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Fluorescence Bioimaging in Translational Research

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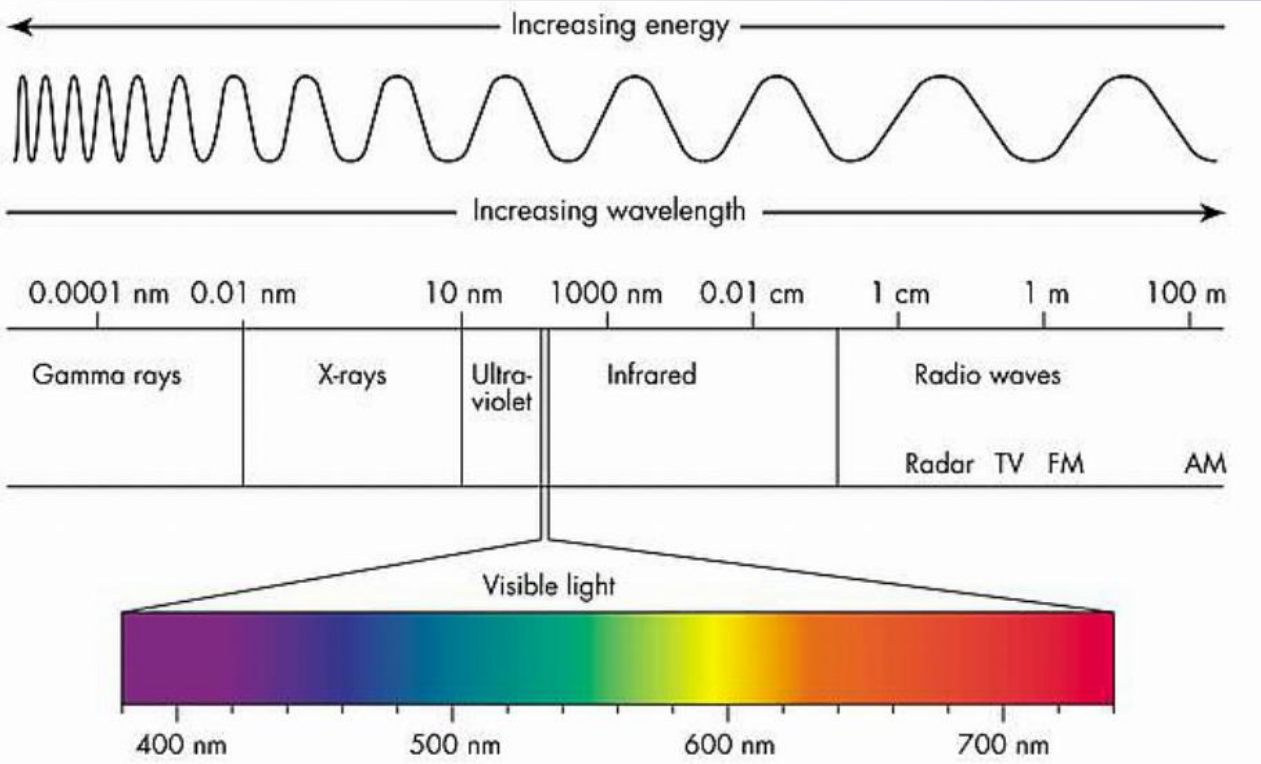
Outline

- What is fluorescence?
- How can we put it in a biological system?
- How can we see it?

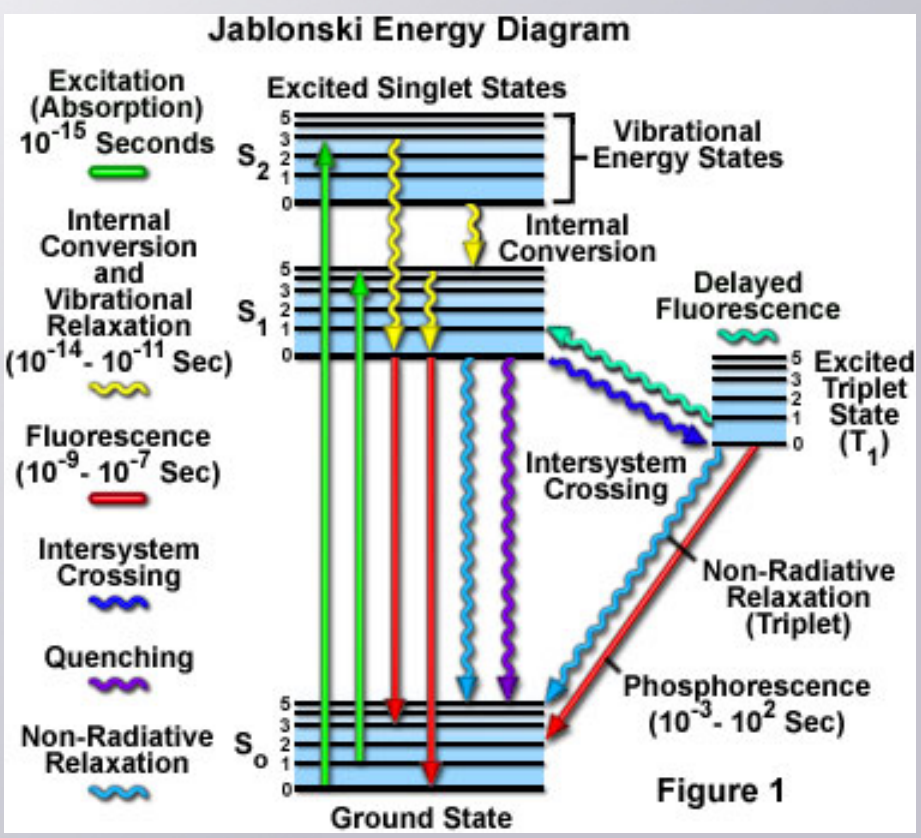
Light

Electromagnetic energy, photons



Fluorescence

- Excitation by photon
- Relaxation
- Emission of a photon at longer wavelength (Stokes shift)



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Fluorescence Spectra

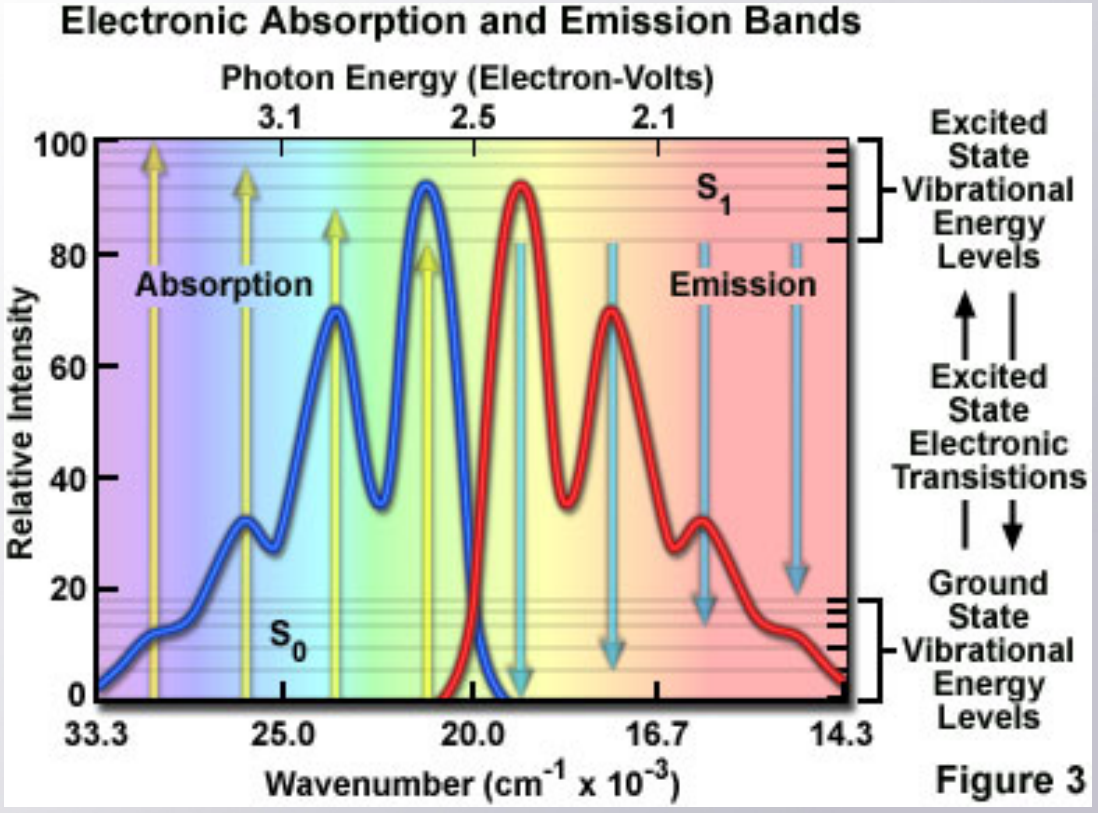

Figure 3

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Fluorescence in Biology

- Autofluorescence
  - aromatic amino acids, NADPH
  - in cells, arises from mitochondria and lysosomes
  - in animals, keratin and collagen
- Fluorophore attached to antibodies for staining specific structure (dead cells/tissue)
- Fluorescent proteins expressed genetically (live cells/tissues/animals)

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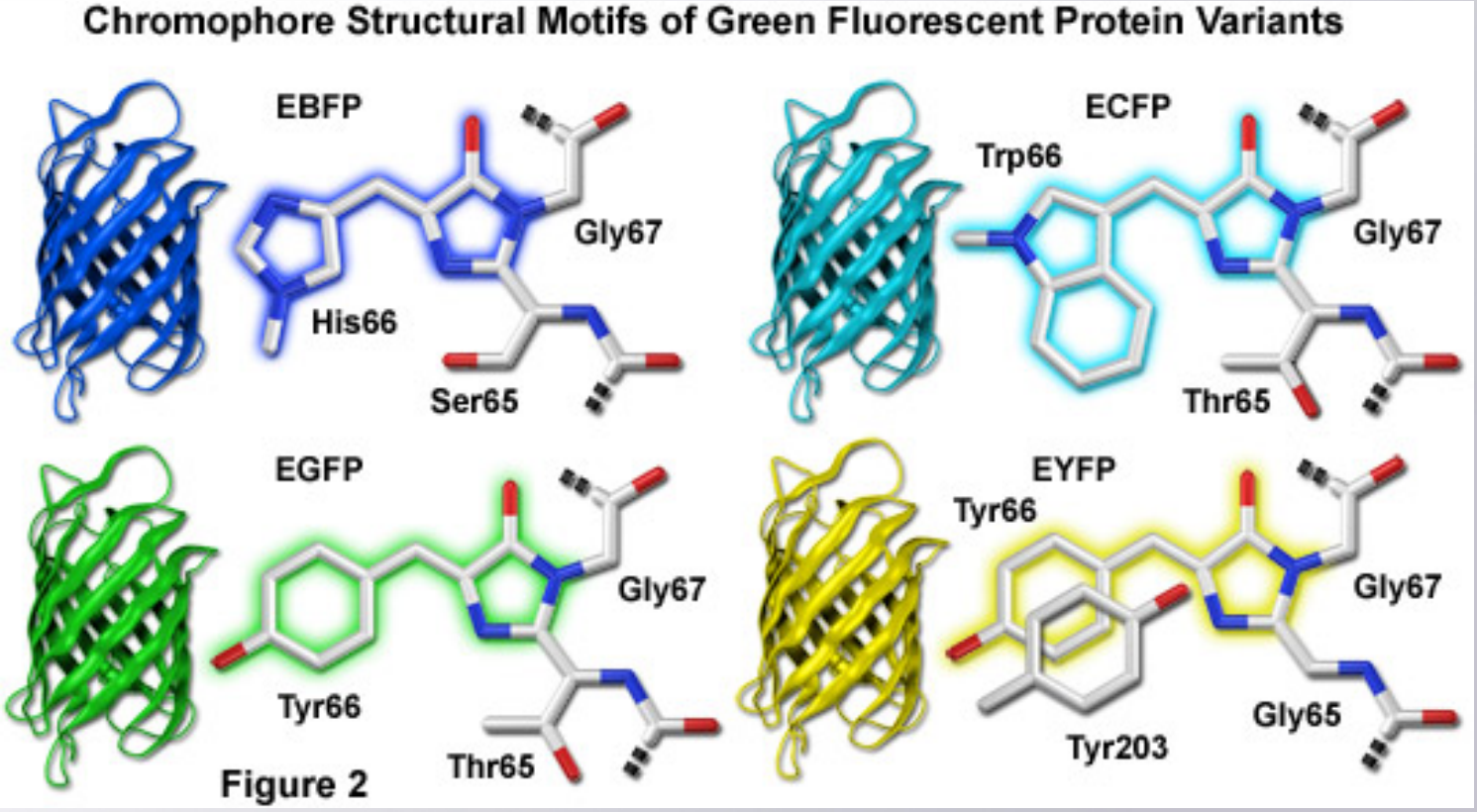
Fluorescent Proteins



named after color of light that is emitted
e.g., GFP Green Fluorescent Protein

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Use of Fluorescent Proteins (FP)

- Transfection - Insert FP genetic code into cellular DNA upstream of a protein of interest
- Protein of interest becomes “labeled” with FP

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Images of FP Labeled Organelles/Structures

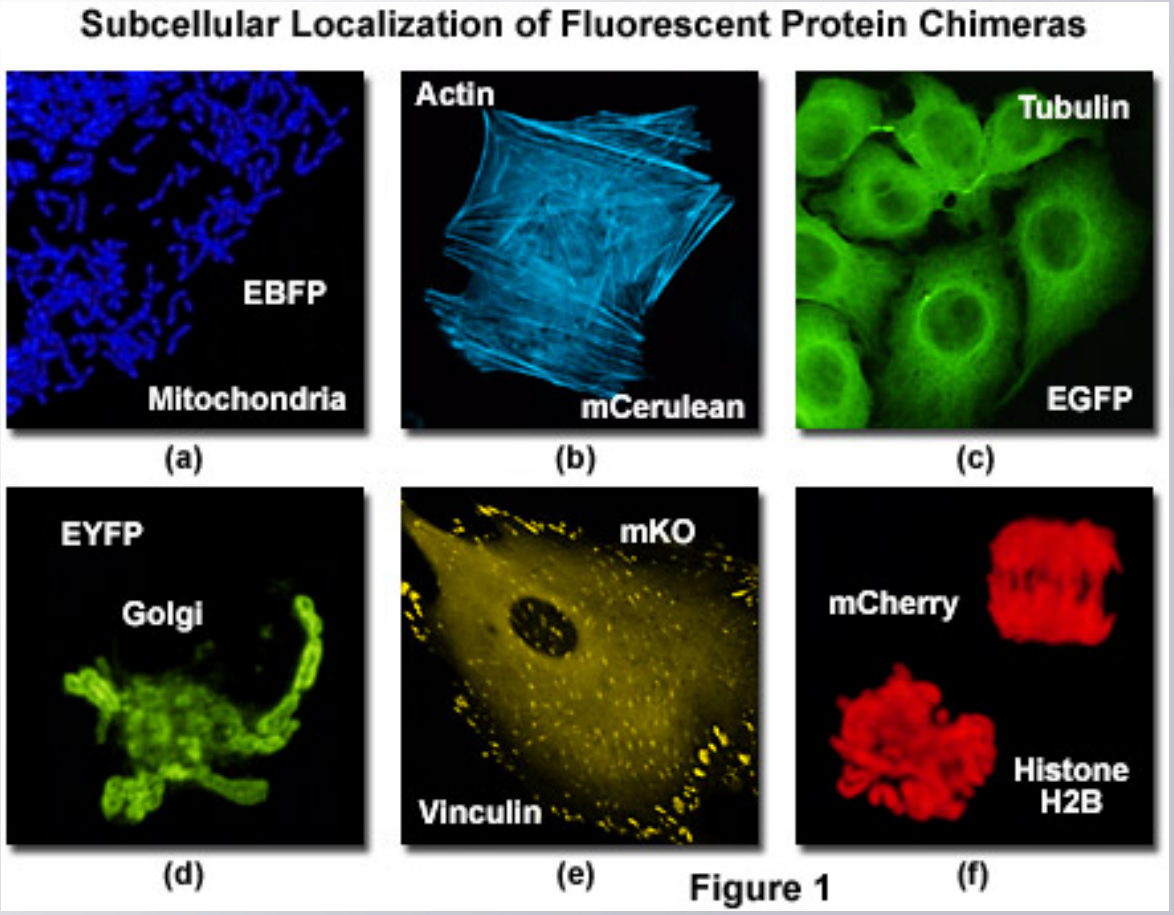

Figure 1

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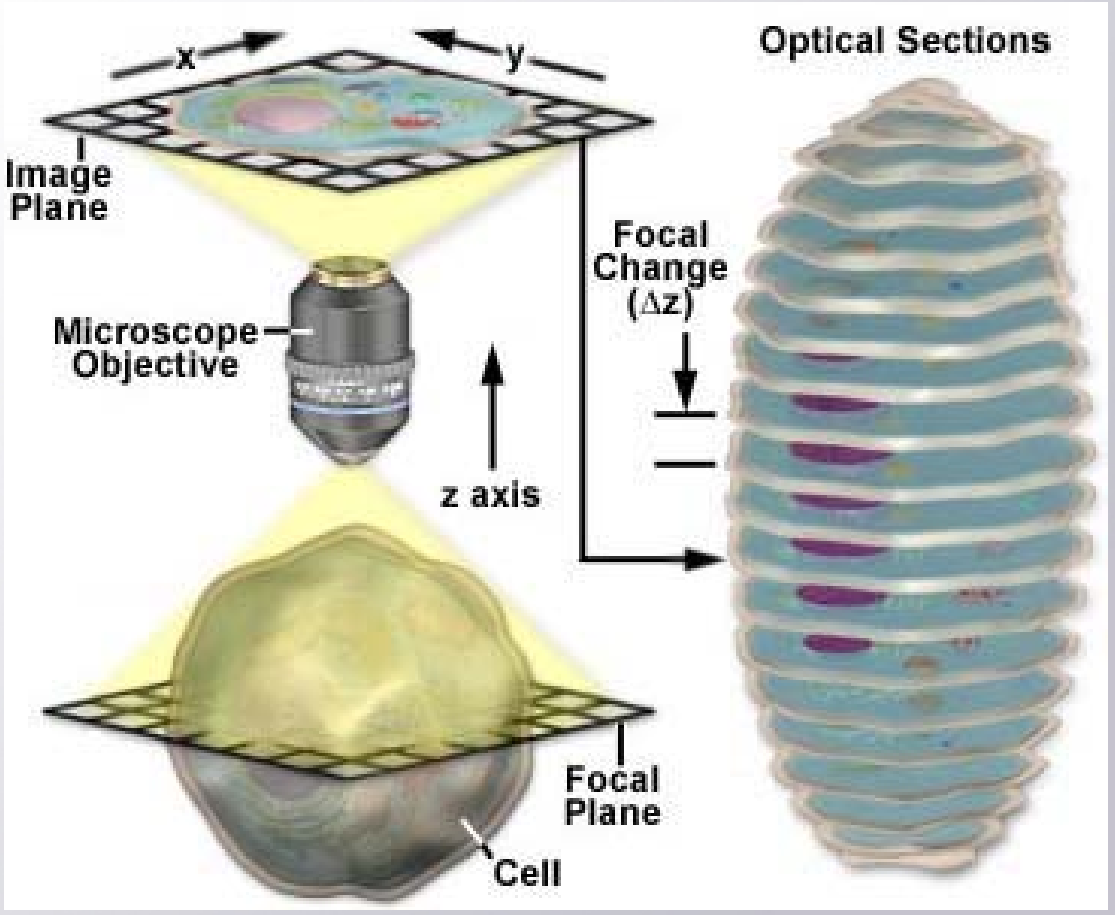
Optical Sectioning



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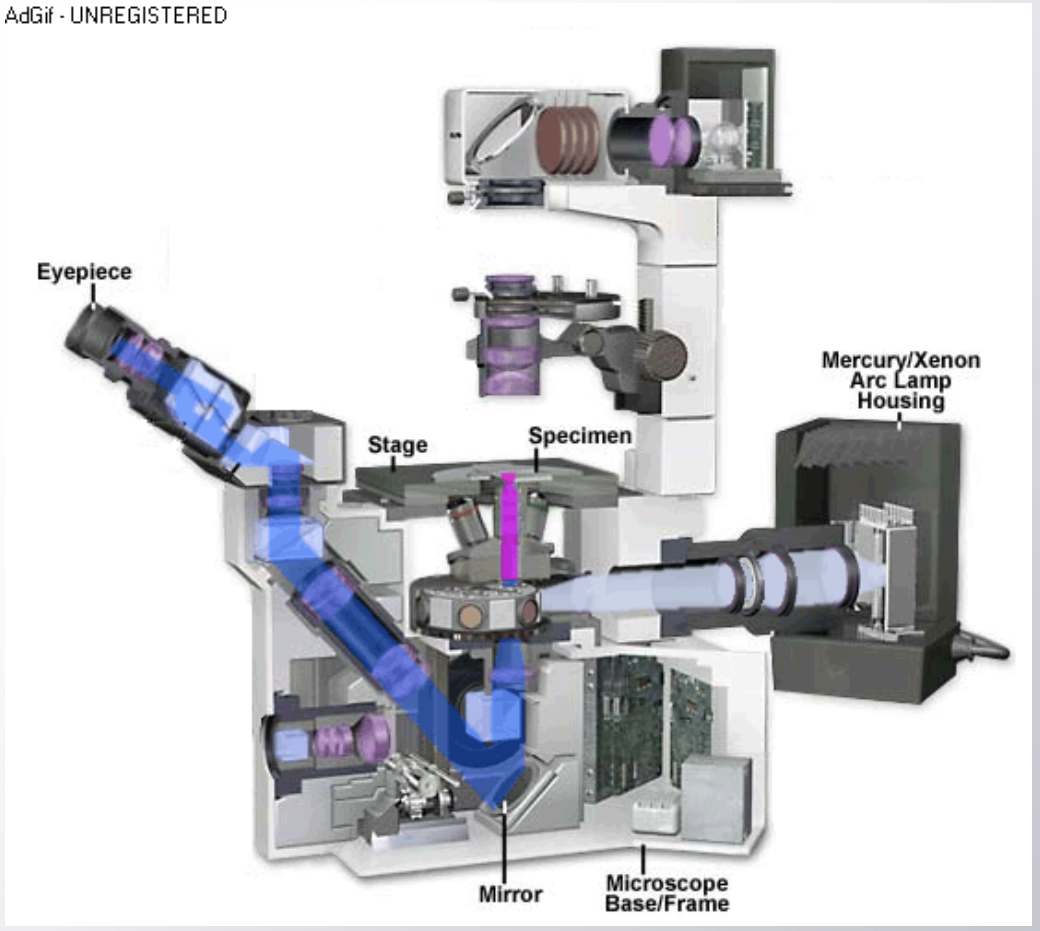
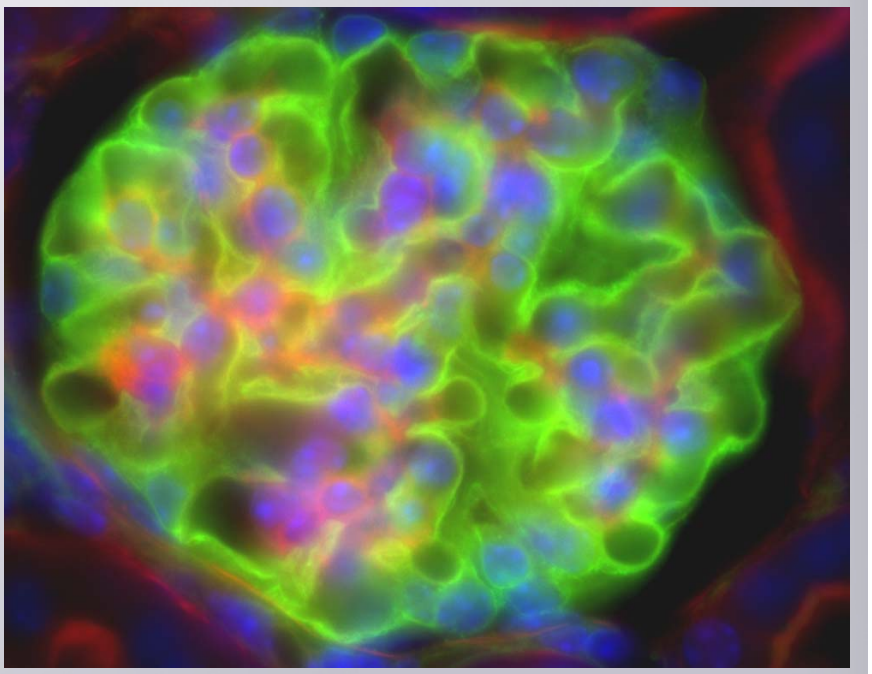
Fluorescence Microscope





Lateral (XY) resolution: ~ 0.25 mm

Axial (Z) resolution: > 1 mm

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Live Cell Imaging