Introduction to Electron Microscopy

1. Orientation of the course
2. Freeze-fracture

9:10 - 9:20 KS Lu, Orientation

9:20 - 10:20 SM Wang, Buffer & rotary shadowing

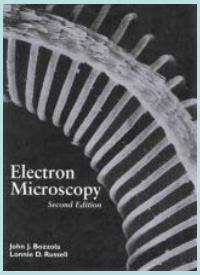
10:30 – 11:20 KS Lu, Introduction to EM, Freeze-fracture

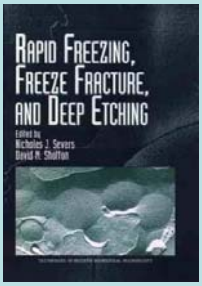
11:20-12:10 CL Chien, Fixation & perfusion

Reference:

Bozzola J.J. and Russel L.D. (1999) ***Electron microscopy Principles and Techniques for Biologists.***

Second edition, Jones and Bartlett Publisher, Sudbury, Massachusetts, USA



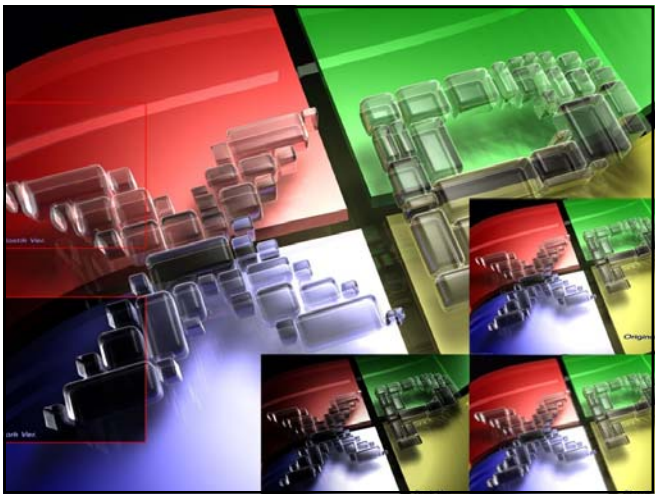


Electron Microscopy: Principles and Techniques for Biologists. second edition, By John J. **Bozzola** and Lonnie D. **Russell**, 1999, Jones and Bartlett Publishers, Inc.

Rapid Freezing, Freeze Fracture and Deep Etching (*Techniques in Modern Biomedical Microscopy 5)* By: **Severs,** Nicholas J.; **Shotton,** David M. 03/10/1995 John Wiley & Sons Inc

Schedule for Hand-in Your Work

- 5/25 Reading micrograph
- 5/18 Photographic and Darkroom techniques
- 5/04 Electron microscope operation
- Specimens: **brain, kidney, liver**
- 5/04 Thick sections on slide (at least three sctions stained with toluidine blue)
- 5/04 Thin sections on grids (at least two grids, stained with uranyl acetae & lead citrate)
- 5/25 Three EM pictures from your own specimens



Guidelines

- Technique-oriented, Practice-oriented
- **Safety (under supervision), Efficient, Work hard,**
- Take notes in every step,
- You must go through all the different procedures by yourself,
- Do all the work by your own,
- Hand in all the work on time

Linear Equivalents		
1	Angstrom	= 0.1 nanometer (nm)
10	Angstroms	= 1.0 nanometer [formerly millimicron (m μ)]
1000	nanometers	= 1.0 micrometer (μ m) [formerly micron (μ)]
1000	micrometers	= 1.0 millimeter (mm)
1000	mililimeters	= 1 meter (m)

1. Resolution (resolving power)
2. *Numeral Aperture*
3. Focal depth
4. Field depth

Resolution of eye versus instrument	
	Distance between Resovable points
Human naked eyes	0.2 mm
Birght field microscope	0.2 μm
SEM	0.2 nm
TEM	0.2 nm
Theoretical	0.05 nm
Tissue section	1.0 nm

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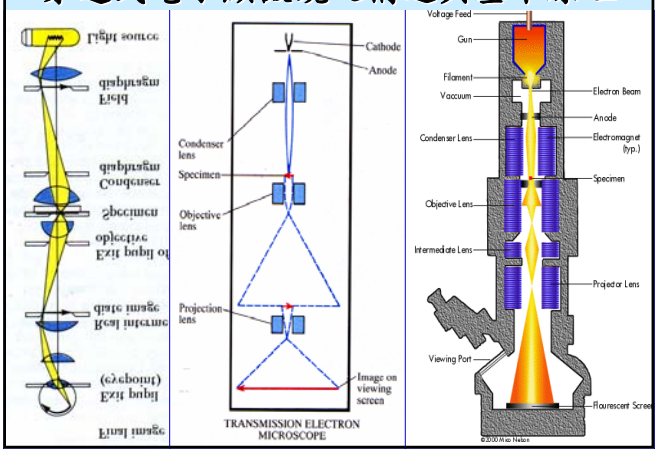
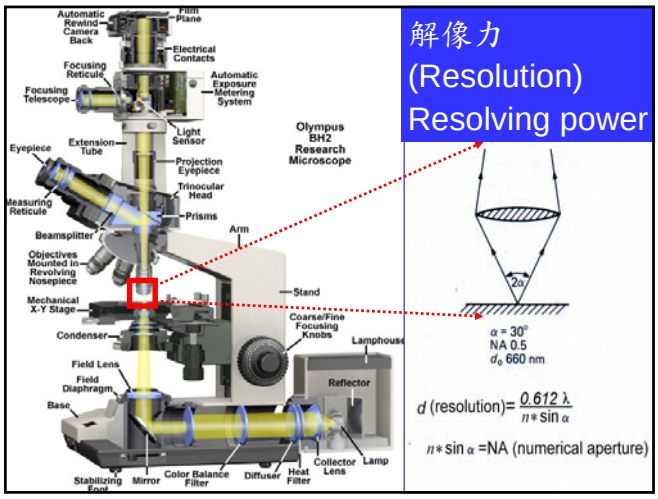
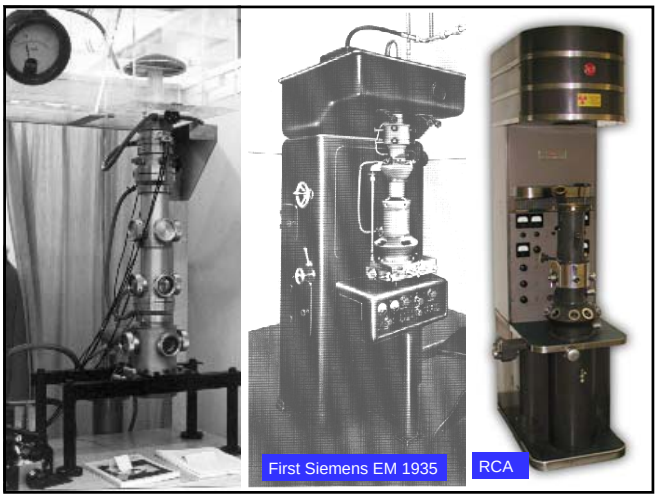
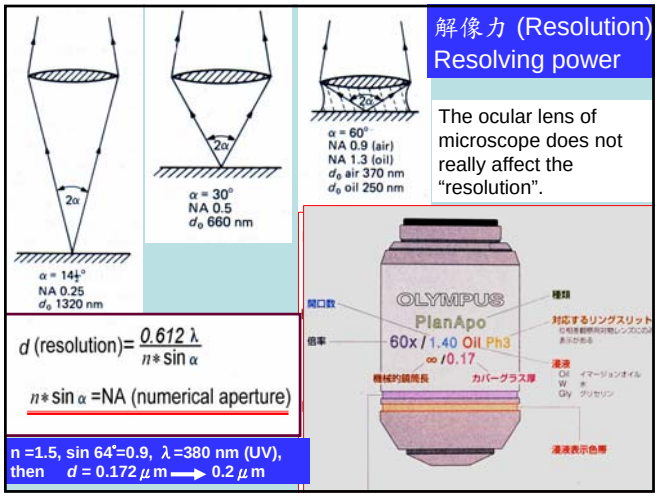
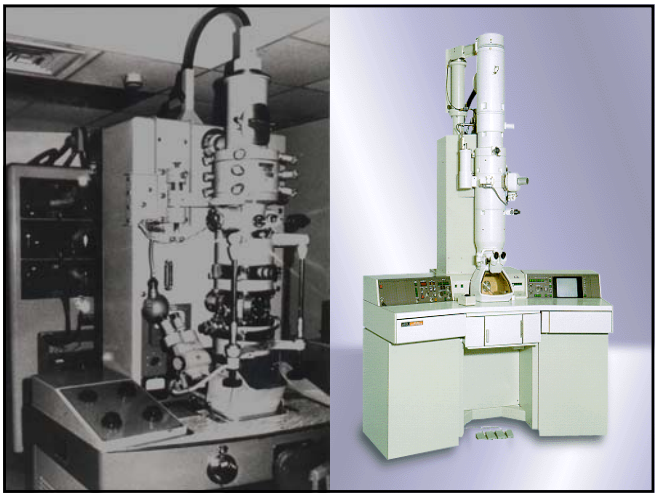
穿透式電子顯微鏡之構造與基本原理



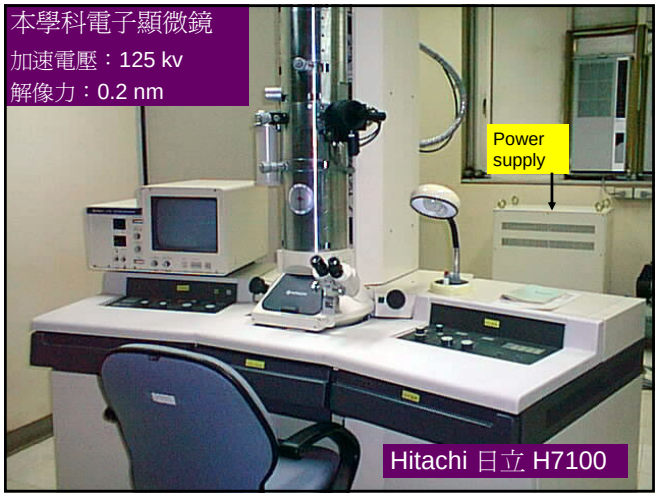


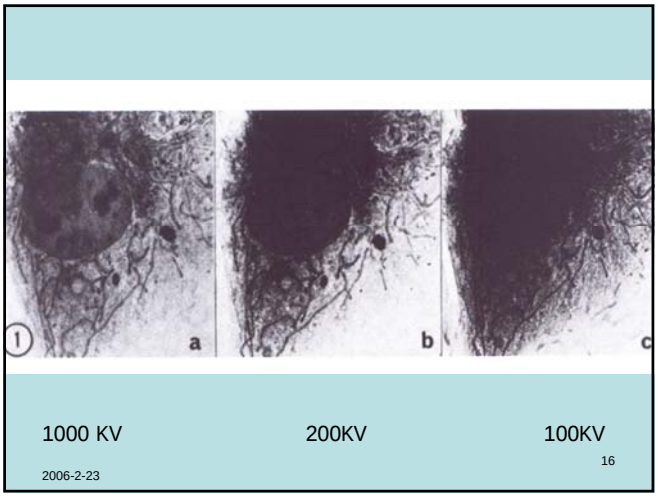






本學科電子顯微鏡
加速電壓：125 kv
解像力：0.2 nm





Ultra-High voltage Electron Microscope
(1 MV, 1000KV)

University of Colorado, Boulder, Colorado

超高圧電子顕微鏡
JSM-ＡＲＭ1300/1000

◆点分解能：
0.10nm (1,300kV)/
0.13nm (1,000kV)
◆加速電圧：
200～1,300kV
(1,000kV)
◆倍率：x150～1,500,000

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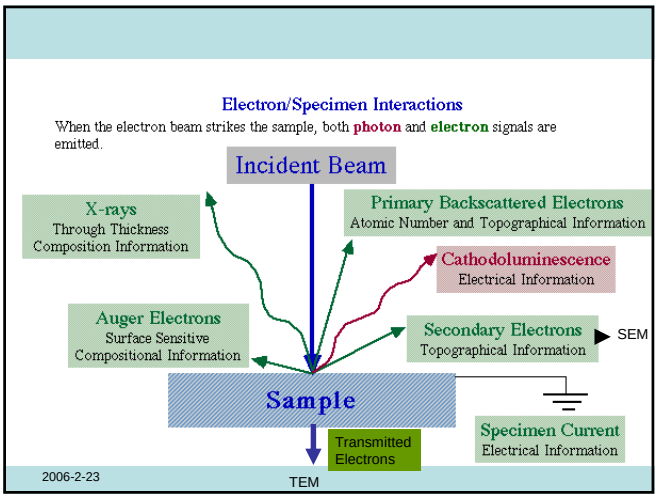
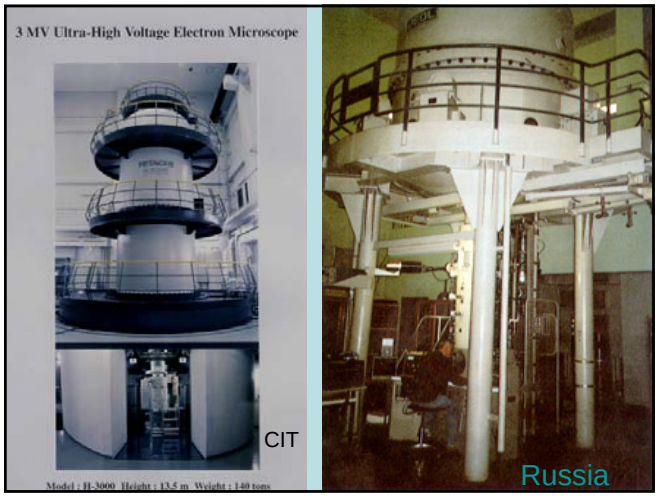
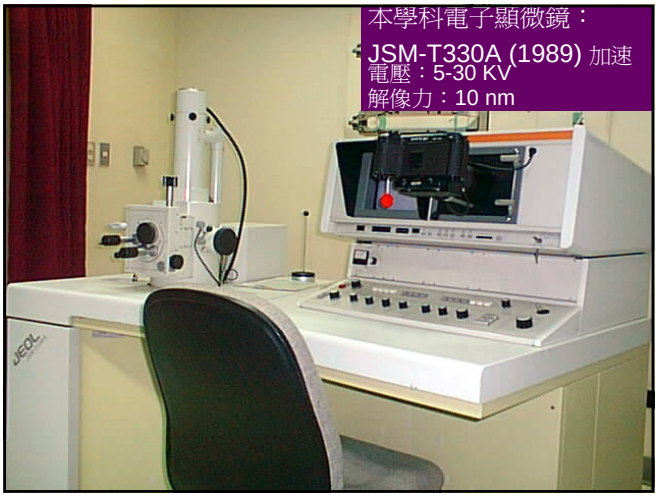


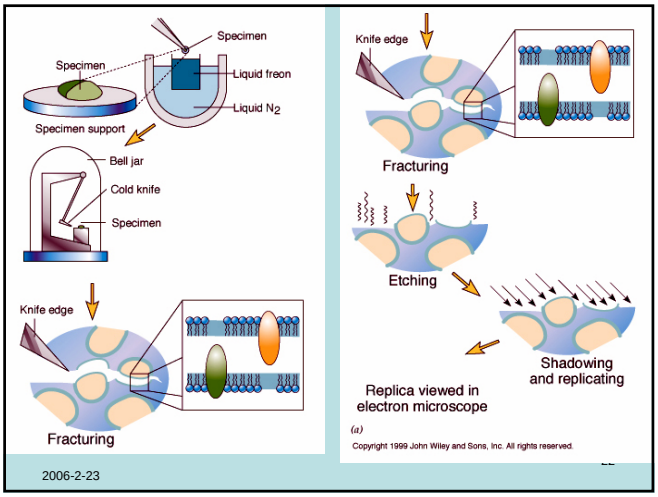


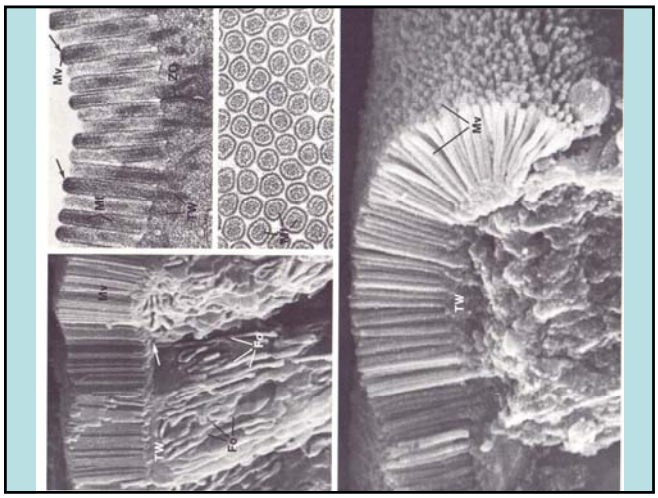


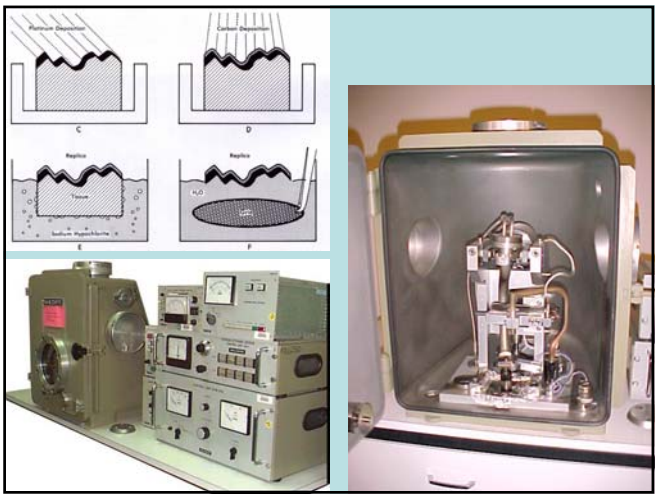
◆分解能：
0.6nm
◆加速電圧
0.5～30
◆倍率：
x10～
950,000
◆試料寸法
23mm(長さ
×
6mm(幅)x
3mm(高さ
◆試料移動
X方向
24mm、
Y方向8mm
Z方向0.6mm
傾斜±30°

Field Emission 走查電子顕微鏡

JSM-6000F

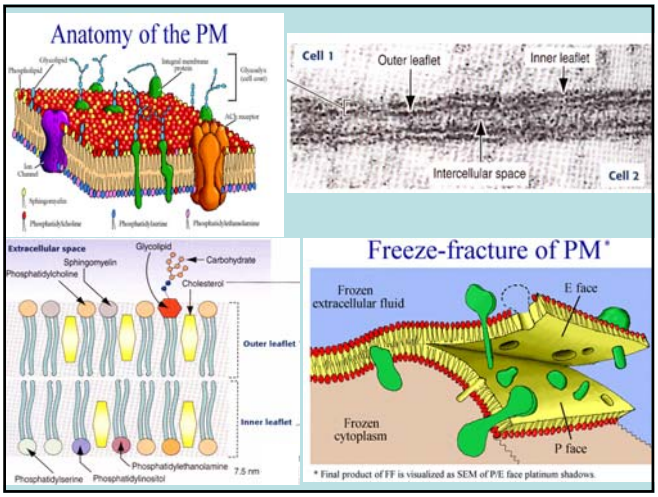






Freeze Fracture Technique

1. Fixation
2. Cryoprotection
3. Freezing
4. Freeze-fracturing
5. Shadowing
6. Cleaning (of replica)
7. TEM observation



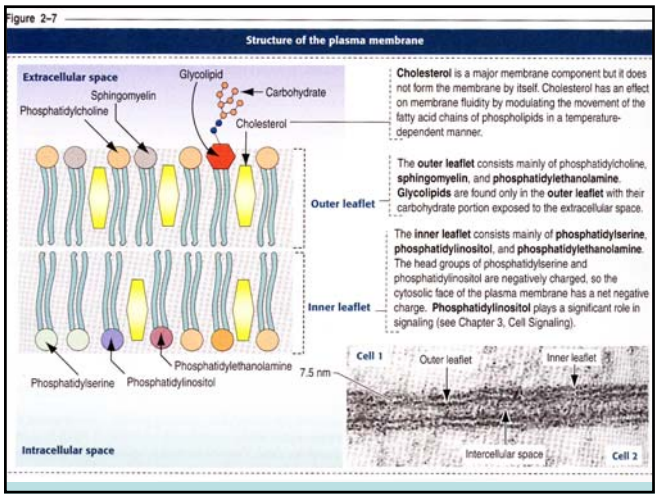
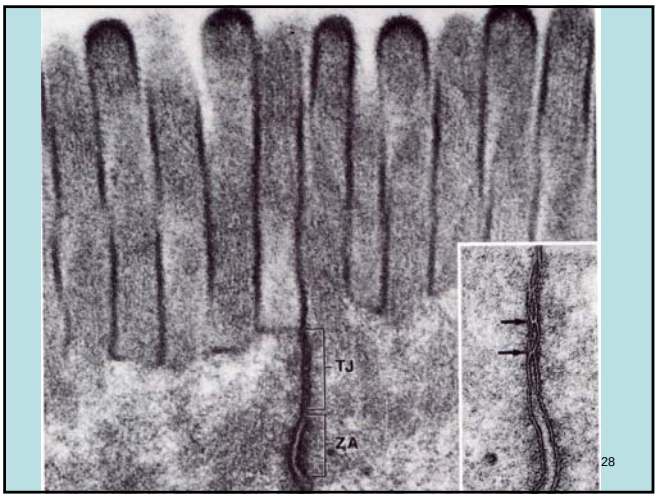


Figure 2–7
Structure of the plasma membrane
Extracellular space
Glycolipid
Sphingomyelin
Carbohydrate
Phosphatidylcholine
Cholesterol
Cholesterol is a major membrane component but it does not form the membrane by itself. Cholesterol has an effect on membrane fluidity by modulating the movement of the fatty acid chains of phospholipids in a temperature-dependent manner.
Outer leaflet
The outer leaflet consists mainly of phosphatidylcholine, sphingomyelin, and phosphatidylethanolamine. Glycolipids are found only in the outer leaflet with their carbohydrate portion exposed to the extracellular space.
Inner leaflet
The inner leaflet consists mainly of phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine. The head groups of phosphatidylserine and phosphatidylinositol are negatively charged, so the cytosolic face of the plasma membrane has a net negative charge. Phosphatidylinositol plays a significant role in signaling (see Chapter 3, Cell Signaling).
Phosphatidylethanolamine
Phosphatidylserine
Phosphatidylinositol
7.5 nm
Cell 1
Outer leaflet
Inner leaflet
Intercellular space
Cell 2
Intracellular space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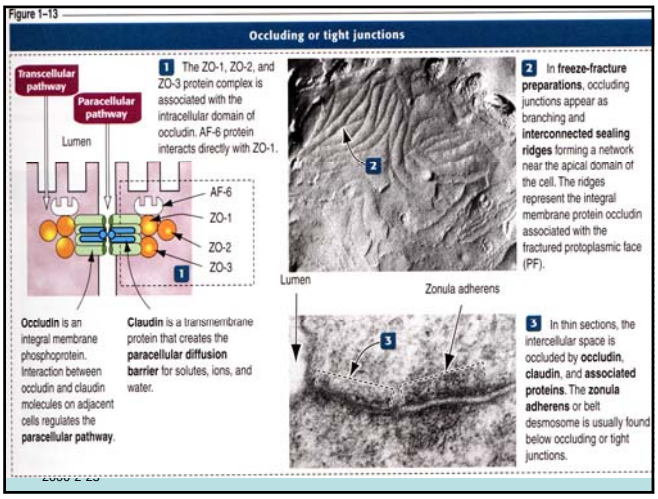
Figure 1–13
Occluding or tight junctions
Transcellular pathway
Paracellular pathway
Lumen
1 The ZO-1, ZO-2, and ZO-3 protein complex is associated with the intracellular domain of occludin. AF-6 protein interacts directly with ZO-1.
AF-6
ZO-1
ZO-2
ZO-3
Occludin is an integral membrane phosphoprotein. Interaction between occludin and claudin molecules on adjacent cells regulates the paracellular pathway.
Claudin is a transmembrane protein that creates the paracellular diffusion barrier for solutes, ions, and water.
2 In freeze-fracture preparations, occluding junctions appear as branching and interconnected sealing ridges forming a network near the apical domain of the cell. The ridges represent the integral membrane protein occludin associated with the fractured protoplasmic face (PF).
Lumen
Zonula adherens
3 In thin sections, the intercellular space is occluded by occludin, claudin, and associated proteins. The zonula adherens or belt desmosome is usually found below occluding or tight junctions.

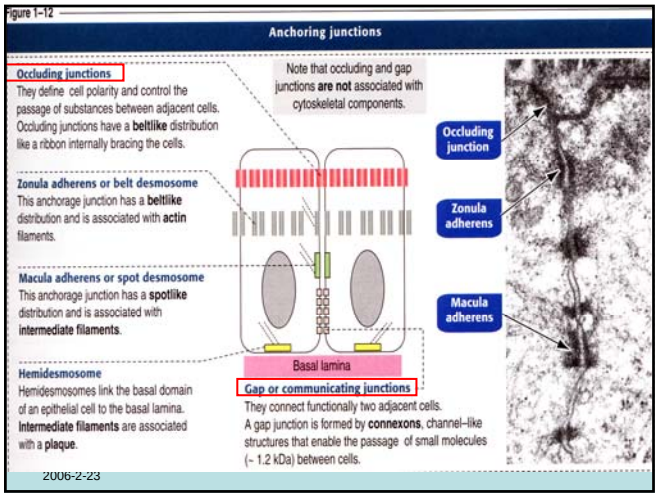
Figure 1–12
Anchoring junctions
Occluding junctions
They define cell polarity and control the passage of substances between adjacent cells. Occluding junctions have a beltlike distribution like a ribbon internally bracing the cells.
Note that occluding and gap junctions are not associated with cytoskeletal components.
Occluding junction
Zonula adherens or belt desmosome
This anchorage junction has a beltlike distribution and is associated with actin filaments.
Zonula adherens
Macula adherens or spot desmosome
This anchorage junction has a spotlike distribution and is associated with intermediate filaments.
Macula adherens
Basal lamina
Hemidesmosome
Hemidesmosomes link the basal domain of an epithelial cell to the basal lamina. Intermediate filaments are associated with a plaque.
Gap or communicating junctions
They connect functionally two adjacent cells. A gap junction is formed by connexons, channel-like structures that enable the passage of small molecules (~ 1.2 kDa) between cells.
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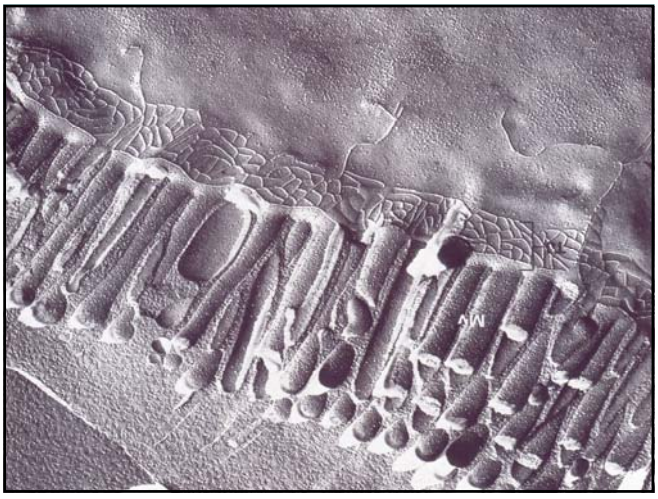

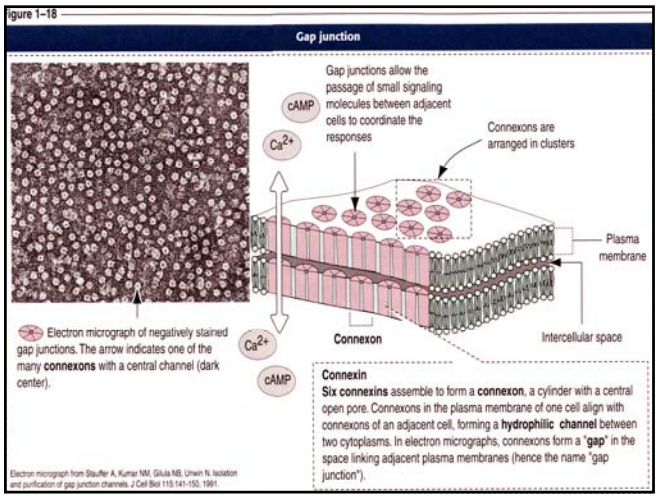

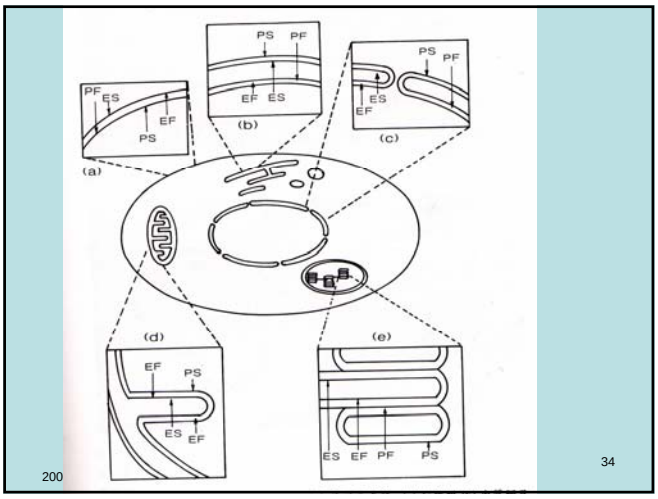

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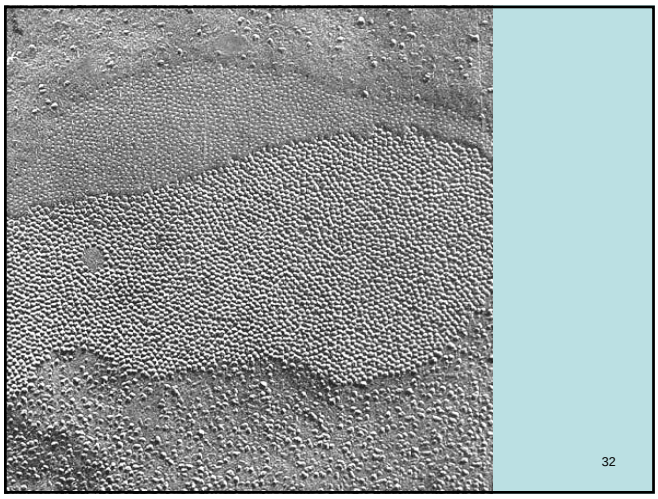

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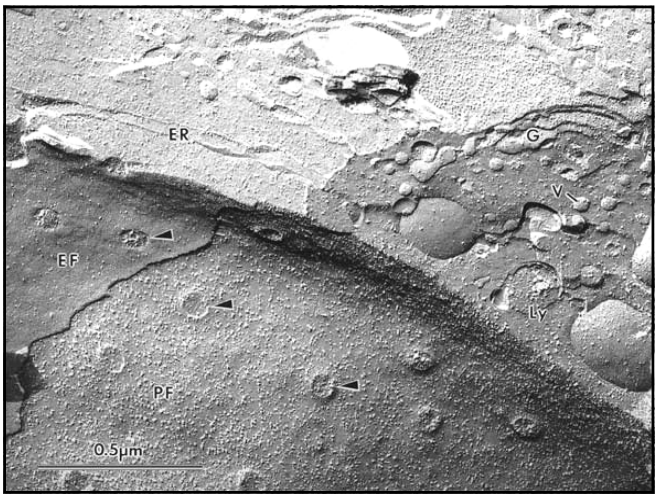

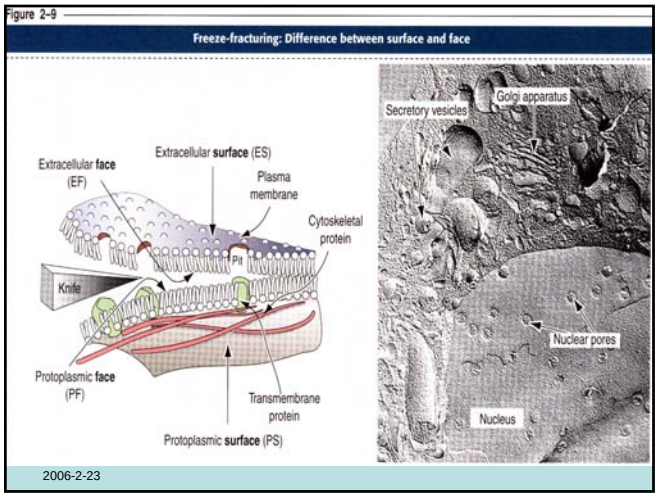

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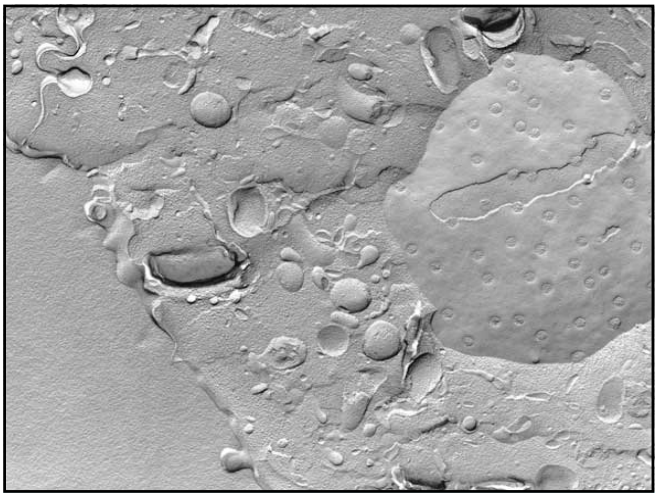

Fracture Plane Is the Only Plane and Intramembranous:

A. Intramembranous particles (IMP), 8.5nm in diameter

B. The membrane fracture face:

1. complementary replica
2. surface labelling - ferritin and *freeze-etching*
3. thin sections

C. Particles in membranes:

1. lack of E-face pit
2. the nature of the particles

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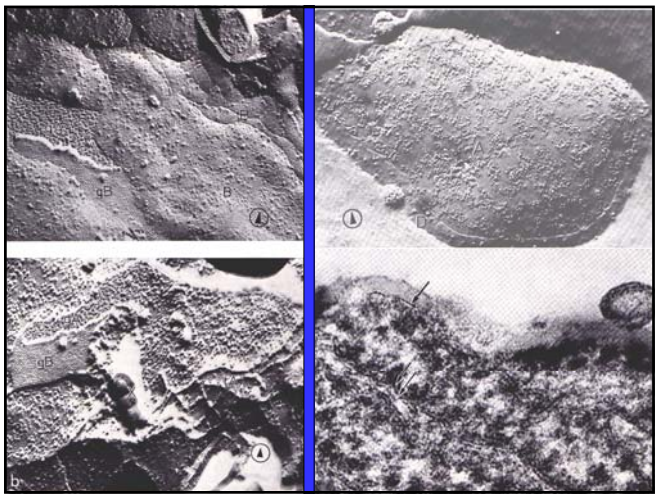

summary

- Guidelines of the course
- Resolution of microscope --
- EM basic principle – TEM (HVEM), SEM
- Freeze-fracture
  - Procedures,
  - P-face, E-face and P-surface and E-surface
  - Anchoring junctions,
    - Tight junction – strand and groove
    - Gap junction – connexons,
  - Fracture plane is unique and intramembnranous

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