Research Techniques I: Microscopy and Cell Imaging

Seeing at the cellular and subcellular level

1

Several Types of Microscopy

- Light microscope
- Fluorescence microscope
- Confocal microscope
- Two-photon microscope
- Electron microscope

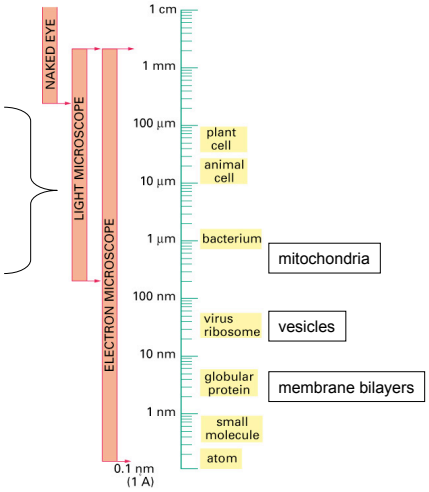

Figure 9–2. Molecular Biology of the Cell, 4th Edition.

2

The Light Microscope (LM)

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

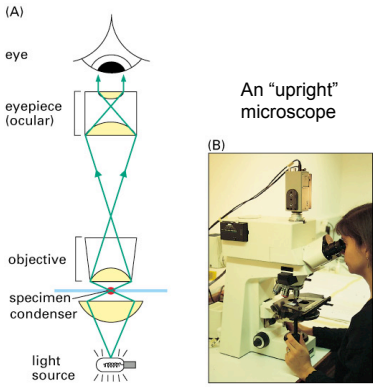

Figure 9–3. Molecular Biology of the Cell, 4th Edition.

3

Four types of LM

1) Bright field
- transmitted light.

2) Phase contrast
- converts phase differences into changes in brightness.

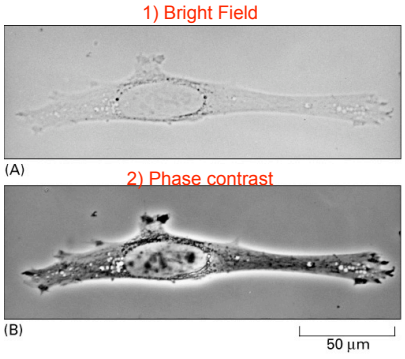

Figure 9–8 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

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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
- Observable with phase contrast.

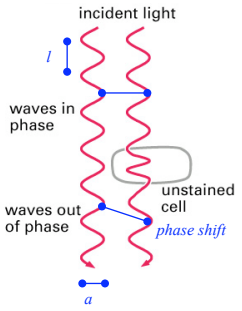

Figure 9-7B. Molecular Biology of the Cell, 4th Edition.

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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)

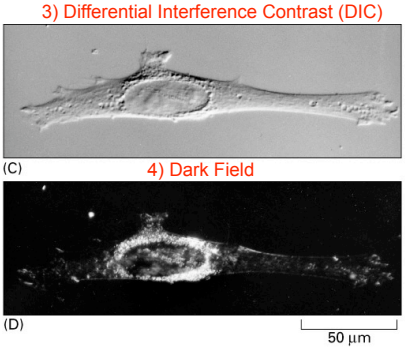

Figure 9–8 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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Phase and DIC Compared

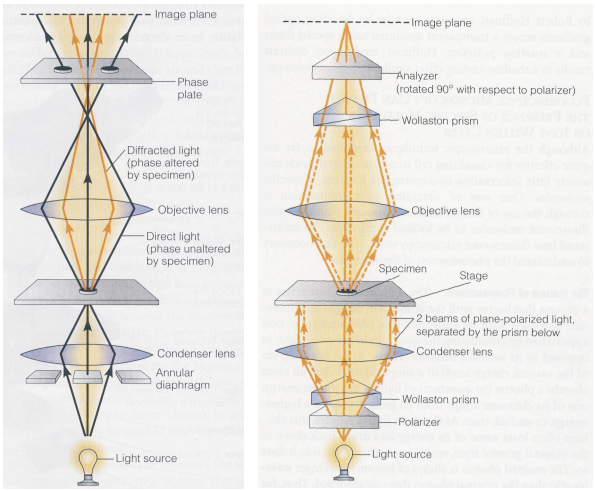

Figure A6, 8. Becker et al. 2006 World of the Cell.

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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.

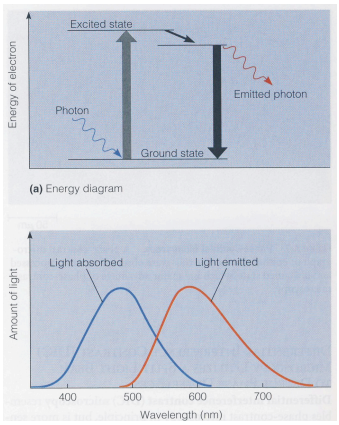

Figure A10. Becker et al. 2006 World of the Cell.

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Fluorescence Microscopy

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

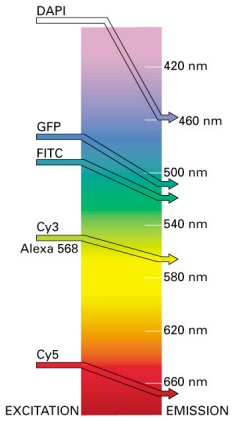

Figure 9–13. Molecular Biology of the Cell, 4th Edition.

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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.

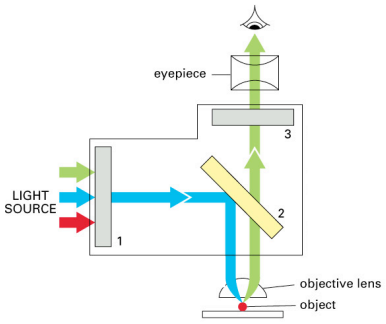

Figure 9-12. Molecular Biology of the Cell, 4th Edition.

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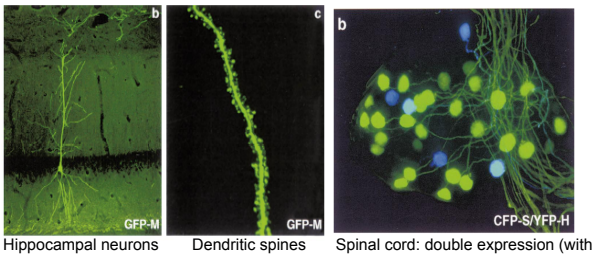
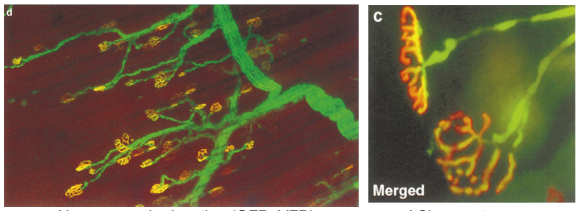
GFP in Research

- 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

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

Tissue Preparation

- To observe cells in tissue, in most cases tissues must be histologically prepared.
- Fixation: exposure to chemical reagents (aldehydes, acids, alcohols) to preserve and stabilize. May produce unwanted effects.
- Sectioning: cutting of thin (1–10 μm) tissue sections on a microtome.
- Staining: if applicable, involves exposure to dyes, e.g. hematoxylin, eosin.

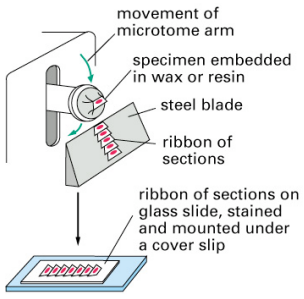

Figure 9–10. Molecular Biology of the Cell, 4th Edition.

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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.

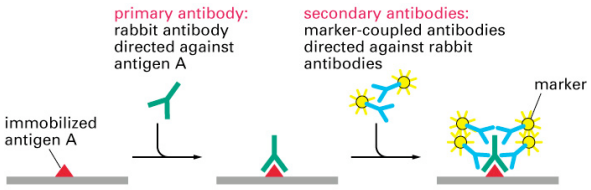

Figure 9–16. Molecular Biology of the Cell, 4th Edition.

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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.

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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".

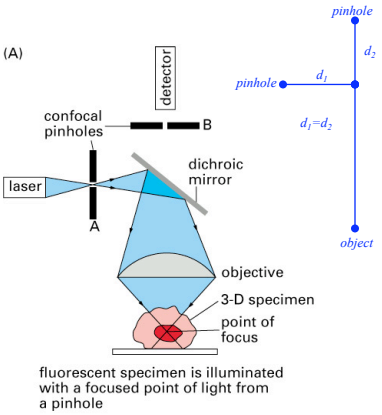

Figure 9–18 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

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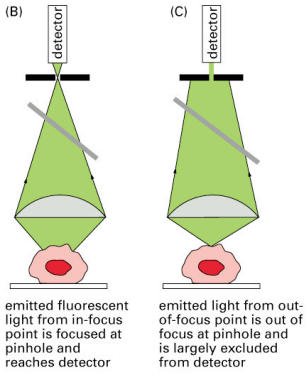
The Confocal Microscope

- 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.



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Comparison of Techniques

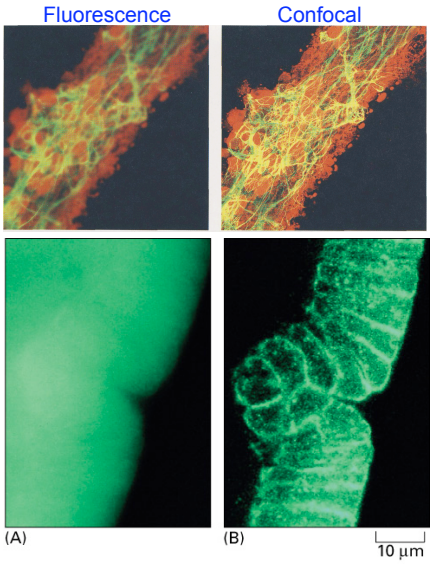

Figure A15. Becker et al. 2006 World of the Cell.

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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.

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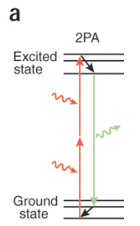
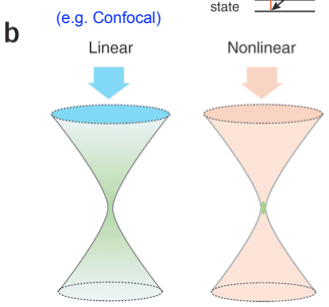
Two-Photon Microscopy

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



- Signal is dependent on photon density, so absorption is spatially confined.
- In confocal, single photon absorption occurs throughout exc. light cone.



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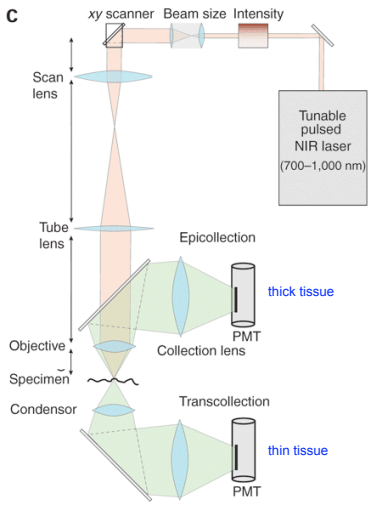
From Helmchen and Denk. 2005. *Nature Meth* 2:932

Two-Photon Microscopy

- Differs from confocal by excitation laser and detection pathway.
- Rapid high-energy pulses (100 fs; 100 MHz).
- Beam expansion tube and scan lens focus light onto sample.
- Signal collected by detector depending on sample thickness.
- No pinholes needed, as in confocal.



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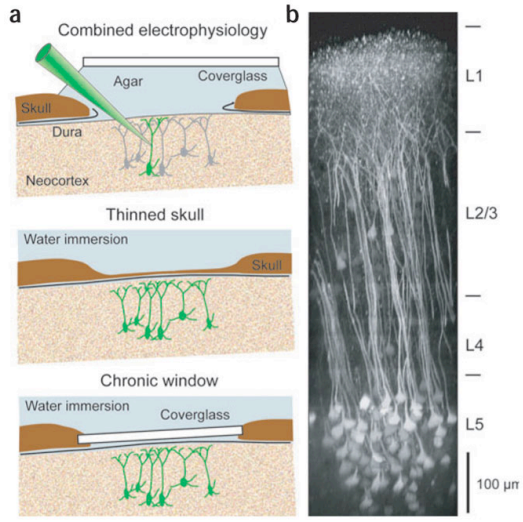
From Helmchen and Denk. 2005. *Nature Meth* 2:932

Two-Photon Microscopy

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



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

Things to Consider…

1. What are the primary differences between phase contrast and DIC?
2. Think about appropriate applications in which you would use regular fluorescence, confocal and two-photon microscopy.

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