ISTA 352

Lecture 32

Biomedical Image Analysis

Microscope Review

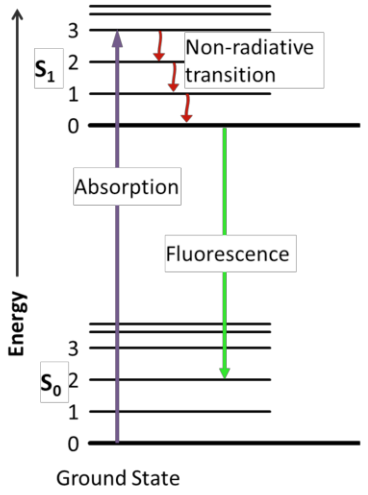
- Illumination methods
  - Optical transmission
  - GFP tagging (see next slide)
- Carrier of information
  - Photons
  - Electrons
  - Physical probes
- Capture method
  - Single field versus scanning
- Fluorescent microscopy with confocal optical path

Green Fluorescent Protein (GFP) tagging

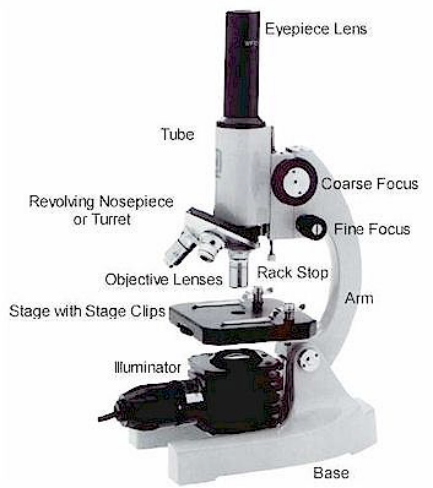
- Genetically modified organisms
  - Insert a gene from a jellyfish that encodes a protein (GFP)that fluoresces under UV light
    - Extremely useful for imaging
    - Also for creating novel pets (GloFish)

Green Fluorescent Protein (GFP) tagging

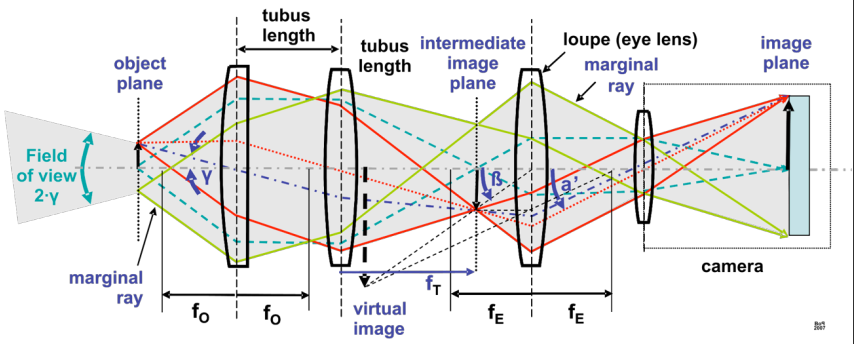
- Reminder (?) about fluorescence
  - Photons are absorbed by molecules, increasing their energy level, and than the "excited" molecule emits on or more photons
  - Typical case is that emitted photons are lower in energy (larger wavelength) than the absorbed ones
    - Example is "black light" where UV light (not visible) leads to glowing surfaces.
- The GFP glows green when appropriate stimulated.



Basic Optical Microscope



Basic Optical Microscope

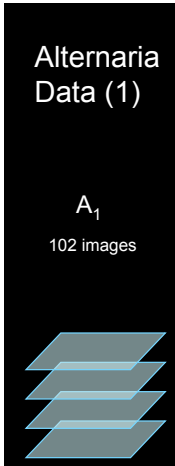


From WikiMedia commons

Alternaria Data (1)

A_1

102 images

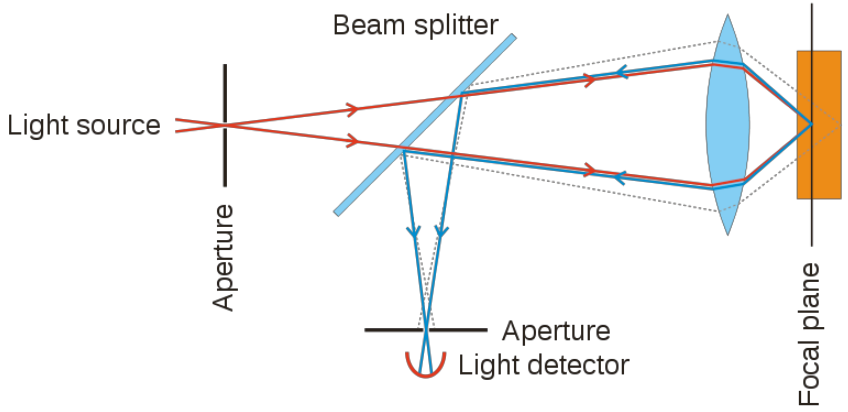


Confocal Microscope

- Recall the depth of field formula

$$F^{\#} = \frac{f}{D}$$ where f is focal length and D is aperature

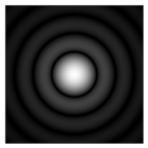
- A confocal microscope uses optical trickery to arrange a small aperture
  - This blocks out of focus light
  - Provides sharp images for a point at a specific focal plane
  - A lot of light is blocked, so we scan, point by point, to make an image



Confocal microscopy

WikiCommons

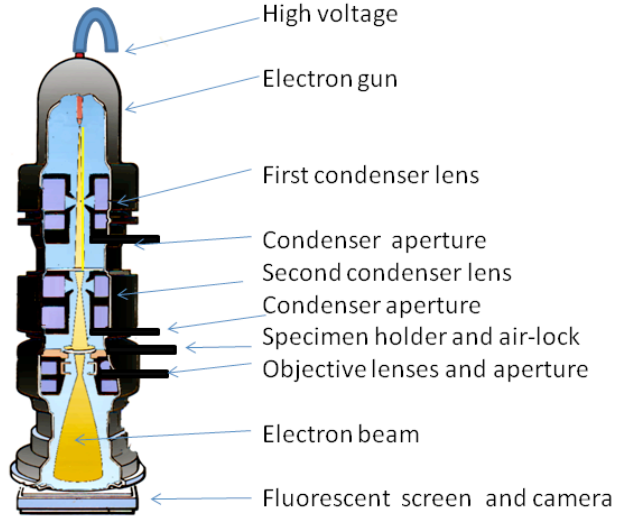
Electron Microscope



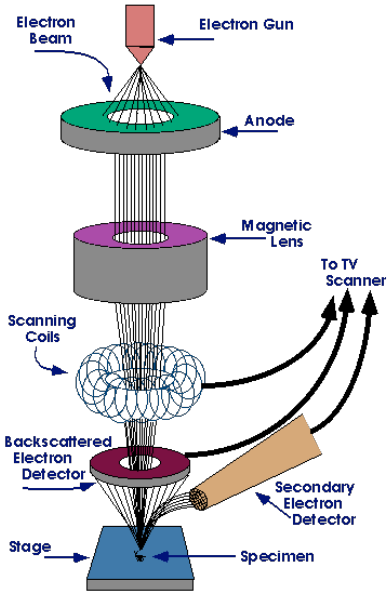
- Recall the Airy disc

$$x = (1.22)\frac{\lambda f}{d}$$ where λ is wavelength, f is focal length, and d is aperature

- Smaller wavelength means higher resolution
- Electrons at energies usable for imaging have much smaller wavelength than visible light (even x-rays).
- Electron beams are focused with magnets.



Transmission Electron Microscope



Scanning electron microscope (SEM).

Electrons are reflected from the surface, rather than transmitted.

The pathway shown is for a **single** pixel, then we move on to the next one (scanning)

Magnification is a function of the scan step size

Caption for picture on the next slide (taken with a scanning electron microscope)

Pollen from a variety of common plants: sunflower (*Helianthus annuus*), morning glory *Ipomoea purpurea*, hollyhock (*Sildalcea malviflora*), lily (*Lilium auratum*), primrose (*Oenothera fruticosa*) and castor bean (*Ricinus communis*). The image is magnified some x500, so the bean shaped grain in the bottom left corner is about 50 µm long.