Observing Microbes

- Human eyes have limited resolution
  - 150 µm (1/7 mm, 1/200 inch)
- Microscope needed to see smaller objects
  - Eukaryotic microbes
    - Protozoa, algae, fungi
    - 10–100 µm
  - Prokaryotes
    - Bacteria, Archaea
    - 0.4–10 µm
Bacterial Shapes

- Cocci = spheres
  - in bunches
  - in chains
  - quartets

- Bacilli = sticks, rods
  - alone
  - in chains

- Vibrios = bent rods

- Irregular

Spiral
Staining

- Fix cells to hold in position
- Stain with dye
  - Reacts with chemical structure of organism
    - Gram stain reacts with thick cell wall
  - Increases absorbance
    - Easier to find in low-contrast conditions
Bright-Field Microscopy

- Increasing resolution
  - Multiple lenses
    - Correct each other's aberrations
    - Compound microscope
  - Need to focus two lenses
    - Objective
    - Condenser
Bright-Field Microscopy

- Increasing resolution
  - Use shorter wavelength light
    - UV, X-rays
      - But images aren’t visible to human eye
  - Lessen contrast
    - Lenses with higher contrast give less resolution
      - But need enough contrast to see object
  - Immersion oil
    - Collects more light from specimen
  - Wider lens closer to specimen
    - Higher numerical aperture (NA)
      - $NA = n \sin \theta$
Dark-Field Microscopy

- Light shines at oblique angle
  - Only light scattered by sample reaches objective
  - With enough light, some bounces off object
    - Even objects smaller than wavelength of light
- Makes visible objects below resolution limit
  - Flagella, very thin bacteria
Phase-Contrast Microscopy

- Light passes through and around sample
- Light through sample is refracted
  - Changes phase of light
  - Light waves out of phase cancel
- Sample appears dark against light background
  - Shows internal organelles of eukaryotes
Differential Interference Contrast (DIC) Microscopy

- Polarized light passes through specimen
  - Sample boundaries bend light
  - Second polarized lens blocks light
  - Bent light affects brighter or darker than background

Head of microscopic worm (C. elegans)

Bacterium

Pharynx (mouth)

Cell nuclei

10 µm
Fluorescence Microscopy

- Fluorophores absorb high-energy light
  - Short wavelength
- Emit lower-energy light
  - Longer wavelength
- Label molecules of interest in cell
  - Marker for position of molecules within cell
Fluorescently Labeling Molecules

- Attach directly to some molecules
  - DAPI binds DNA

- Attach labeled antibody to molecules
  - Antibody binds specific molecules
    - Fluor covalently bound to antibody
Electron Microscopy

- Electrons behave like light waves
  - Very high frequency
  - Allows very great resolution
    - A few nanometers
- Sample must absorb electrons
  - Coated with heavy metal
  - Electron beam and sample are in a vacuum
  - Lenses are magnetic fields

Electron source (tungsten filament)
Condenser lens
Specimen
Objective lens
Projection lens
Image plane
Fluorescent screen
Transmission EM

- Sample is fixed to prevent protein movement
  - Aldehydes to fix proteins
  - Flash-freezing
  - High-intensity microwaves
- Fixed sample is sliced very thin
  - Microtome
- Sample is stained with metal
  - Uranium
  - Osmium
Transmission EM

- High resolution
  - Can detect molecular complexes
    - Ribosomes
    - Flagellar base
    - Strands of DNA
  - Need many slices to determine 3D structure
Figure 01: Metal shadowing.

Figure 03: Negative contrast method of visualizing particles by electron microscopy.
Figure 04A: Image of negatively stained bacteriophage T7 helicase/primase in the presence of dTDP.

Figure 04B: Tomato bushy stunt virus particles

Part B courtesy of Robert G. Milne, Plant Virus Institute, National Research Council, Turin, Italy.
Scanning EM

- Sample is coated with heavy metal
  - Not sliced
  - Retains 3D structure
  - Gives 3D image
- Only examines surface of sample
Visualizing Molecules

- **X-ray crystallography**
  - Locates all atoms in a large molecular complex
  - Sample must be crystallized

- **Nuclear magnetic resonance (NMR)**
  - Measures resonance between chemical bonds
  - Can locate all atoms in a small protein
  - Shows atomic movement of proteins in solution
    - Proteins embedded in membranes
X-Ray Crystallography

- X-rays have tiny wavelength
  - Resolution less than 1 Angstrom
    - 0.1 nm = width of a hydrogen ion
- No lenses to focus X-rays
  - Shoot X-rays at crystallized sample
    - Many molecules in identical conformation
    - X-rays diffract according to position of atoms
    - Compute position of atoms from pattern of scattered X-rays
X-Ray Crystallography

- Can detect position of thousands of atoms in a complex of proteins

“Ribbon” shows position of “backbone” of amino acids in proteins