Research Techniques I: Microscopy and Cell Imaging

Seeing at the cellular and subcellular level

Several Types of Microscopy

- Light microscope
  - Conventional (e.g. bright field, phase contrast etc.)
  - Fluorescence microscope
  - Confocal microscope
  - Two-photon microscope
- Electron microscope

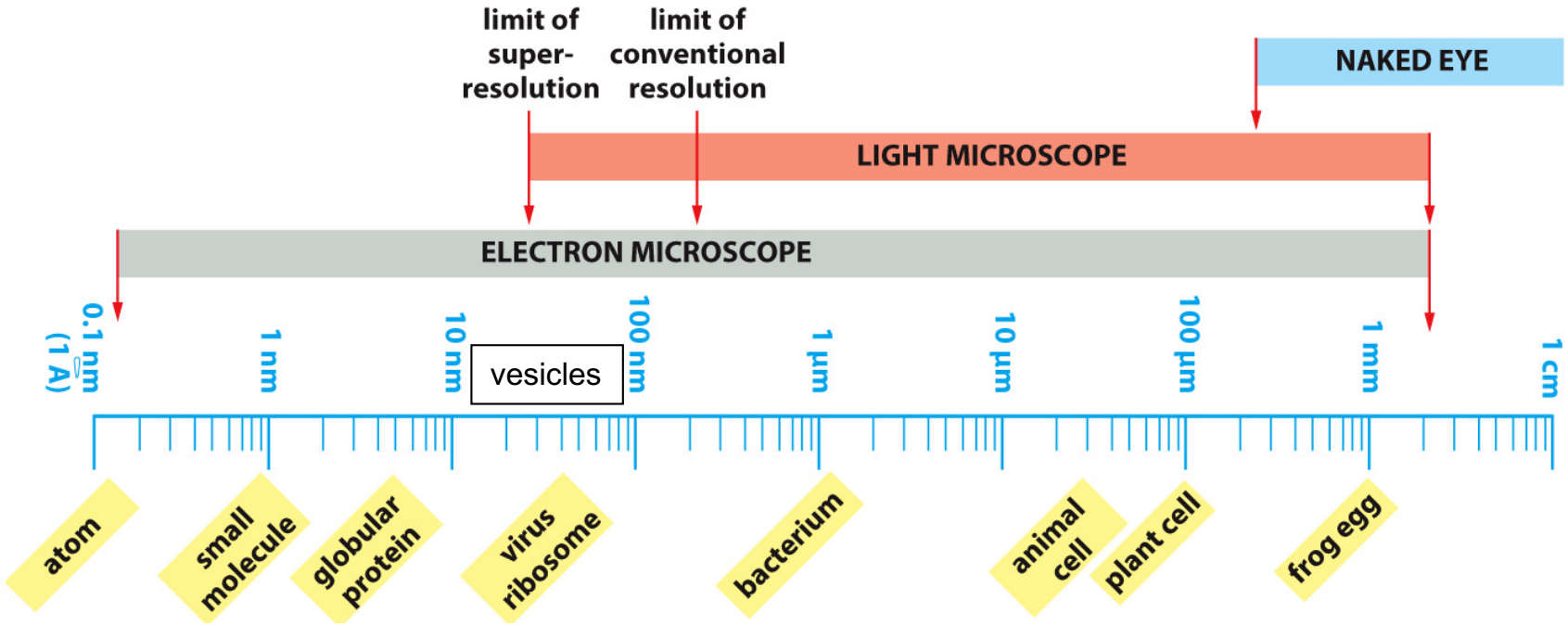


Figure 9-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

membrane bilayers

mitochondria

The Light Microscope (LM)

- Utilizes basic light path.
- Used for live or fixed cells and tissue.
- Tissues: *upright microscope*.
- Isolated cells: *inverted microscope*.

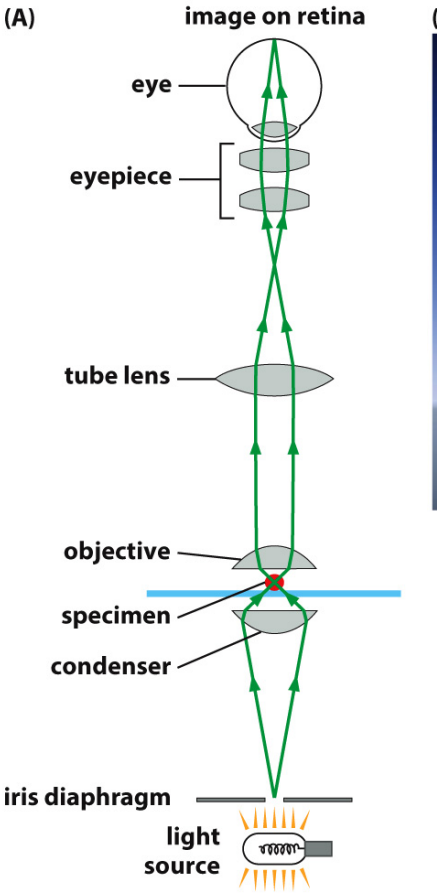


Figure 9-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

An “upright” microscope

Four types of LM

1) Bright field

- transmitted light.

2) Phase contrast

- converts phase differences into changes in brightness.

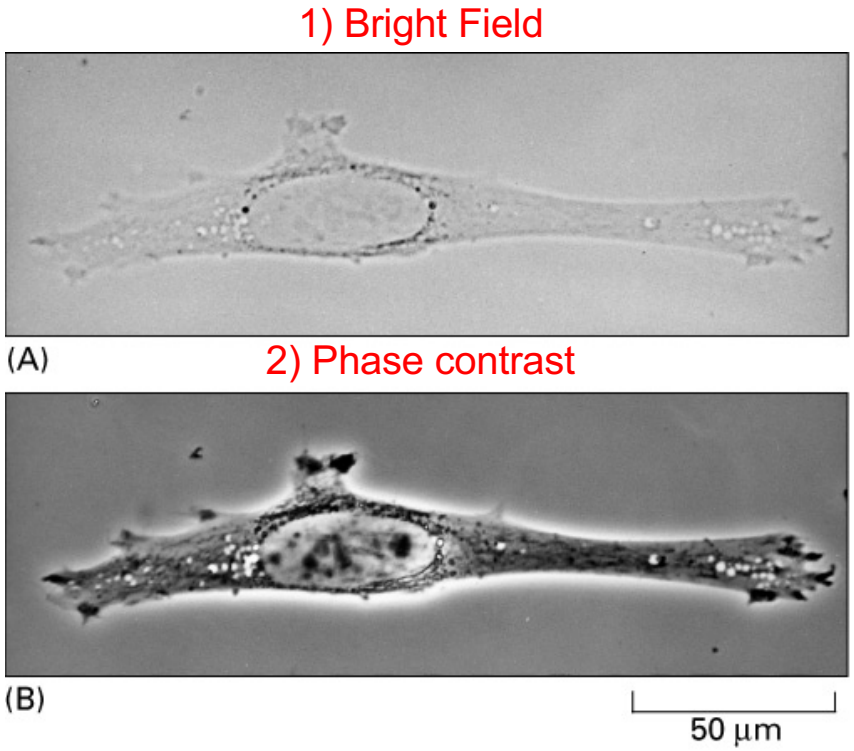


Figure 9–8 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Four types of LM

- Wave properties of light can be exploited.
- In unstained cells, a *phase shift* will occur as light travels through the cell.
- Phase alignment is related to increased brightness.
- Observable with phase contrast.

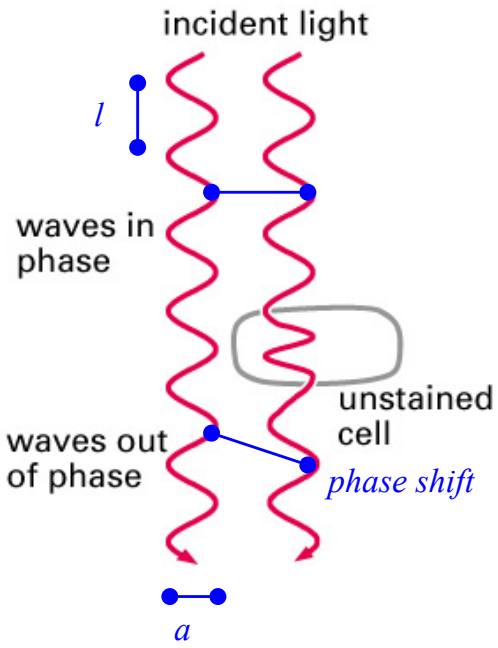


Figure 9-7B. Molecular Biology of the Cell, 4th Edition.

Four types of LM

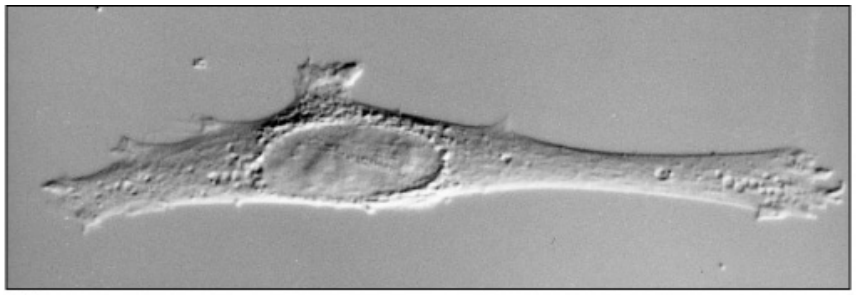
3) Differential interference contrast

- similar principles as with phase contrast.
- more definition.

4) Dark field

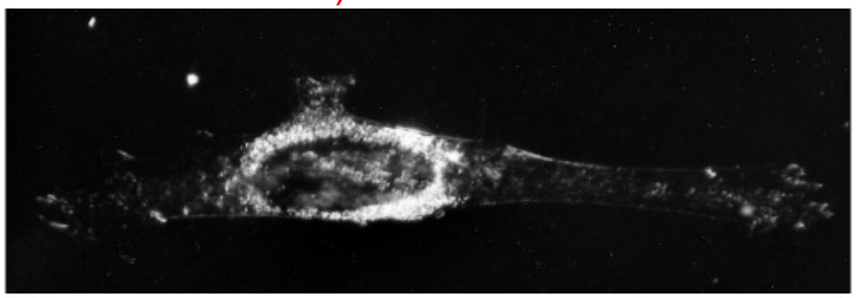
- lateral light source shows only scattered light.

3) Differential Interference Contrast (DIC)



(C)

4) Dark Field



(D)

50 µm

Figure 9–8 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Phase and DIC Compared

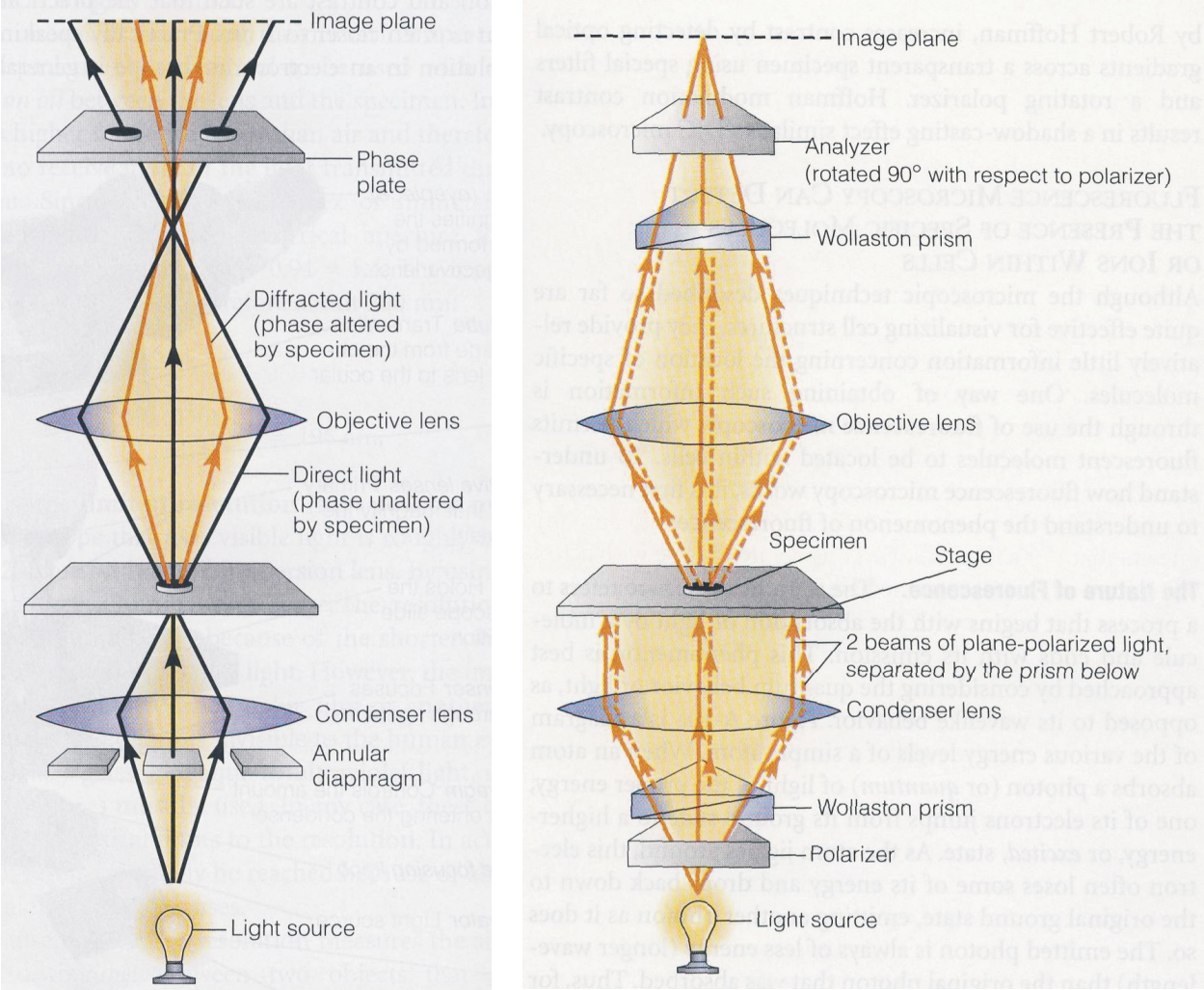
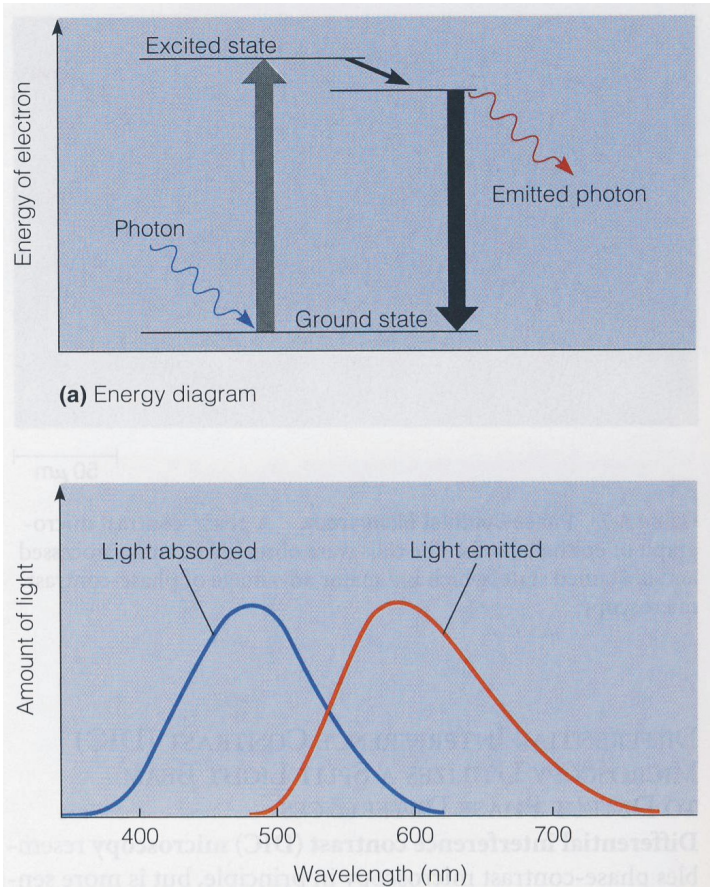


Figure A6, 8. Becker et al. 2006 World of the Cell.

Fluorescence Microscopy

- Useful for detection of specific molecules or ions.
- Works on the principle that some molecules absorb and emit photons of light at specific wavelengths.
- Atomic *absorption* of a photon is followed by *emission* at a longer wavelength, and a light signal is detected.



wavelength shift

Figure A10. Becker et al. 2006 World of the Cell.

Fluorescence Microscopy

- A variety of fluorescent molecules are used in fluorescence microscopy (e.g. DAPI, GFP, FITC).
- Note spectral characteristics of each dye/molecule.

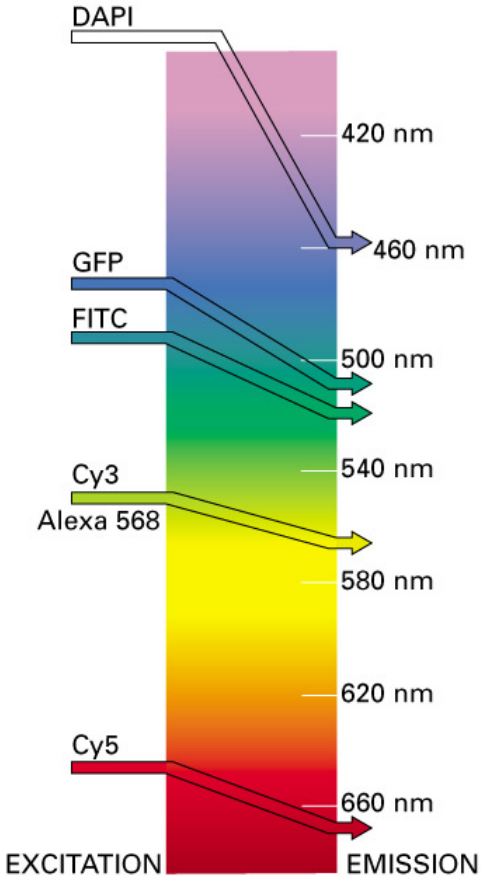


Figure 9–13. Molecular Biology of the Cell, 4th Edition.

Fluorescence Microscopy

- Fluorescence microscope is optically similar to LM.
- High energy lamps (Hg) provide bright source.
- Filters reduce light of unwanted wavelengths.
- Chromophores excited at specific wavelengths.

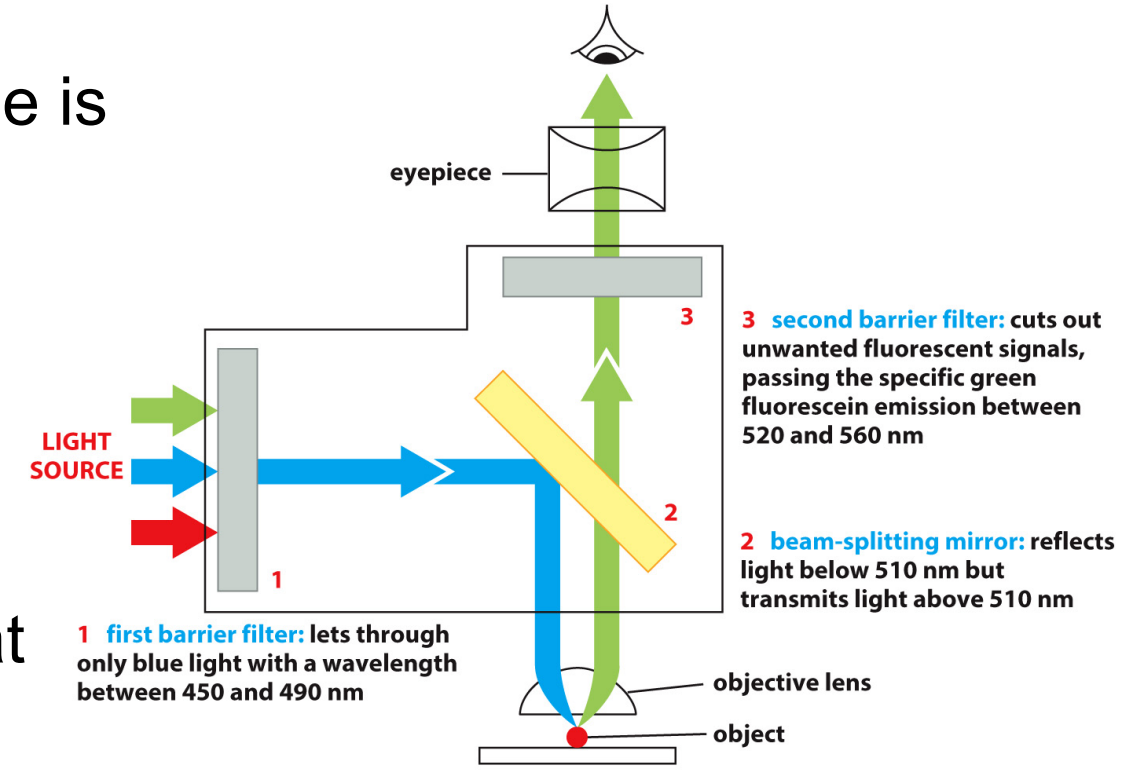
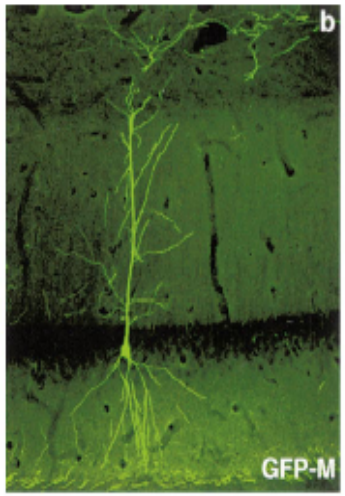


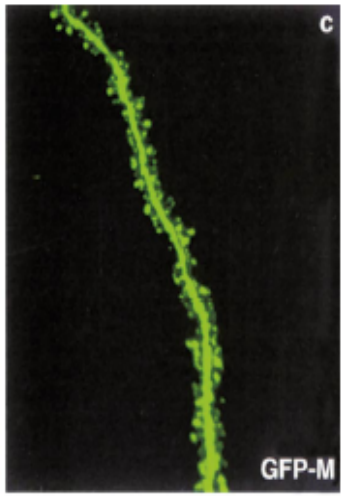
Figure 9-12b Molecular Biology of the Cell 6e (© Garland Science 2015)

GFP in Research

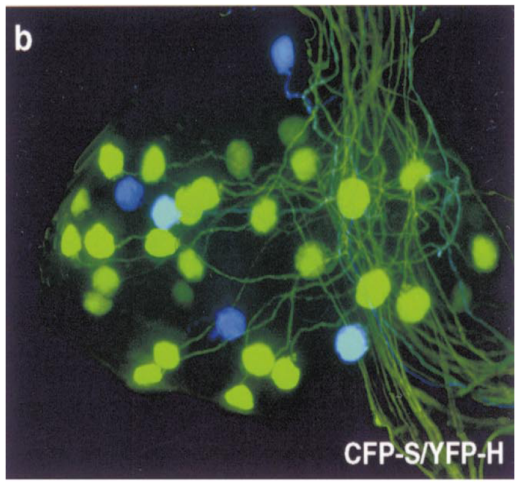
- Transgenic mice generated to express GFP and other “FPs”.
- Permits selective labelling and imaging of cells in *live* specimens.



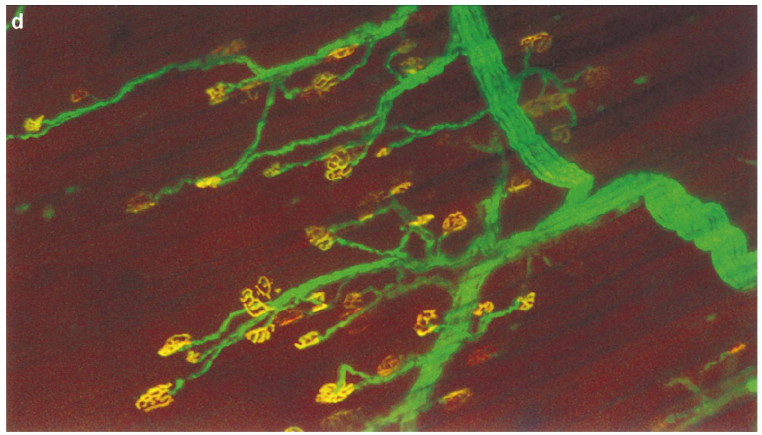
Hippocampal neurons



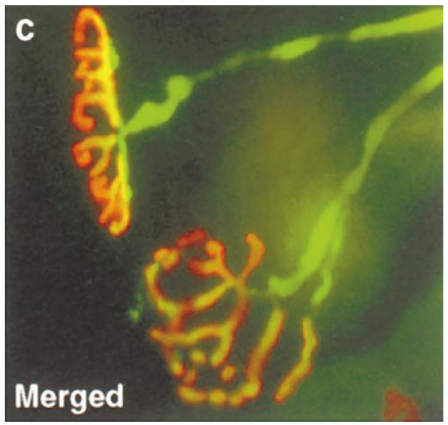
Dendritic spines



Spinal cord: double expression (with YFP)



Neuromuscular junction (GFP+YFP)



ACh receptors

From Feng et al. 2000. *Neuron* 28:41-51.

Tissue Preparation

- To observe cells in tissue, in most cases tissues must be histologically prepared (e.g. not for GFP).
- Fixation: exposure to chemical reagents (aldehydes, acids, alcohols) to preserve and stabilize. May produce unwanted effects.
- Embedding: plastic or polyethylene glycol.
- Sectioning: cutting of thin (1–10 μm) tissue sections on a microtome.
- Staining: if applicable, involves exposure to dyes, e.g. hematoxylin, eosin, antibodies.

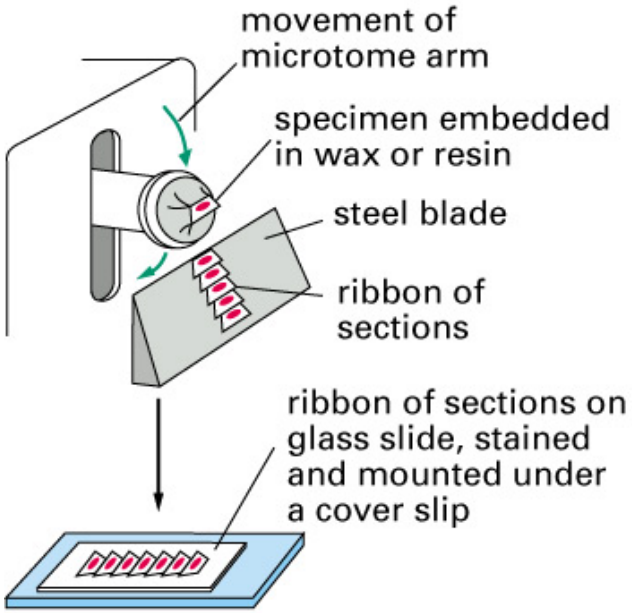


Figure 9–10. Molecular Biology of the Cell, 4th Edition.

Immunofluorescence

- Antibodies are produced in host animal and collected.
- Fixed tissue is *permeabilized* and treated with *primary antibody* directed against a specific *antigen.*
- Antibody binds to antigen on or within cell.
- *Secondary antibody* conjugated with fluorescent marker binds to primary antibody.
- *Indirect immunohistochemistry* labels cell structures.

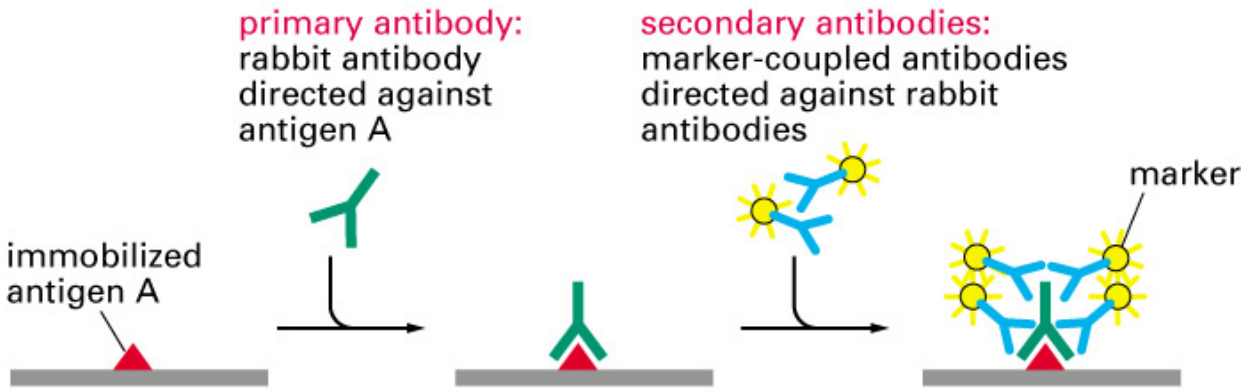


Figure 9–16. Molecular Biology of the Cell, 4th Edition.

The Confocal Microscope

Advantages:

- Technique that provides clear images with reduced "background" signal.
- Particularly useful for applications involving thick sections or *whole-mount preparations*.

Disadvantages:

- Costly.

The Confocal Microscope

- "Confocal" refers to equidistance between light source and object, and object and detector.**
- Utilizes fluorescence and high energy lasers (He-Ne and Ar).
- *Pinhole* focuses light at a single point in specimen, producing an *optical section* with low background "noise".

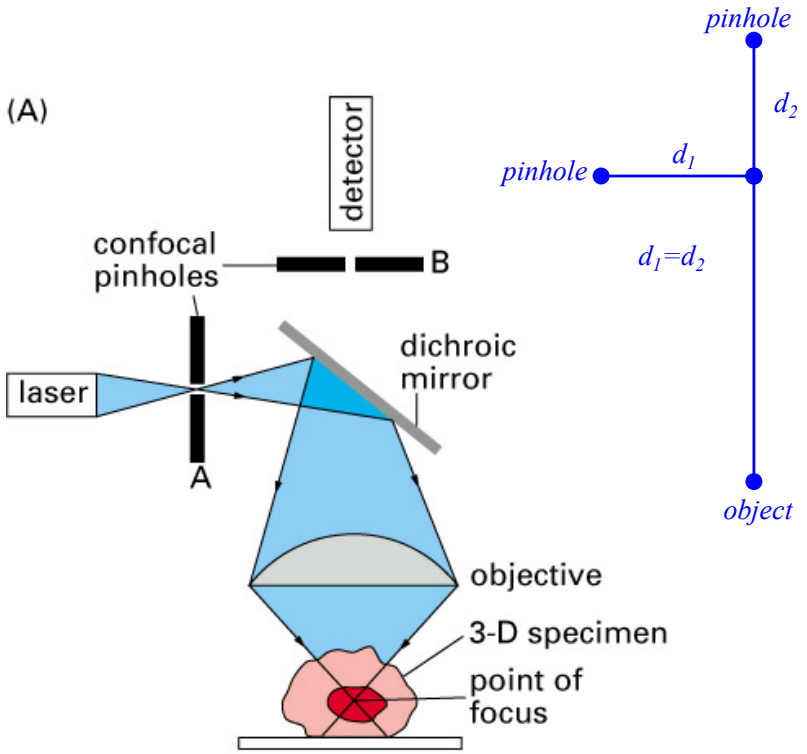


Figure 9–18 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

The Confocal Microscope

- Only light focused at the pinhole will enter the detector.**
- This allows the confocal to provide clear images a few µm into tissue.
- In addition to the x and y axes, imaging may occur in the z axis.
- 3D reconstructions possible.

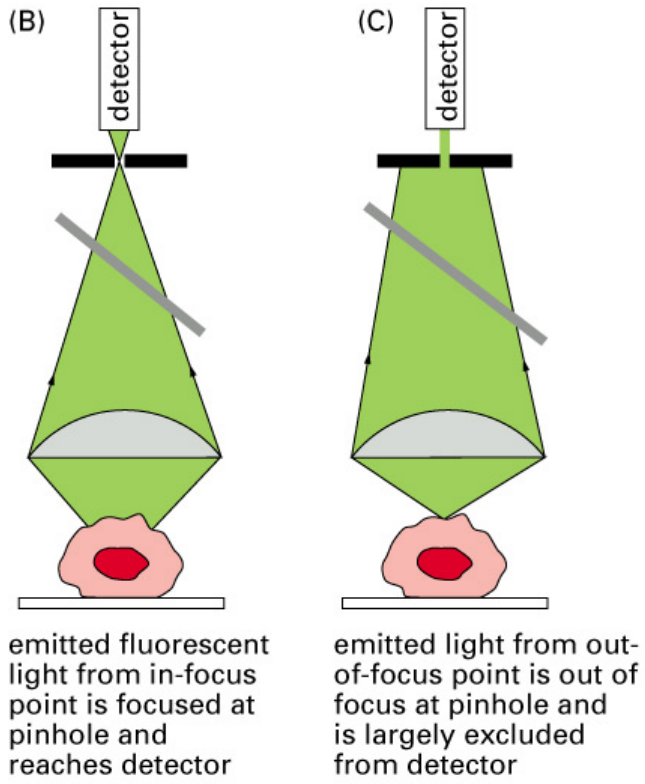


Figure 9–18 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Comparison of Techniques

Glia (red)
Neurons (green)

Actin (green)
in *Drosophila*
embryo

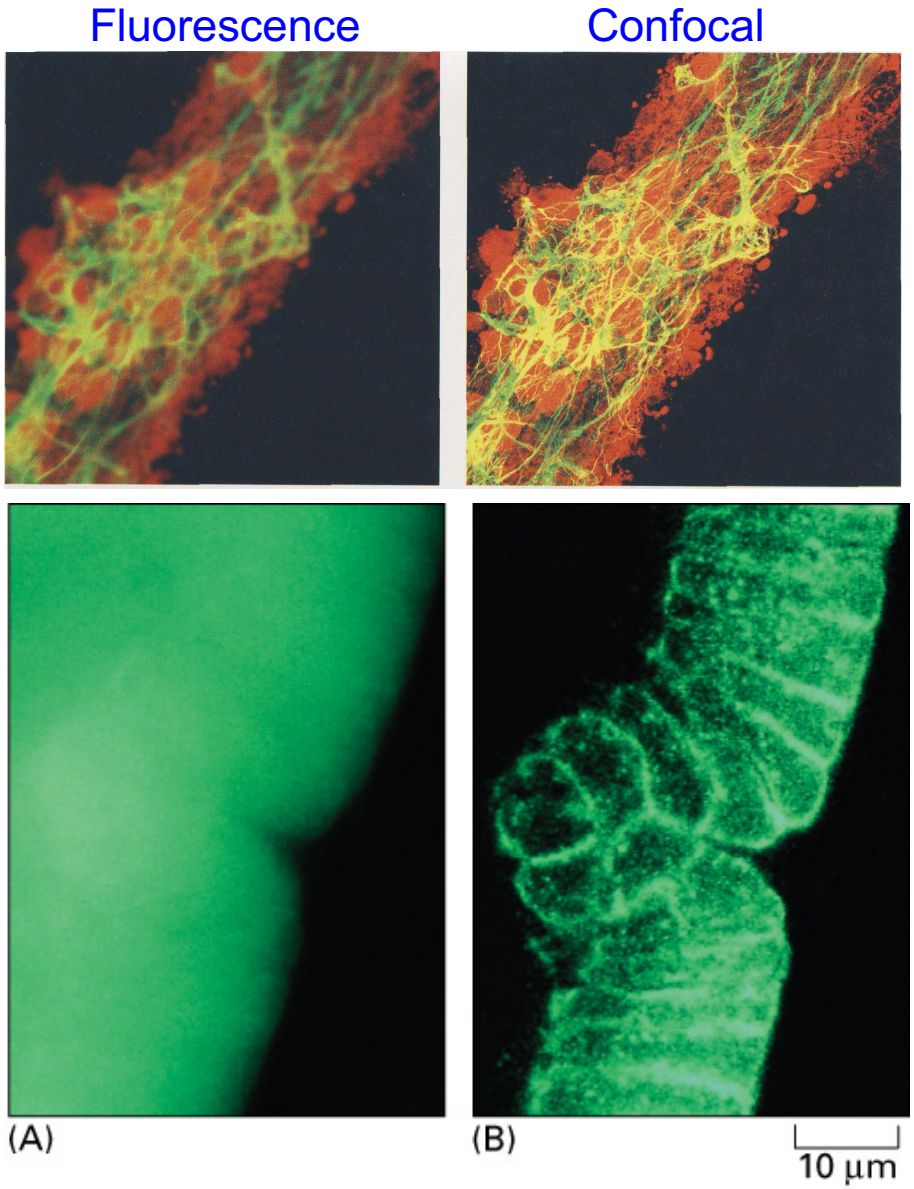


Figure A15. Becker et al. 2006 World of the Cell.

Figure 9–19. Molecular Biology of the Cell, 4th Edition.

Two-Photon Microscopy

(non-linear)

Advantages:

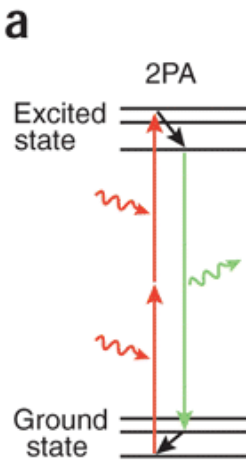
- Non-linear technique that uses higher-order light-matter interactions from multiple photons to generate contrast.
- Allows deep tissue imaging (up to 1 mm depth possible).
- In this process, absorption occurs in the near IR region, and NIR light penetrates deep into tissue.

Disadvantage:

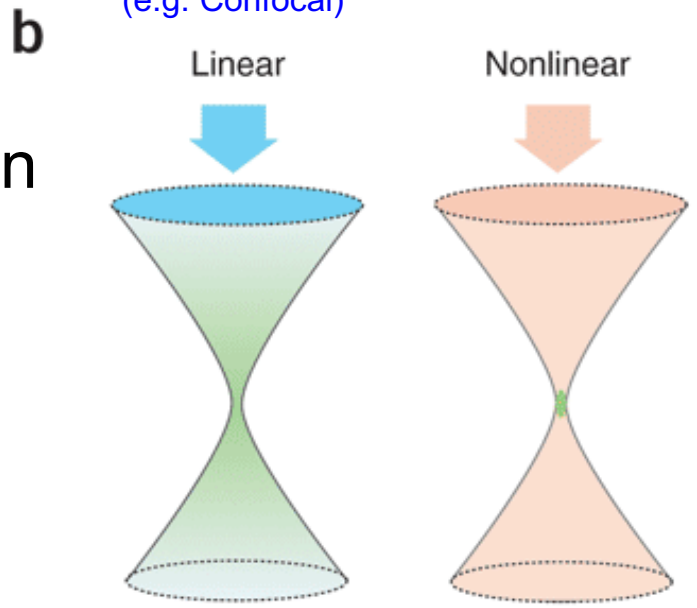
- Very costly.

Two-Photon Microscopy

- Two-photon absorption involves “simultaneous” (~0.5 fs) arrival at excitable molecule.
- Excitation and emission occurs, as in fluorescence.



(e.g. Confocal)



- However, signal is dependent upon photon density, so absorption is spatially confined.
- In confocal, single photon absorption occurs throughout exc. light cone.

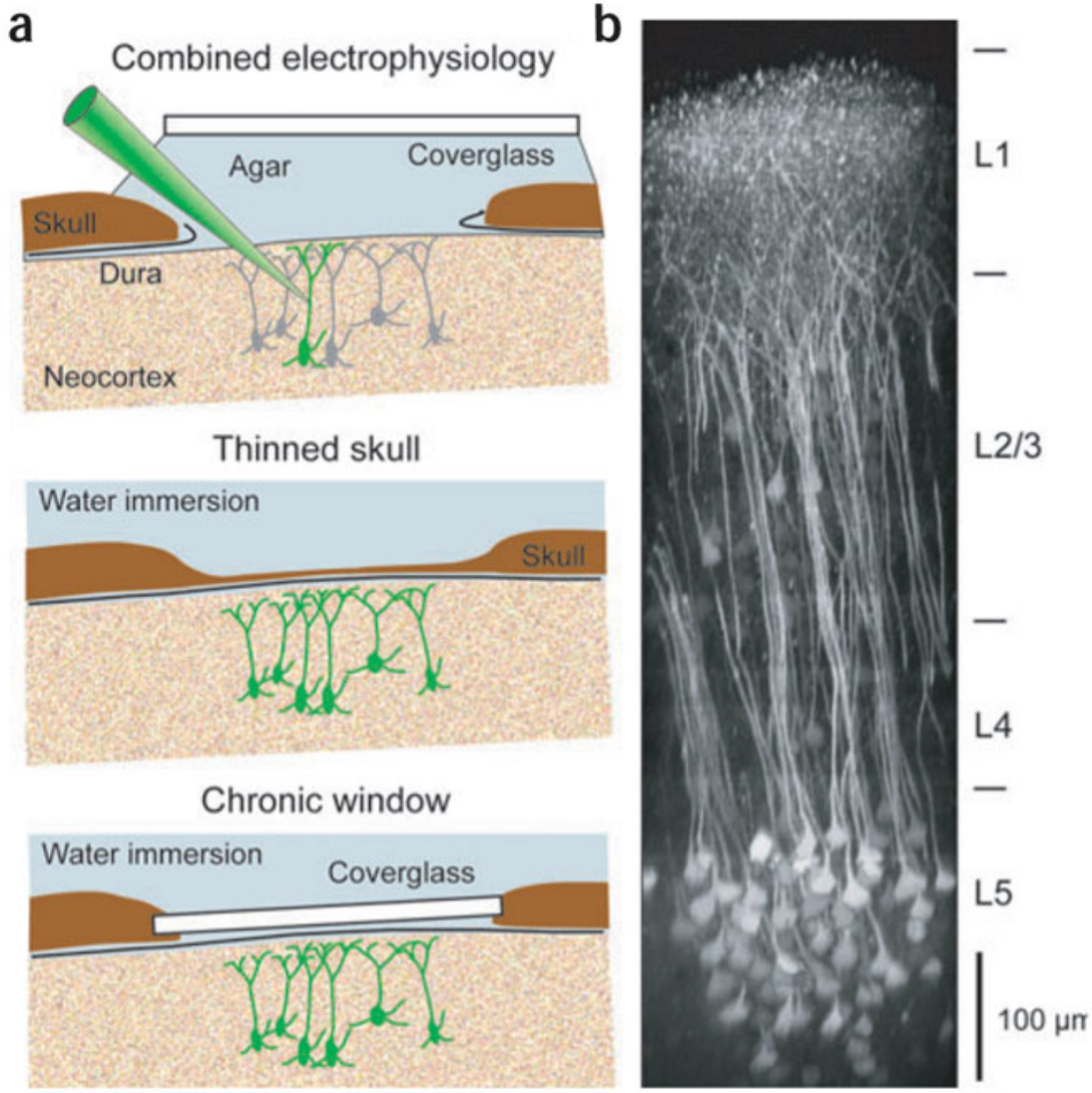
From Helmchen and Denk. 2005. *Nature Meth* 2:932

Two-Photon Microscopy

- Differs from confocal by excitation laser and detection pathway.
- Rapid high-energy laser pulses (100 fs; 100 MHz) are emitted.
- Signal collected by detector depending on sample thickness.
- No pinholes needed, as in confocal.

Two-Photon Microscopy

- Example of *in vivo* deep-tissue imaging.
- Possible configurations.
- Intact neocortex.



From Helmchen and Denk. 2005. *Nature Meth* 2:932

Things to Consider…

1. What are the primary differences between the different types of microscopy discussed so far?
2. Think about appropriate applications in which you would use regular fluorescence, confocal and two-photon microscopy.