# I. Light Microscopy (Histology)

A. Definition

B. Resolution of light microscope

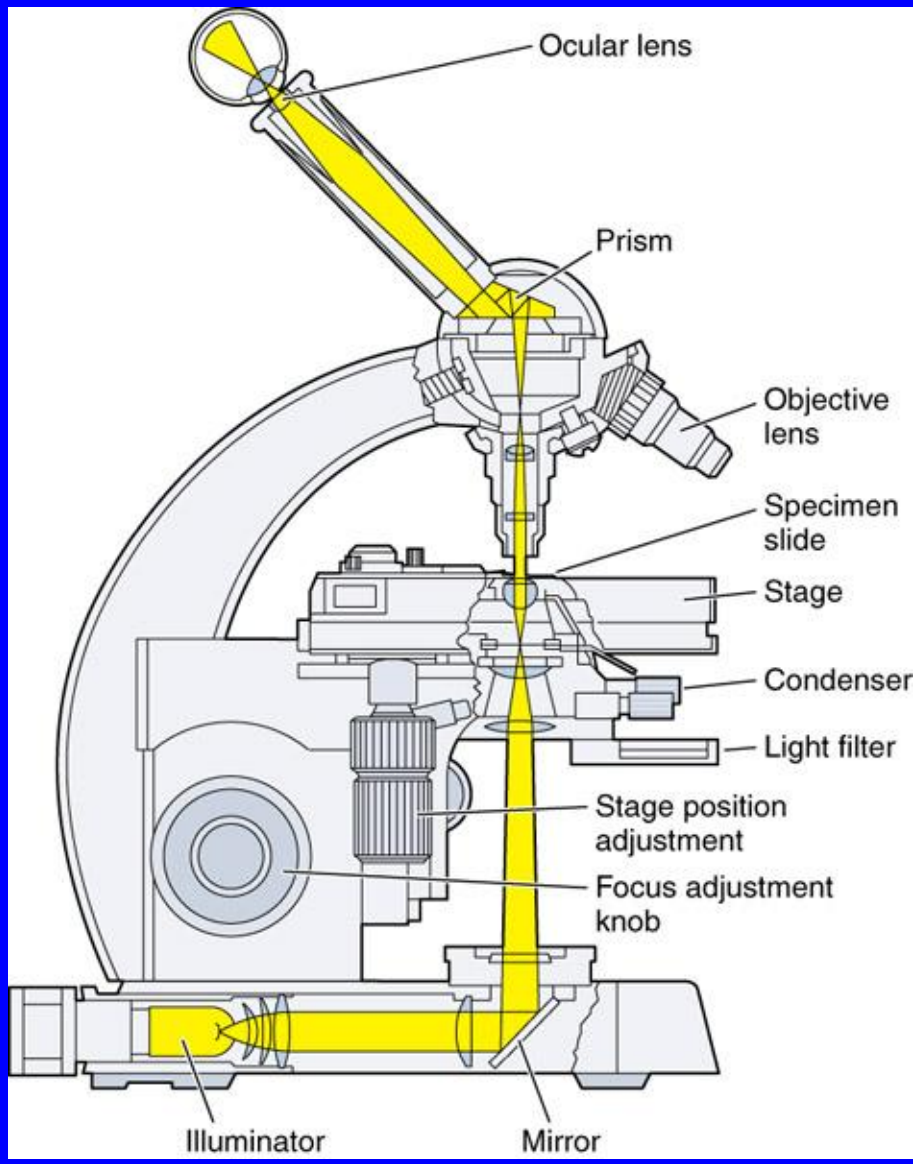
1. 0.2 μm

2. units of measurement

a. mm, μm, nm, A

C. Translucent specimen

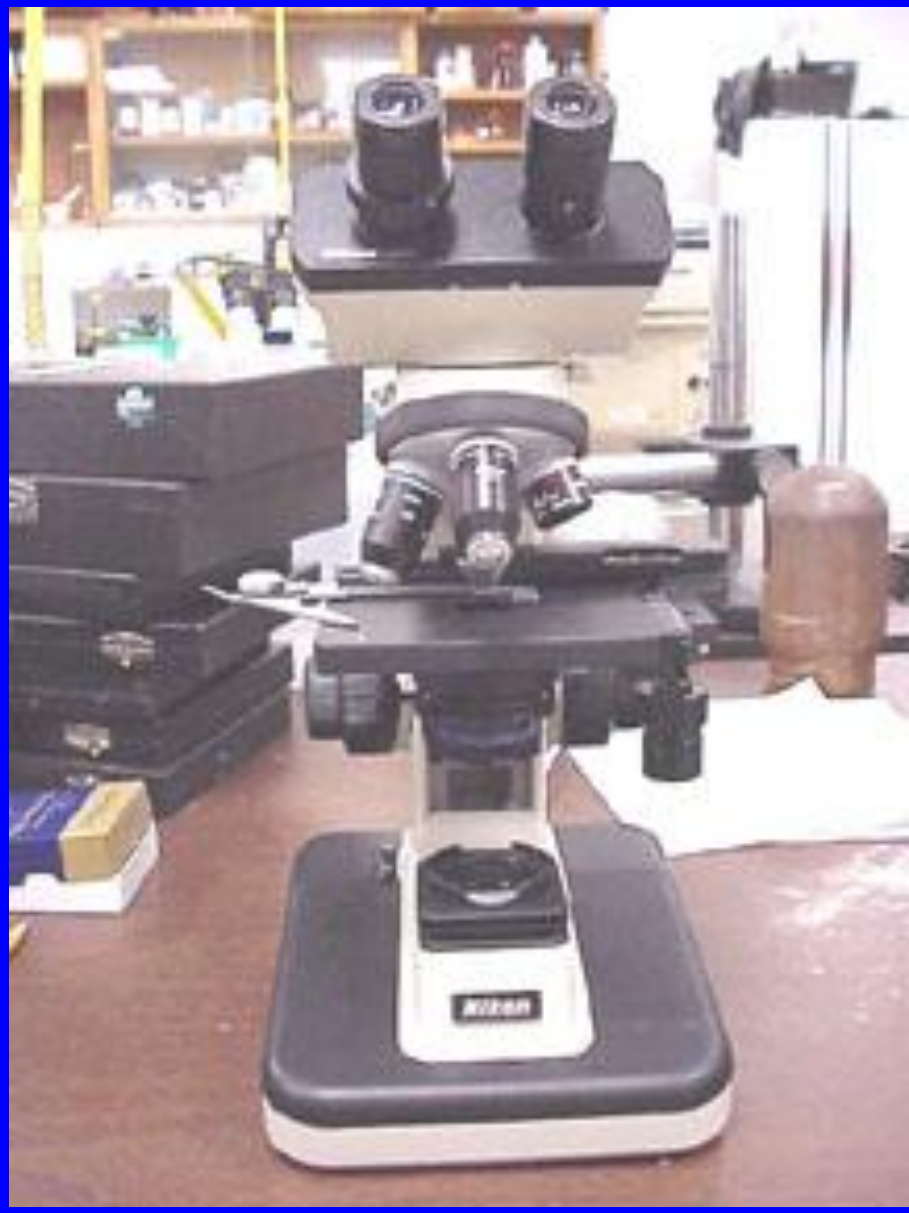
[Resolution of human eye ~100 μm]



# I. Light Microscope

D. Nikon LM

1. 10X oculars, width adjustment
2. Nosepiece
3. 4,10,40,100X objectives
4. Mechanical stage
5. Condensor lens
6. Condensor aperture
7. Light source, rheostat
8. Coarse & fine focus



# I. Light Microscope

E. 5-Headed LM

1. Located in back hallway
2. Available 8-5, M-F, first-come, first-served
3. Do not remove slide box
4. HAS field iris diaphragm
5. HAS separate power supply
6. HAS lighted pointer

# I. Light Microscope

F. 2-Headed LM

1. Located in Rm 6128
2. Available 8-5, M-F
3. Do not remove slide box
4. Has field iris diaphragm
5. Has lighted pointer
6. Has video camera and computer with frame grabber to view/print/save images (not for student use)
7. Priority use for tissue processing lab

# Tissue Processing

# Rgistration

- **Lab.no, Date, Name of patient, age, Sex, Occupation, address, reffereing doctor**

# II. Fixation: preservation of tissue structure

A. Avoid autolysis

B. Common fixatives:

1. formaldehyde,buffered formal-saline 10%
2. glutaraldehyde: for EM
3. 90-100% alcohol: suitable for cytology
4. heat: boiling water, microwave

# The purposes of fixation are

- **A.to inhibit autolytic enzymes and kill microorganisms of decomposition .**
- **B.to preserve tissue as nearly as possible in its original form.**
- **C.to protect tissues against subsequent damage during embding.**

# The purposes of fixation are-continue

D. to give tissue a texture which permits easy sectioning .

E. to render the various constituents receptive of the proposed stains

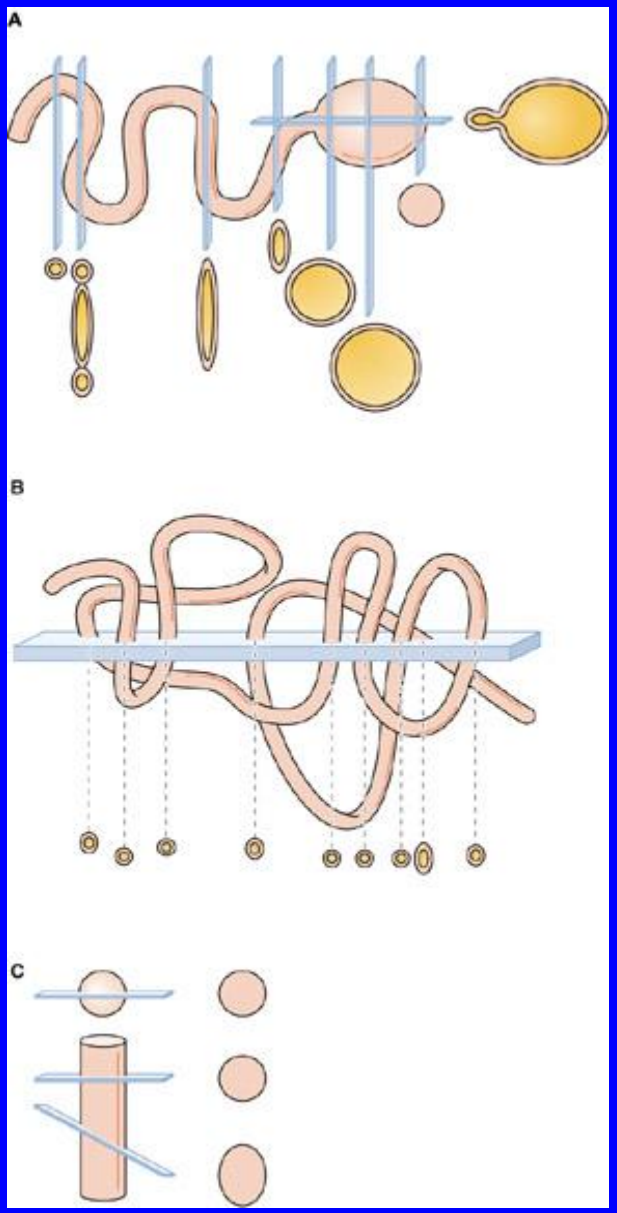
- **Fixation of tissues with different types of fixatives depend on tissues, large specimen should be sliced . loose tissues max. 10 mm penetration , compact tissue max. penetration 5mm, hollow organs injected or packed with wool socked in formalin .**

# Gross examination

- Includes description of the specimen : weight , dimensions , color , texture , cutsection , followed by photography

- Tissue dissection and taking representative sections followed by labeling .
- The pieces taken will placed in tissue cassette .
- Transfer casssette either to automatic tissue processoe or to jars (in case of manual tissue processing )

# Tissue dissection



# III. Dehydration

A. Definition: removal of water

B. Rationale: for paraffin embedding/sectioning

C. Steps

1. wash out fixative
2. graded series of alcohol
    a. 70%, 95%, 100%, 100%
3. replace water by diffusion
4. not too long, not too short

# III. Dehydration

D. Procedure

1. automatic tissue processor

   a. overnight

2. Baths: water, 70,95,100,100 % alcohol

3. Clearing agent: 2 baths of xylene

# IV. Clearing

A. Paraffin solvent

B. Xylene, “clearing agent”

C. Makes tissue appear “clear”

# V. Infiltration

A. Replace xylene with paraffin

B. Immerse in melted paraffin

1. ~55$^{o}$ C MP

C. Remove all bubbles, xylene

D. Procedure

1. Two baths of melted paraffin

# VI. Embedding

A. Orient tissue

1. cross section
2. longitudinal section

B. Dissection orientation

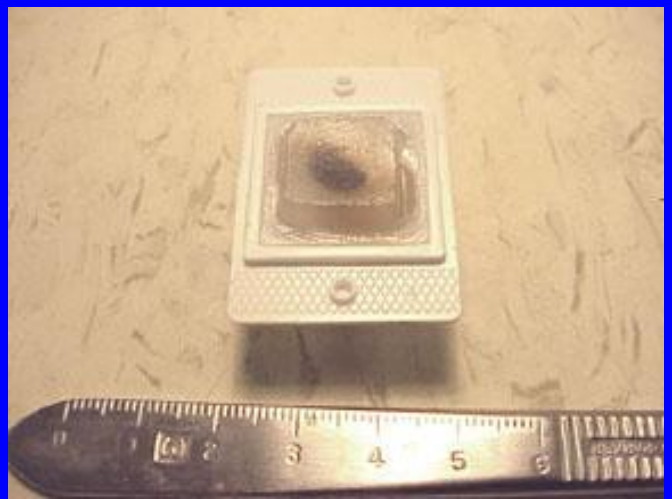
C. Avoid bubbles

Fig. 1-30

# VI. Embedding

## D.Procedure

1. Place tissue cassette in melted paraffin
2. Fill mold with paraffin
3. Place tissue in mold
4. Allow to cool



# VII. Sectioning – Trimming the Block

Untrimmed tissue block

Trimmed block with
excess paraffin
removed and block face
in a trapezoid shape

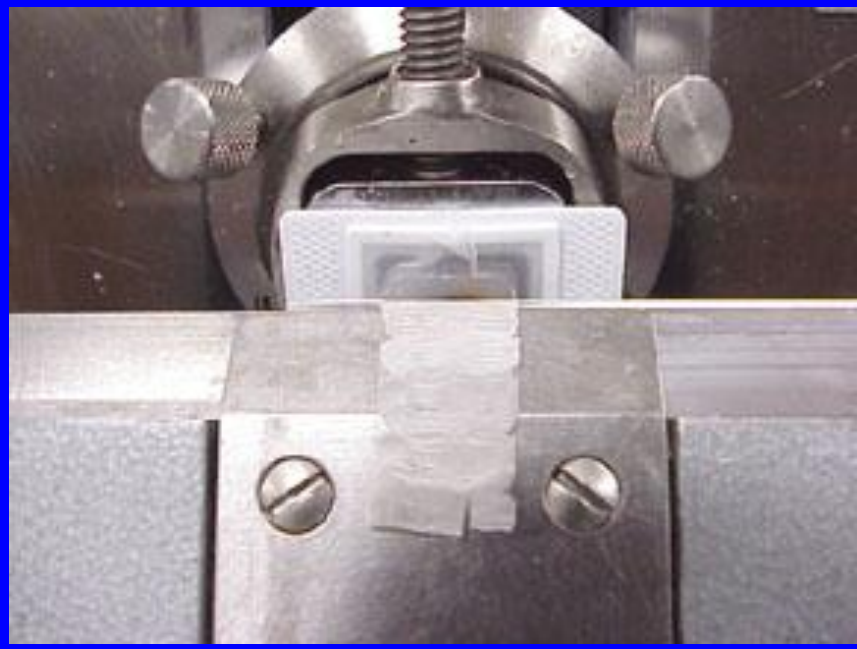
# VII. Sectioning

A. Rotary microtome
   1. 5-10 μm
   2. resolution vs. staining
B. Cryostat
C. Freezing microtome
D. Vibratome

# VII. Sectioning

## E. Procedure

1. Place tissue block in microtome with wide edge of trapezoid lowest, and parallel to knife
2. Advance blade toward block



# VII. Sectioning

NOTE: Many of the figures in the text are of plastic embedded sections cut at 1 μm thickness, and thus showing better resolution than 5-10 μm paraffin sections seen in lab.

# VIII. Mounting sections

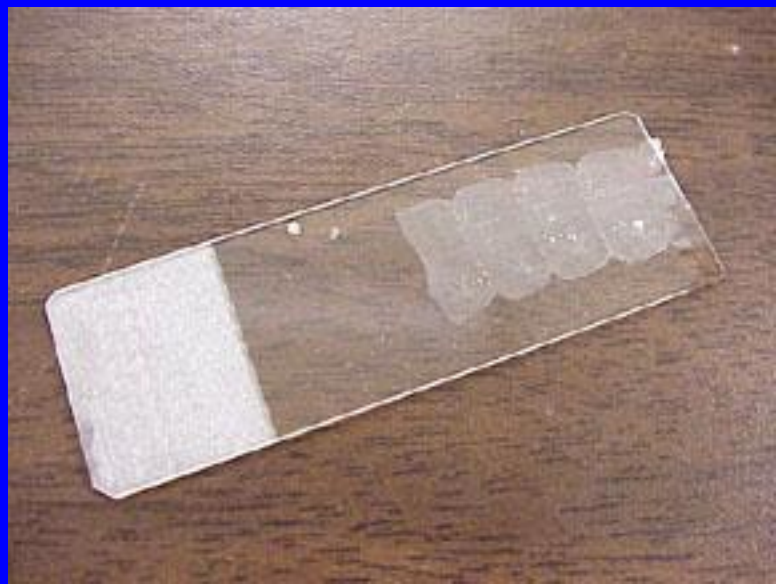
A. 40$^{o}$ C water bath

1. Flattens paraffin section
2. Permits mounting on slide

B. Gelatin & albumin

C. Glass slides

D. Oven / air dry



# IX. Staining

A. Basic dye: hematoxylin

1. basophilic structures: DNA, RNA
2. differentiation: sodium bicarbonate

B. Acid dye: eosin

1. acidophilic (eosinophilic) structures
   a. mitochondria, collagen

C. Water soluble dyes (paraffin sections)

D. Clearing agent (remove paraffin)

E. Rehydrate

F. Stain (trial & error timing)

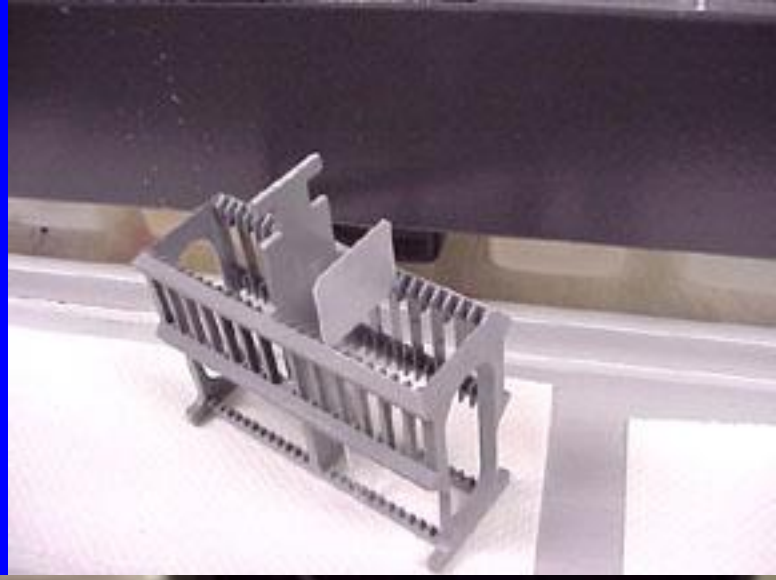
# IX. Staining

Most of the stains are water soluble and don't mix with paraffin , so staining should started with de-waxing by using solvents (Xylene). Then rehydration of tissue using descending concentrations of alcohol (100%, 90%,70%,  water )

# IX. Staining

G. Procedure

1. Slide rack
2. Solutions
   a. rehydration
   b. stain
   c. dehydration



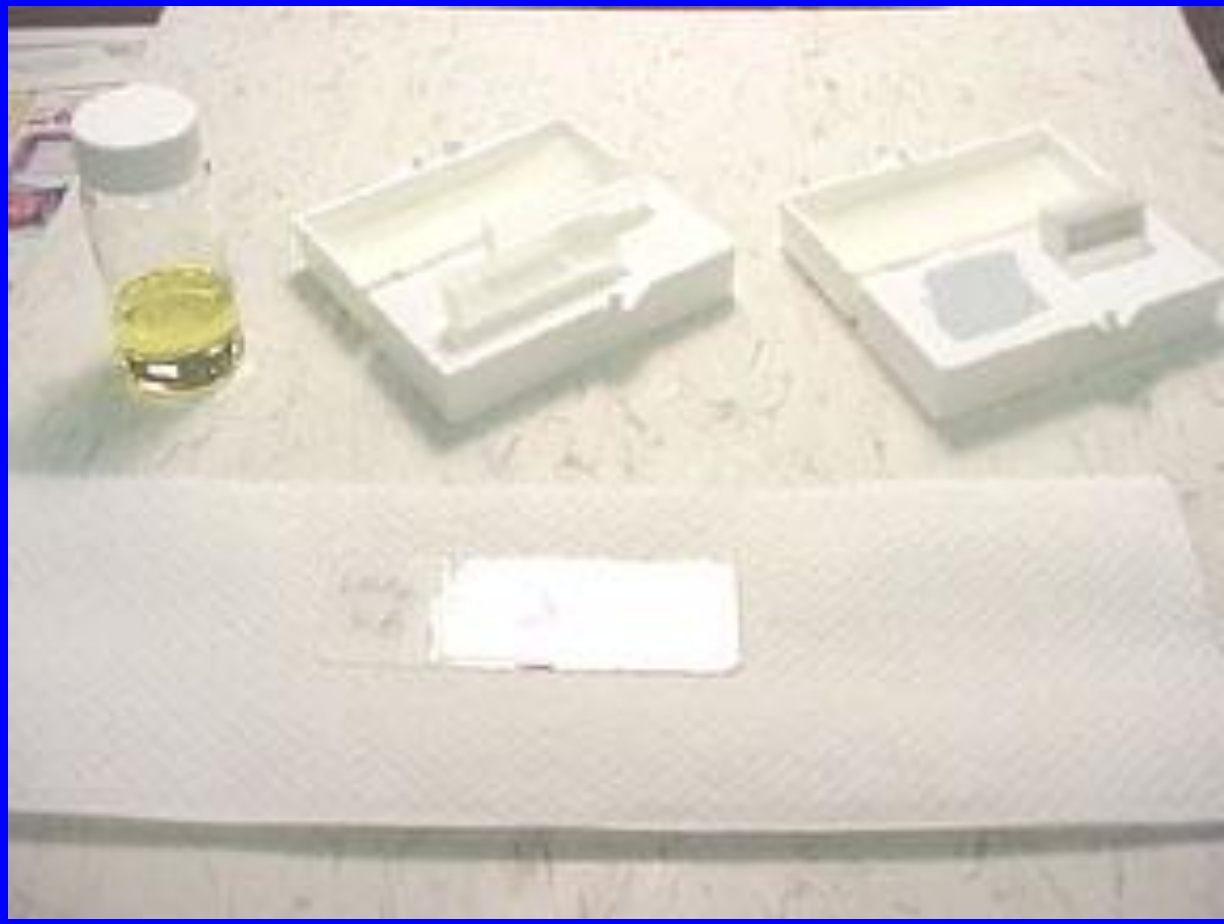
# X. Coverslipping

A. Coverslip & mounting medium (not miscible with water)

B. Dehydrate

C. Clearing agent

D. Permount



# XI. Pitfalls

A. Poor fixation (poor structural details)

B. Inadequate dehydration

C. Contaminated xylene (milky)

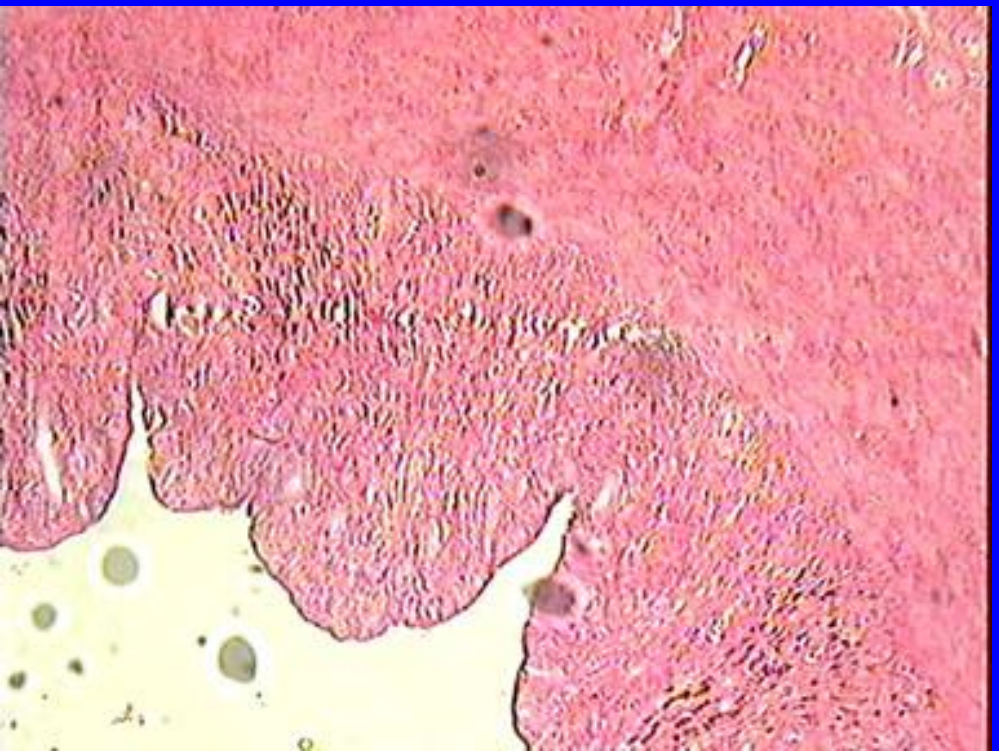
D. Poor infiltration (bubbles, poor support)

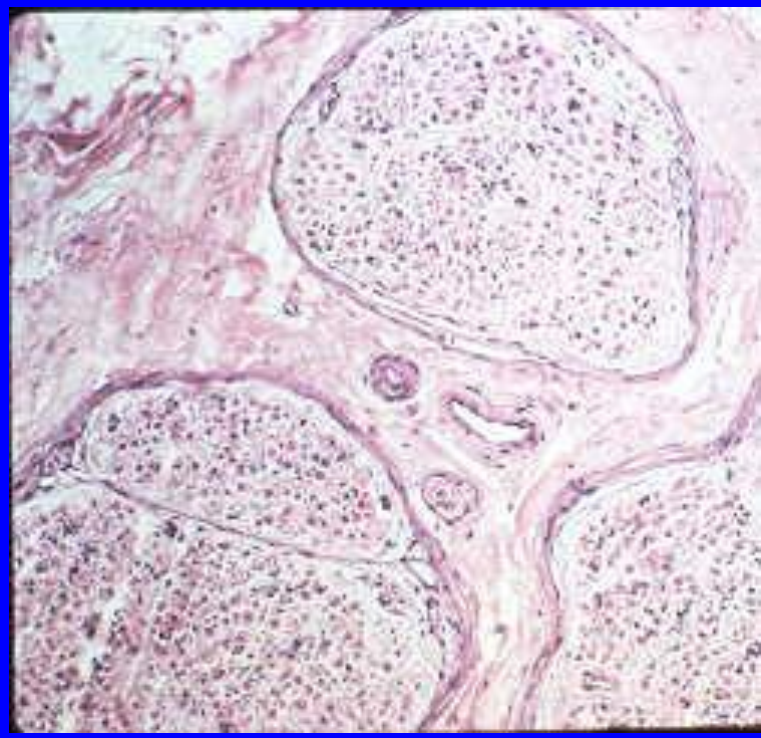
E. Embedding: orientation, bubbles

# XI. Pitfalls

F. Poor sectioning

1. knife marks (scratches perpendicular to knife edge)
2. compression (waves parallel to knife edge)



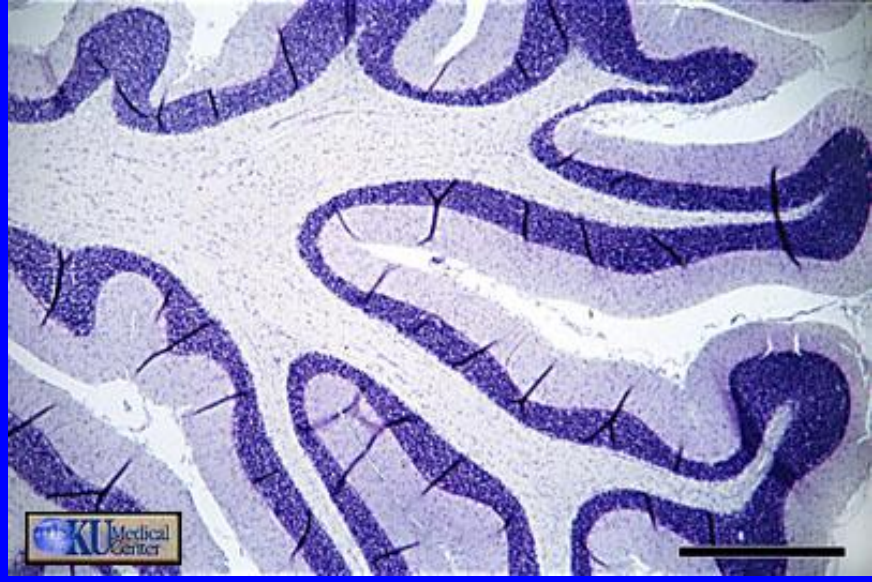


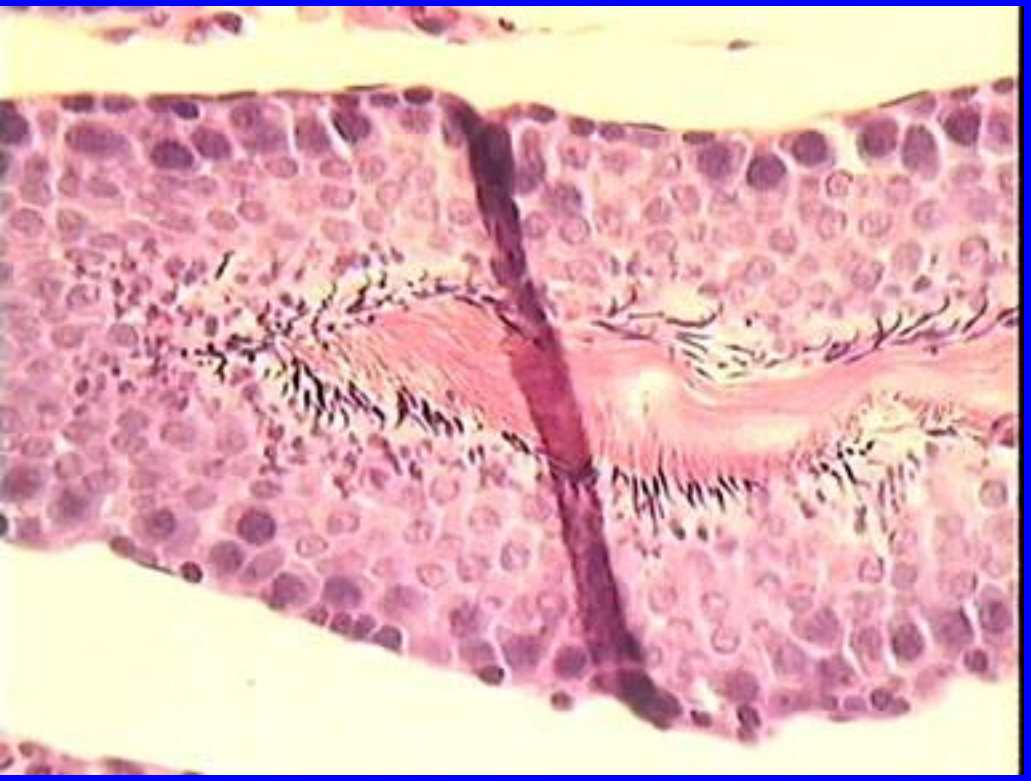
# XI. Pitfalls

G. Mounting sections

1. folds & tears

2. excess albumin (stain)

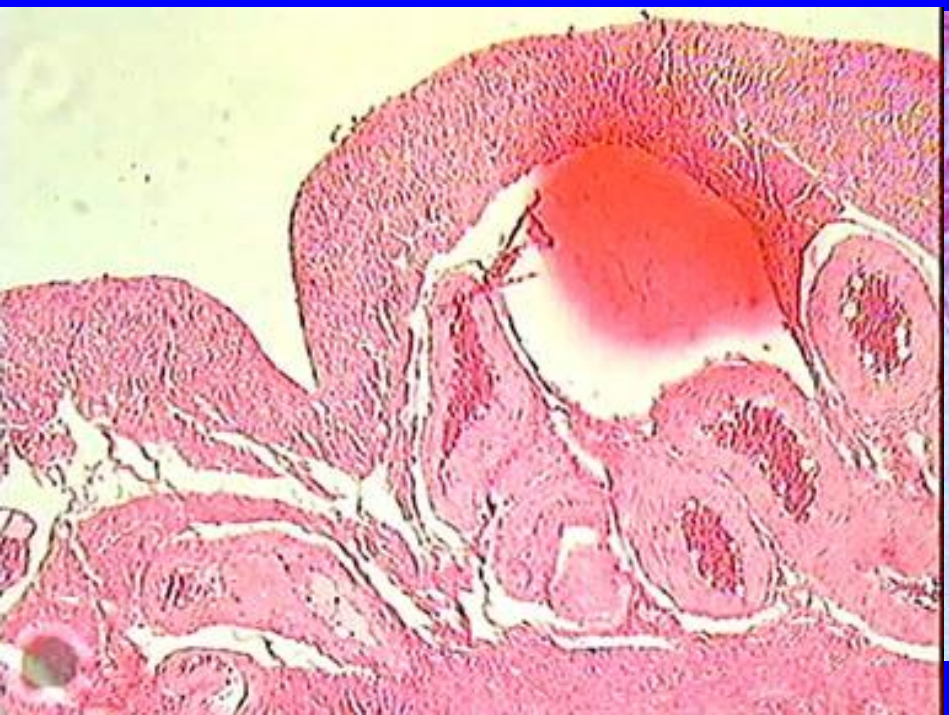


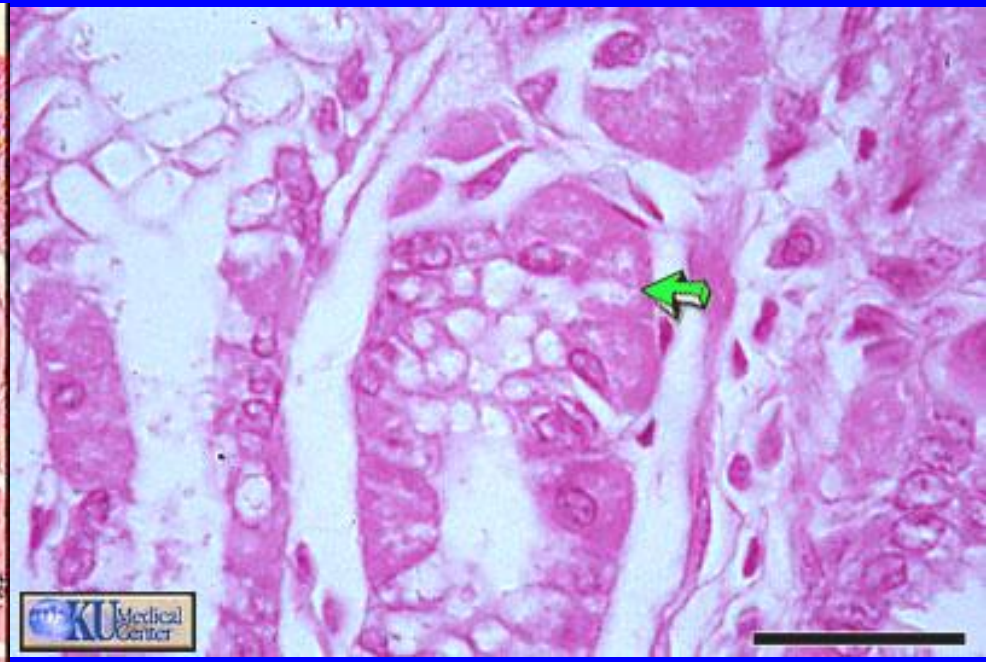



# XI. Pitfalls

H. Staining

1. inadequate rehydration (uneven staining)
2. too dark or too light (timing off)
3. inadequate agitation

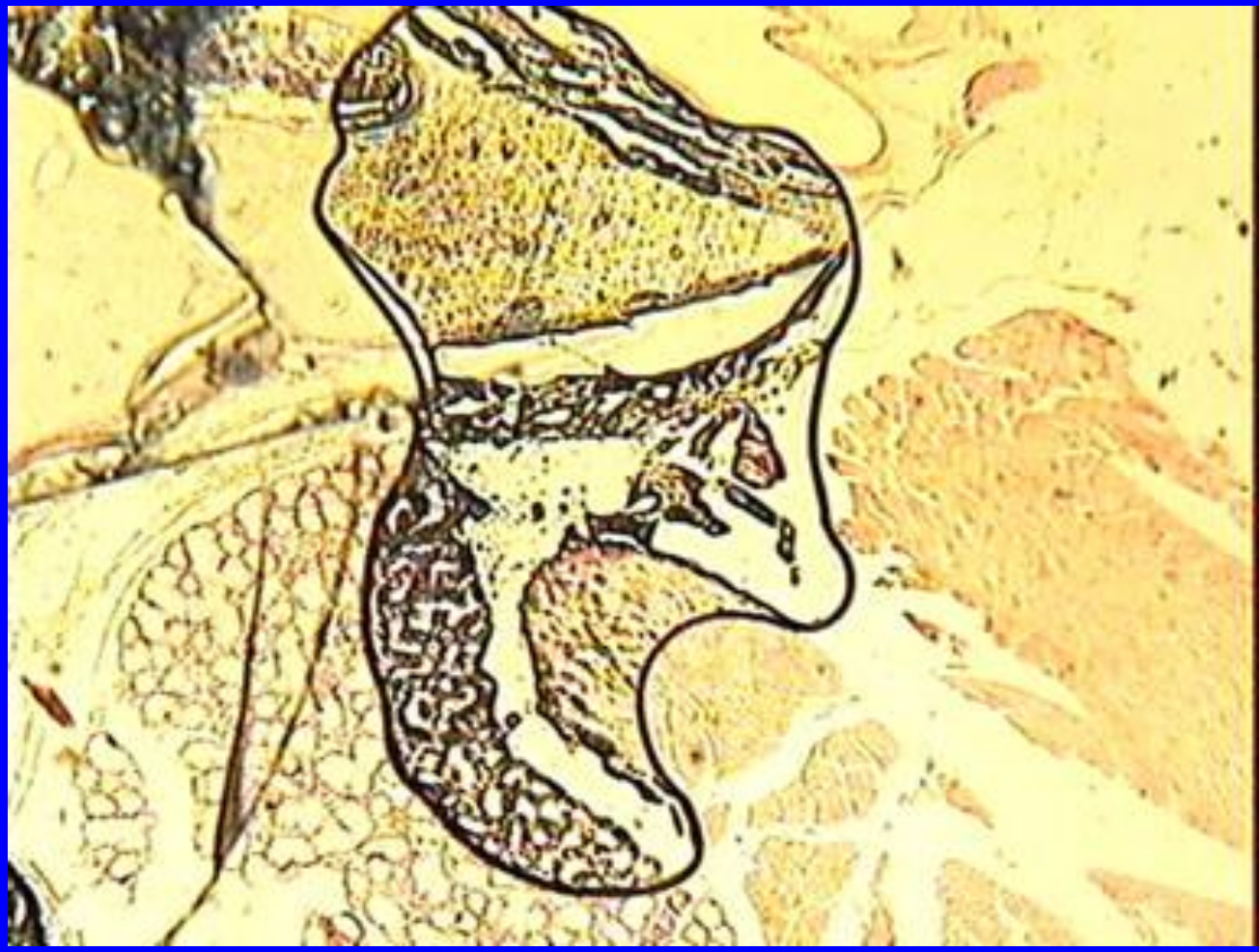




# XI. Pitfalls

## I. Coverslipping

### 1. Bubbles

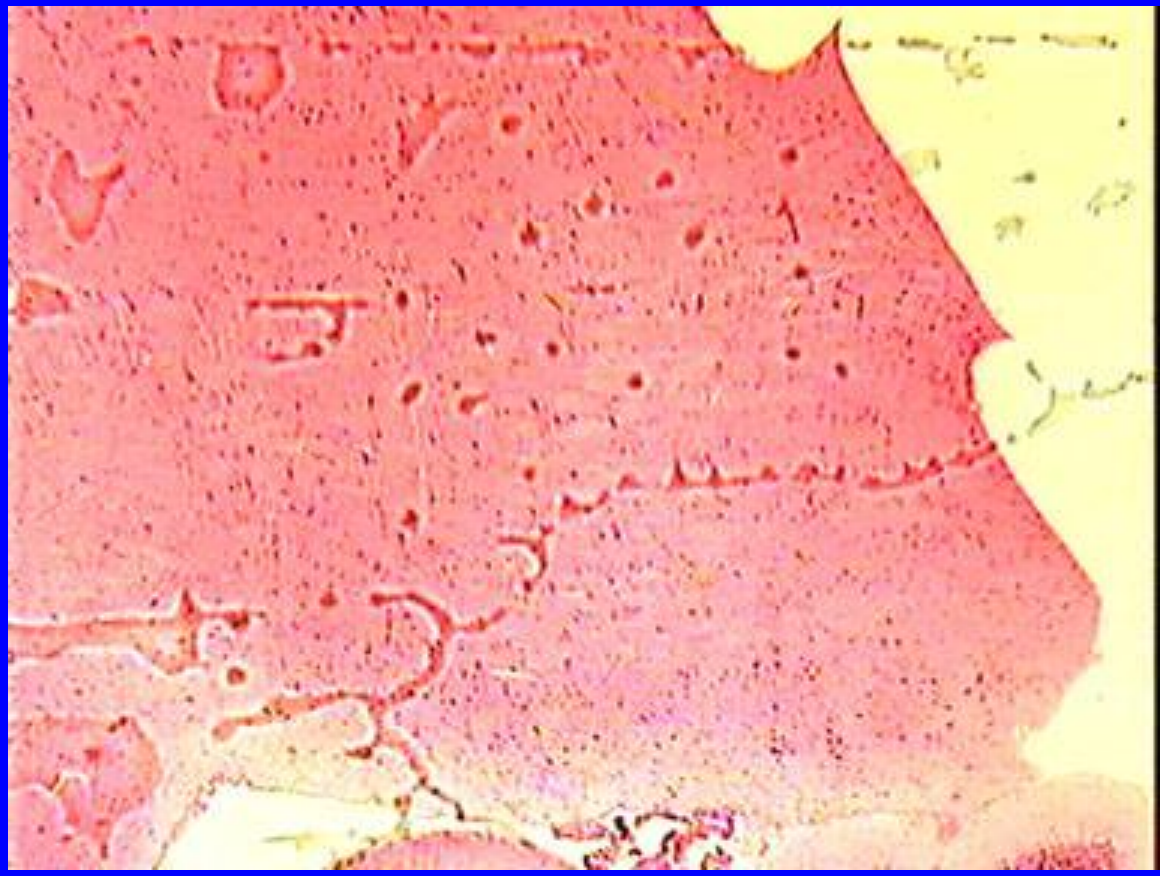


# XI. Pitfalls

I. Coverslipping

2. excess Permount

3. two coverslips



# Electron Microscopy & Immunohistochemistry (IHC)

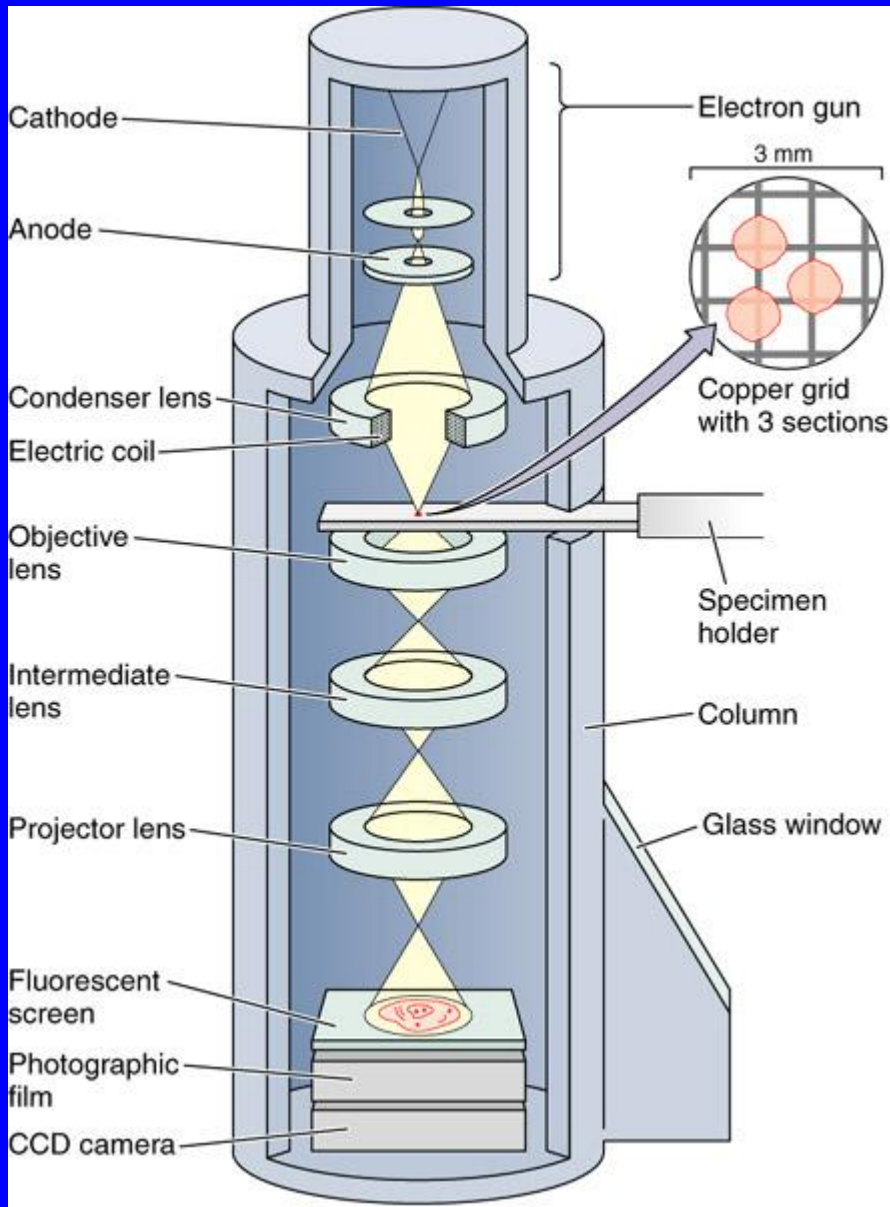
Students are responsible for all EMs in the text.

# I. Electron Microscope

A. TEM (1.5)

B. Similarities with LM

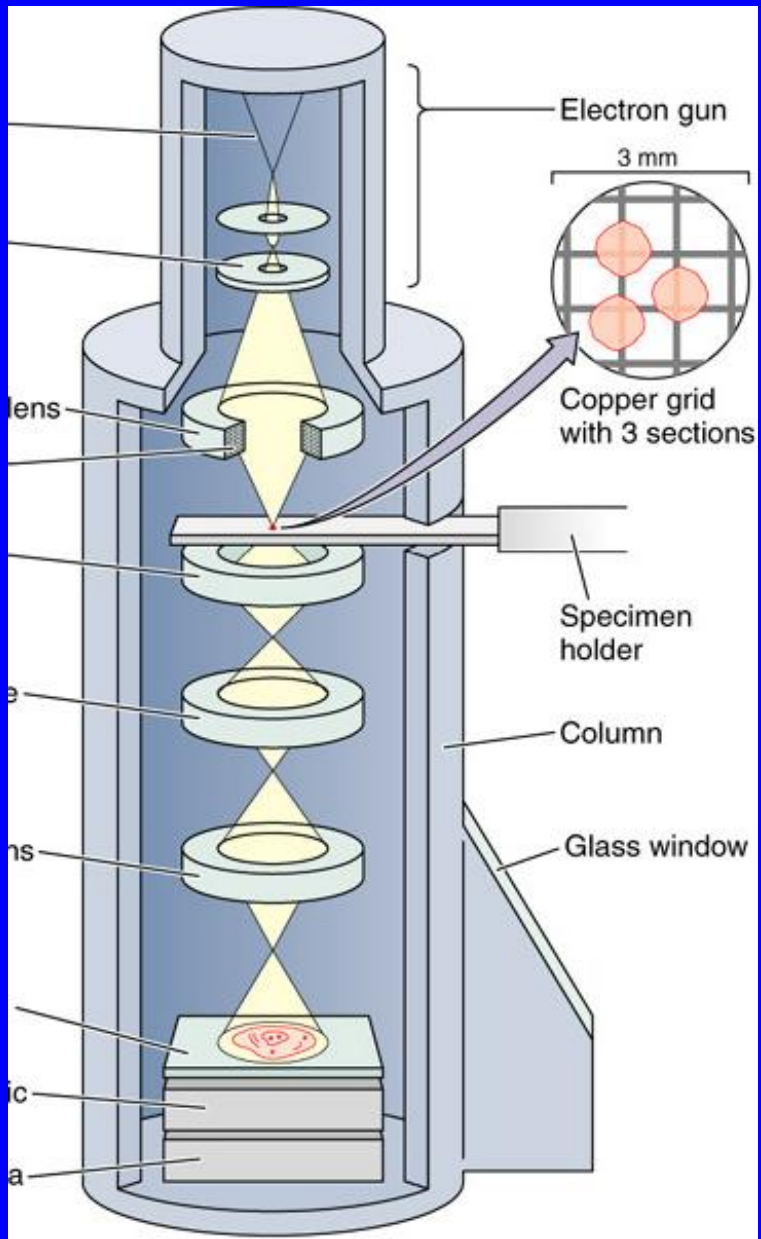
1. electron source (vacuum) [light]
2. condenser (electromagnetic) lens
3. specimen chamber [stage]
4. objective lens
5. projector lens
6. fluorescent screen
7. camera



# I. Electron Microscope

C. Differences from LM

1. vacuum (no living material)
2. electron penetration
   a. 0.02 - 0.1 μm sections
3. resolution: 0.2 nm
   a. magnification ~5k-1 million
   b. small field of view
4. BW



# I. Electron Microscope

# II. EM Fixation

A. buffered glutaraldehyde & osmium tetroxide

B. smaller sample size (~1$mm^3$)

# III. EM Embedding & Sectioning & Staining

A. plastic resin

B. polymerize (cure)

C. ultramicrotome (0.02 - 0.1 μm sections)

   1. diamond knife

   2. fresh glass knife

D. copper grids

E. electron dense stains

   1. lead citrate

   2. uranyl acetate

F. Demonstration: tissue block, diamond knife, copper grid

# III. EM Viewing

A. Advantages (1.8)

1. high resolution

a. cell organelles

b. plasma membrane

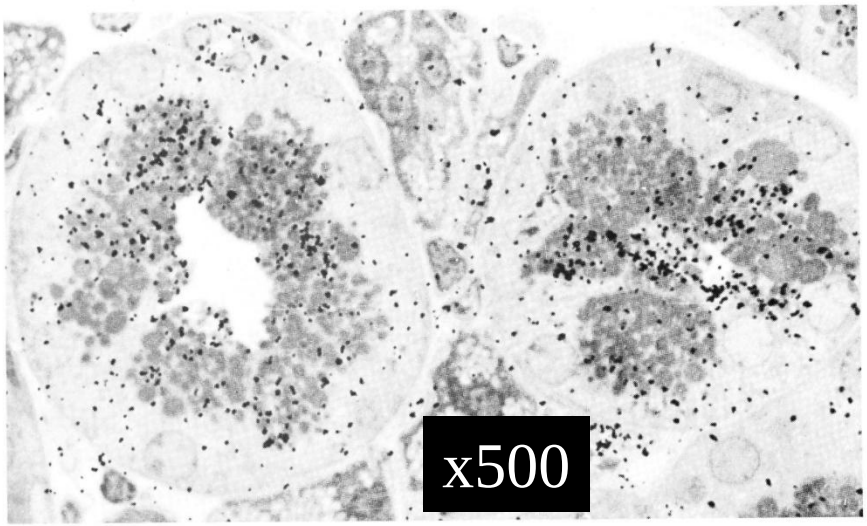
B. Disadvantages

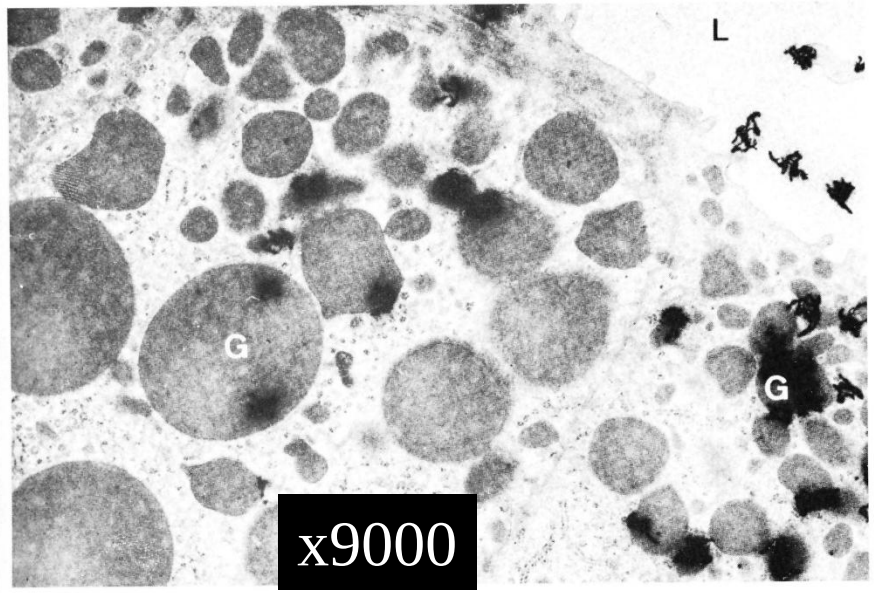
1. small sample

2. small field of view

3. 2-D image

4. static image



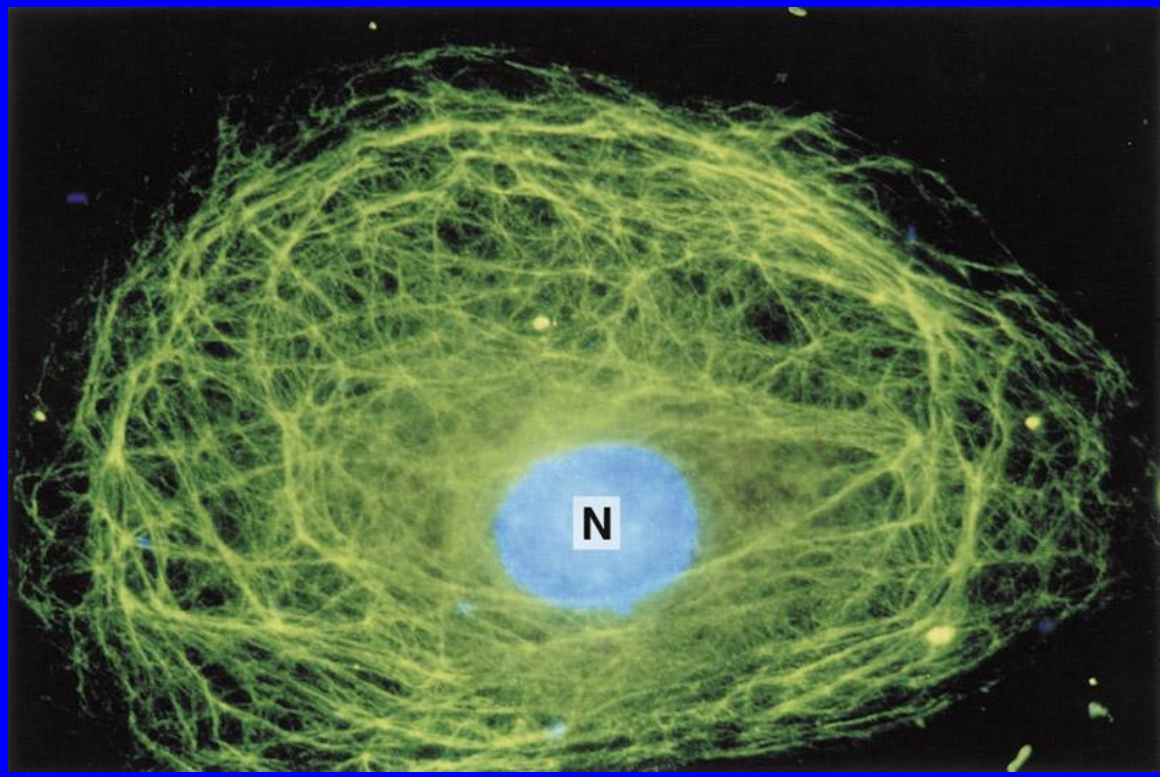



# I. Immunohistochemistry (IHC)

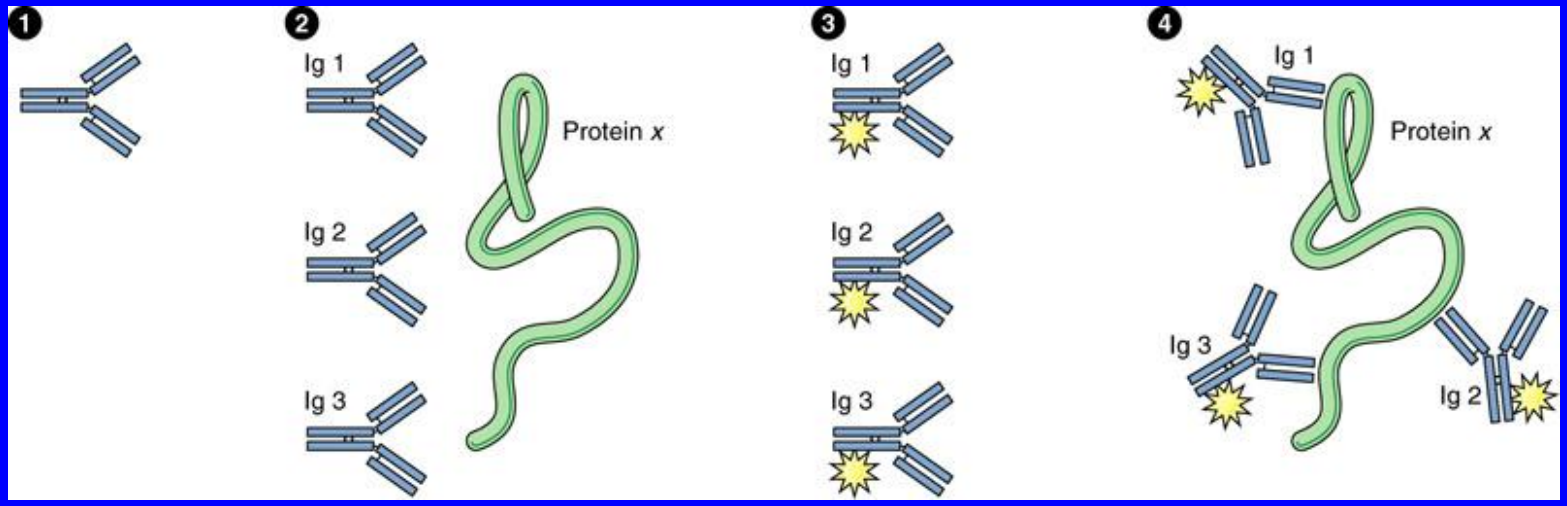
A. Identification & localization of specific molecules (1-18)

B. Antigen-antibody reaction

1. high affinity
2. specific
3. ex.: intermediate filaments in mouse cell



# II. Direct Labeling of antibodies




A. Fluorescent molecules (1-23)

1. fluorescein, rhodamine

B. HRP (horseradish peroxidase)

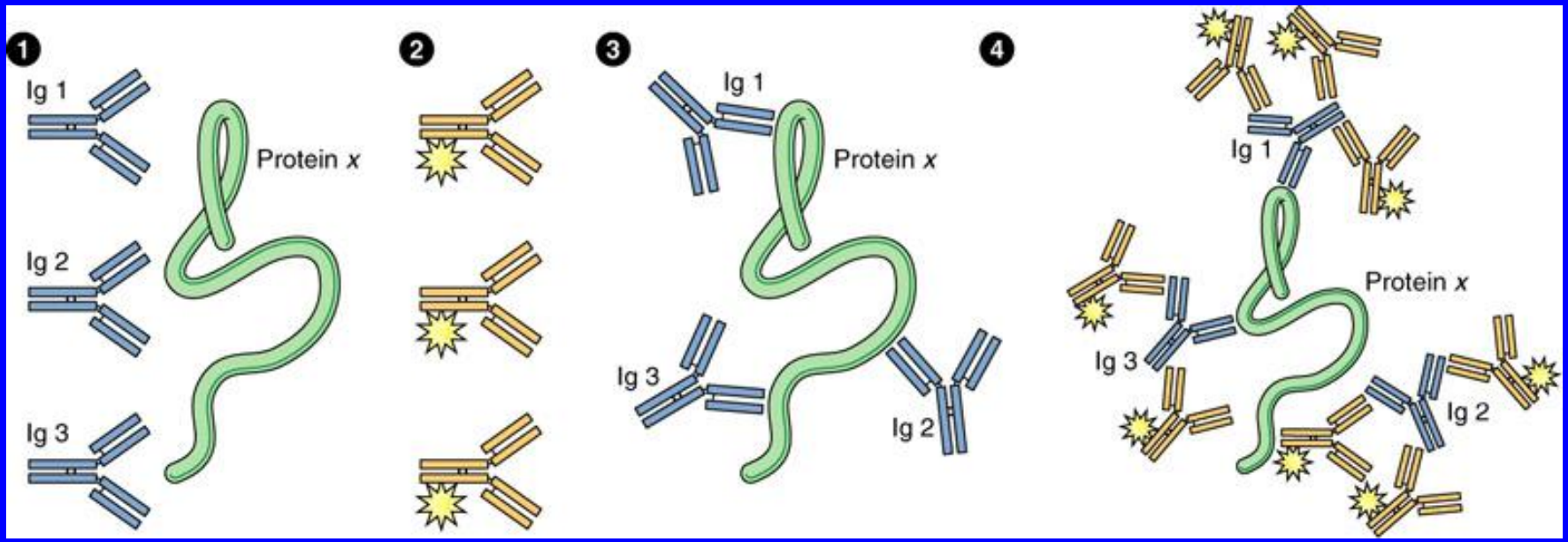
1. histochemical reaction

2. peroxidase + chromagen

C. Gold particles

# IV. Indirect Immunohistochemistry

1-21: Primary Ab attaches to Ag; Secondary Ab tagged with HRP attaches to primary; HRP reacted to form visible ppt




1-24

# IV. Indirect IHC

Ab to nNOS labeling neurons and processes in the superior colliculus in a P11 rat. From summer 2002.