Slide 1

Microscopy

Lecture 4

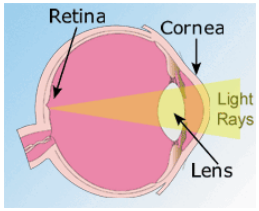
Slide 2

Learning Outcomes

- Introduction to microscopes
- Compound light microscope
- Electron microscopes
- Microscopy terms
- Preparing specimens

Slide 3

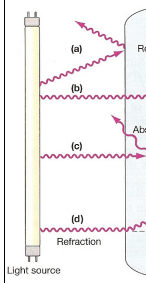
Your Retina

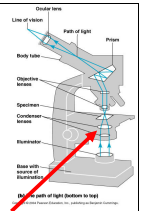


- This is where the image if formed
- Detects light and colour
- Light shone through specimen
  - Specimen interferes with light
- The image you see is how the specimen has affected the light

Slide 4

The Path of Light





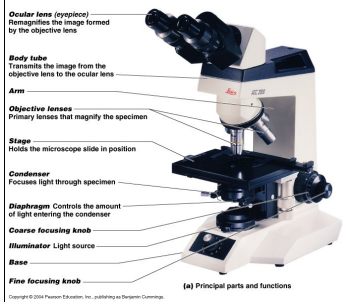
Light focused on specimen by condenser

Specimen alters beam of light

- Transmission
- Reflection
- Refraction
  - Scattering
- Absorption

Slide 5

Compound Light Microscope

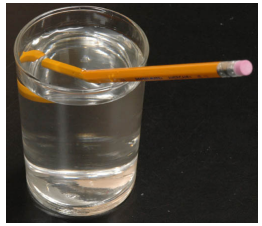


- Source of illumination
  - Light you will see
- Specimen
  - transparent
- Lenses
  - Gather scattered light
  - Magnify image

Slide 6

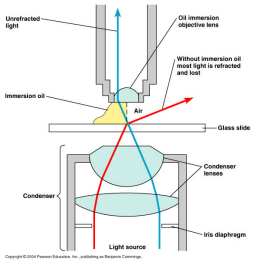
Refraction

- Refraction = bending of light as it passes through different substances
  - Air –water
  - Air-glass
- Specimens also refract light
  - Light will not pass through thick specimens
  - **glycocalyx**



Oil Immersion

- Light refracted when it changes medium
  - water →air
- Immersion oil same refractive index as glass → no refraction
- Maximum magnification with light
- 100x objective lens x 10x ocular = 1000x



Numerical Aperture

- How much scattered light is gathered by the lens
- Limit of Resolution
- Calculated by dividing wavelength of light by sum of NA objective lens plus NA condenser

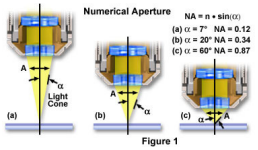


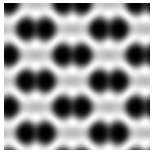
Figure 1

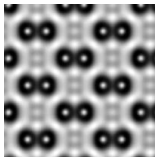
Slide 9

- Magnification
- Increase in apparent size of object
- Curvature of lenses bend light waves
- Maximum level 1000 X using light
- Greater magnifications limited by resolution
  - Cannot be resolved

Slide 10

Talking About Resolution…

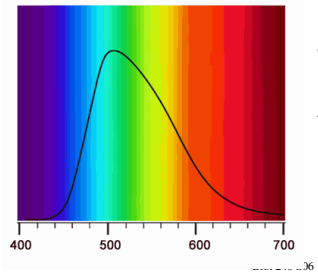




- The ability of the lenses to distinguish between two points a specified distance apart.
- Function of the wavelength of light

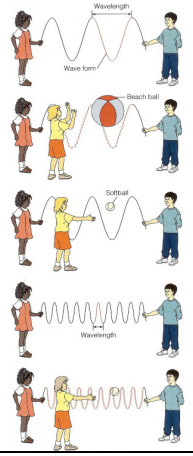
Slide 11

Wavelenghts of Light



- Wavelength in nm
  Blue light shorter wavelength than red
  400nm v 730 nm

Slide 12

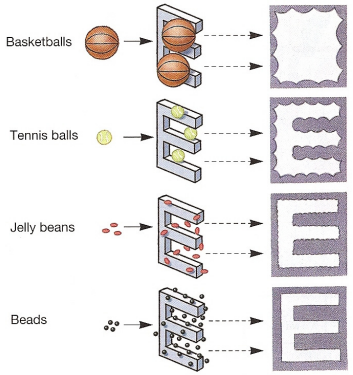


Wavelength and Resolution

- The shorter the wavelength
- The smaller the object that can be seen
- The greater the resolution
- Wavelength of light
- 400-700nm
- Bacteria ~ 2um (2000nm)
- Limit of resolution
- 0.2 um (200nm)

Slide 13

Wavelength and Resolution

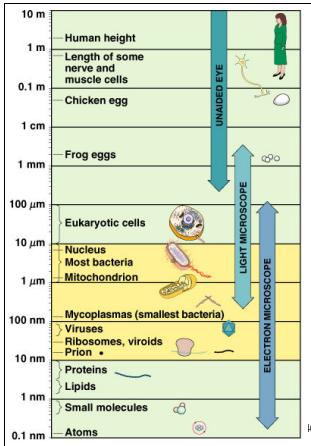


- Longer wavelengths too large to pass through small gaps
  - Poor resolution
- Short wavelengths pass through small gaps
- Electron beam 2nm
- Limit of resolution
- 2.5 nm

13

Slide 14

Relative sizes



- Eukaryote cells ~20um
- Bacteria ~ 2um
- *Mycoplasmas* 100-200 nm
- *Escherichia coli*, 1.5 µm wide by 4.0 µm long

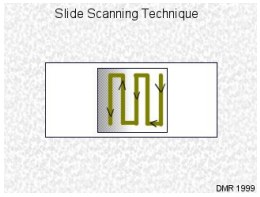
i0 S06

14

Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.

Slide 15

Microscope Terminology



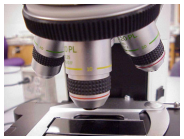
- Field of View
  - How much you can see in focus horizontally
  - Increase magnification reduces field of view
  - Wide field of view allows slide to be scanned
- Depth of field
  - How much is in focus vertically
  - Increase magnification reduces depth of field

Slide 16

Lenses

- Ocular Lenses
  - 10 x magnification
- Diopter
  - Adjust focus for each eye
- Binocular vision

- Objective Lenses
- Parfocal once one is in focus the others are nearly in focus
- Low power
  - Scanning
- High dry
  - Observe eukaryote cells
- Oil immerson
- Observation bacteria
  - Fine detail eukaryote cells
  - Total magnification
- Ocular x objective



Focusing

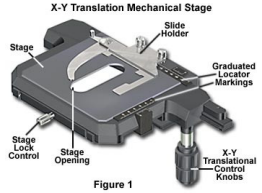
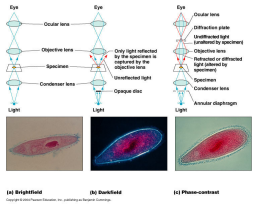


Figure 1

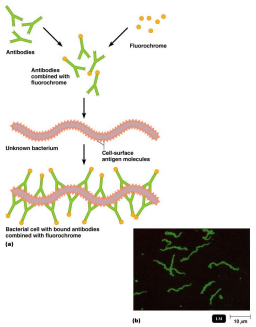
- Coarse focus
  - Move stage for access
- Fine Focus
  - Focus image
- Focal Point
- Distance between specimen and eye/camera
- Stage control knobs
  - Positioning and scanning

Illumination



- Brightfield
  - Normal light microscope
- Darkfield
  - Opaque condenser
- Phase Contrast
  - Living unstained cells

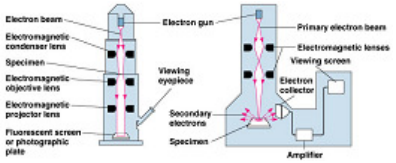
Fluorescent-Antibody Technique



- Fluorescence
  - Absorb short wave light (UV) but emit longer wave (visible) light
- Fluorochrome
  - Dye that fluoresces
- Immunofluorescence
- Flurochrome attached to antibody specific to a particular bacterium
- Antibodies that bind fluorescent under UV light
- Spirochetes too thin to be easily seen using light microscope
- Lyme Disease
  - *Borrelia burgdorferi*

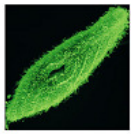
Copyright © 2007 Pearson Education, Inc., publishing as Benjamin Cummings.

Electron Microscopes



- Objects < 0.2um can ONLY be observed by EM
- Beam of electrons focused by magnets
- Shorter wavelength
  - ~2nm
- 100,000 x magnification
- Greater resolving power 0.5 nm

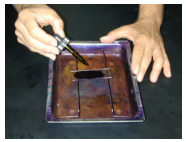
Electron Microscopes



(a) Transmission (b) Scanning

- Transmission
  - Electrons pass through specimen
  - Internal structure
- Scanning
- electrons bounce off surface
  - External structure

Stains

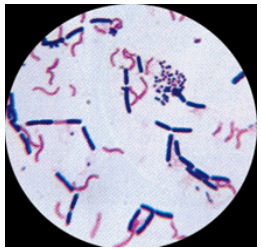


- Microbes colorless
- Stains enable us to see them
- Microbe -ve surface
- Basic Dyes color microbe
  - Crystal violet, Malachite Green, Safranin
- Negative Stains
- Acidic dyes color background
  - Eosin, acid fuchsin, nigrosin

Slide 23

Gram Stain

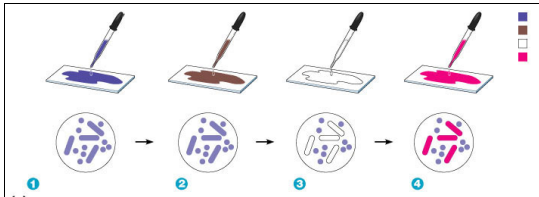
- Gram positive bacteria
- Purple

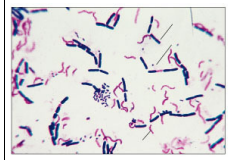


- Gram negative
- Pink

- Effectiveness
- False positive
  - Dead Gram +ve bacteria appear pink

Slide 24



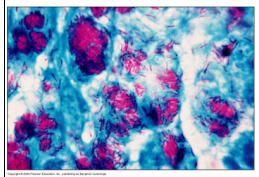


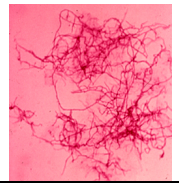
(b)

Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.

Slide 25

Acid Fast Stain

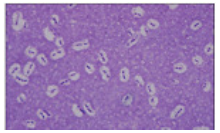


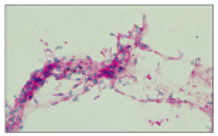


- Primary stain Carbol Fucshion lipid soluble
- Binds strongly to waxy cell walls
- Decolorizer Acid alcohol
- *Mycobacterium*
  - *M. tuberculosis*
  - *M. leprae*
- *Nocardia*

Biol 240 S06 25

Slide 26





Special Stains

- Capsule
- Negative stain binds to glass of slide
- Endospore
- Flagella
- Acid Fast

Biol 240 S06 26