Micro Electro Mechanical Systems for mechanical engineering applications

Lecture 11:
Detection Methods for BioMEMS (1)

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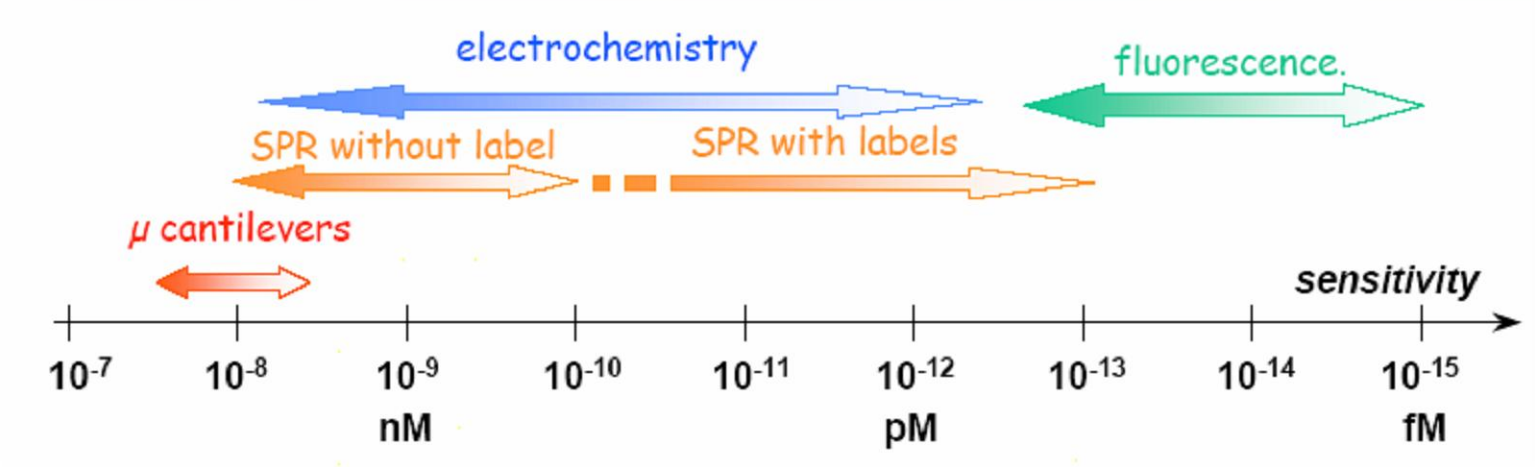
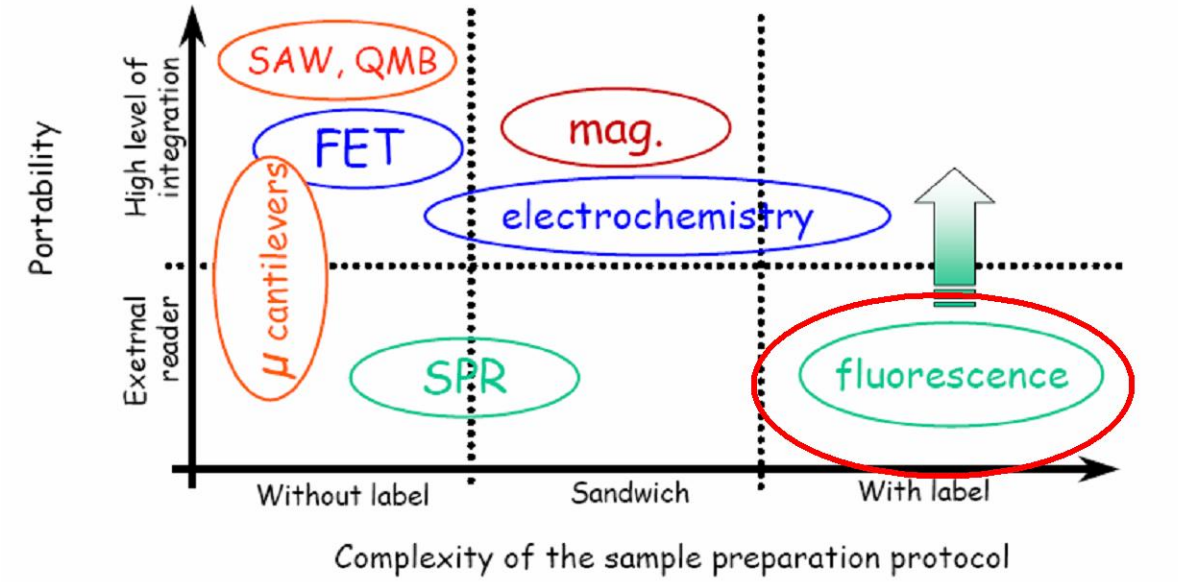
Why do we need detection?

- Diagnostic
  - ✓ High sensitivity (0.1 pM on immunologic test, 0.1 molecule/μm2 for DNA test)
  - ✓ Low cost reading system
- High throughput screening
  - ✓ Speed ,multiparameters, sensitivity (0.1 nM in molecular screening)
  - ✓ The reader cost is not an issue
- Point of care
  - ✓ Easy to use, autonomy , robustness….
  - ✓ Low cost reading system

Detection method?





Why light microscopy (LM)?

- Four LM images of a cultured eukaryotic cell, showing how different things look with different optics

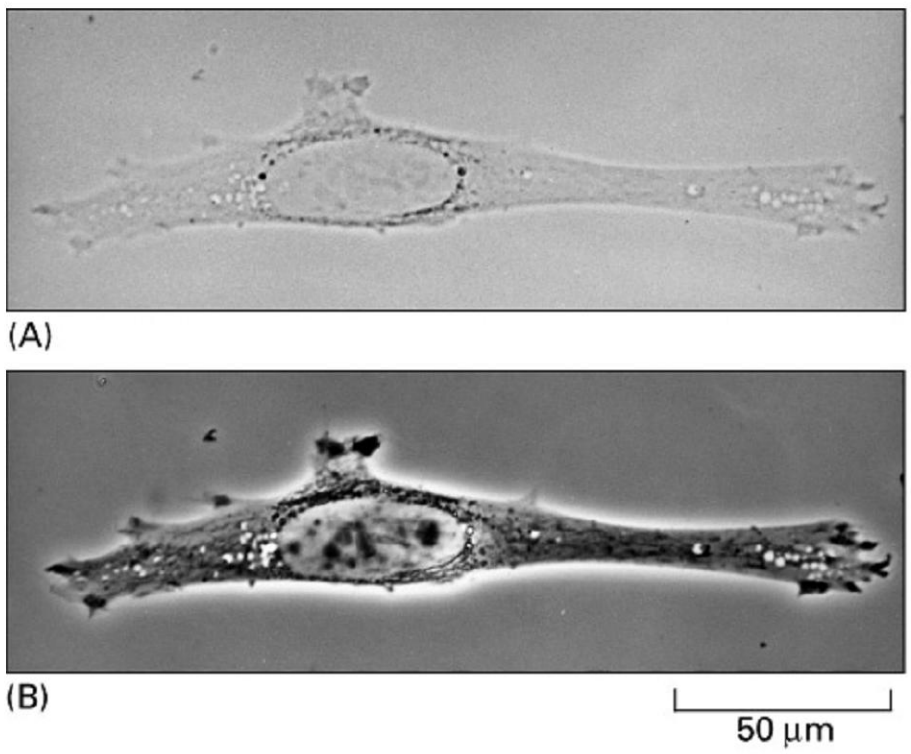


Figure 9–8 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

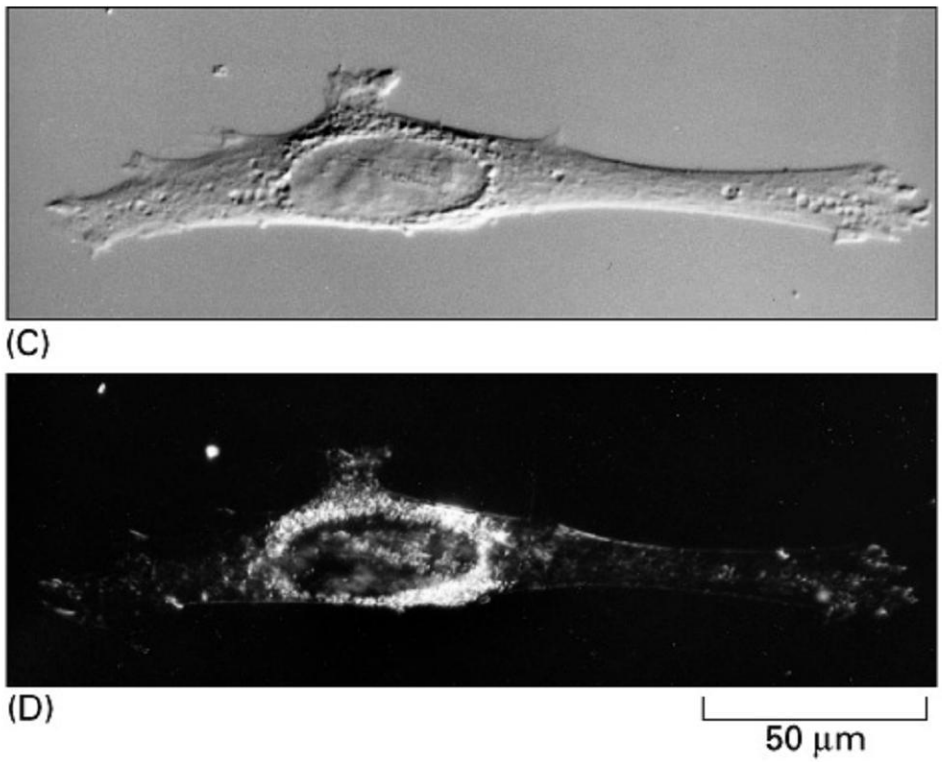
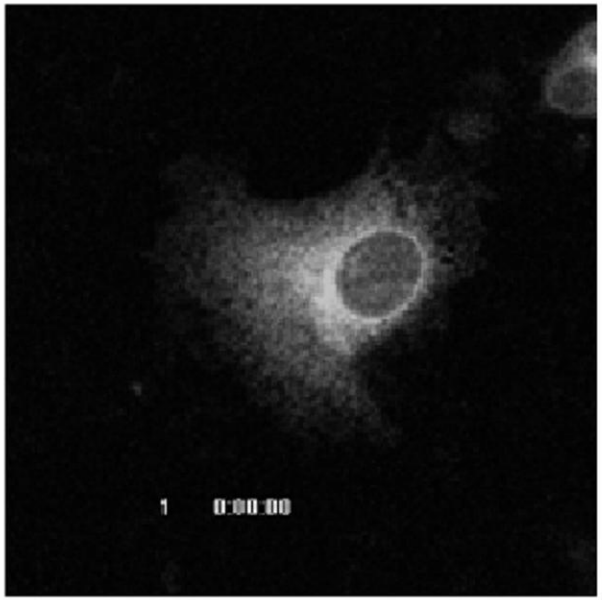


Figure 9–8 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

One Exciting Application of Current Microscopy



Specific Protein! **Real Time!** **Live Cells!**

Microscopes

basic structure and function of a microscope

comparison of different types of microscopes

conventional light microscopes

fluorescence microscopes

Microscopes - 1

basic structure and function of a microscope

comparison of different types of microscopes

conventional light microscopes

fluorescence microscope

Basic Components of a Microscope

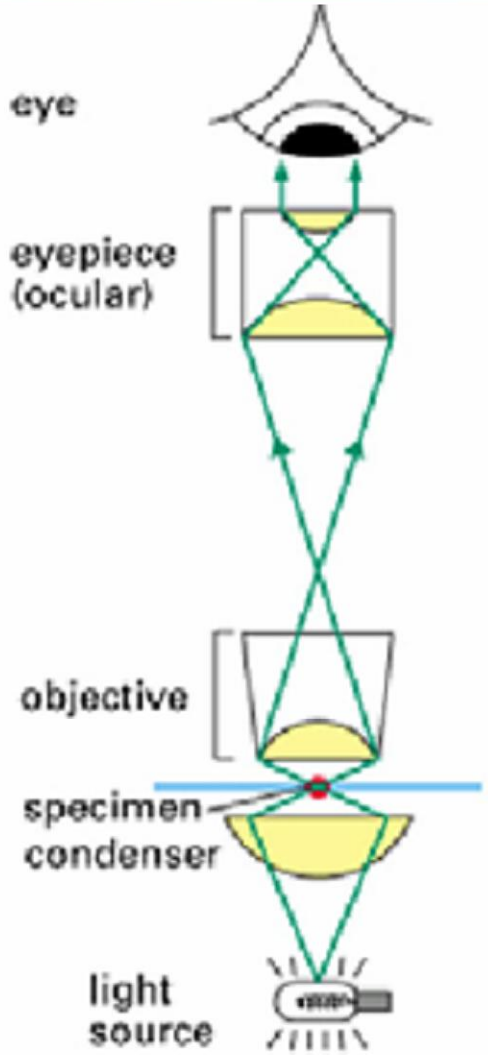


Fig 9-3 Molecular Biology of the Cell 4th Ed.

(B)

Functions of Microscope

- **Magnification**

- **Resolution: the ability to separate clearly two points lying close together in the specimen**

Magnification

(magnification of eyepiece) X (magnification of objective)

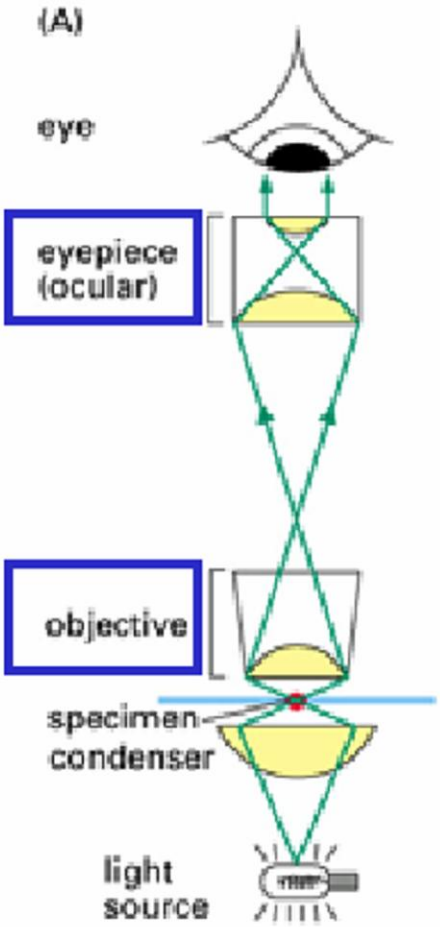
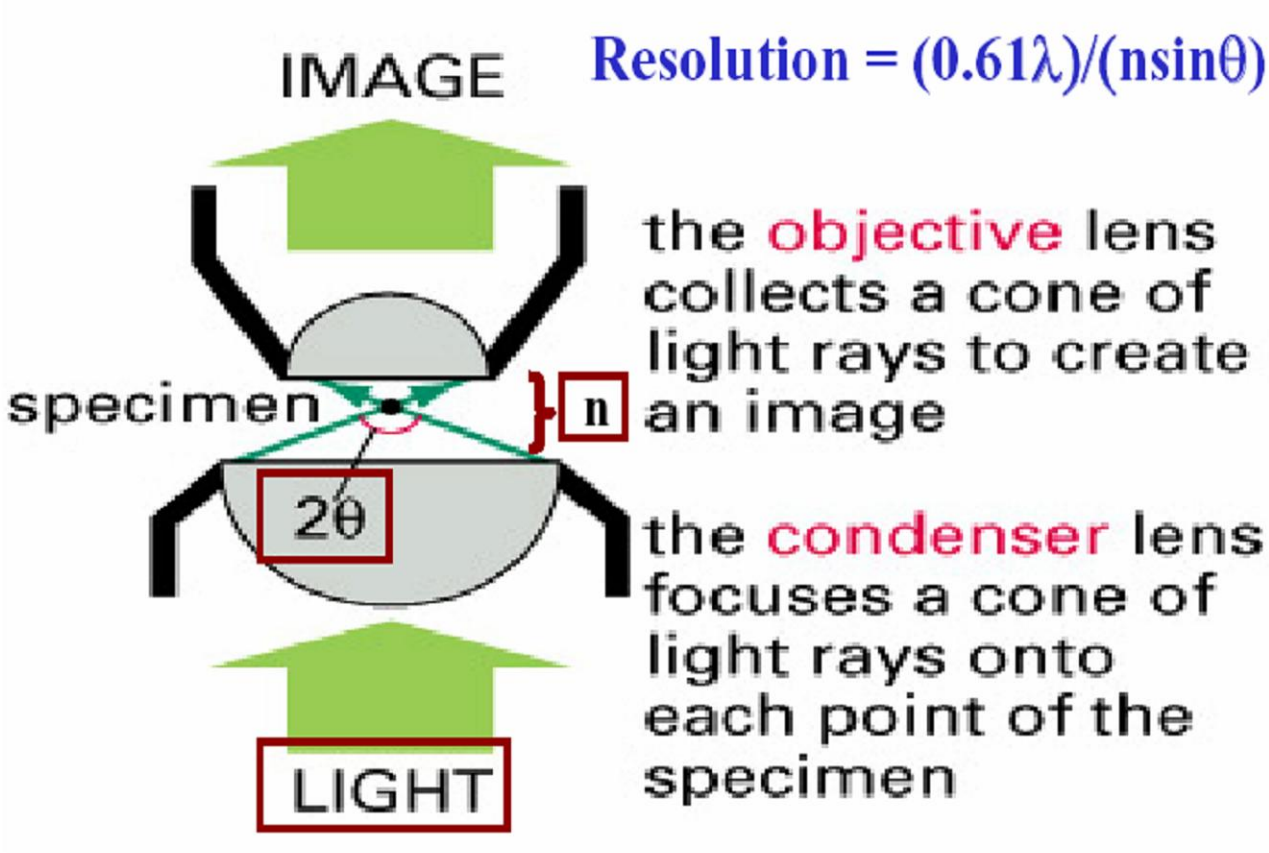


Fig 9-3 Molecular Biology of the Cell 4th Ed.

(B)

Resolution



Resolution = (0.61λ)/(nsinθ)

nsinθ : numerical aperture (NA) of the lens

NA as a measure of light-gathering ability of the lens

Microscopes - 2

basic structure and function of a microscope

comparison of different types of microscopes

conventional light microscopes

fluorescence microscope

Types of Microscopes

Light microscopes:

conventional light microscope

fluorescence microscope

Electron microscopes:

transmission microscope

scanning microscope

Comparison of Imaging Methods

Light microscopes:

conventional light microscope	**white light**
fluorescence microscope	**fluorescence**

Electron microscopes:

transmission microscope	**high-speed electrons**
scanning microscope	**high-speed electrons**

Comparison of Resolving Powers

Light microscopes:	
conventional light microscope	**~200 nm**
fluorescence microscope	**~200 nm**
Electron microscopes:	
transmission microscope	**~0.002nm (0.1-2 nm)**
scanning microscope	**~0.002nm (10 nm)**
Human eyes	**200,000 nm**

Comparison of Sizes of Objects Imaged

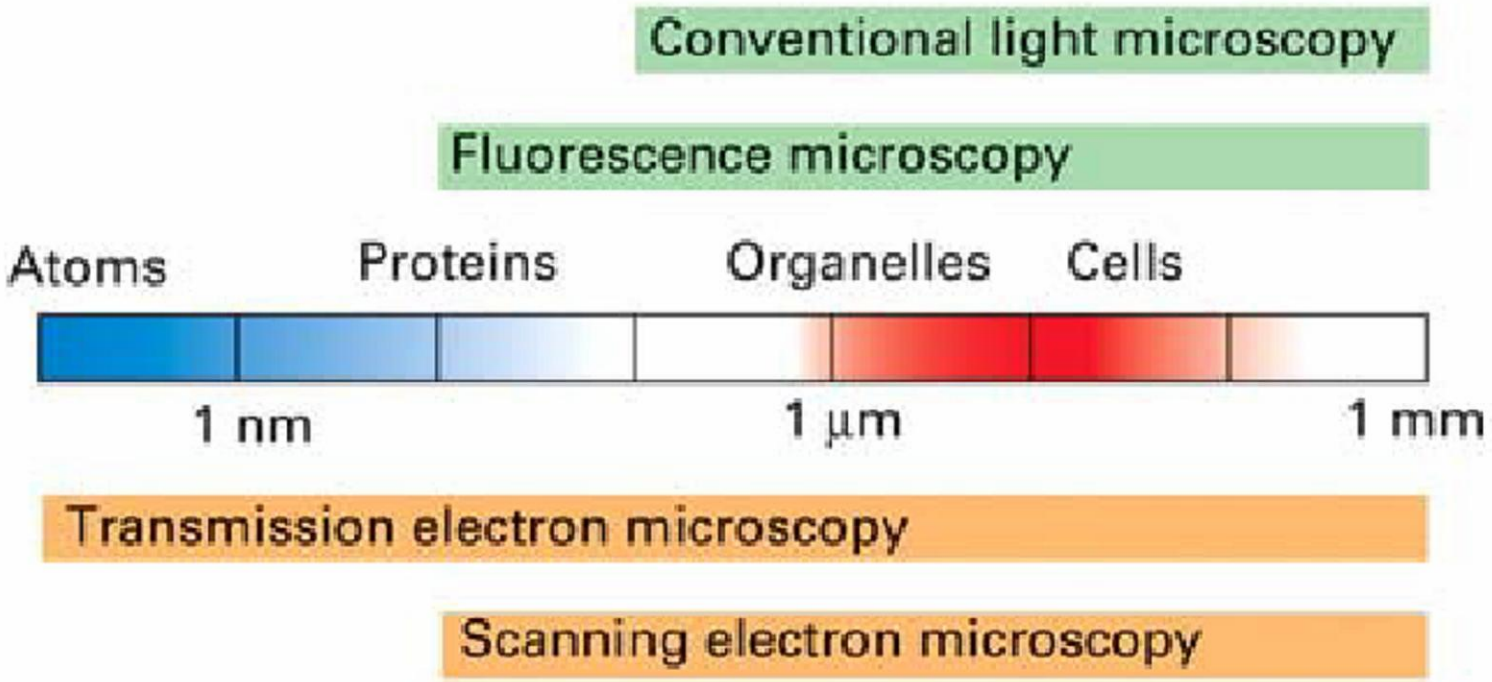


Fig. 5-41 Molecular Cell Biology, 5th Ed.

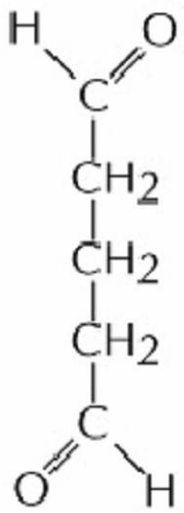
Comparison of Live Cell Imaging

Light microscopes:	
conventional light microscope	**Yes**
fluorescence microscope	**Yes**
Electron microscopes:	
transmission microscope	**No**
scanning microscope	**No**

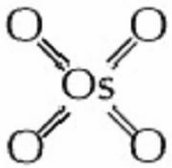
Fixation

to stabilize or lock the macromolecules in their positions

proteins



proteins
lipids



common fixatives used for EM

glutaraldehyde osmium tetroxide

Figure 9–23. Molecular Biology of the Cell, 4th Edition.

Microscopes - 3

basic structure and function of a microscope

comparison of different types of microscopes

conventional light microscopes

fluorescence microscope

Four Types of Conventional Light Microscope

Bright-field microscope

Phase-contrast microscope

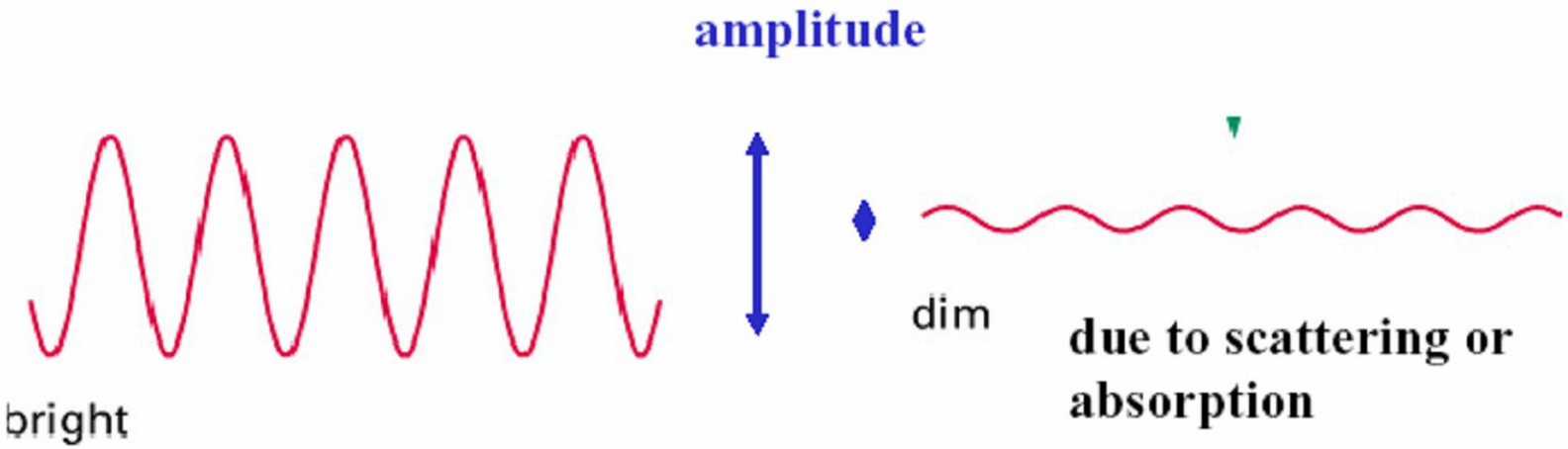
Differential interference contrast (DIC) (Nomarski) microscope

} images formed by transmitted light

Dark-field microscope (complementary to bright-field)

images formed by scattered light

Ways of Generating Contrast (by Amplitude Change)



Problem: live cells are almost transparent

Methods of Contrast Enhancement via Amplitude Change

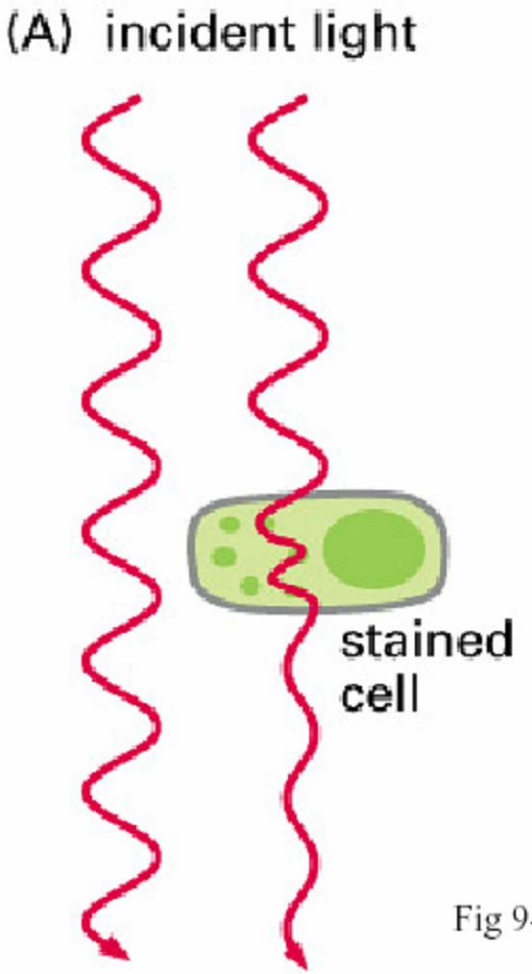


Fig 9-7 Molecular Biology of the Cell 4th Ed.

Ways of Generating Contrast by Phase Change

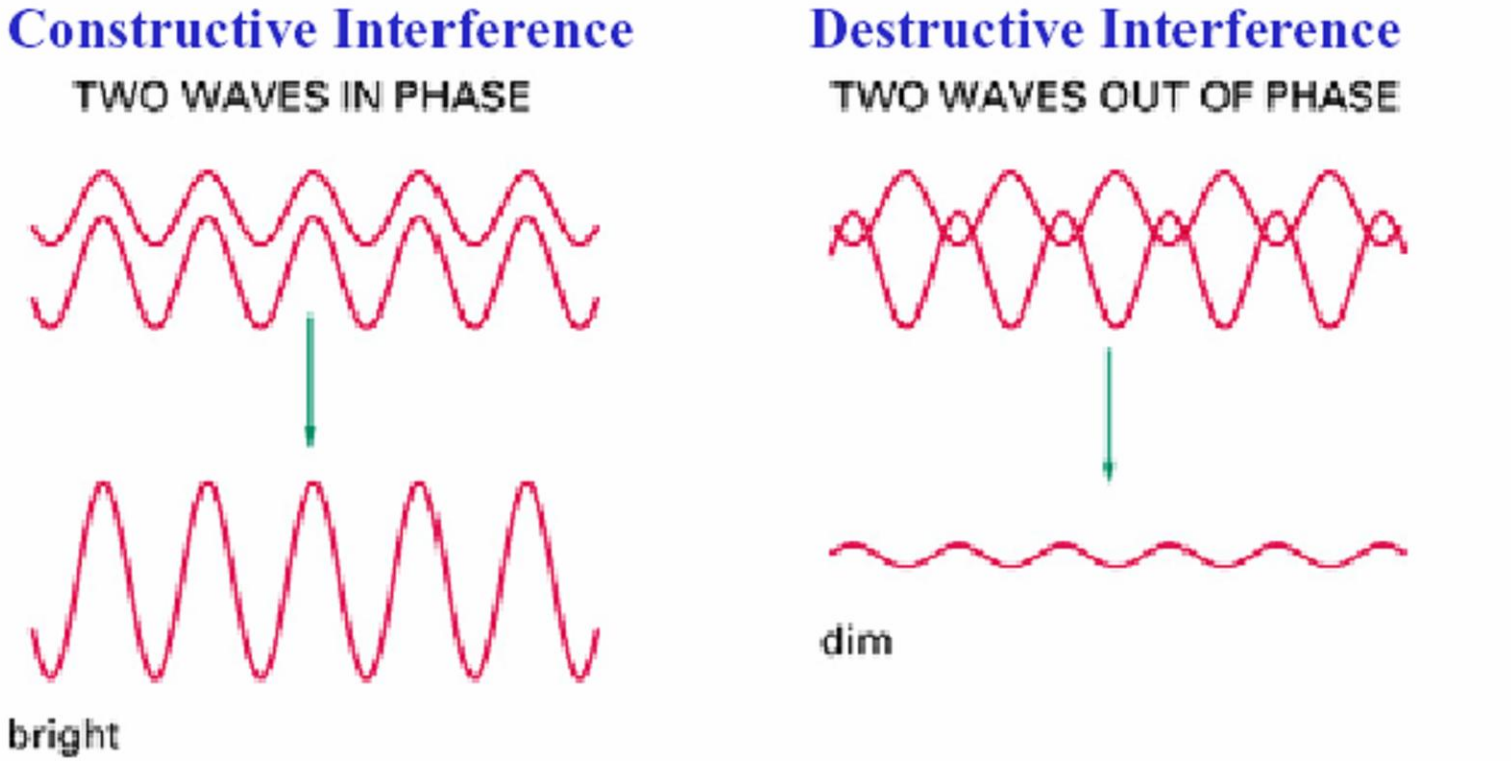
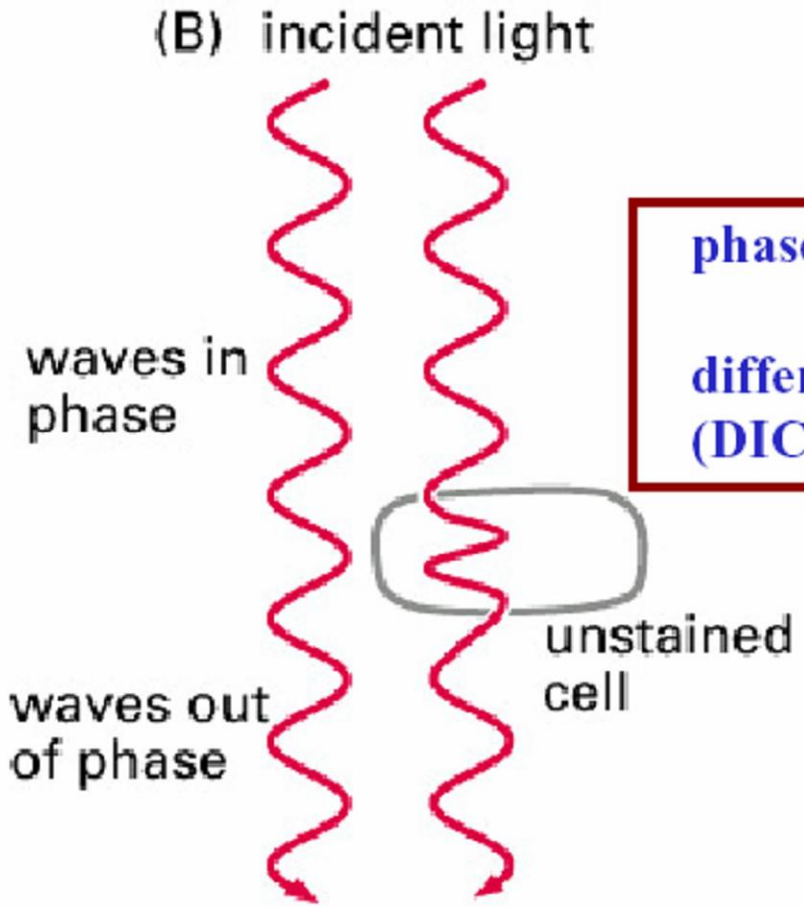


Figure 9–4. Molecular Biology of the Cell, 4th Edition.

Problem: live cells generates only small phase changes

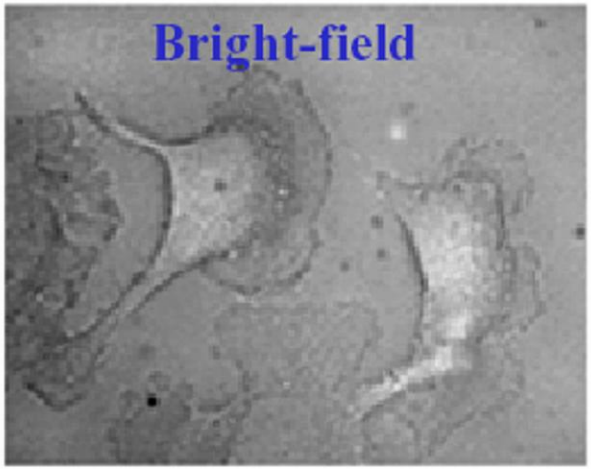
Methods of Contrast Enhancement via Phase Change

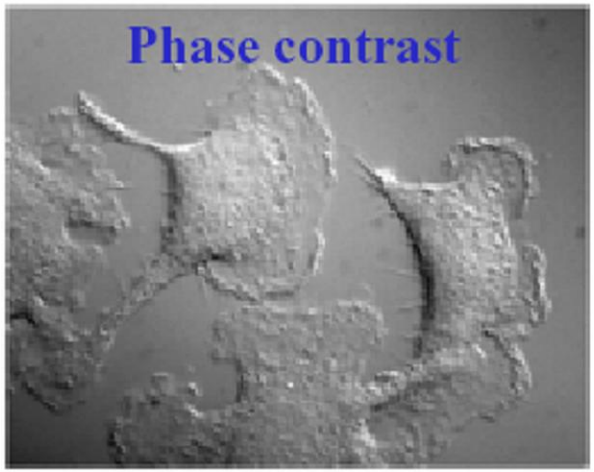


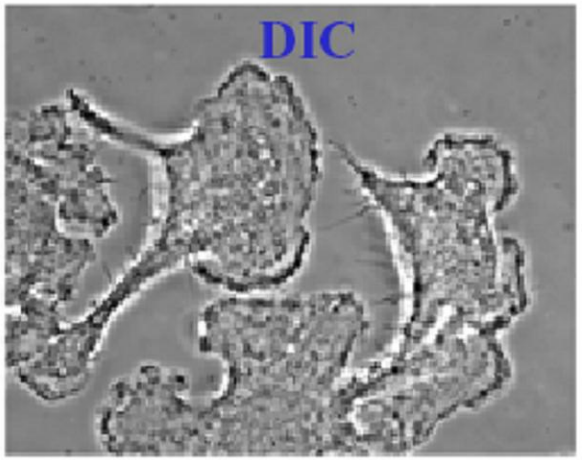
phase contrast microscopy

differential interference contrast (DIC) microscopy

Fig 9-7 Molecular Biology of the Cell 4th Ed.


Bright-field


Phase contrast


DIC

Microscopes - 4

basic structure and function of a microscope

comparison of different types of microscopes

conventional light microscopes

fluorescence microscope

a fluorescent molecule:
absorbs light at one wavelength and
emits lights at a specific longer wavelength

The principle of fluorescence

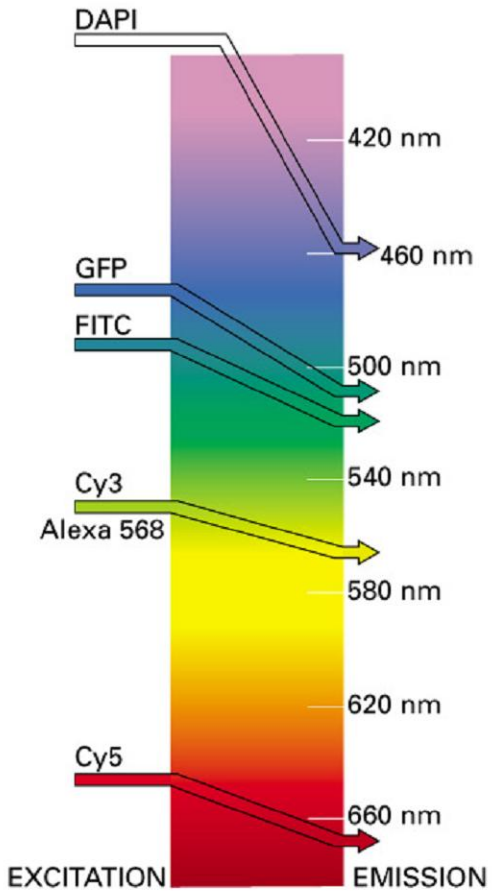


Figure 9–13. Molecular Biology of the Cell, 4th Edition.

The color of light (its wavelength, reflects its energy: blue is more energetic than red. During fluorescence, light is always emitted with a longer wavelength

Principles of fluorescence microscopy

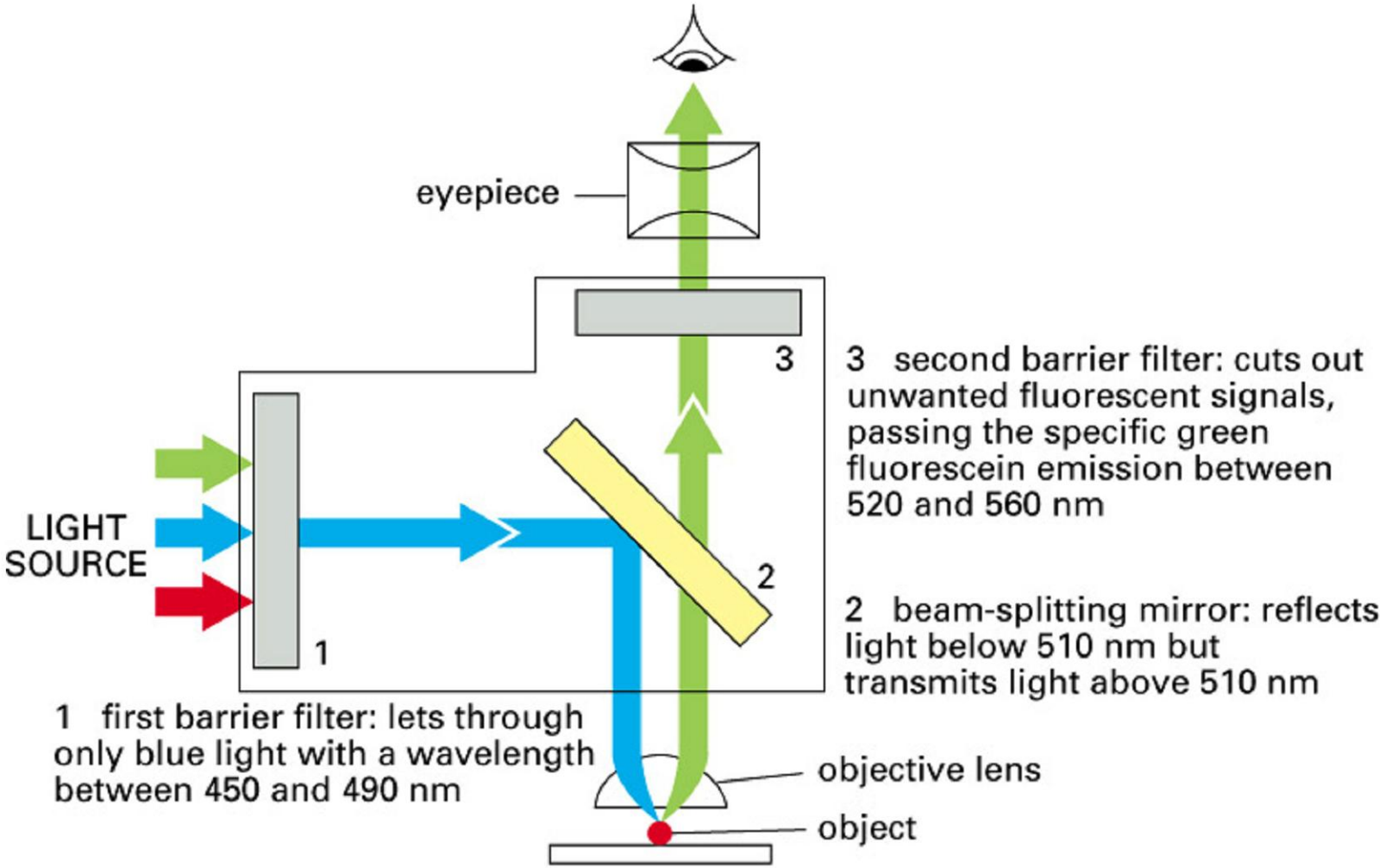
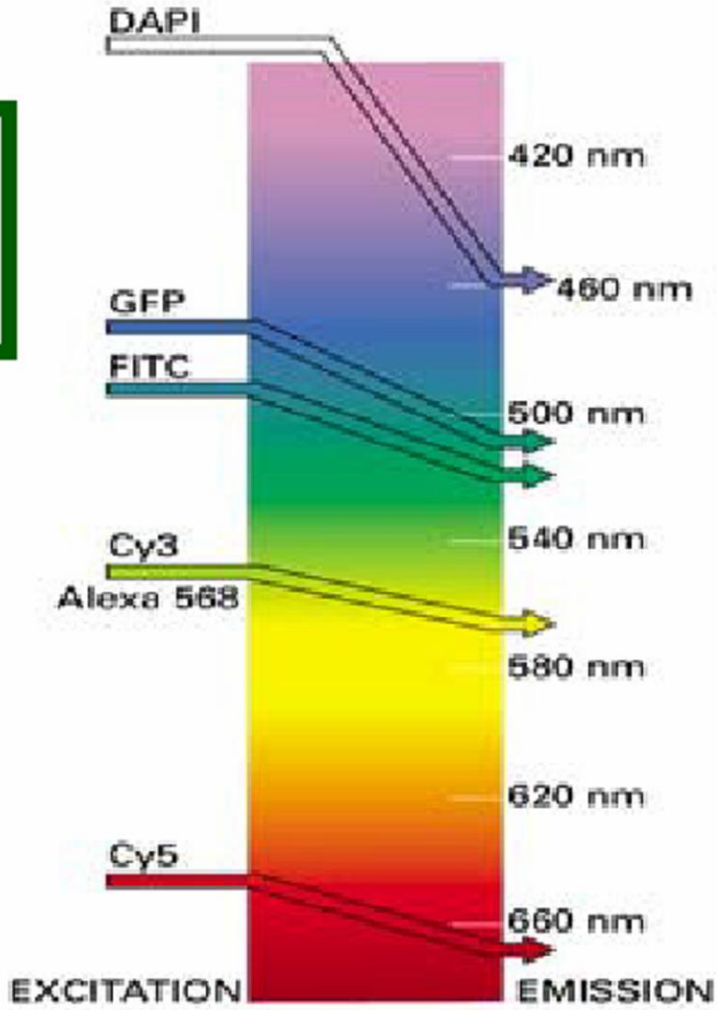


Figure 9–12. Molecular Biology of the Cell, 4th Edition.

Two Major Advantages of Fluorescent Microscopy (1)

Multiple labeling within a cell



Two Major Advantages of Fluorescent Microscopy (1)

- Immunofluorescence of Fixed Cells

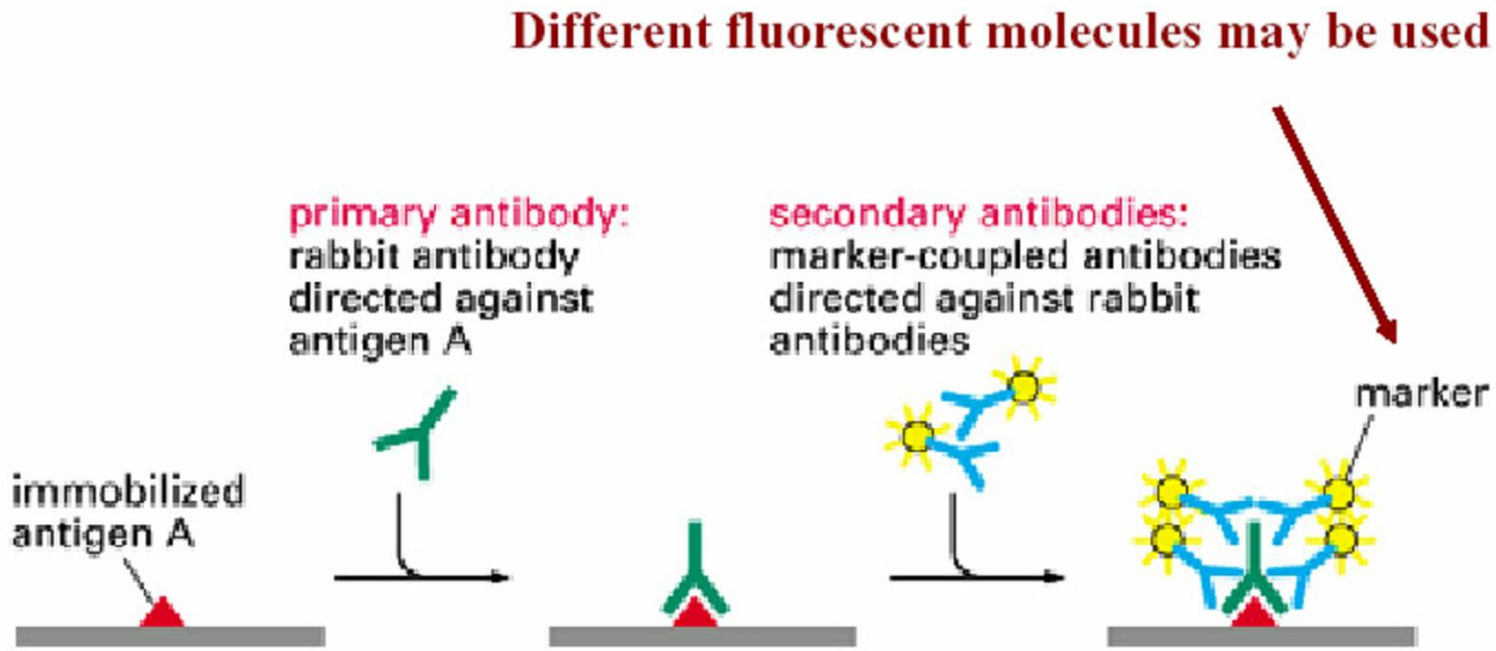
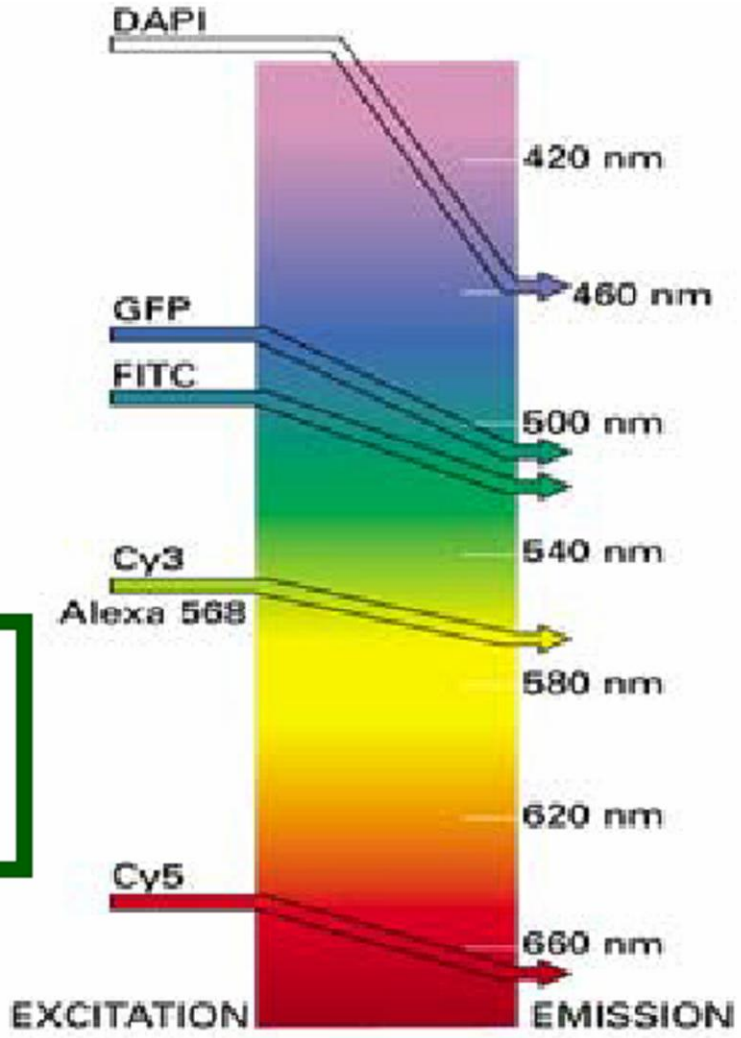


Figure 9–16. Molecular Biology of the Cell, 4th Edition.

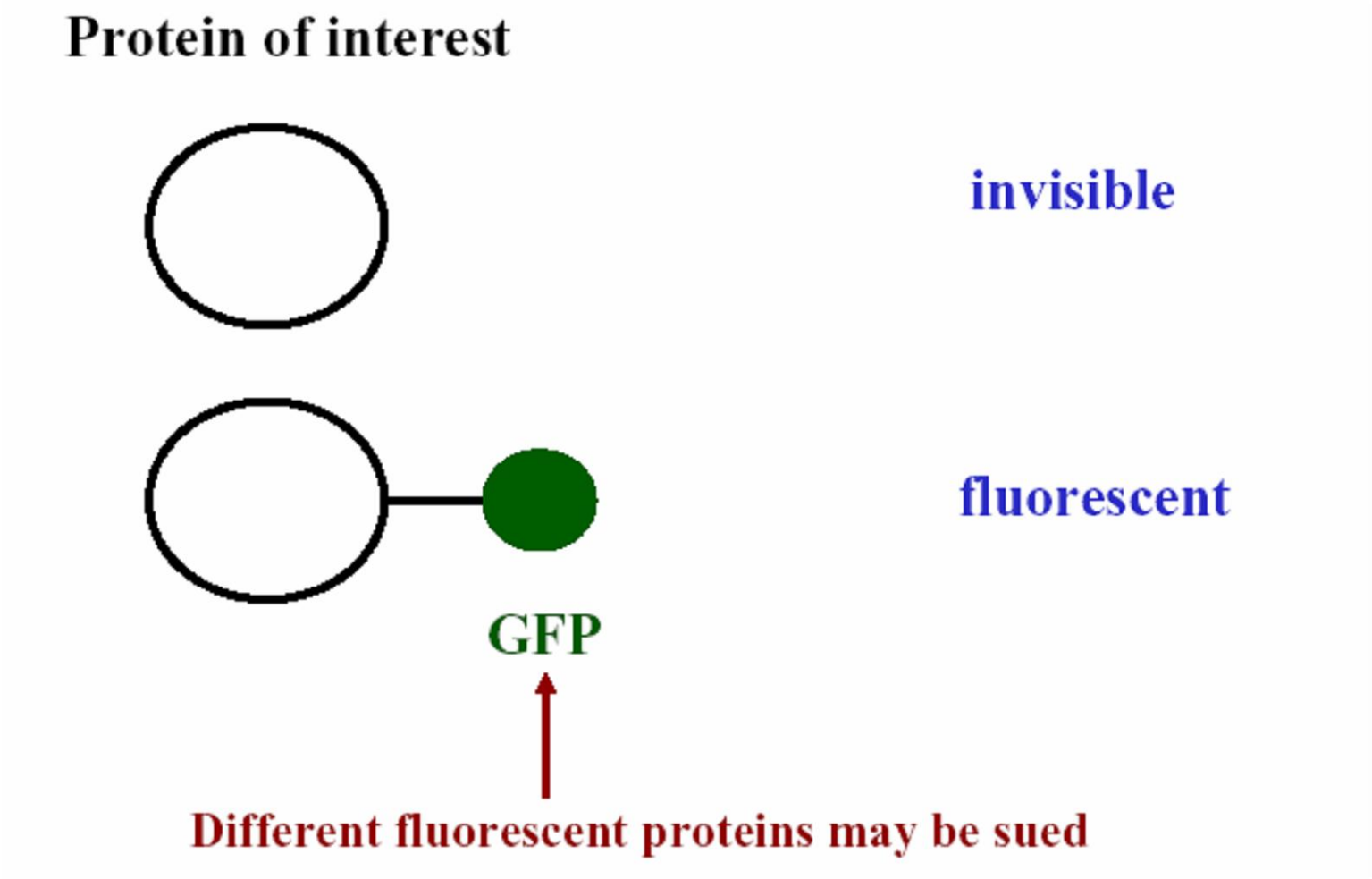
Two Major Advantages of Fluorescent Microscopy (2)

Multiple labeling within a cell

Flexible (fixed or live cells)



Fluorescent Microscopy of Live Cells



Micrograph of a Cell in Mitosis

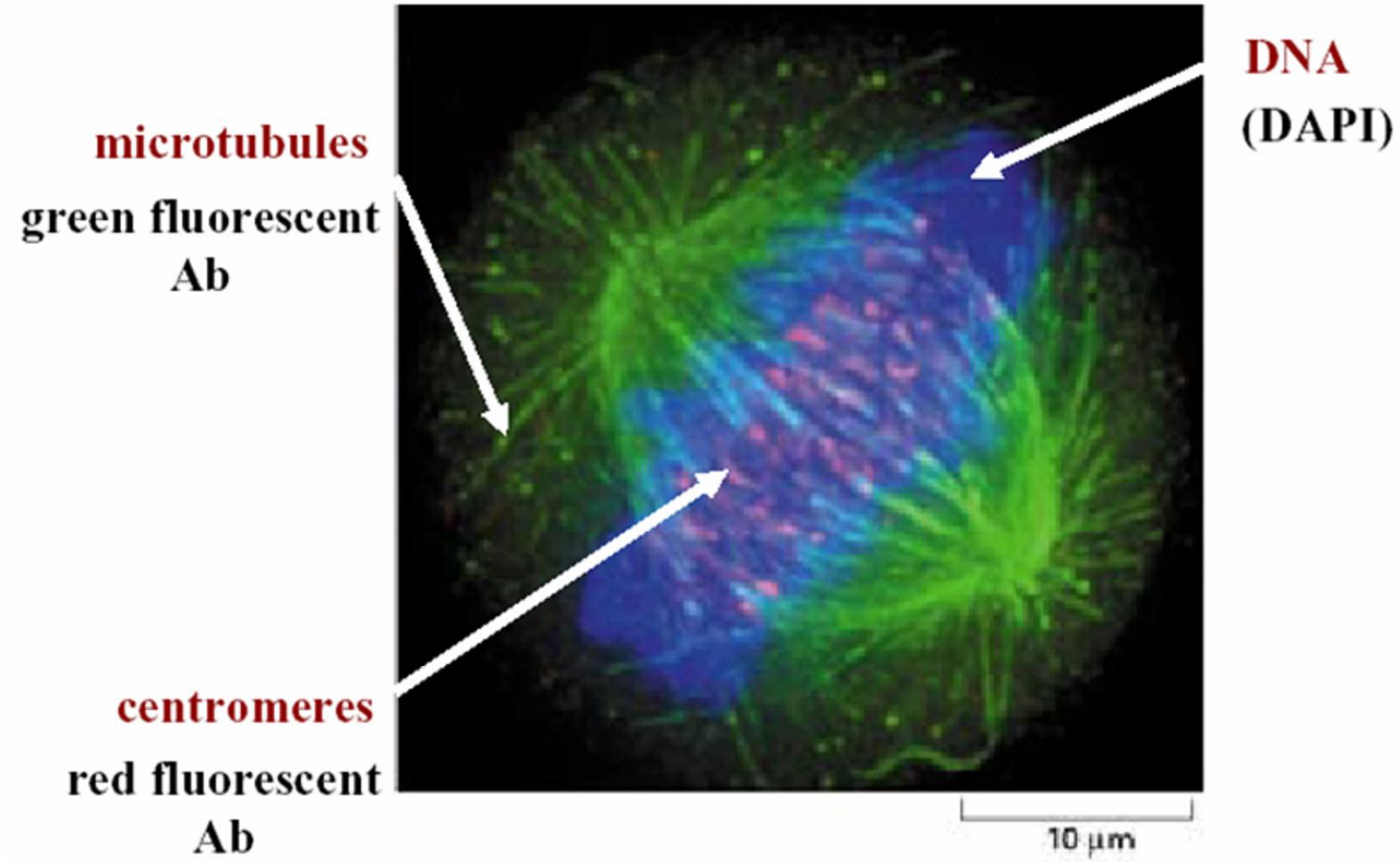


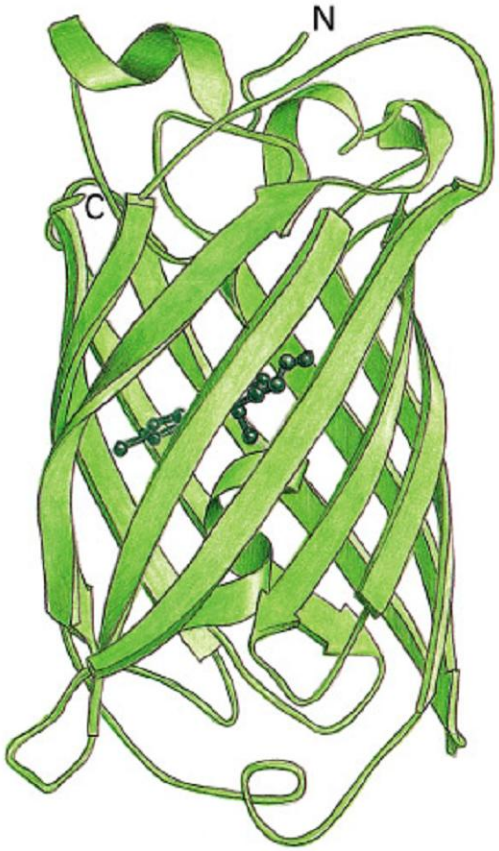
Figure 9-14. Molecular Biology of the Cell, 4th Edition.

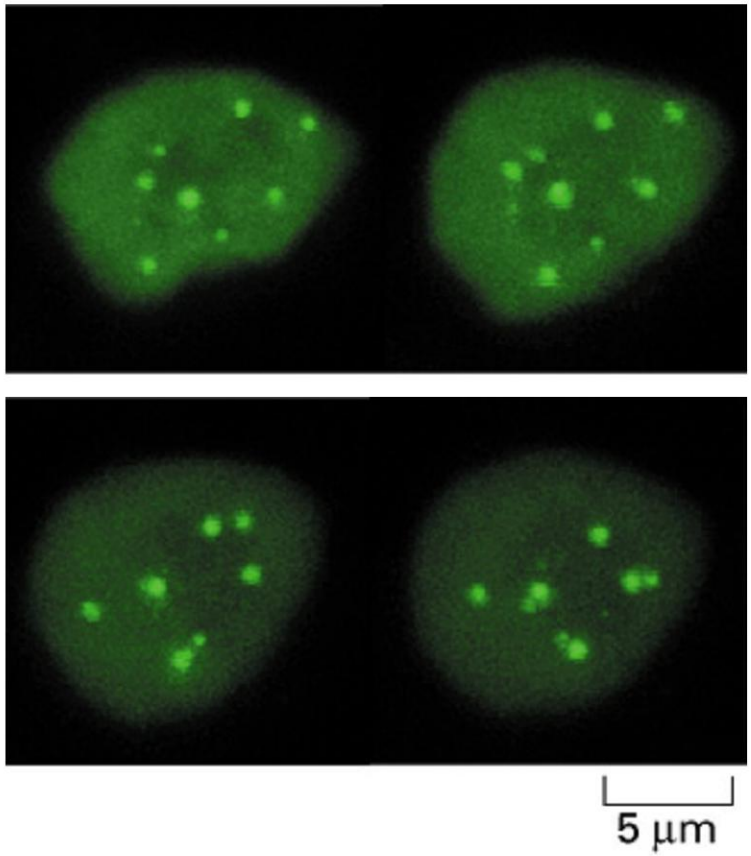
Problem: imaging of 3-D objects due to out-of-focus light

Live-cell imaging can employ dyes that provide fluorescent contrast from specific components of living material

- There are “vital” fluorescent dyes for some bio-materials: Hoechst 33342 for DNA; Rhodamine 123 or “Mitotracker” for mitochondria; DiI, DiO, $DiOC_6$ (lipid-soluble dyes) or lipids with fluorescent labels for ER and Golgi
- The Green Fluorescent Protein (GFP), or its cyan, yellow, and red counterparts, can be used to track spaces. As chimeras with specific proteins they reveal subcellular localization

GFP is a beta-barrel protein that becomes fluorescent through its own chemistry. It is very stable





Comparison of SEM (left) and GFP imaging (right). Inner detail becomes visible

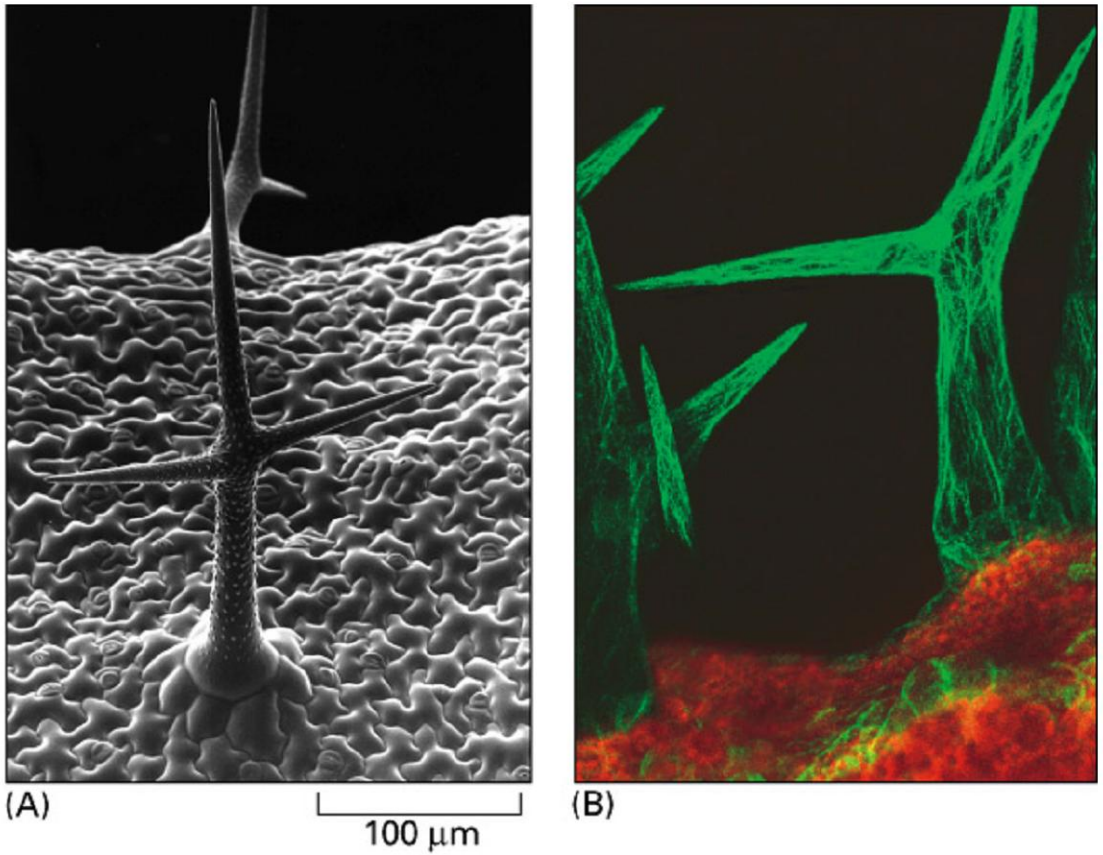


Figure 9–44. Molecular Biology of the Cell, 4th Edition.

A Major Problem Associated with Fluorescence Microscopy

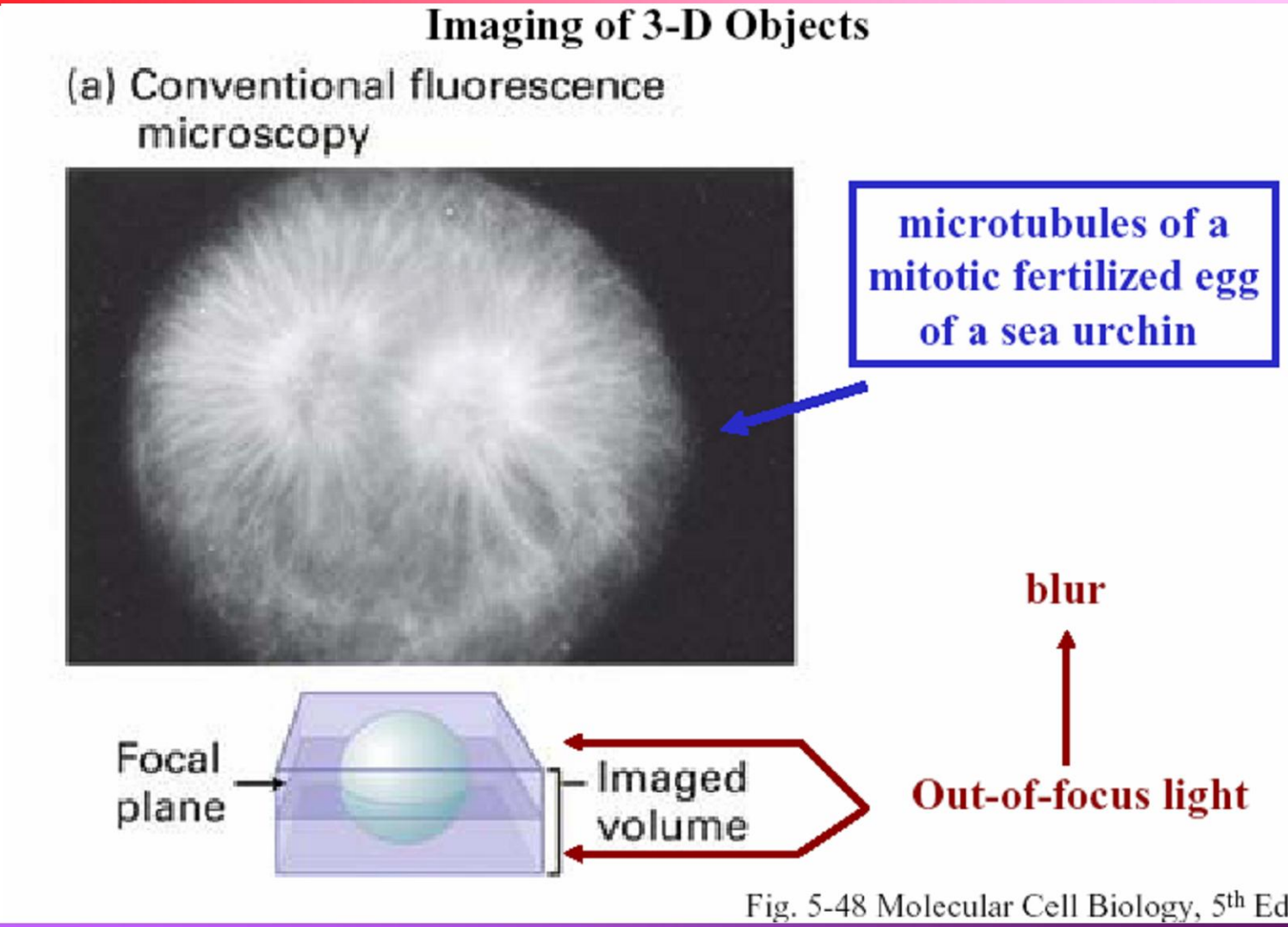


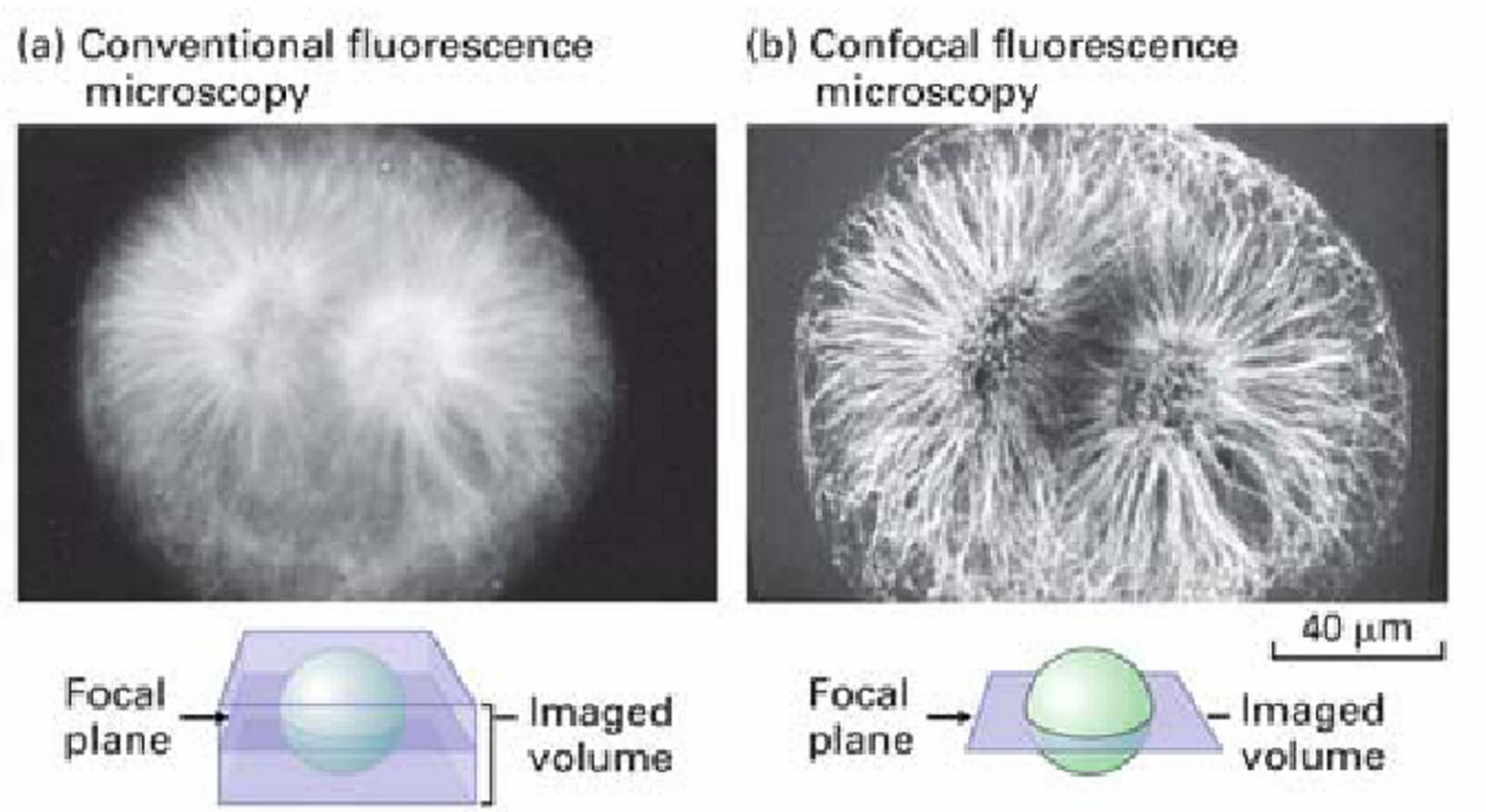
Fig. 5-48 Molecular Cell Biology, 5th Ed.

Two Ways to Reduce the Out-of-Focus Light

Confocal scanning: an optical approach

Deconvolution: a computer approach

1. Confocal scanning (1)



1. Confocal scanning (2)

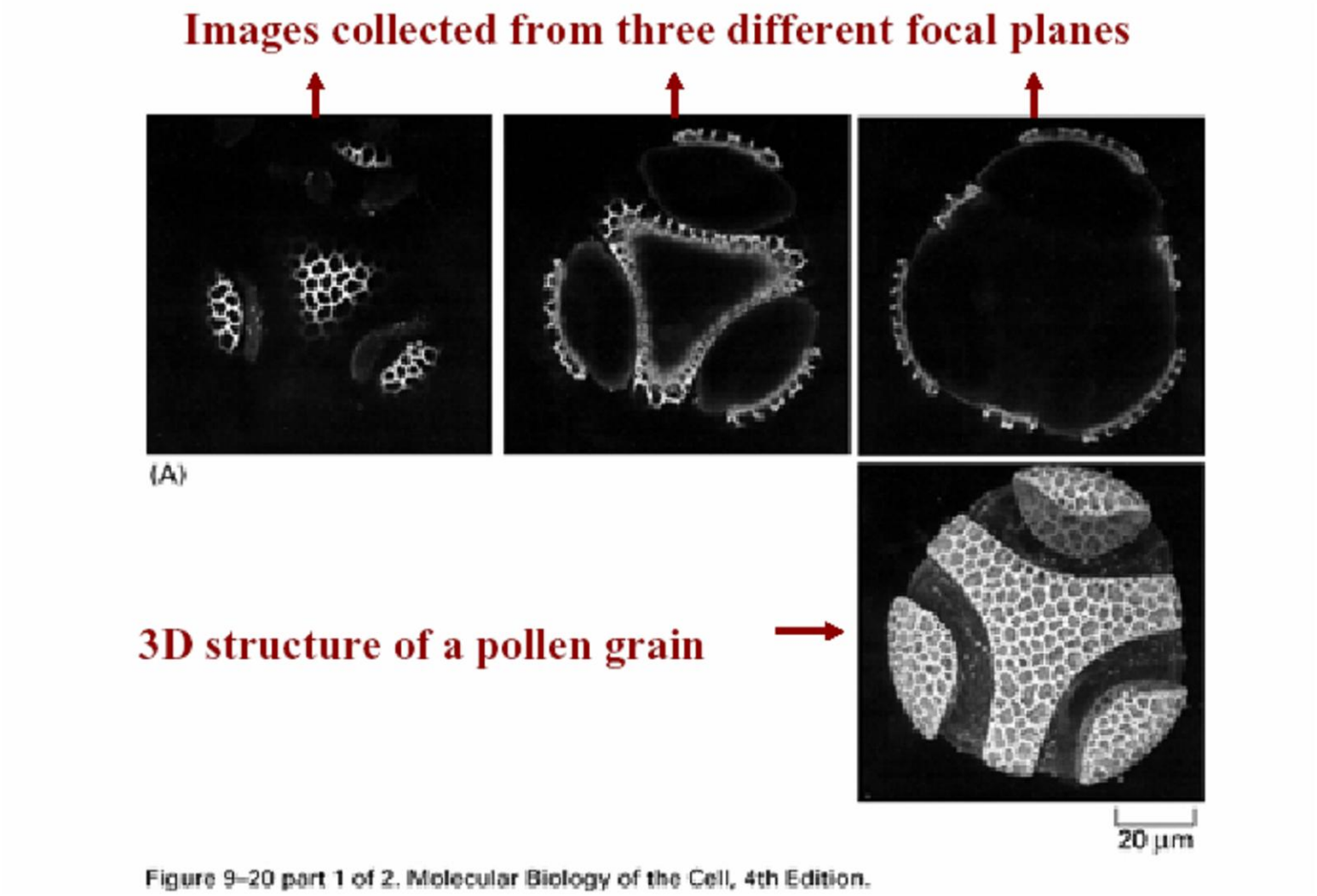


Figure 9–20 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

2. Deconvolution

3D image of the large polytene chromosomes from the fruit fly

conventional fluorescence microscopy

deconvolution fluorescence microscopy

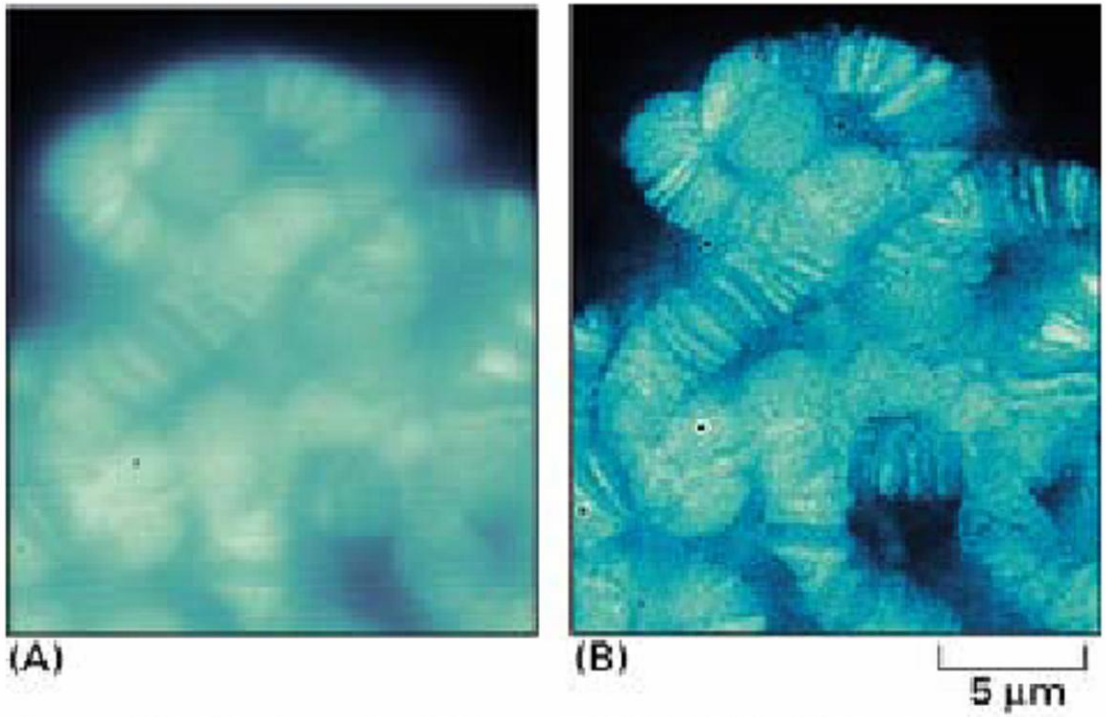


Figure 9-17. Molecular Biology of the Cell, 4th Edition.