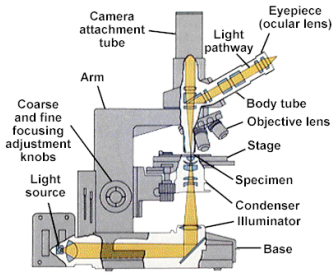
✒Phase-Contrast Microscope

- ☞Exploits the refractive index of cells and the surrounding media

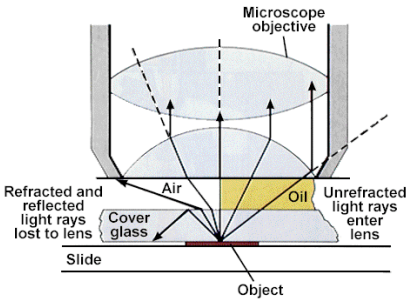
✒Nomarski differential interference contrast microscope (DIC)

- ☞Image appears three dimentional

The Light Microscope



Refracted Light

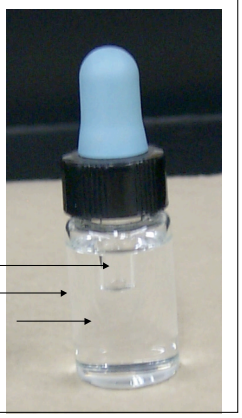


Refractive index

Air with a refractive index of 1.00

Glass has refractive index of 1.52

Mineral oil with a refractive index of 1.52



The Compound Light Microscope

- eye piece or ocular objective (10X)
- Scanning objective (4X)
- low power objective (10X)
- high power objective (40X)
- oil immersion objective (100X)

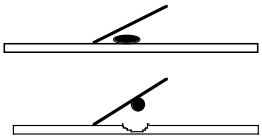
Magnification

- ocular x objective = magnification
- 10 x Scanning = 40X
- 10 x low power = 100X
- 10 x high power = 400X
- 10 x oil immersion = 1000X

Observing microorganisms using a compound light microscope.

- living specimens
- stained specimens
- permanent specimens (also stained)

Wet mount and hanging-drop method



Why do we stain microorganisms?

- They are transparent or translucent.
- To show overall structure.
- To identify internal structures.
- To identify and differentiate between types of microorganisms.

4 steps of staining bacterial cells

- Make a smear.
- Heat/chemically "fix" cells to slide.
- Stain with one or more dyes.
- Rinse off excess stain.

Staining techniques for the light microscope

1. Simple stain -- One dye to reveal basic cell shape & arrangements
2. Negative stain -- color the background around the cells
3. Differential stain -- two or more dyes to distinguish various properties of the organism

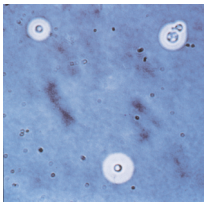
Types of simple stains….

- ✓ Methylene blue
- ✓ Crystal violet
- ✓ Carbolfuchsin
- ✓ safranin

Types of negative stains….

✓Nigrosin
✓India ink

Negative stain



Cryptococcus neoformans

Types of differential stains...

- Gram stain
- Acid fast stain
- Endospore stain
- Capsule stain
- Flagella stain

Types of light microscopy

1. Bright field
2. Dark field
3. Fluorescence
4. Phase contrast
5. Fluorescence
6. Confocal (laser)

Differential staining

1. Primary stain
2. Mordant (fixes the primary dye)
3. Decolorizing rinse --critical step
4. Counter stain

Gram stain

1. Crystal violet
2. Iodine
3. Alcohol rinse
4. Safranin

Gram reaction

Step	Positive	Negative
Crystal violet		
Iodine		
Alcohol		
Safranin		

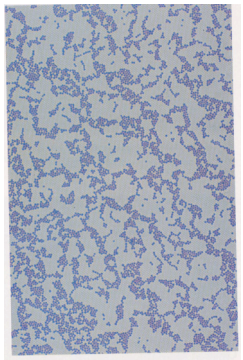


FIGURE 3.7 A Gram stain of a 24-hour culture of *Staphylococcus aureus*, yielding uniform results. All cells are purple, a Gram-positive reaction (3600×).

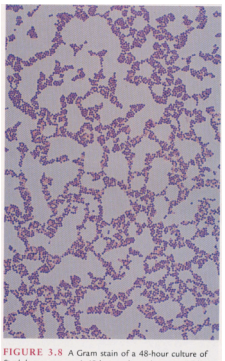


FIGURE 3.8 A Gram stain of a 48-hour culture of *Staphylococcus aureus*. A 48-hour culture does not yield uniform results. Even though most cells are purple, a Gram-positive reaction, some cells are red, a Gram-negative reaction (3600×).

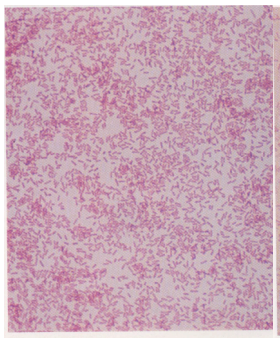


FIGURE 3.10 A Gram stain of a 24-hour culture of *Escherichia coli*, yielding uniform results. All cells are red, a Gram-negative reaction (3600×).

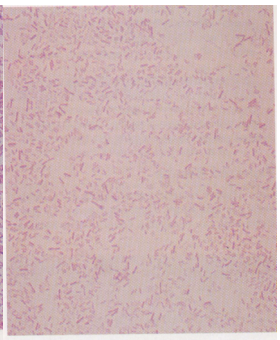


FIGURE 3.12 A Gram stain of a 72-hour culture of *Escherichia coli*. Only a few cells are red, and most cells stain only lightly (3600×).

Acid fast reaction

Step	Positive	Negative
Carbolfuchsin		
Acid alcohol		
Methylene blue		

Acid-fast stain

FIGURE 3.23 An acid-fast stain of *Mycobacterium tuberculosis*, an acid-fast rod. Acid-fast rods appear red (3600×).

Special Stains

- ✓Endospore stain
- ✓Flagella stain
- ✓Negative stain
- ✓Capsule stain

Flagellum stain

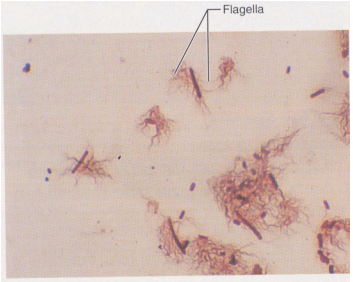


FIGURE 3.36 Peritrichous flagella of *Proteus vulgaris* (3600×).

Negative stain (bacillus)

FIGURE 3.5 A negative stain of the rod *Bacillus* (3600×).

Diplobacilli

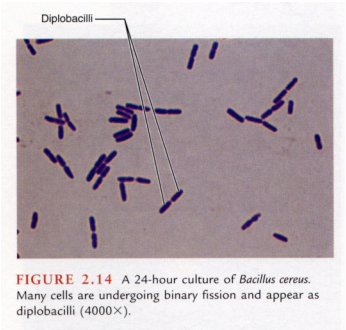


FIGURE 2.14 A 24-hour culture of *Bacillus cereus*. Many cells are undergoing binary fission and appear as diplobacilli (4000×).

Diplococci

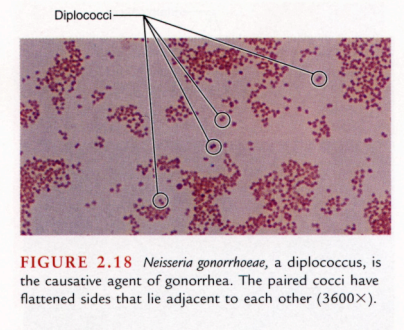


FIGURE 2.18 *Neisseria gonorrhoeae*, a diplococcus, is the causative agent of gonorrhea. The paired cocci have flattened sides that lie adjacent to each other (3600×).

Staphylococci

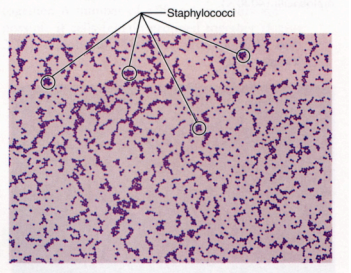
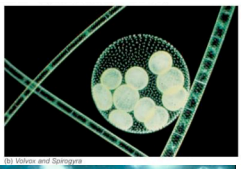


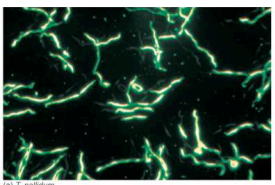
FIGURE 2.22 Cocci in clusters (staphylococci) in *Staphylococcus aureus*. These clusters are due to cell division occurring in more than three planes (2500×).

The Dark-Field Microscope

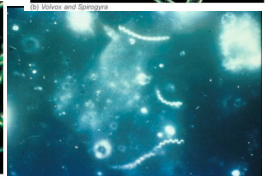
- ✓ produces a bright image of the object against a dark background
- ✓ used to observe living, unstained preparations
- ✓ Used to identify *Treponema pallidum*



(b) Volvox and Spirogyra



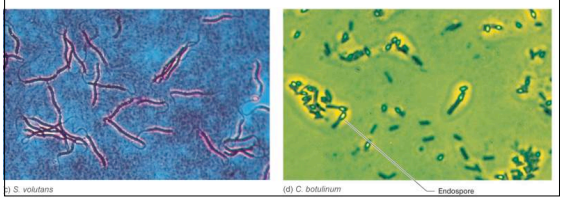
(a) *T. pallidum*



The Phase-Contrast Microscope

✓enhances the contrast between intracellular structures having slight differences in refractive index

✓excellent way to observe living cells



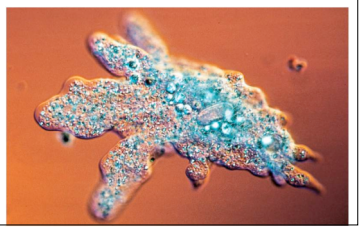
The Differential Interference Contrast Microscope

✓ Two beams of polarized light

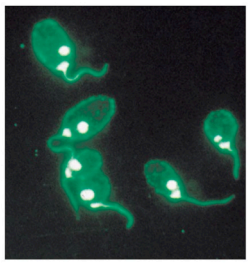
✓ creates image by detecting differences in refractive indices and thickness of different parts of specimen

✓ excellent way to observe living cells

Amoeba proteus

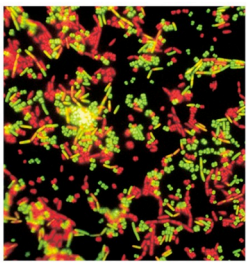


Fluorescence Microscopy



Kinetoplasts stained

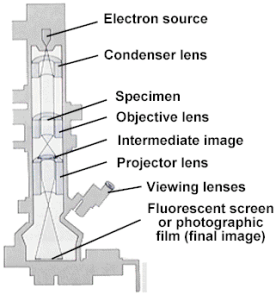
Crithidia luciliae



Live verses dead bacteria

Micrococcus luteus & Bacillus cereus

Electron Microscope



Electron microscopy

Electrons are generated, condensed, and focused onto an object. An image is created and magnified on a fluorescent screen.

Staining for electron microscopy

1.Heavy metals

2.Radioactive materials

Why?

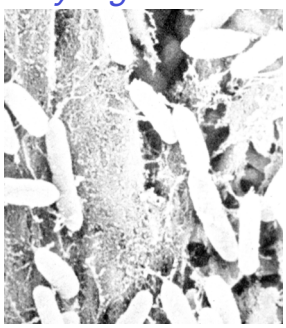
It is difficult for electrons to pass through very dense materials.

Types of electron microscopy

1. Transmission EM
2. Scanning EM
3. Scanning-Tunneling EM
4. Immuno-EM
5. Atomic force Microscopy

Pseudomonas syringae

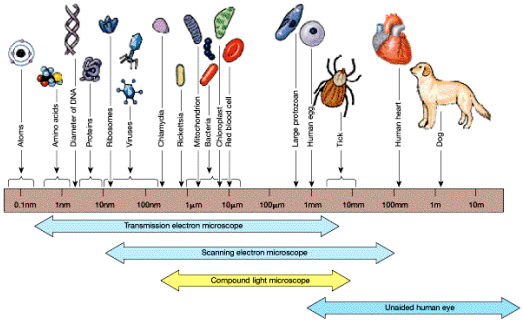
- Gram negative bacteria
- Aerial surfaces of plants
- Produces antifungal molecules



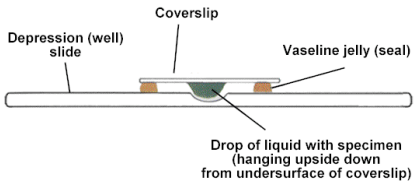
Magnification (EM)

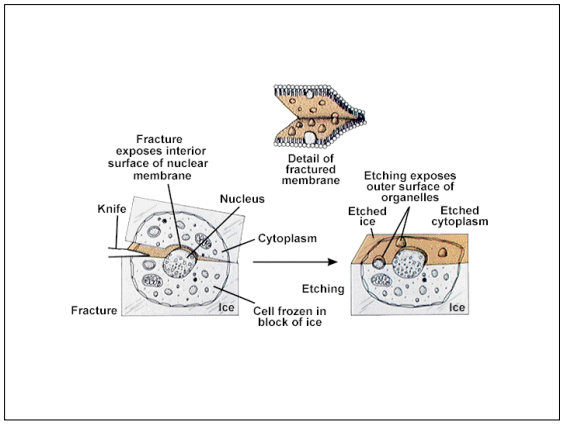
Light microscopy: 10-2000X

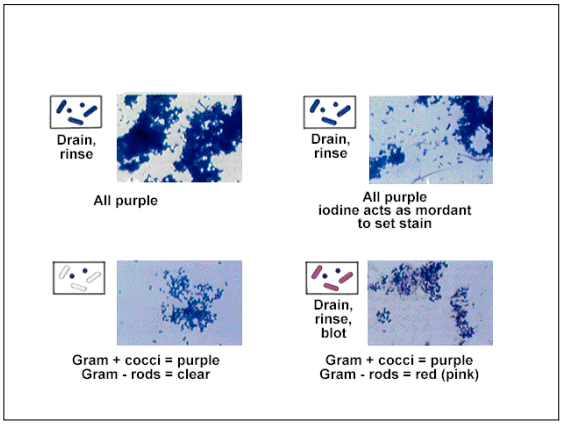
Electron microscopy:
10X-200,000-400,000X

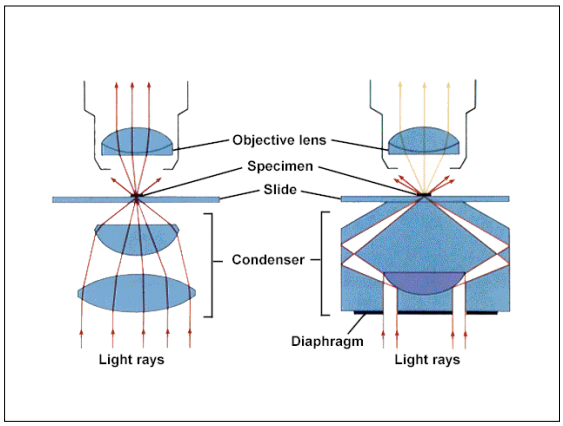


Air Force One




Fracture exposes interior surface of nuclear membrane
Detail of fractured membrane
Etching exposes outer surface of organelles
Knife
Nucleus
Etched ice
Etched cytoplasm
Cytoplasm
Etching
Fracture
Ice
Cell frozen in block of ice
Ice


Drain, rinse
All purple
Drain, rinse
All purple
iodine acts as mordant
to set stain
Gram + cocci = purple
Gram - rods = clear
Drain, rinse, blot
Gram + cocci = purple
Gram - rods = red (pink)


Objective lens
Specimen
Slide
Condenser
Diaphragm
Light rays
Light rays