How to study cells

Tools used to answer biological questions. The methods are important, but it's what we discover with them that's really interesting.

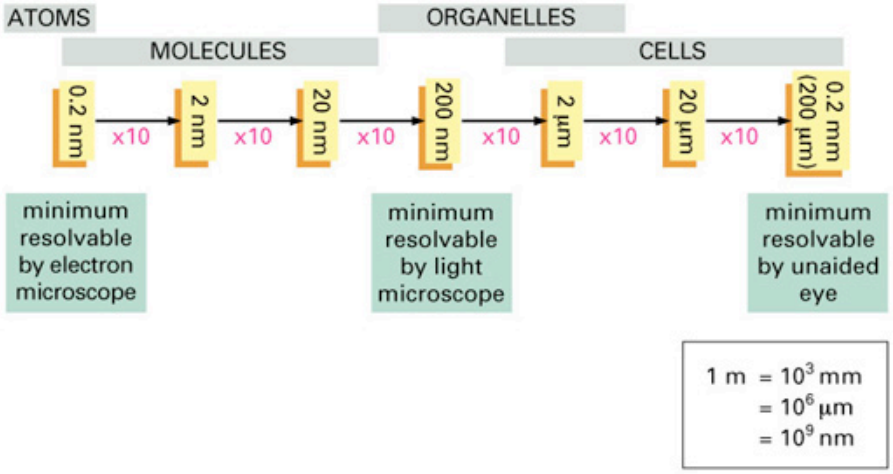
1. Microscopy (chap 1)

Role of microscopy in Cell Biology

- Invention of light microscopes in or just before the 1600s - cells became visible for the first time
- sole tool to study cells for centuries
- light microscopy is still a valuable tool to cell biologists

What microscopes do:

- designed for magnification, resolution, & contrast
- wavelength of light limits resolution
- sizes of cells and cell components:



Types of microscopy:

1. <u>light microscopy</u>
   a. conventional wide field
   b. phase contrast, differential interference contrast
   c. darkfield

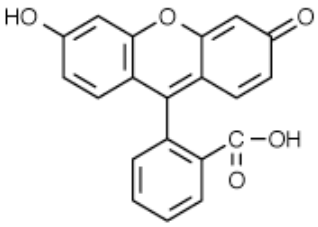
- staining: another important method for generating contrast
- preparing cells/tissues for staining:
  - fixation
  - sectioning

   d. fluorescence microscopy

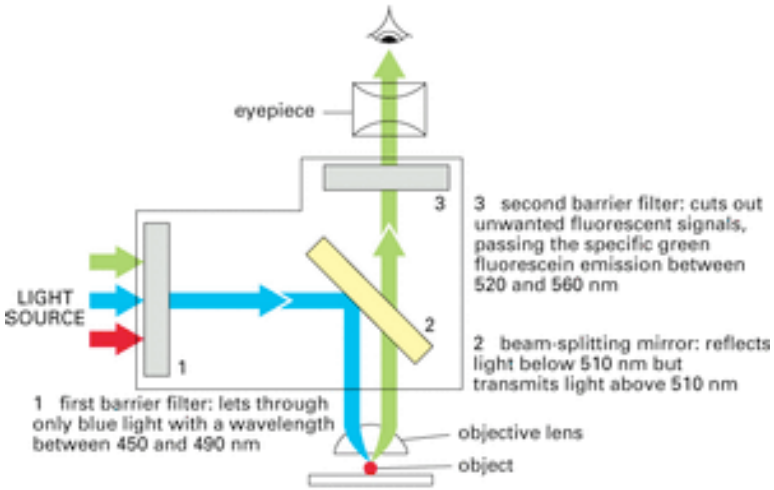
fluorescent dyes (see figure) can be used to identify molecular structures.

- Viewing fluorescent cells:
  (1) Epi-fluorescence illumination (see figure)
  (2) Laser-scanning confocal microscopy (see figure)
- Immunofluorescence - use of antibodies
- Indirect immunofluorescence
- Fluorescent proteins

fluorescein



epifluorescence microscopy



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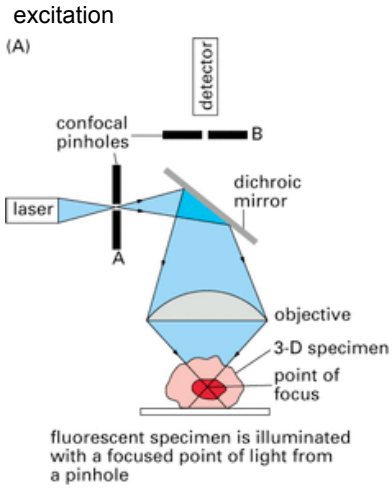
confocal microscopy



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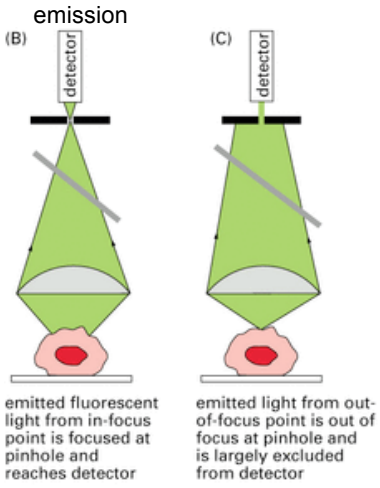

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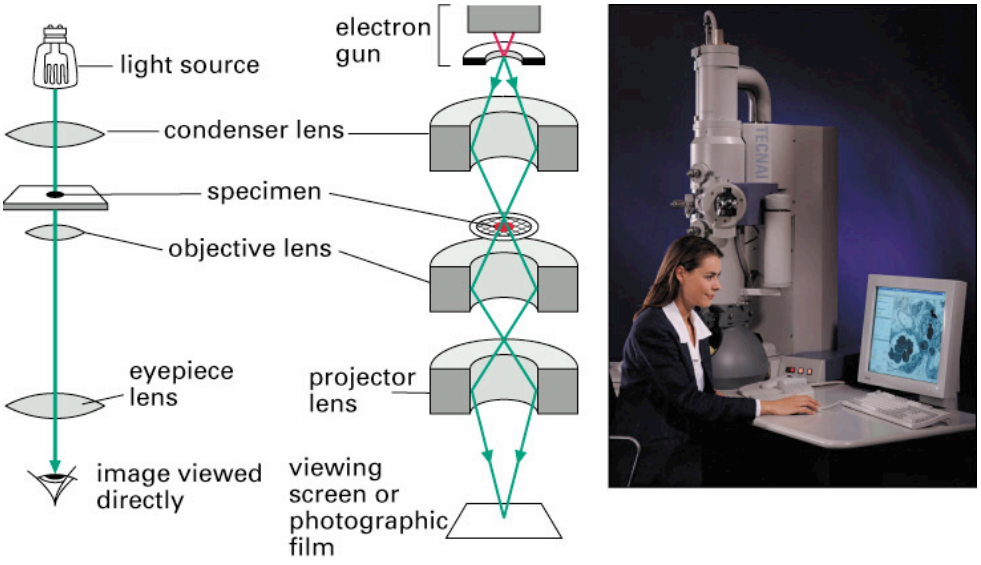
2. Electron Microscopy
-TEM (Transmission electron microscopy)
-SEM (Scanning electron microscopy)

light ***TEM***



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SEM

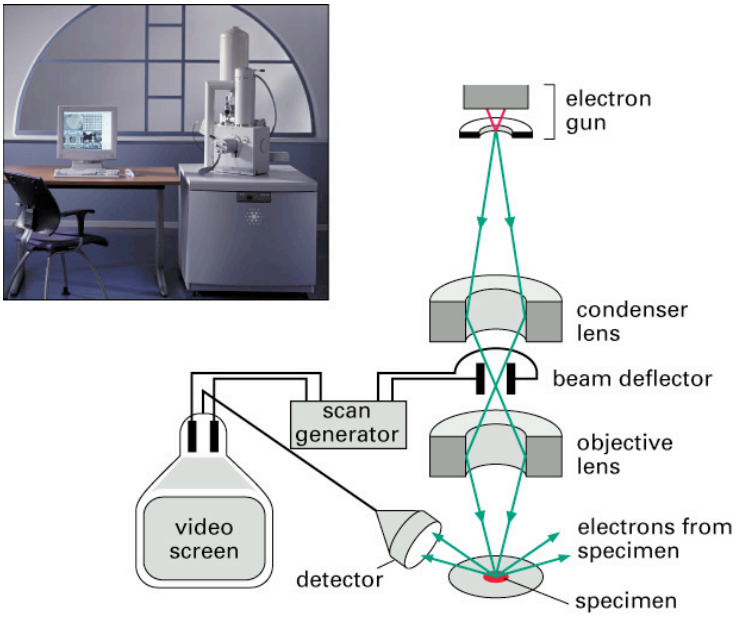
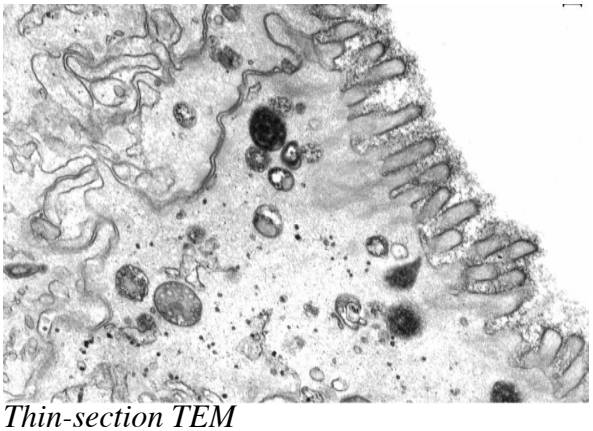


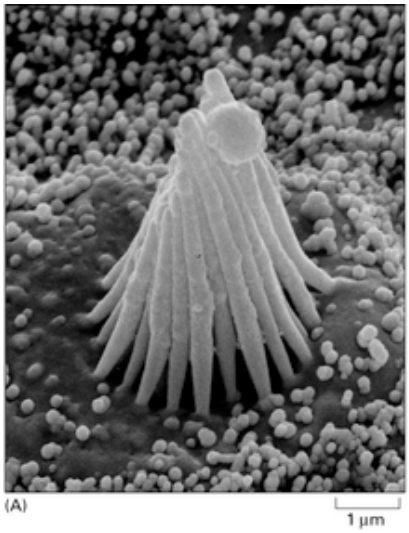
Figure 9–29. Molecular Biology of the Cell, 4th Edition.

Comparing limits of resolution

Light microscopy	Typical Wavelength	Limit of Resolution
Conventional wide field	500nm	200nm
Epifluorescence	500nm	200nm
Laser scanning confocal	500nm	200nm
Electron Microscopy		
TEM	0.01nm	1-2nm
SEM	0.01nm	10-20nm
X-Ray Crystallography		
Diffraction analysis	0.15nm	0.1nm



Thin-section TEM



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SEM

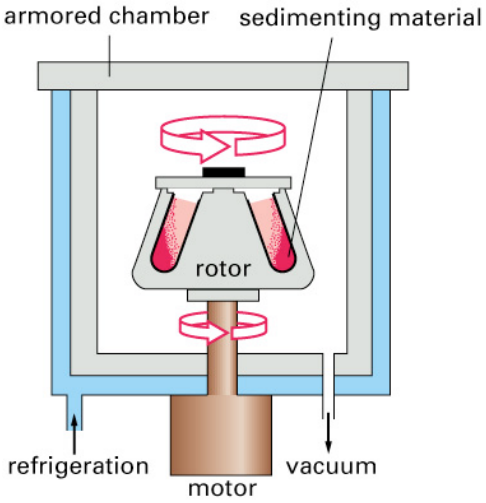
2. Isolation and growth of cells

- Direct manipulation of cells
- Cell culture: primary cultures, cell lines

3. Isolating cellular components

Cellular components can be studied in isolation
- breaking cells
- centrifugation

centrifugation



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3 methods of centrifugation:

(1) differential centrifugation

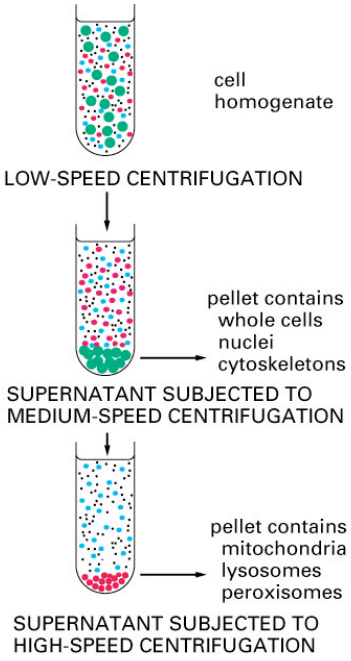
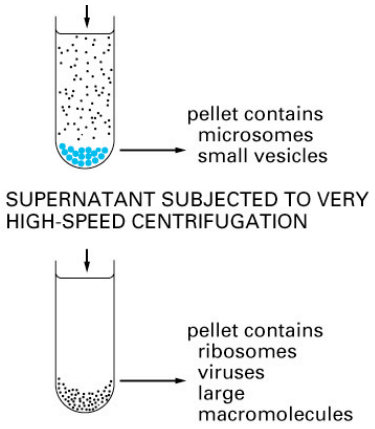


Figure 8–8 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



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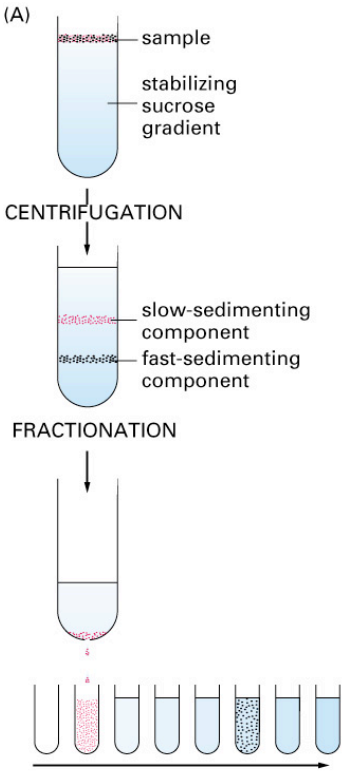
(2) velocity sedimentation



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(3) equilibrium sedimentation

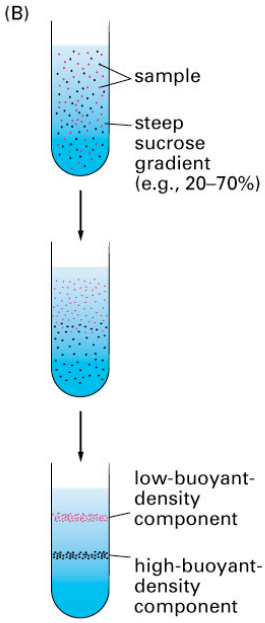

Figure 8–9 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

4. DNA, RNA & Protein methods

DNA & RNA methods such as reverse transcription, cloning, PCR, etc. learned in 103 & 220 are important tools for studying cells; will not be reviewed here. Protein methods will be discussed in the next lecture.