LECTURE 1 – MOLECULAR AND CELLULAR BIOLOGY

What do cells do?

- Cell proliferation, specialization, interaction, movement. (universal mechanism of animal development)
- Cancer is progressive accumulation of mutations

Imaging technologies

- Resolving power
  - Smallest distance you can see two points of an image
- Resolution
  - Lower the wavelength the better
  - Numerical aperture if objective lens is measure of light gathering capacity of lens- higher the better
- Light microscope
- Specimen Preparation (fix and section)
  - Fixing preserves cells within the tissue
  - Sectioning- thin transparent slices of tissue
  - Two main techniques
    - Frozen
      - Tissue quickly frozen, sectioned in cryostat, fixed and stained
    - Paraffin Sections
      - Fixation
      - Dehydration
      - Embedding (paraffin wax)
      - Sectioning (microtome)
      - Mounted on glass slides
      - Stained

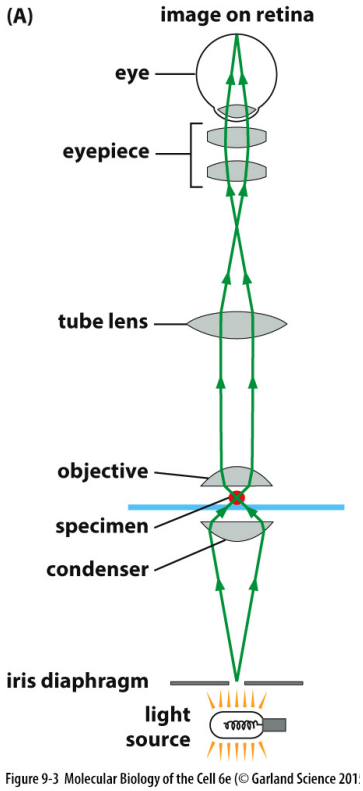
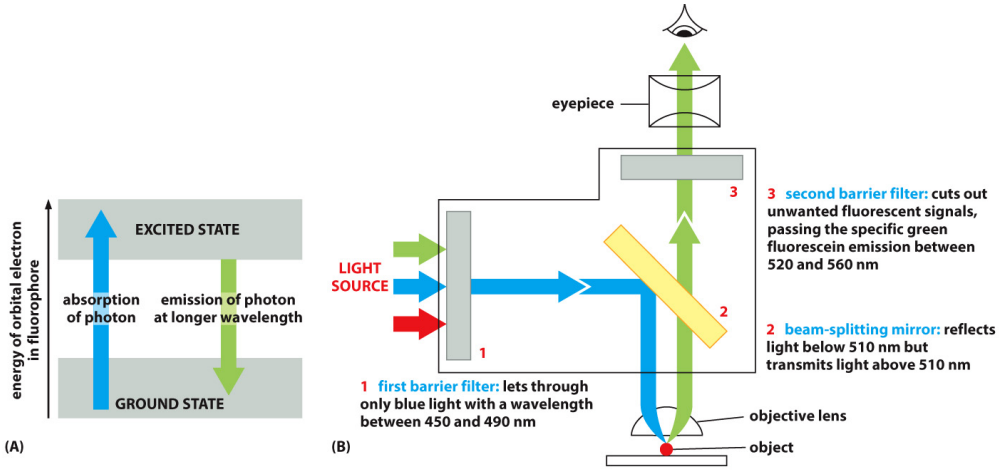


Figure 9-3 Molecular Biology of the Cell 6e (© Garland Science 201

Fluorescence Microscopy

- Sample is light source
- Florescence by dyes, proteins, antibody-dyes, auto florescence
- High intensity light to excite fluorescence
- Molecules emit a longer wavelength than absorbed



Fluorescent probes

- DAPI gives off blue colour
    - binds to A-T rich regions of DNA
- Immunofluorescence
    - Coupling florescent dye (fluorescein or rhodamine) to antibody molecule
- Green fluorescent protein
    - GFP from jelly fish
    - Placed under transcriptional control of promotor of a gene
    - Peptide location signal to organelles
    - GFP DNA coding sequence added at end or start

Electron Microscopy

- Fine structure
- Accelerated electrons
- Resolution better

- Transmission electron microscopy
    - Fixation
        - Glutaraldehyde in phosphate or cacodylate buffer
    - Processing
        - Post fixation
        - Dehydration
        - Infiltration
    - Embedding
        - Epon-araldite
    - Sectioning
        - Glass or diamond knives
        - TEM sections thinner than 10 x resin sections for LM and 100 thinner than paraffin
        - Sections floated off edge into water and picked by metal grids