

## Chapter 3 - Characterization of Microorganisms

### Relative Size

**Micrometer ( $\mu\text{m}$ )** -  $10^{-6}$  m

**Nanometer (nm)** -  $10^{-9}$  m

**Angstrom (A)** -  $10^{-10}$  m

**Protozoan** - 100 - 200  $\mu\text{m}$

**Bacteria** - 2  $\mu\text{m}$

**Virus** - nm range

**Human Eye** - 1 cm to 1mm

**From largest to smallest** = Algae, Fungi, Bacteria, Virus

Microscopy - the use of microscopes

Magnification - level at which structures are still clearly distinguishable and not blurred

Resolving Power - ability to distinguish images of 2 close objects as separate

### Binocular Compound (Light) Microscope

lenses - ocular, objectives

total Magnification - ocular x objective

resolution -  $\lambda$  (wave length) / (2 x numerical aperture)

### Compound (2 lenses) (light) Microscope

also called: Light Microscope, Bright Field Microscope, Compound Microscope

lenses

ocular

objectives - light rays are brought together here to form a image.

illuminator

condenser - focuses light on the specimen

magnification - 1000X

resolution - 0.2  $\mu\text{m}$

**Light microscope light path** = light source, condenser, specimen, objective, body tube, ocular(reversed image)  $\rightarrow$  2x magnified and 1x reversed

**Resolving power** - smaller = better;  $\lambda/(2 \times \text{NA})$ ; NA = Numerical Aperture

**Resolution** = ability to distinguish two points as two distinct separate points, measured as distance (the smaller the # or distance the better the resolution.

To increase resolution:

1. Shorter  $\lambda$  (wave length)
2. Larger Numerical aperture (NA)
3. oil - helps keep the light rays together as they pass between the specimen & objective  
refractive index of the glass and oil are the same
4. staining

**Focal point** = point at which the light rays converge to focus your vision

**Focal distance** = the distance between where the light source and the focal point

**Parfocal** = stays in focus when changing objectives

**Field of Vision** = the area you view through a microscope

### Types of Compound (light) Microscopes

**Brightfield** - white light around specimen enters objective

- field illuminated
- object is darker
- organism stained or killed
- Resolution - 0.2  $\mu\text{m}$
- Magnification - 1000x to 2000x

**Darkfield** - special ring w/i the condenser of a brightfield microscope or an actual darkfield microscope

- field dark
- object illuminated
- organism unstainable or living
- only the light bent by the specimen enters the objective ∴ specimen is light and surrounding field is dark
- good for specimens that cannot be stained or alive cells
- magnification limit = 1000x to 2000x
- resolution = 0.2 μm

### Phase Contrast

- condenser with annular (ring) diaphragm
- ring-shaped diffraction plate in objective
- can see internal structure without staining
- organism living (better than darkfield)

### Fluorescent

- fluorescent stained (gives off longer λ) = fluorochrome - FITC → fluorescein isothiocyanate (green)  
→ auramine O (yellow)
- UV light (absorbed = fluorescent)
- fluorescent antibody technique:
  1. tag antibodies with FITC
  2. Antigen binds with antibodies

**Types of Electron Microscopes** - for objects smaller than 0.2 μm

light source - electrons

focused - by magnets in condenser

slide - cooper grid slide not glass

**Transmission** - highest magnification and resolving power

- specimen must be frozen, sliced, and stained
- magnification =  $10^4$  -  $10^5$ x (high)
- resolution = 2.5 nm (high)
- photographic plate: object sometimes distorted
- beam transmitted through specimen

**Scanning** - intermediate in magnification and resolving power

- 3D picture (intact cells/surface)
- freeze fracture
- magnification =  $10^3$  -  $10^4$ x
- resolution = 20 nm
- beam passes over the surface of specimen and collected on other side

**Staining** - coloring with dye

- smear
- heat fix
- stain (washed & blotted) + dried

stains are composed of color ions called chromophores

1. basic stains (+) ions or (+) stains and stain the specimen
  - crystal violet
  - methylene blue
  - safranin (red)
2. acidic stains (-) ions or (-) stains and stain the background
  - eosin (blue/black)
  - nigrosin (very black)

**Staining Techniques** - used to show the overall structure of microorganisms, to I.D. their internal structures, and to help I. D. and separate similar organisms

**Simple stain** - (use of one dye)

Begin staining with a process called heat fixing:

1. Slide with bacteria
  2. Air dry
  3. Pass through flame - to denature the proteins so that they stick to the glass  
Denature - to break down or unravel the protein making it non functioning
  4. Add positive stain or negative stain
- apply basic dye
  - use mordant (intensifies stain)
  - (+) stain for organism stain
  - (-) stain for background stain

**Differential Stain** - differentiates between bacteria's with thick peptidoglycan wall (Gram +) and those that have thin peptidoglycan wall (Gram -)

- Gram Stain developed by Hans Christian Gram - first using steps 1-3 from above then:

1. Crystal violet (1 min): primary stain
2. Wash & add mordant (1 min) - iodine → enhances binding of primary stain to peptidoglycan
3. Wash with EtOH to decolorize Gram (-) → strips color out of gram (-)
4. (+) safranin as counterstain (secondary stain):  
G(+) = purple  
G(-) = pink (or red)

**Acid-Fast Stain** - use on mycobacteria (ex. TB) waxy material in cell wall - high lipid content in outerwall that prevents the absorption of Gram Stains. Medically Important - because some antibiotic are resistant to either (+) or (-) gram stains and some are not.

Process:

1. (+) Carbofuchsin (red)
2. Heat
3. Cool & Wash with acid - OH, decolorizer
4. counterstain with Methylene blue  
Acid-fast = red (TB or mycobacteria will fall in this category)  
Non-Acid-fast = blue

## Bacteria Differences

**Size and Shape (morphology)**

1. Diameter - 0.2 - 2.0  $\mu\text{m}$
2. Length - 2 - 8  $\mu\text{m}$
3. Shapes
  - coccus (spherical)
  - bacillus (rod)
  - spiral (twisted)
4. Shape arrangement (using coccus)
  - diplo = two
  - strepto = chain
  - tetrad = four
  - staphylo = cluster
5. Spiral Shapes
  - vibrio = J shaped
  - spirilla = on complete spiral
  - spirochete = more than one spiral

**Staining Characteristics** - specified under the staining techniques

**metabolism**

**genetics**