

# Research Techniques II: Microscopy and Cell Imaging

Seeing at the cellular and  
subcellular level

(continued...)

1

## Electron Microscopy

- Technique with a long history.
- We will discuss two major types:
  - 1) Transmission electron microscope (TEM).
  - 2) Scanning electron microscope (SEM).
- Uses bombardment of electrons rather than light.
- Advantage:

Resolution 2,000× that of LM. 0.1 nm = 1.0 Å (Ångströms). Lower resolution in some biological preparations (2 nm).
- Disadvantage:

Very time-consuming preparation. But for some experiments, this is the only appropriate technique.

2

# Transmission EM

- General configuration analogous to LM.
- Electrons emitted at filament or cathode.
- Accelerated by high voltage ( $10^5$  V) in a vacuum.
- Magnetic coils focus electron beam like a lens.
- Sample may be stained, producing “electron dense” images.

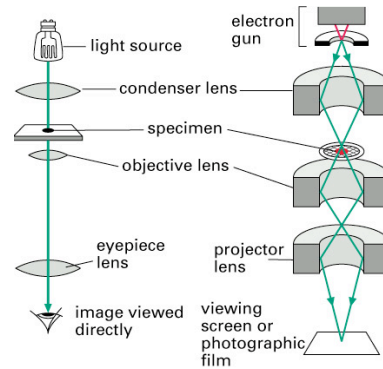


Figure 9-22. Molecular Biology of the Cell, 4th Edition.

3

# Preparation for TEM

- Lengthy procedure that takes days to weeks.
- Tissue must be fixed in *glutaraldehyde*.
- Addition of  $OsO_4$  increases electron density.
- *Dehydration* and *infiltration* with a plastic resin gives extra support.
- *Ultrathin* sections (50 – 100 nm) must be cut with a diamond knife.
- Sections cannot be handled directly.
- Placed on copper grids.

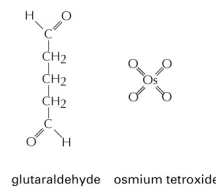


Figure 9-23. Molecular Biology of the Cell, 4th Edition.

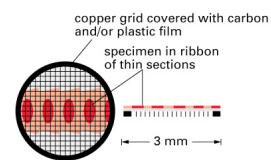


Figure 9-24. Molecular Biology of the Cell, 4th Edition.

4

# TEM Micrograph

A mitochondrion



Figure 14-8 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

A mitochondrion in serial sections

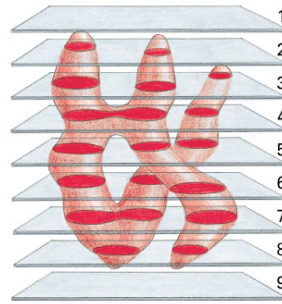


Figure 9-27. Molecular Biology of the Cell, 4th Edition.

5

# Immunogold EM

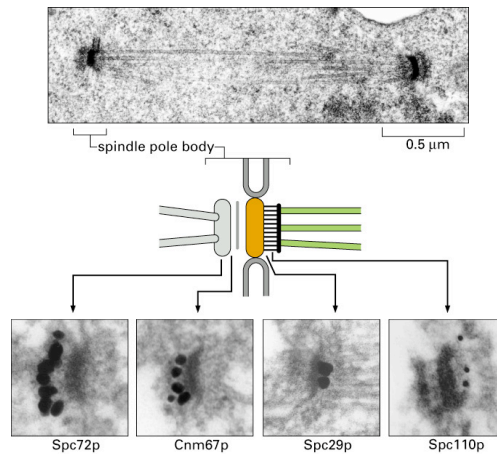


Figure 9-26 | Molecular Biology of the Cell, 4th Edition.

6

# Scanning Electron Microscope

- Produces 3D images of *surface structures*.
- Used to study whole cells and tissues, rather than intracellular structures.
- Principles of preparation and operation similar to TEM
- Cells/tissues coated with heavy metal.
- *Scattered* electrons from the specimen surface are collected.

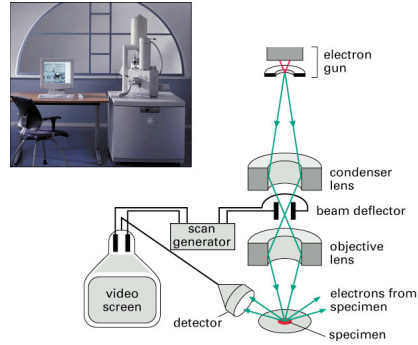
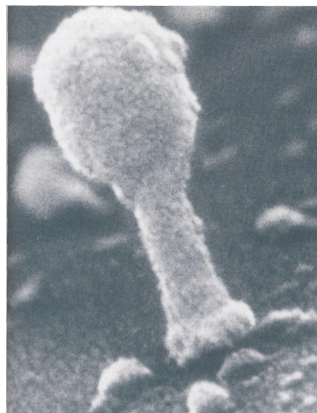


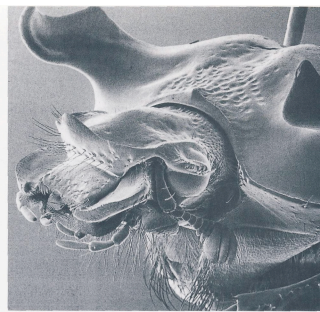
Figure 9-29. Molecular Biology of the Cell, 4th Edition.

7

# SEM Micrographs



T4 Bacteriophage  
0.1  $\mu\text{m}$

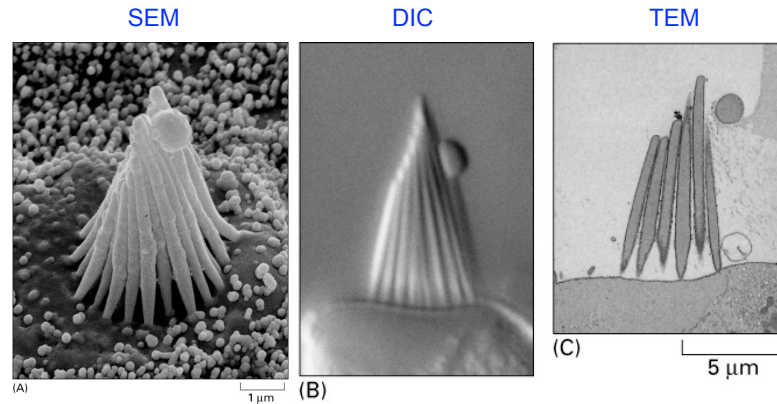


Insect head  
2.0 mm

Figure 18.19. Karp, 2008 Cell and Molecular Biology.

8

## EM and LM Compared



Stereocilia of hair cell from frog inner ear.

Molecular Biology of the Cell, 4th Edition.

9

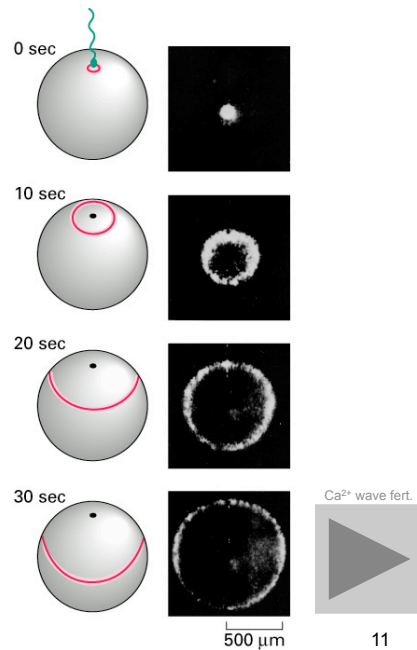
## Ion Imaging

- Changes in intracellular ion concentrations (e.g.  $\text{Ca}^{2+}$  and  $\text{H}^+$ ) are physiologically important.
- Ion-selective indicators emit light depending on local ion concentrations.
- These reveal rapid intracellular dynamics.

10

## Ca<sup>2+</sup> Imaging

- Intracellular Ca<sup>2+</sup> is low.
- Bioluminescent *aequorin* injected into a fish egg reveals Ca<sup>2+</sup> wave propagated during fertilization.
- Other synthesized indicator molecules produce signal (e.g. Fura-2, Fluo-4)



## Ca<sup>2+</sup> Imaging

- *Ratiometric* imaging exploits differential wavelengths associated with ionic binding.
- Dyes can be injected, or “AM” analogues can be used to cross cell membrane.  
= *acetoxymethyl ester*

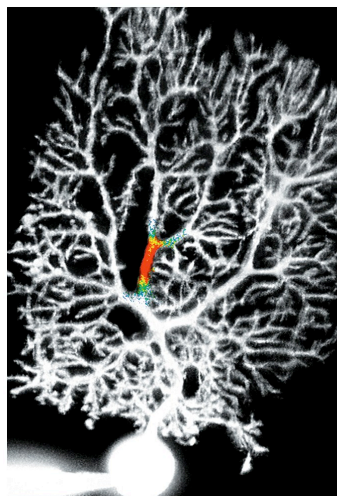


Figure 15-25 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



12

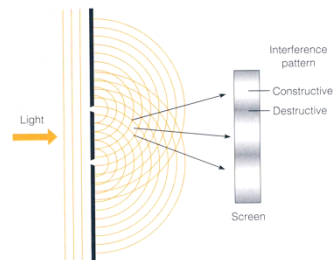
# X-ray Crystallography

‘Seeing’ at the molecular level

13

# X-ray Crystallography

- Structure - function
- Macromolecules
- Atomic resolution
- X-rays (0.1 nm)
- Crystallized proteins
- Bombardment and diffraction.
- e.g. Interference patterns



Becker. 2006 World of the Cell

Problems:

- Time-consuming (first structure...22 yrs!).
- Large amount of material required.
- Insoluble protein crystallization (e.g. membranes)?

14

# X-ray Crystallography

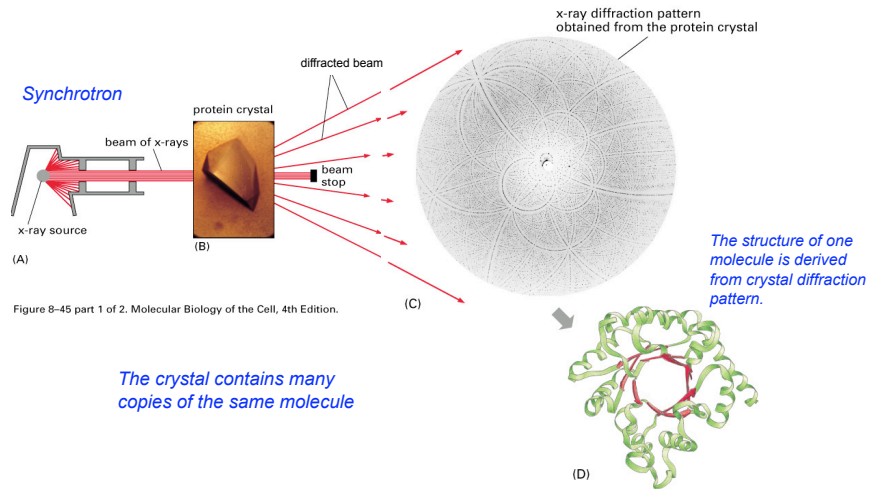
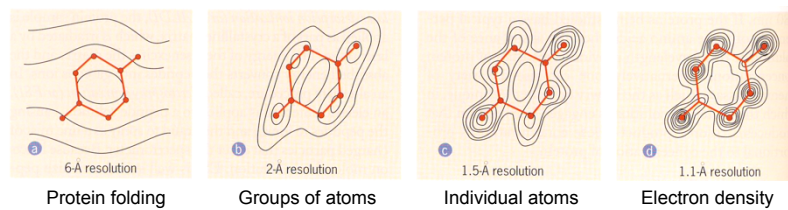


Figure 8-45 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 8-45

Molecular Biology of the Cell, 4th Edition.

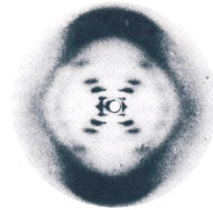
# X-ray Crystallography



Karp. 2008. Cell and Molecular Biology

# X-ray Crystallography

- Diffraction pattern
- Space-filling model of DNA double helix.



From Becker. 2006 World of the Cell

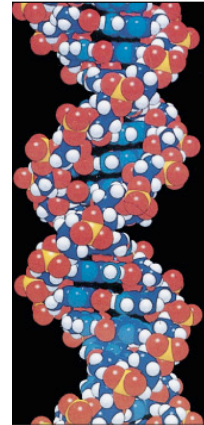


Fig. 4-5A. Alberts et al.

Nobel Prize 1962



Francis Crick

James Watson

Maurice Wilkins

17

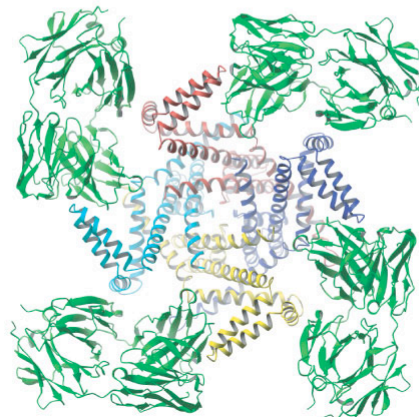
# X-ray Crystallography

- Structure of K<sup>+</sup> ion channel by X-ray crystallography.
- Monoclonal antibodies (green) used to “hold” protein.

Nobel Prize 2003



Roderick MacKinnon



3.2 Å resolution

Jiang et al. 2003. *Nature* 423:33-41.

18

## Things to Consider...

1. Think about applications in Cell Biology in which specific techniques of microscopy are most appropriate. For example, would you use an electron microscope to study intracellular  $\text{Ca}^{2+}$  dynamics?
2. What are the major differences between each technique that we've discussed? For example, use of light or electrons, linear vs. non-linear, advantages and disadvantages.