Practical training in Histology and Embryology

HISTOLOGY

- structure and ultrastructure of normal cells and tissues,
- **cytology and general histology**
- **special histology** = microscopic anatomy of individual organs

- relevance: oncology, surgery, hematology, pathology, forensic,…

EMBRYOLOGY

– prenatal (intra uterine) development

- **General embryology** (until 2nd month – EMBRYO)

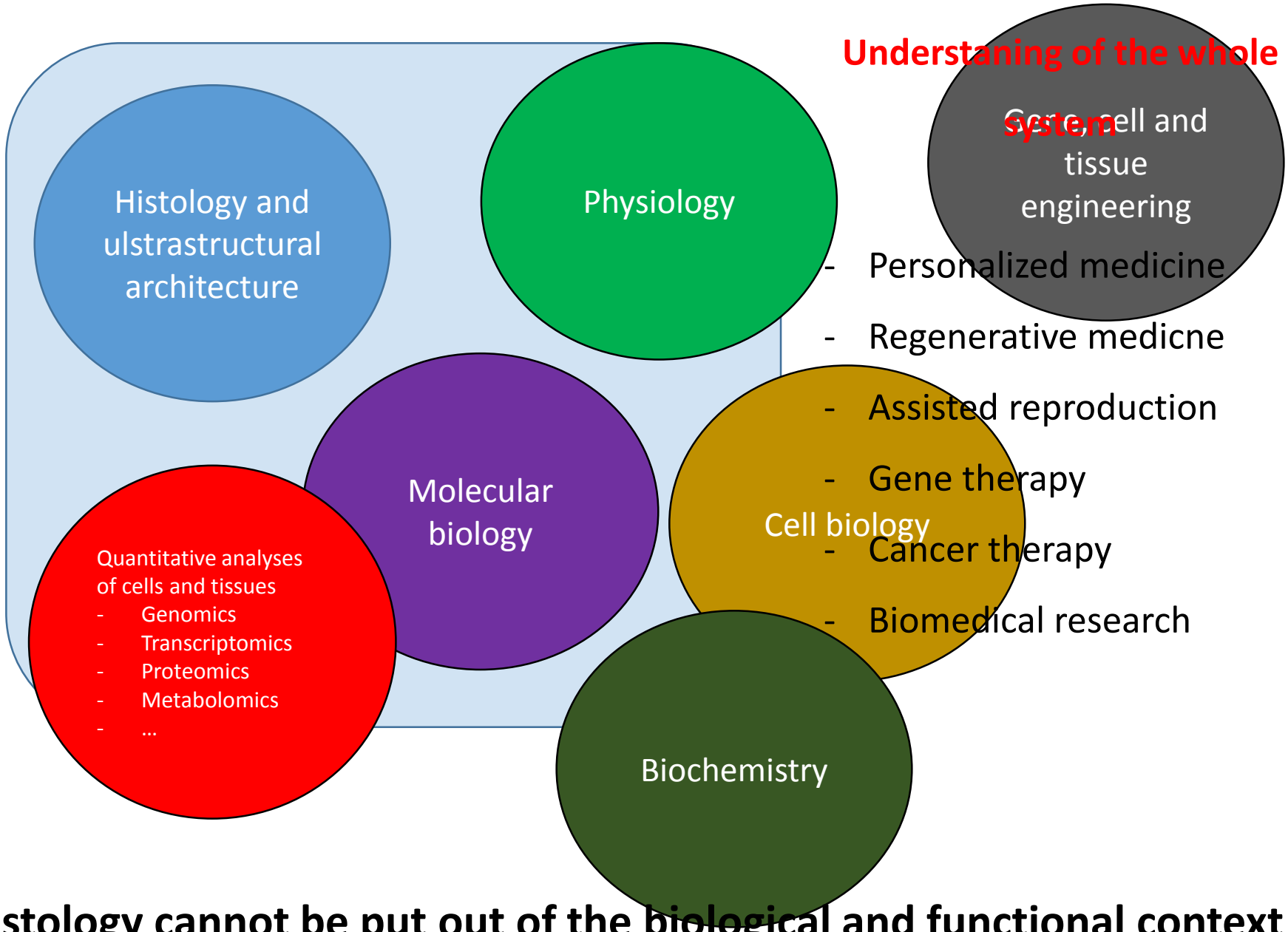
  gametogenesis and early embryonic development

- **Special embryology** (since 3rd month to birth – FETUS)

  organogenesis

- **Teratology** – defects in organ development, malformations, anomalies; prenatal screening – ultrasonography, amniocentesis, genetic and karyotype screening

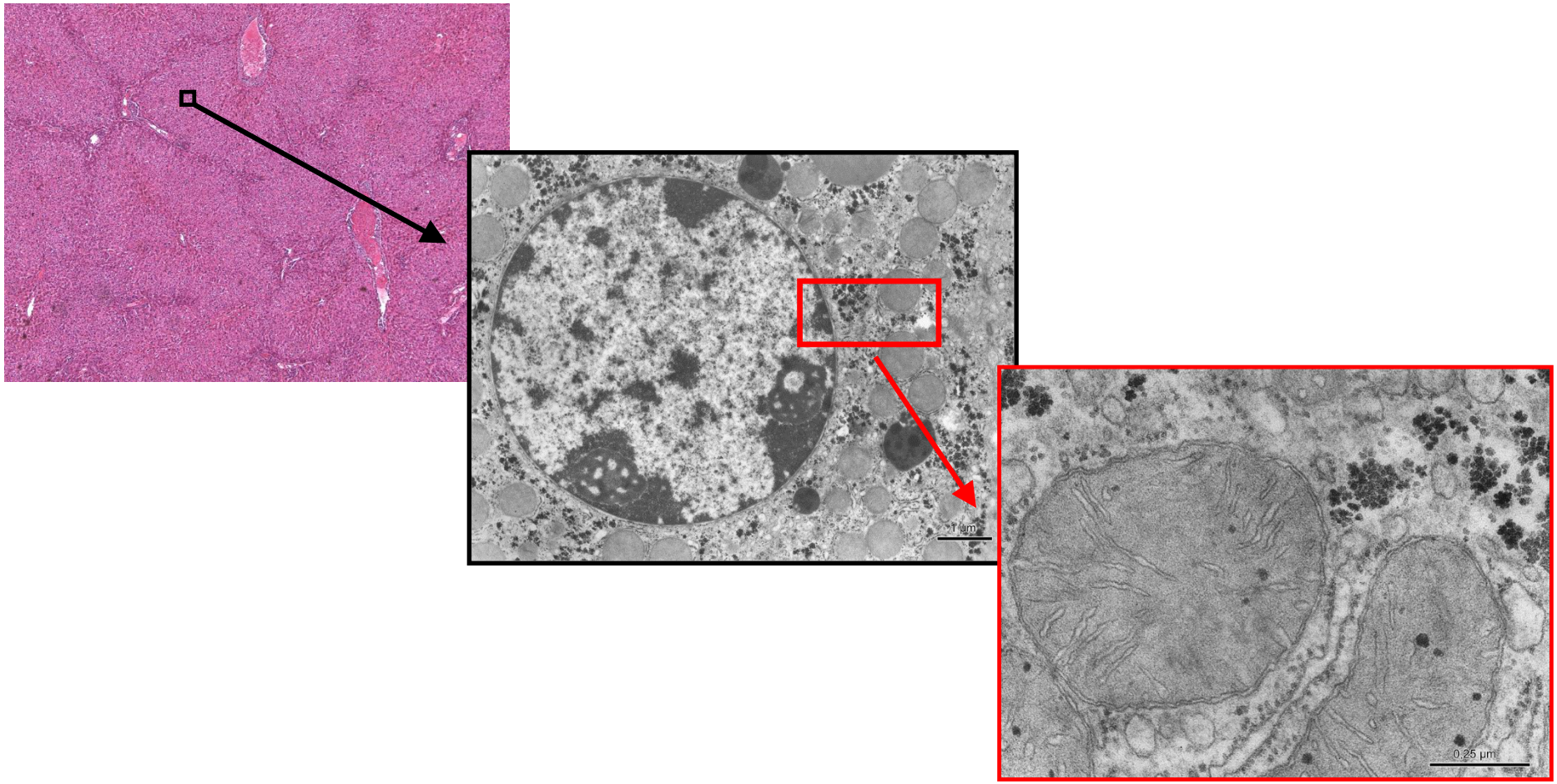
- Relevance: gynecology and obstetrics, pediatrics, assisted reproduction



Histology cannot be put out of the biological and functional context

Histology

- Resolution of naked eye – 0,1 mm
- Resolution of light microscopy – 10 nm
- Resolution of electron microscopy – 0,1 nm



Tissue processing for the light microscopy (LM)

(making of permanent preparations – slides)

- **SAMPLING** (obtaining of material – cells, tissue pieces)
- **FIXATION** of samples (tissue blocks)
- **RINSING** (washing) of samples
- **EMBEDDING** of samples - embedded blocks
- **CUTTING** of blocks - sections
- **AFFIXING** of sections
- **STAINING** of sections
- **MOUNTING** of sections

SAMPLING

- A small piece of organ (tissue) is sampled and quickly put into the fixative medium.

- Biopsy during surgical dissection of organs in living organism

  = excision

  = puncture (liver or kidney parenchyma, bone marrow)

  = curettage (uterine endometrium, adenoid vegetation)

- Necropsy from dead individual (sections); in experiments laboratory animals are used and tissue have to be sampled as soon as possible after the break of blood circulation

- The specimens shouldn't be more than **5 – 10 mm^3** thick and fixation should follow immediately.

FIXATION

- Definition: denaturation and stabilization of cell proteins with minimum artifacts)

- The reason of fixation: freshly removed tissues are chemically unstable – dry, shrink, undergo hypoxia, autolysis and bacteriological changes

- To stop or prevent these changes and preserve the structure tissue samples have to be fixed. During the fixation, all tissue proteins are converted into inactive denaturized (stable) form.

- 3 main requirements on fixatives:
  - good preservation of structure
  - quick penetration into tissue block
  - no negative effects on tissue staining

- Fixatives: solutions of different chemicals

  - organic fixatives – ALDEHYDES – formaldehyde (*most frequently used for LM*)

    – glutaraldehyde (*used for EM*)

    – ALCOHOLS – 96 – 100 % (absolute) ethylalcohol

    – ORGANIC ACIDS – glacial acetic acid, picric acid,

    trichloracetic acid

  - inorganic fixatives – INORGANIC ACIDS – chromic acid, osmium tetraoxide (OsO4)

    – SALTS OF HEAVY METALS – mercuric chloride HgC_{l2}

  - compound fixatives – mixtures (two or more chemical components to offset

    undesirable effects fo indiviual (simple) fixatives.

    FLEMMING´s fluid – with OsO_4

    ZENKER´s and HELLY´s fluid, SUSA fluid – with $HgCl_2$

    BOUIN´s fluid – with picric acid

    CARNOY´s fluid – with alcohol

Performance: fixatives are carried out at room temperature, the duration varies between **12 – 24 hours**, specimen must be covered by 20 – 50 times fixative volume:

Ratio of tissue block volume to fixative volume 1 cm^3 : 20 – 50 cm3

RINSING and EMBEDDING

- All samples should be washed to remove the excess of fixative; the choice of rinsing medium is determined by type of fixative: running tap-water or 70-80% ethanol

- Relevance of embedding: tissues and organs are brittle and unequal in density, they must be hardened before cutting

Embedding media

- water soluble – gelatine, celodal, water soluble waxes
- anhydrous – paraffin, celoidin

EMBEDDING into PARAFFIN

- dehydration – to remove water from fixed samples by ascending series of ethanol is used (50%, 70%, 90%, 96%. each step - 2 – 6 hours
- clearing – the ethanol must be replaced with organic solvatant that dissolves paraffin – benzene or xylene
- infiltration – melted paraffin wax (56°C) is used; 3 x 6 hours.

- casting (blocking out) – moulds (plastic, paper or metal chambers) are used for embedding.

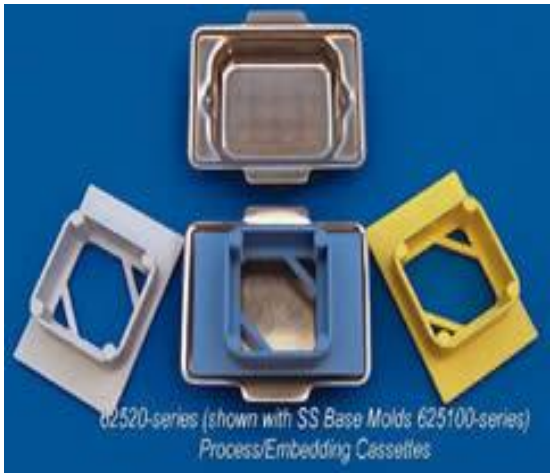
- The moulds are filled with melted paraffin, tissue samples are then placed inside and immediately immersed in cold water to cool paraffin quickly down.
- These paraffin blocks are ready for trimming

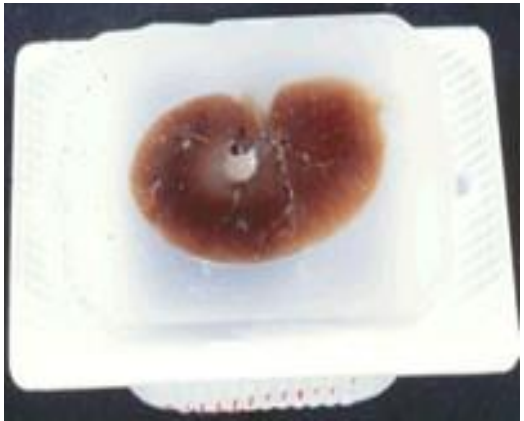
Leica TP 1020

Automated device for tissue dehydration

Paper chambers

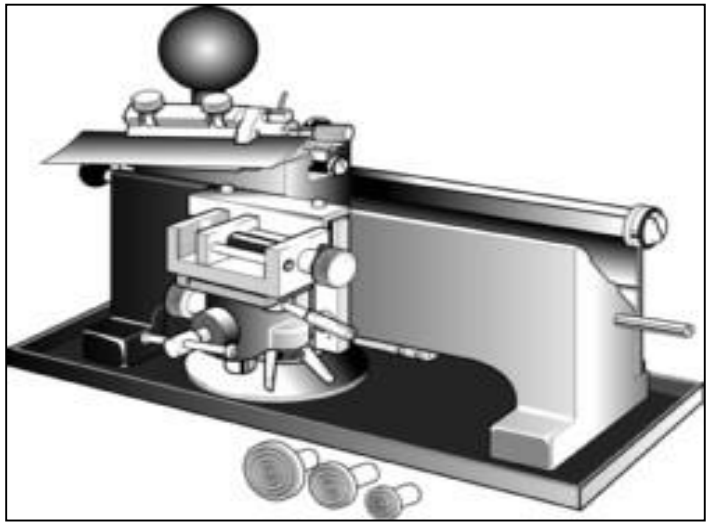
- **metal**



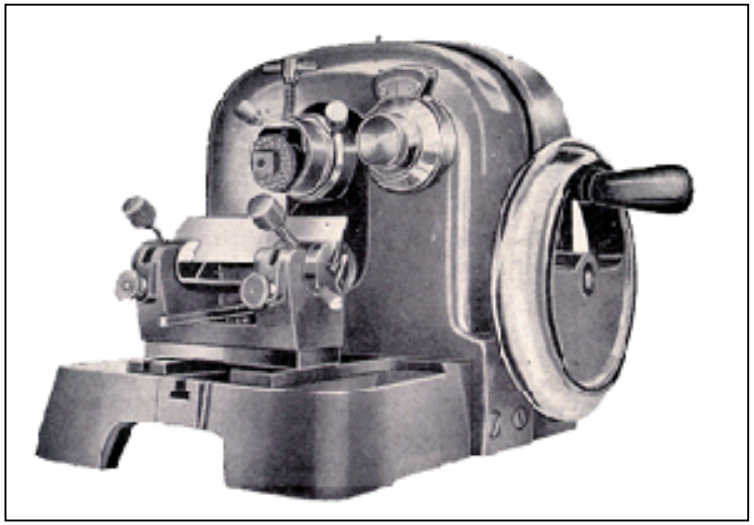


CUTTING

- Microtome – a machine with automatic regulation of section thickness: 5 – 10 µm is optimum.

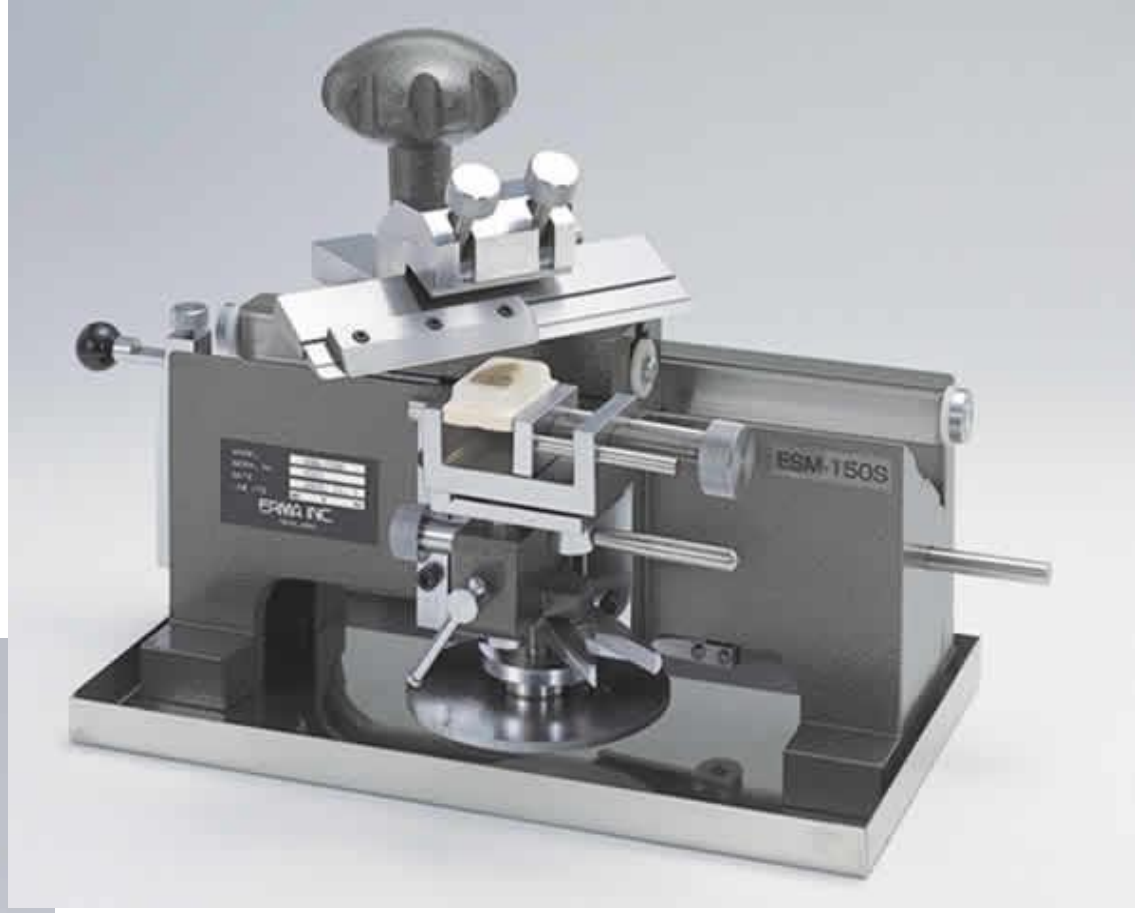


sliding microtome – block is fixed in holder, knife or razor moves horizontally



rotary microtome – knife is fixed, block holder moves vertically

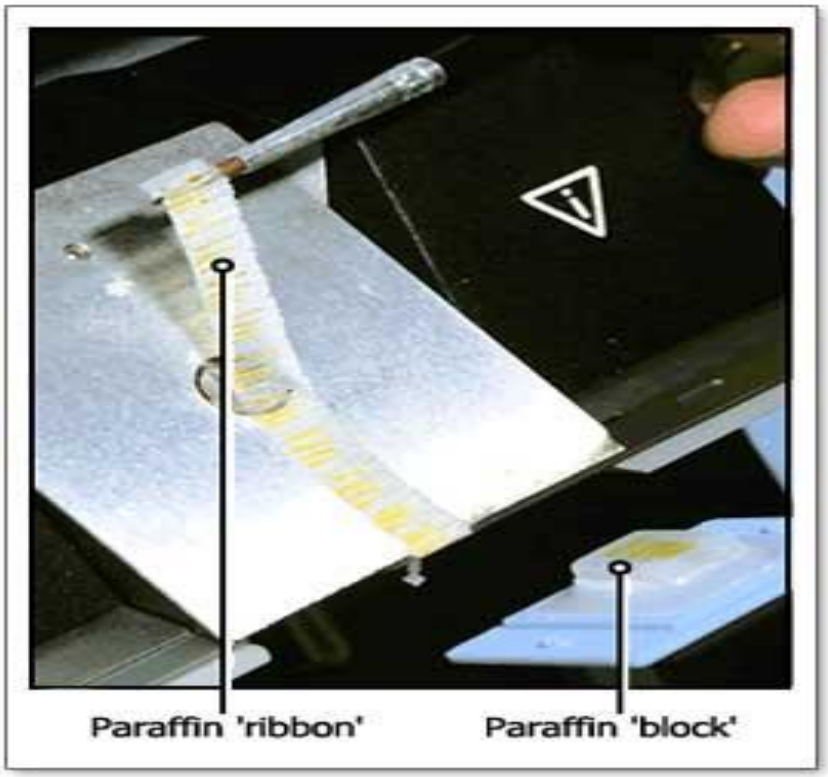
Sliding microtome

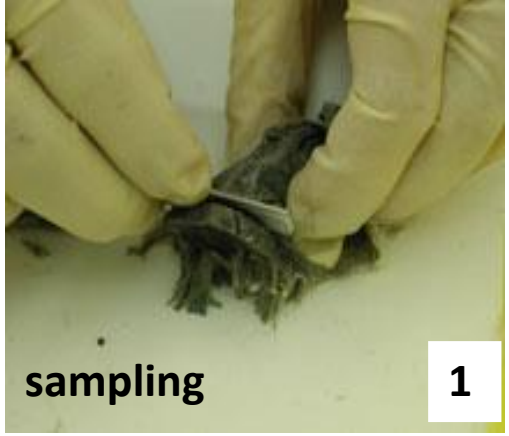


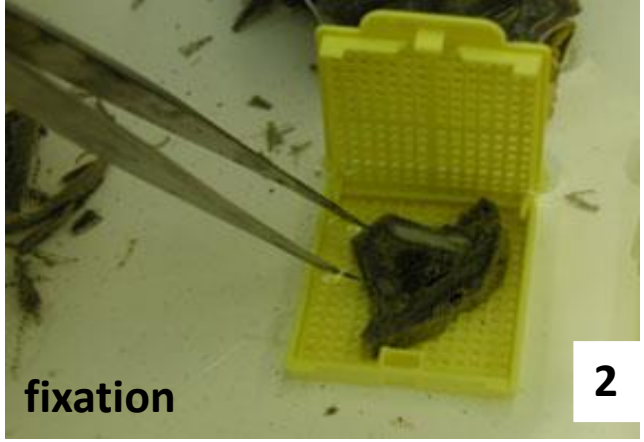
Rotary microtome

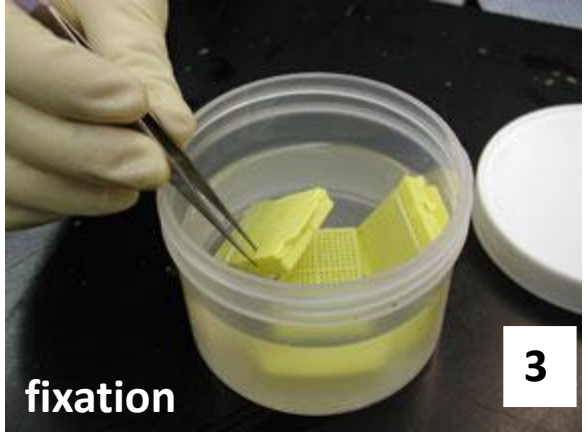
Freezing microtome (**cryostat**)
= rotary microtome housed in freezing box
(– 60º C)

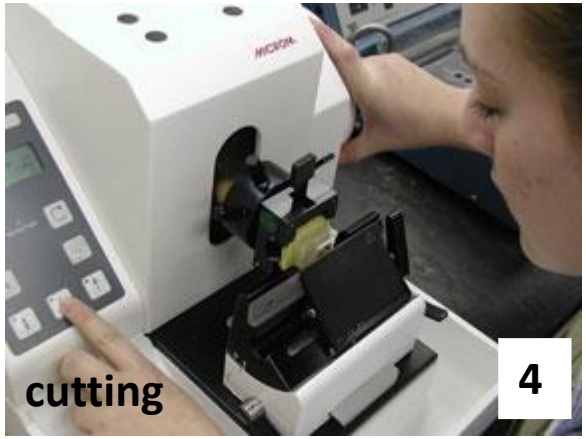
Cutting of frozen tissue without the embedding

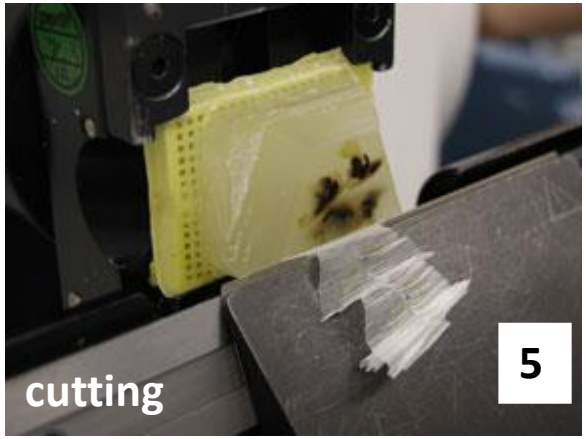


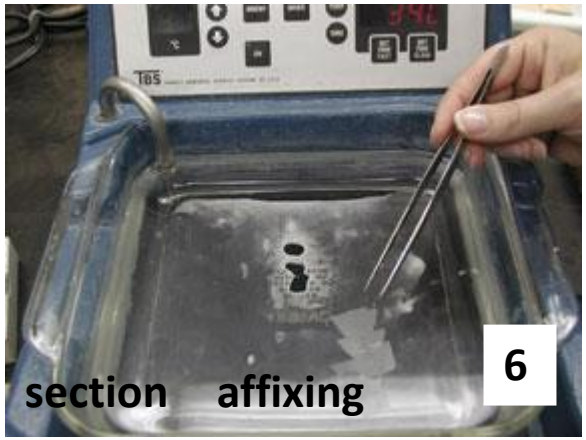

sampling
1

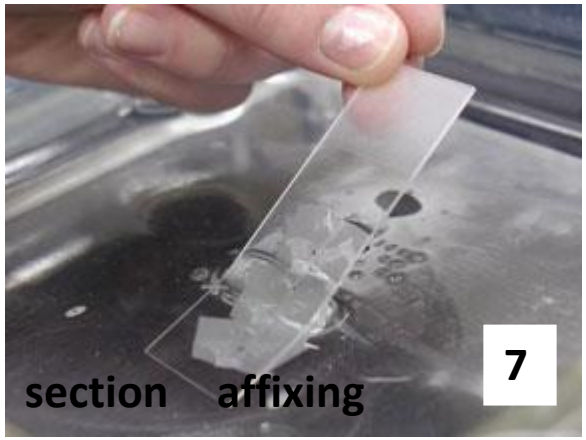

fixation
2

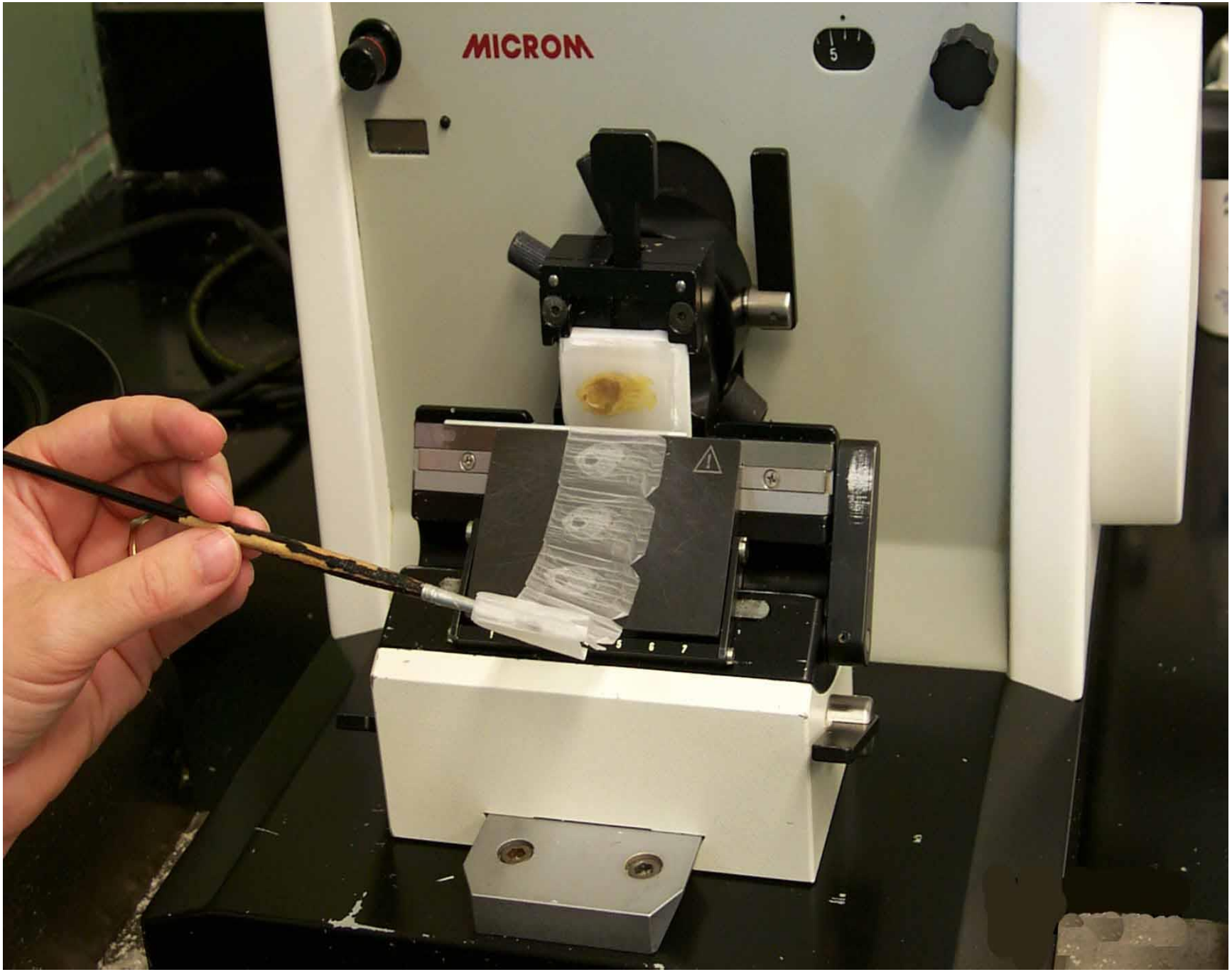

fixation
3

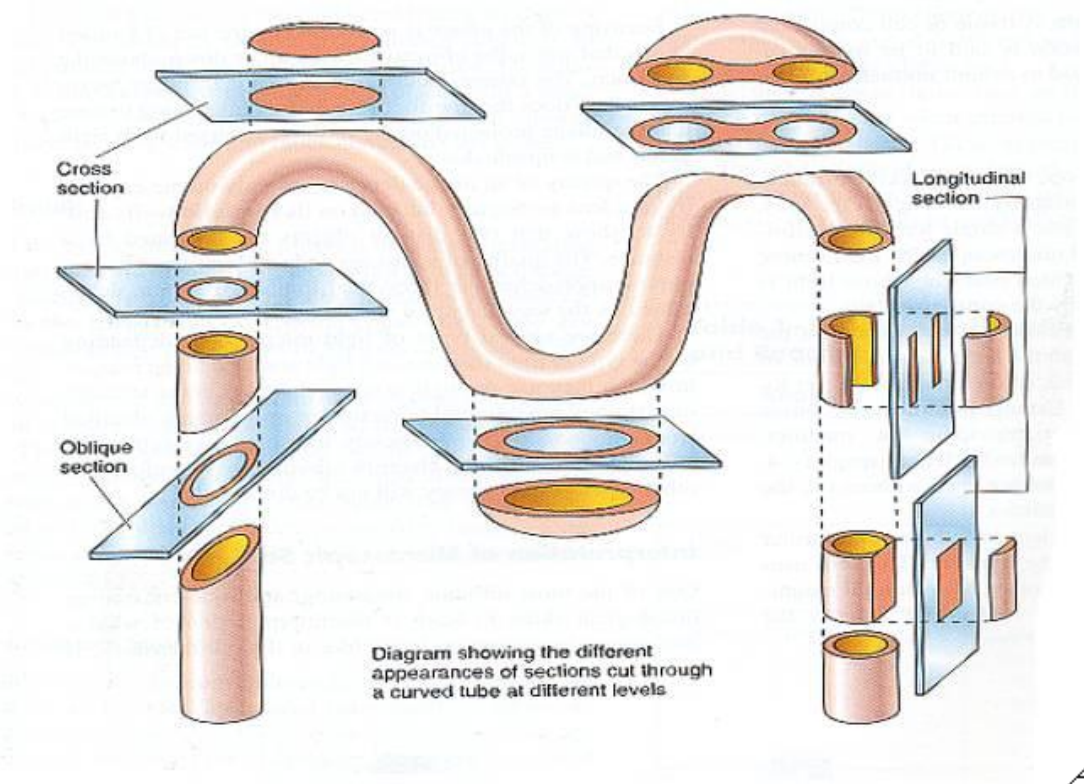

cutting
4

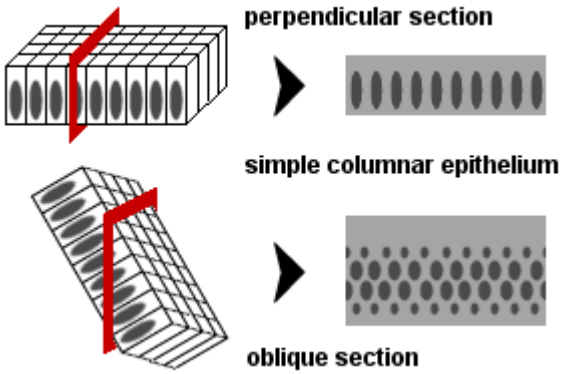

cutting
5


section affixing
6


section affixing
7


MICROM


Cross section
Longitudinal section
Oblique section
Diagram showing the different appearances of sections cut through a curved tube at different levels


perpendicular section
simple columnar epithelium
oblique section

AFFIXING

- Mixture of glycerin and egg albumin or gelatin

- Section are transferred from microtome razor or knife on the level of warm water (45º C), where they are stretched; then they are put on slides coated with adhesive mixture; excess of water is drained and slides are put in incubator (thermostat, 37º C) over night to affixing of sections.

Stretching of sections on warm water

Stretching on a warm plate

STAINING

- Different cell or tissue structures are not apparent without staining.
- Cellular structures exhibit different affinity to staining dyes

alkaline dyes (basic or nuclear) – react with anionic groups of cell and tissue components

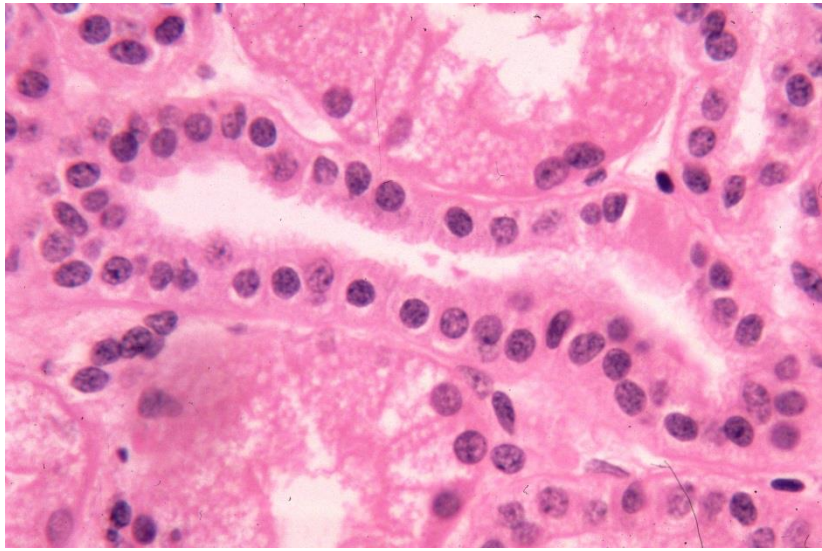
basophilia – basophilic structures in the cell

acid dyes (cytoplasmic) – react with cationic groups

acidophilia – acidophilic structures in the cell

neutrophilia – no reaction

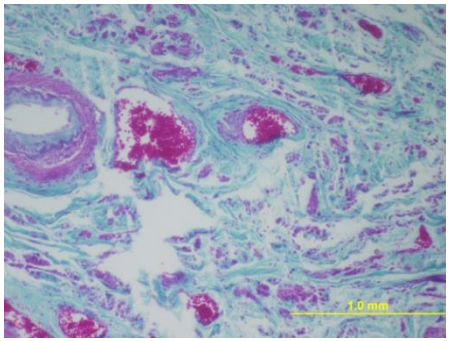
HE – the most frequent used method

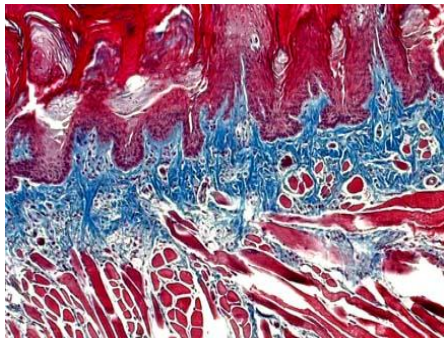


Staining methods:

routine – HE, AZAN

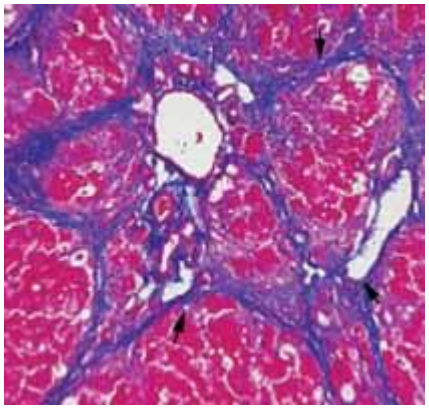
(demonstrate all components of tissue)





special

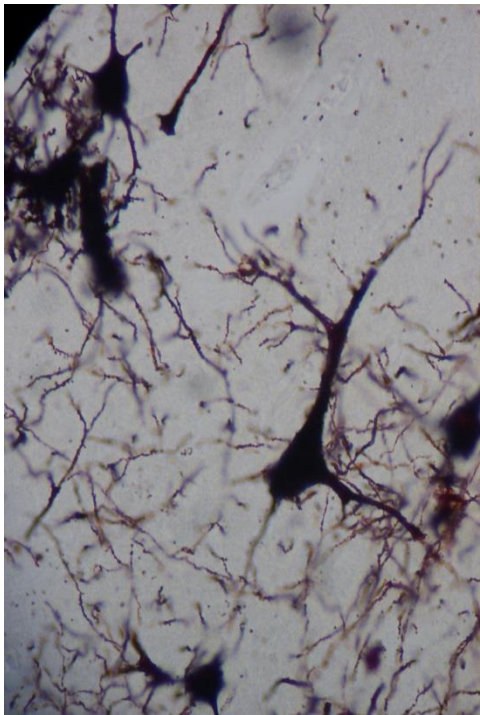
visualizes only special structures



Lipid droplets
detected by oil red

impregnation

by silver salt for detection
of nerve or reticular fibers



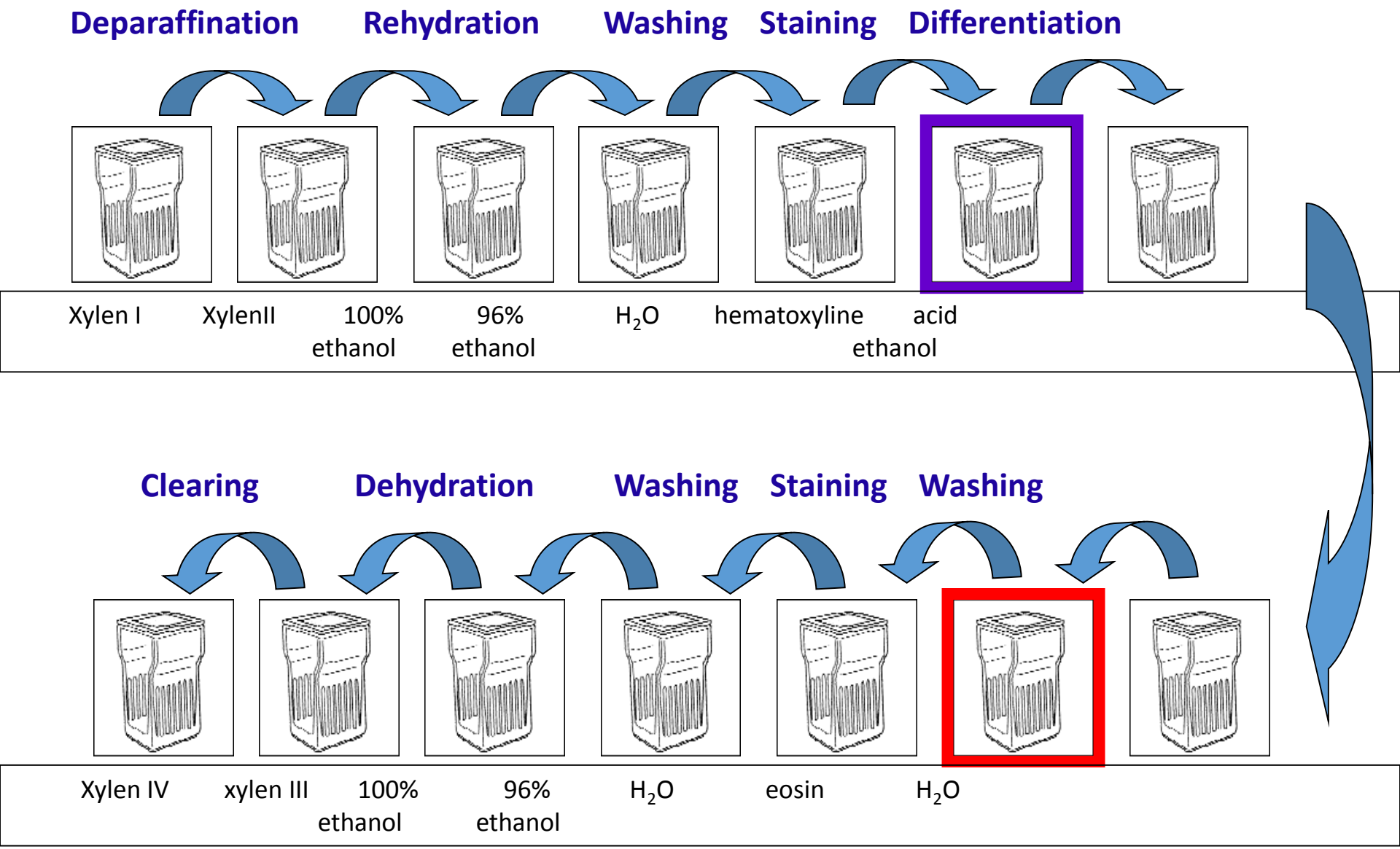
ROUTINE STAINING with HEMATOXYLINE – EOSIN (HE)

Hematoxyline – basic (nuclear) dye

Eosin – acid (cytoplasmic dye

- Staining procedure:
- paraffin must be removed (dissolved) by xylene
- sections are rehydrated in descending series of ethanol (100% →96% →80%)
- staining with hematoxyline
- differentiation in acid ethanol and water (excess of dye is removed)
- staining with eosin
- rinsing in water (excess of dye is removed)
- dehydration in graded ethanol series (80% →96% →100%)
- clearing in xylene

HEMATOXYLINE – EOSIN (HE)



Staining results:

- **HE** = ***H****ematoxyline* – ***E****osin*

  nuclei – bright clear blue or dark violet

  cytoplasm and collagen fibers – pink

  muscle tissue – red

- **HES** = ***H****ematoxyline* – ***E****osin* – ***S****afron*

  connective tissue – yellow

- **AZAN** = ***AZ****ocarmin* – ***AN****iline blue* – *orange G*

  nuclei – red

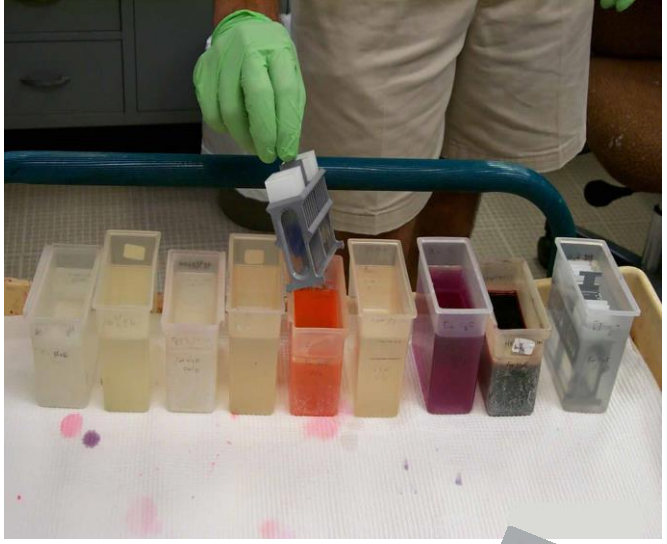
  erythrocytes – orange

  muscle – red

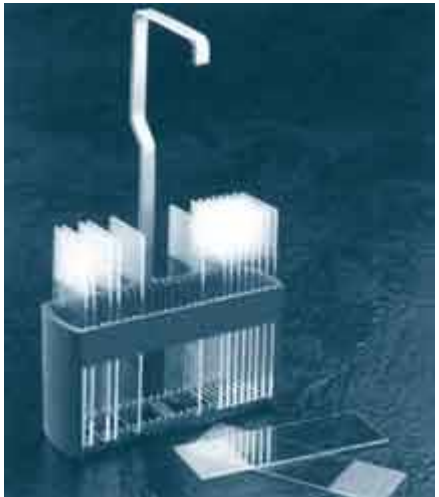
  collagen fibers – blue

Staining tools:

cuvette



flask



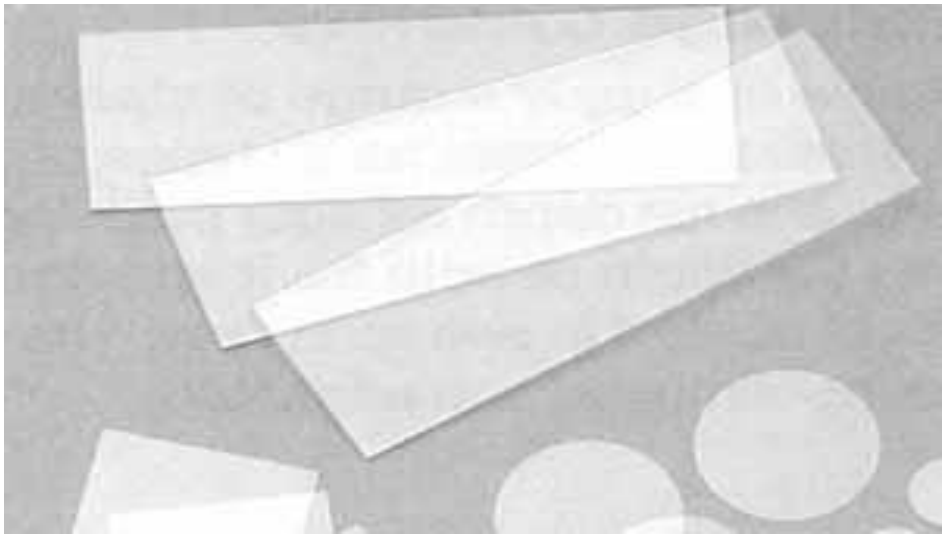
slides holder
(basket)

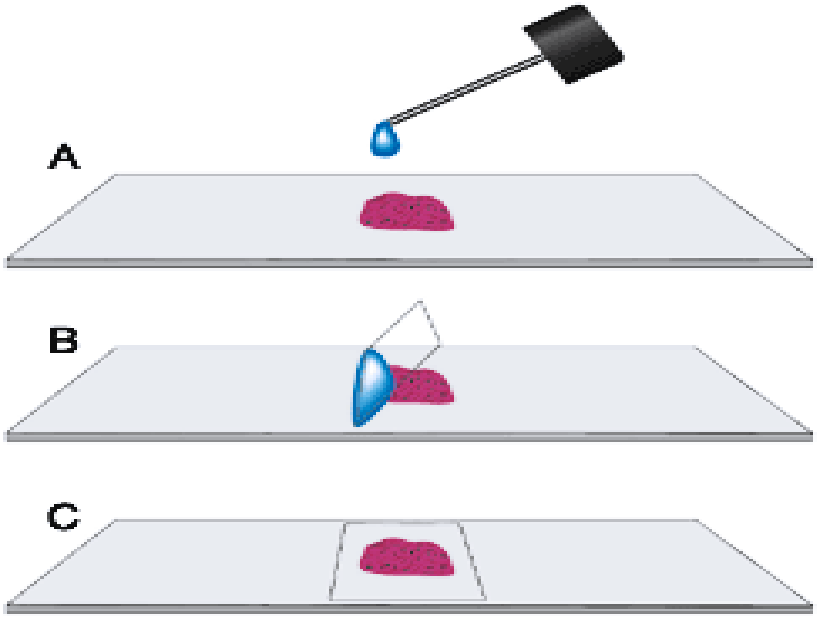
Automatic slide stainer

staining set of boxes with media

MOUNTING

- Finally, preparates are closed with coverslip (coverglass) to form a permanent preparate. Small amount of mounting medium must be placed between stained section and the coverslip.

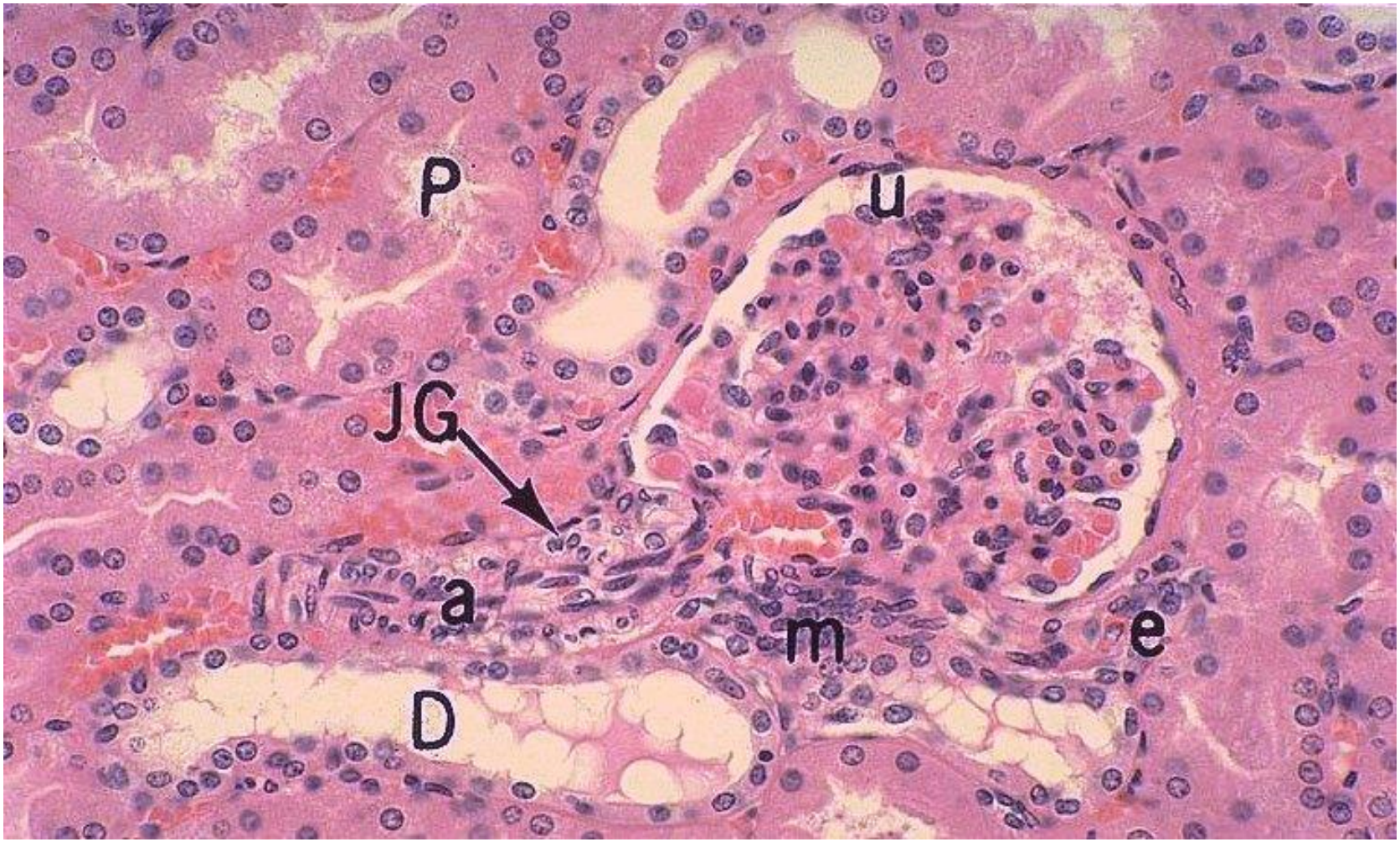




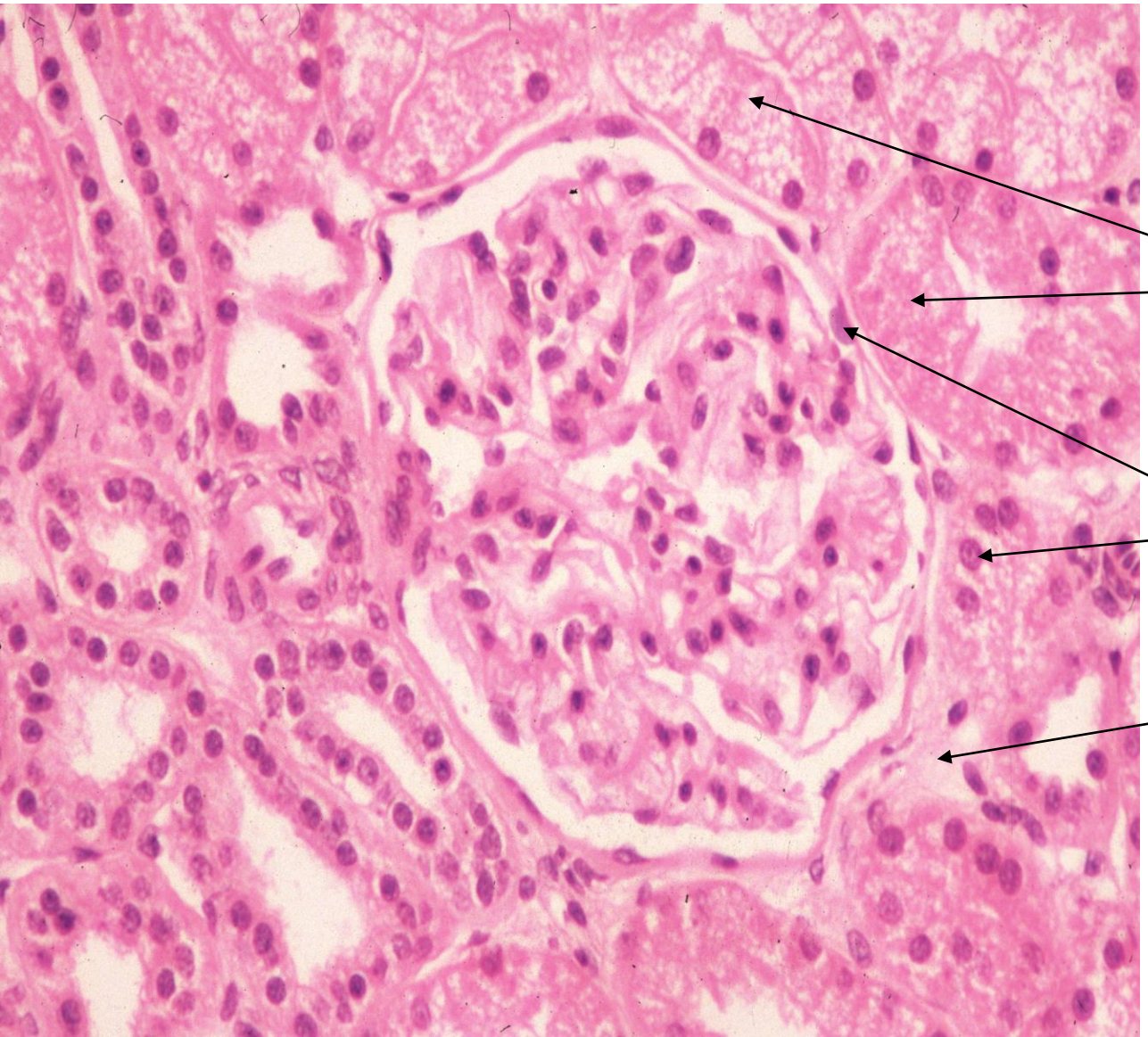
- **Mounting media**: soluble in xylene – **canada balsam**
  soluble in water – glycerin-gelatine, arabic gum

Permanent histological slides for study in the light microscope

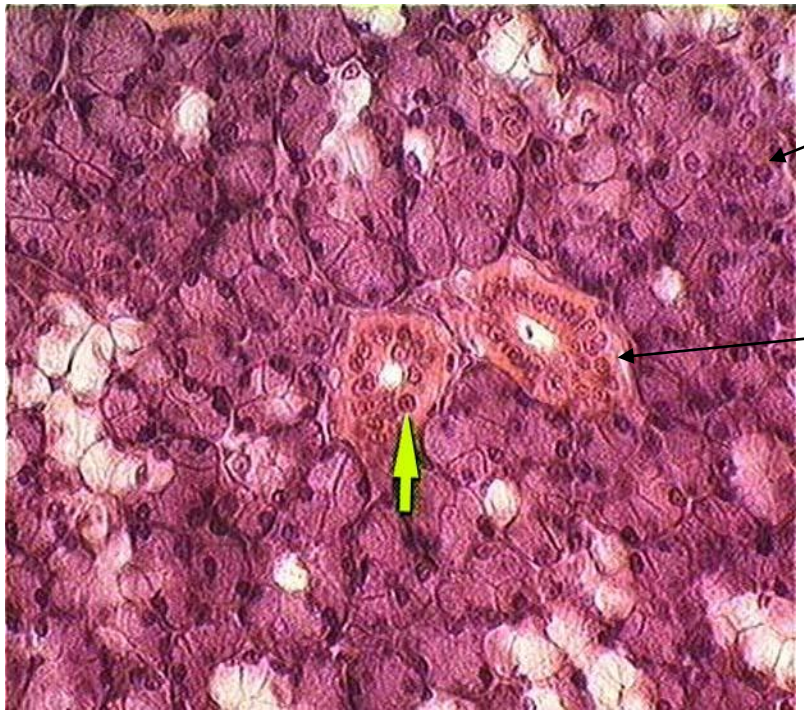
Hematoxyline and eosin (HE)



Hematoxyline and eosin (HE)



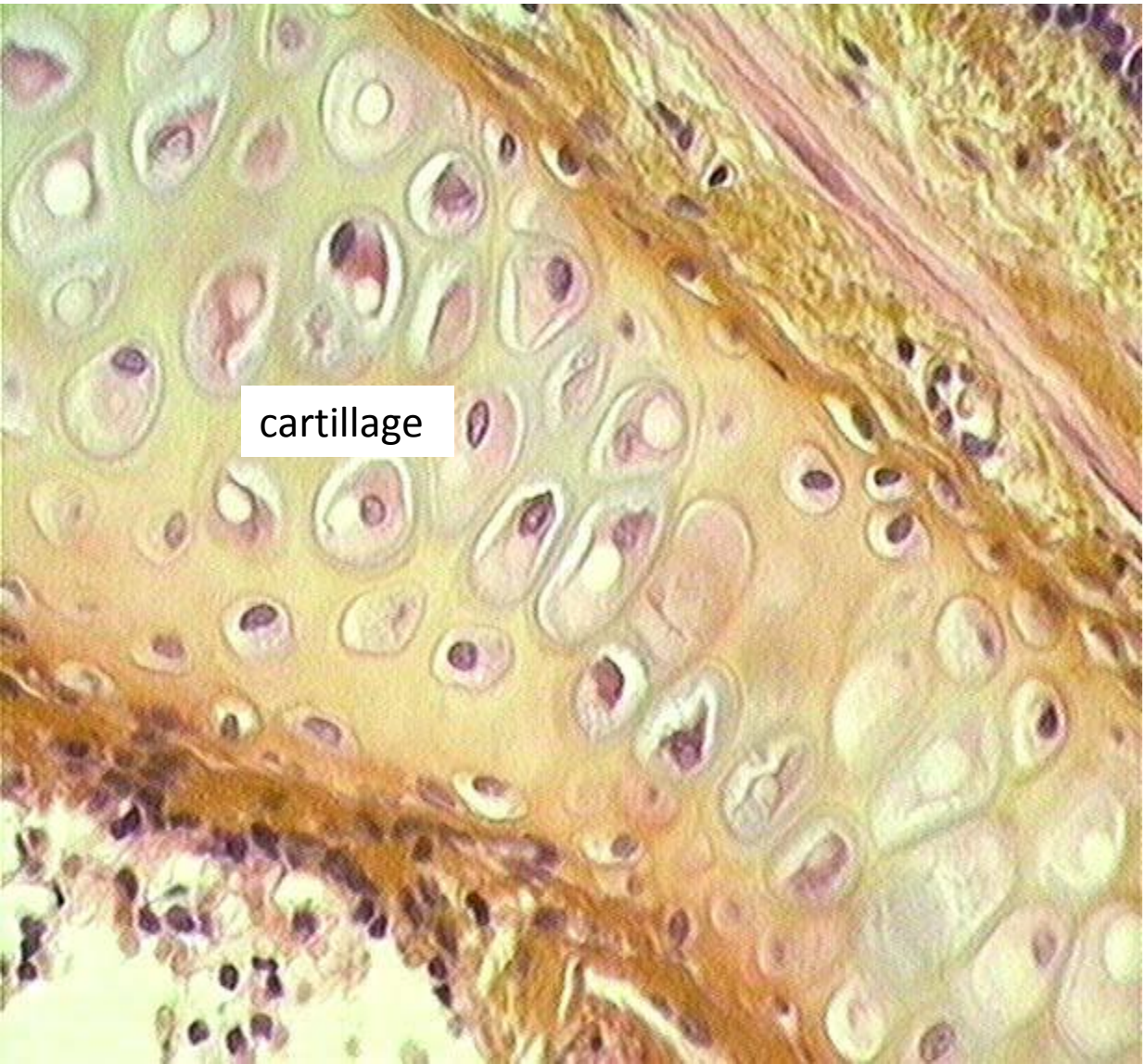
Hematoxyline and eosin (HE)



basophilic cytoplasm of glandular cells *(contains ribosomes with RNA)*

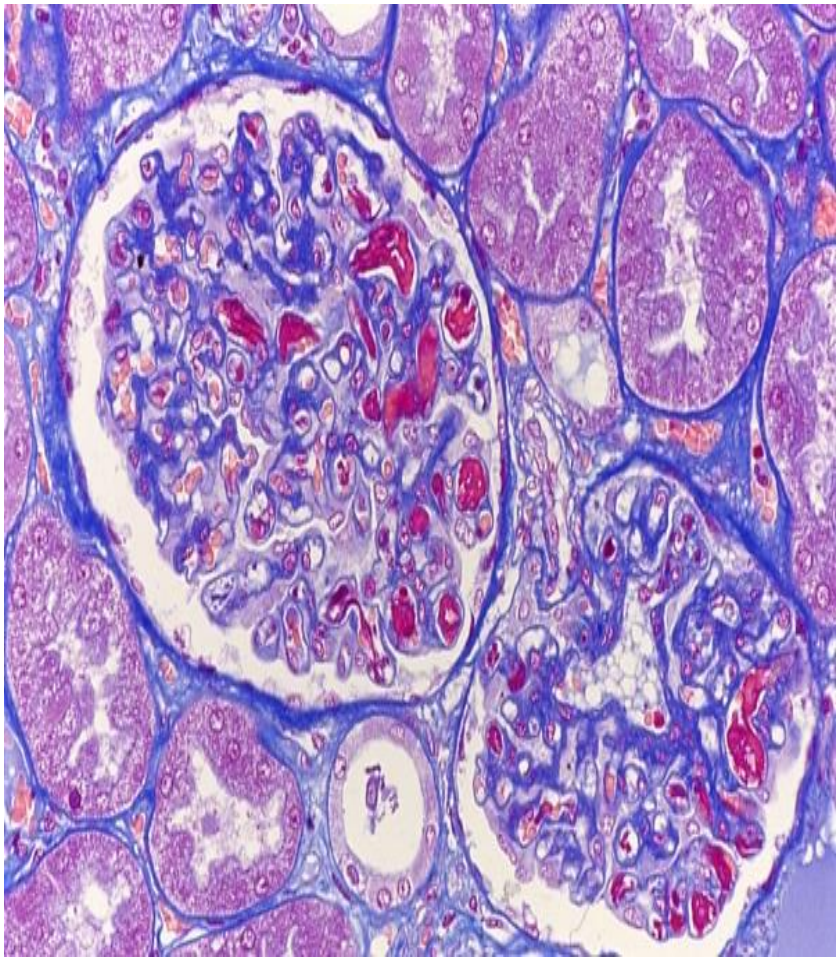
acidophilic cytoplasm of epithelial cells

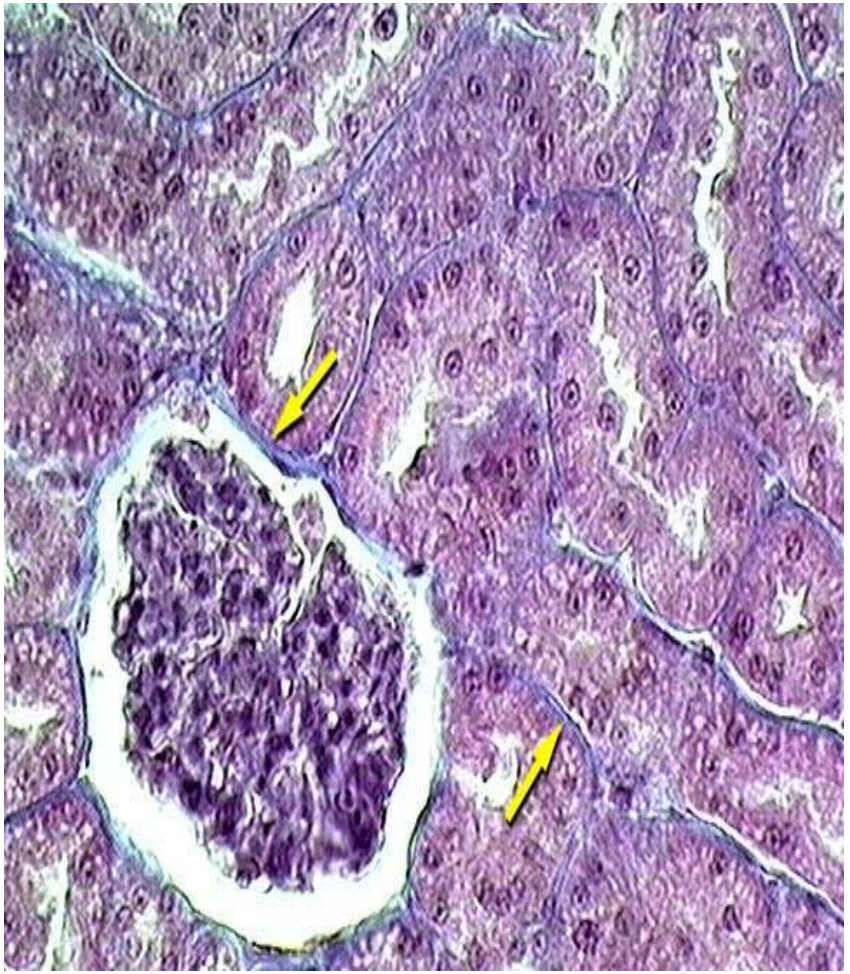
Hematoxyline, eosin and saffron (HES)



Collagenous fibers
of connective tissue
are yellow after staining
with saffron

Azocarmine and aniline blue (AZAN)

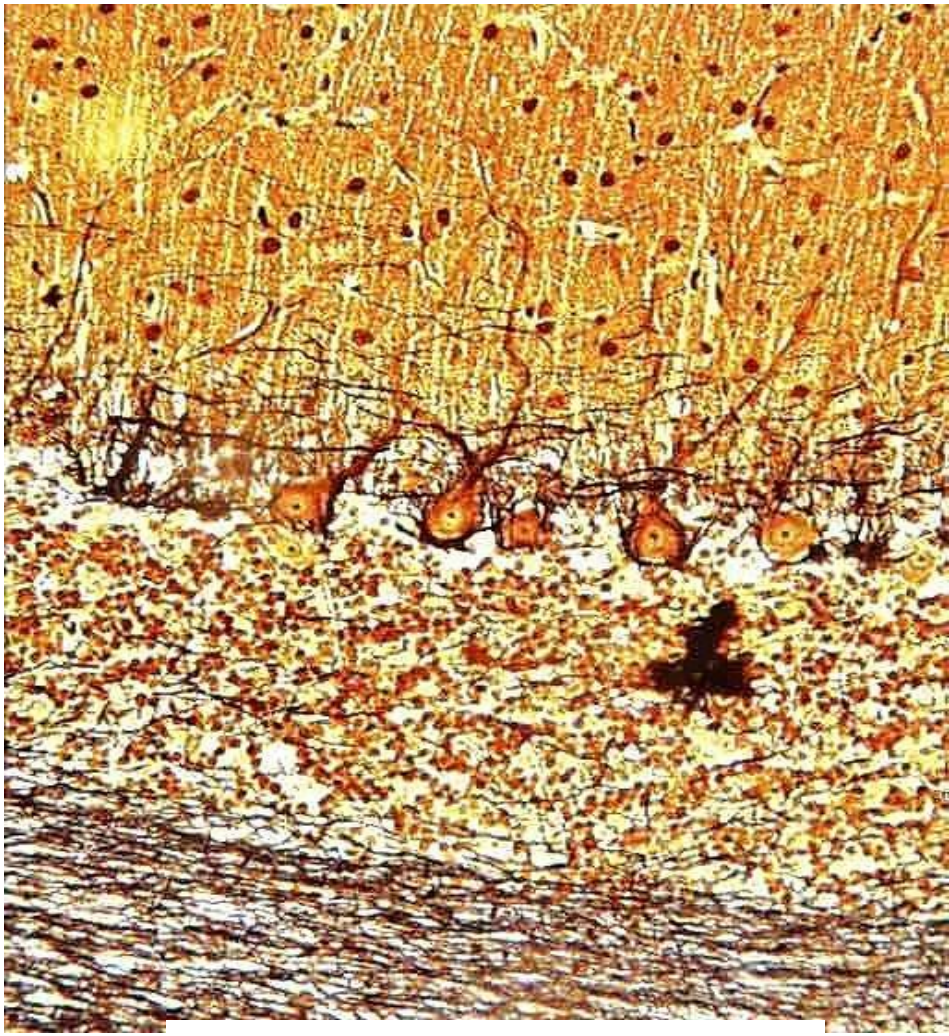




Kidney – collagen connective tissue

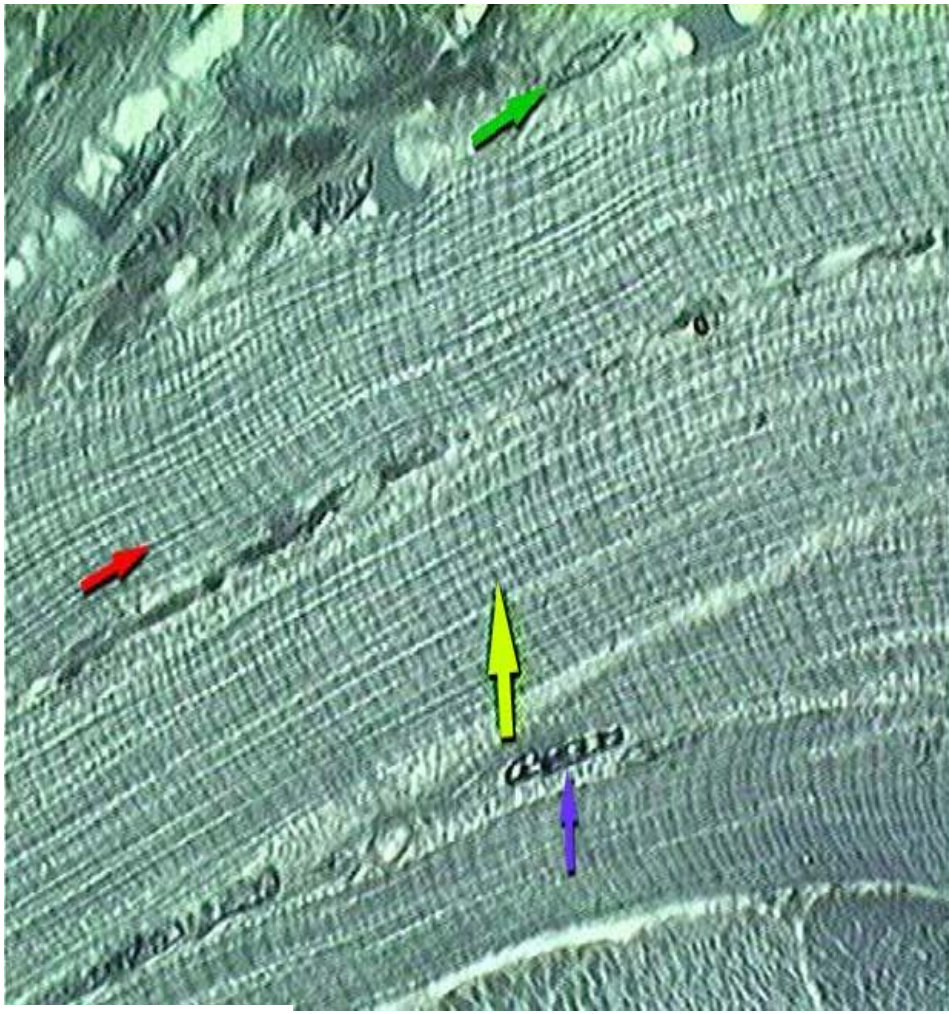
Impregnation of tissue with silver

Lien - reticular fibers



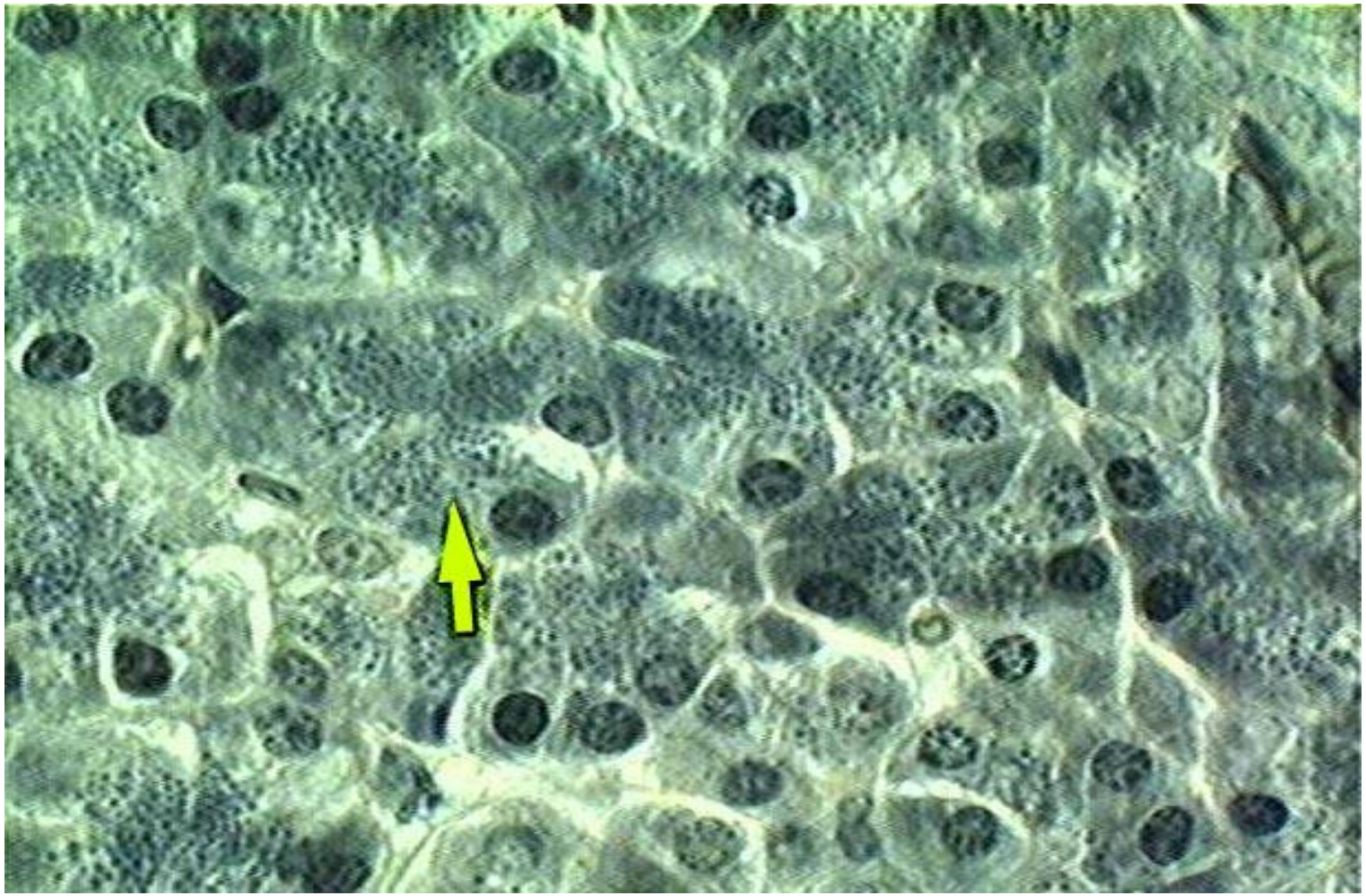
Cerebellum – nerve fibers

Iron hematoxyline

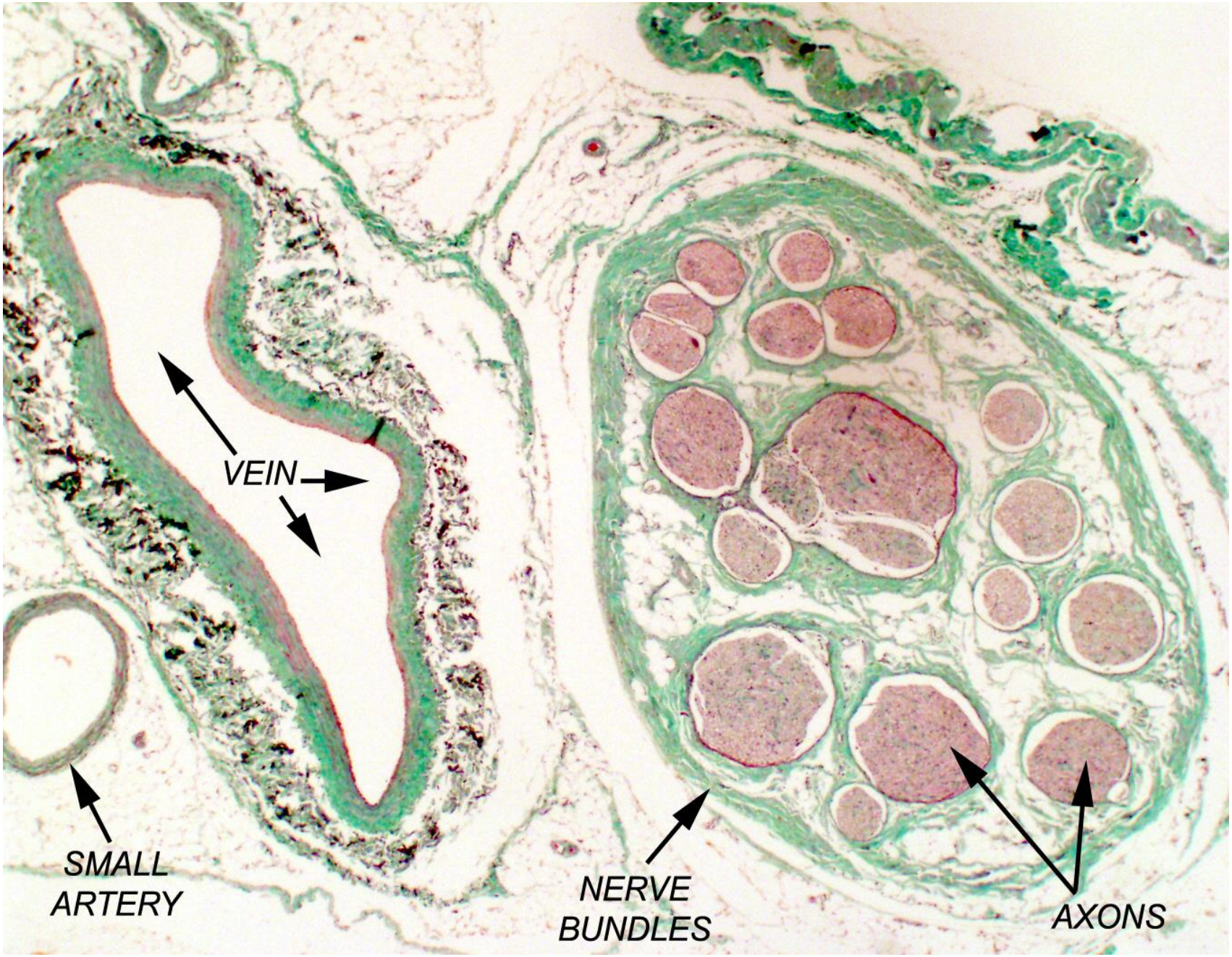


Skeletal muscle cells (fibers)

Iron hematoxyline



Mitochondria in hepatocytes


VEIN
SMALL ARTERY
NERVE BUNDLES
AXONS

Histochemistry and Immunohistochemistry

- Relevance:

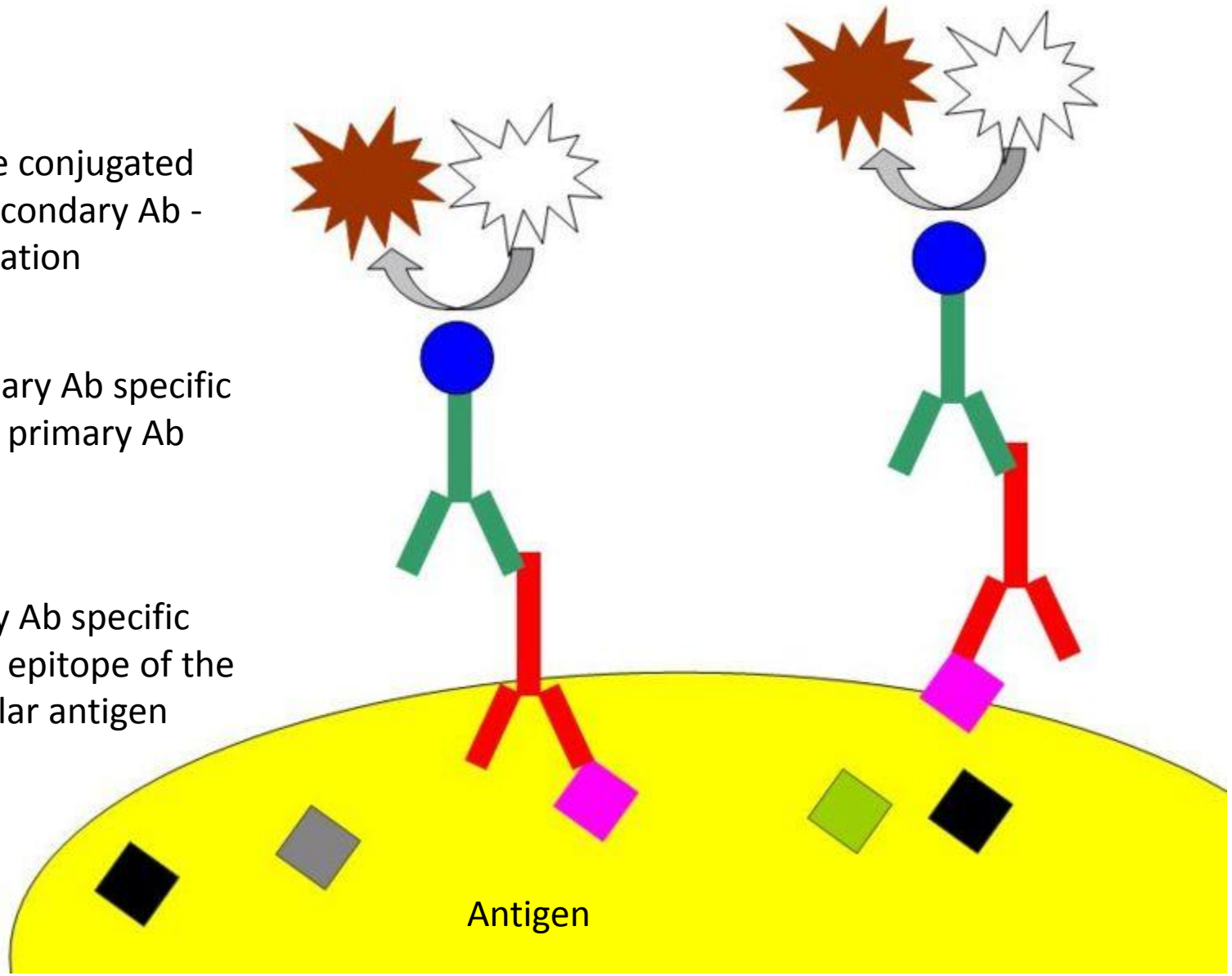
various chemical compounds detected „in situ“ (proteins, AA, NA, saccharides, lipids, enzymes, pigments, inorganic substances – Fe, Ca, Zn)

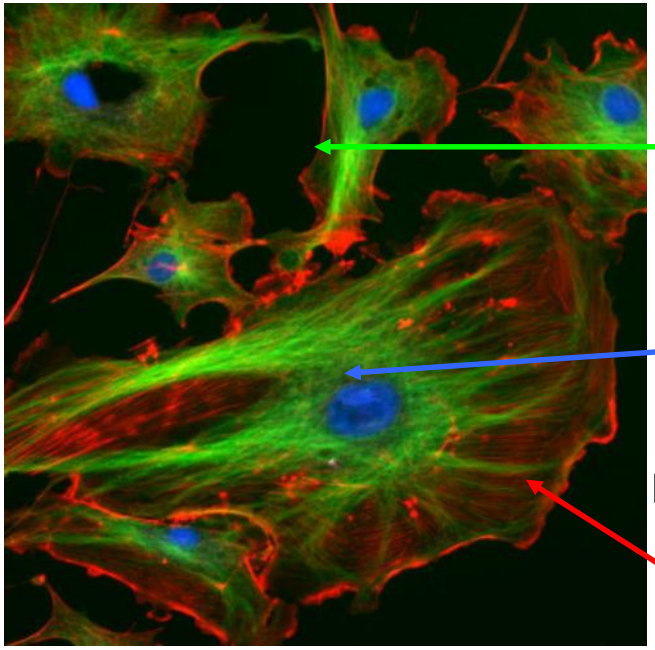
Various epitopes detected by immunotechniques

Enzyme conjugated with secondary Ab - visualization

Secondary Ab specific against primary Ab

Primary Ab specific against epitope of the particular antigen

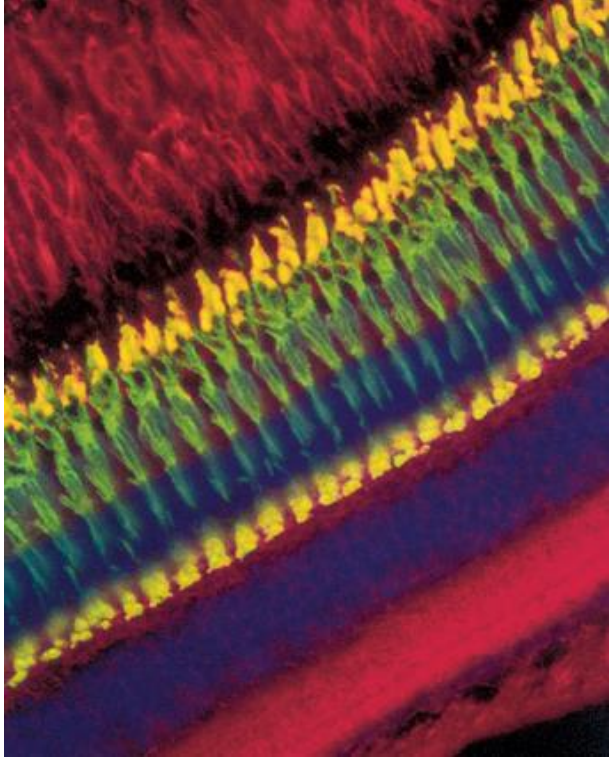


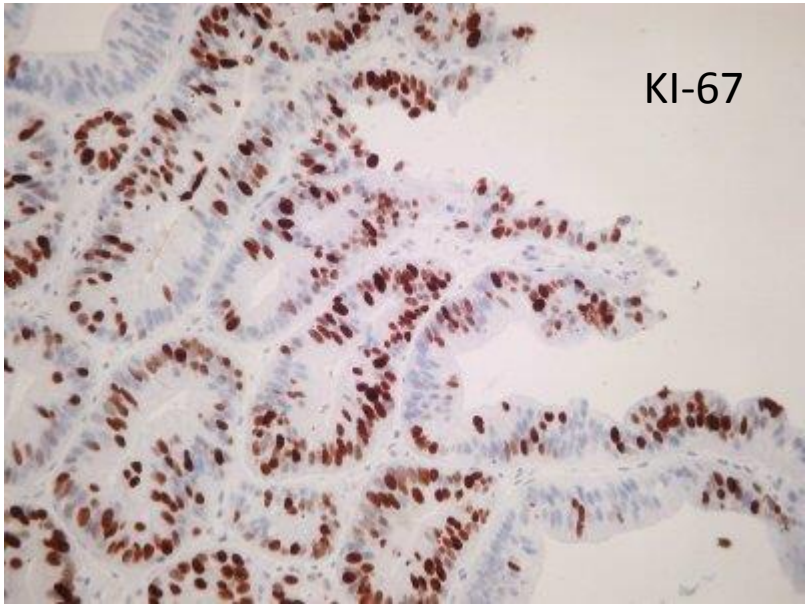


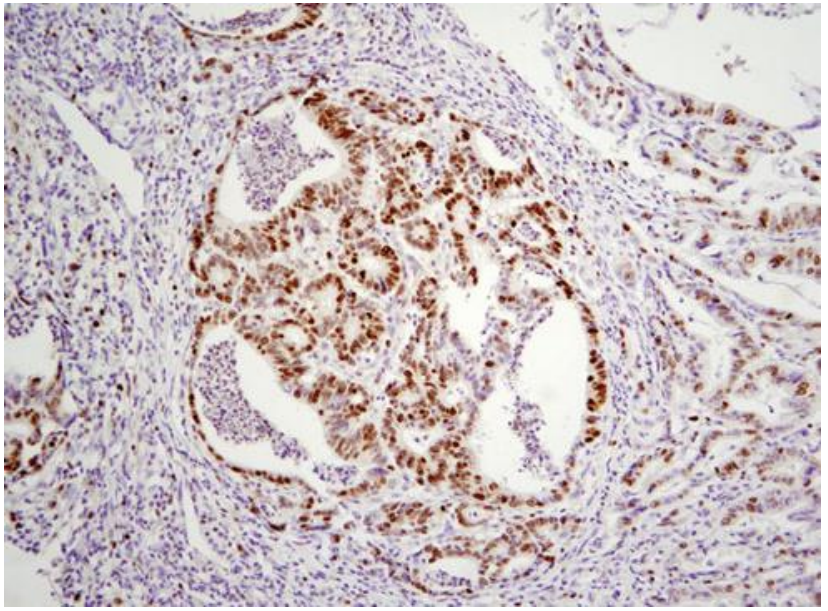
Actin (cytoskeleton)

DAPI (nucleus)

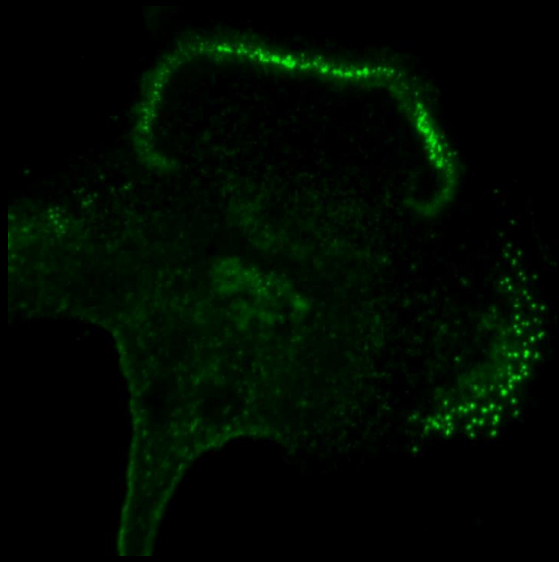
Microtubules (cytoskeleton)

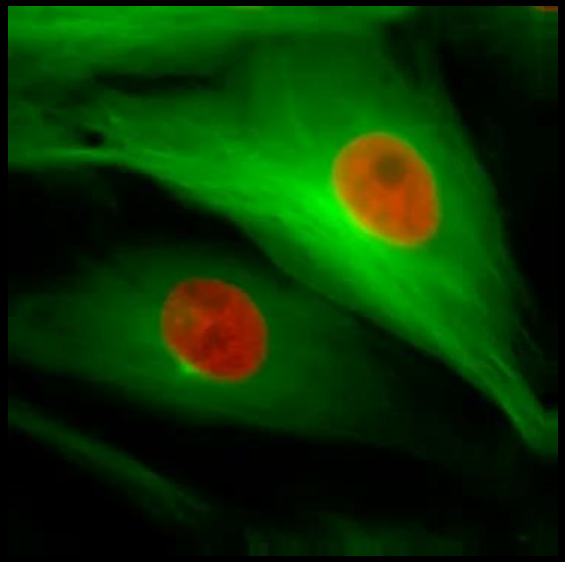


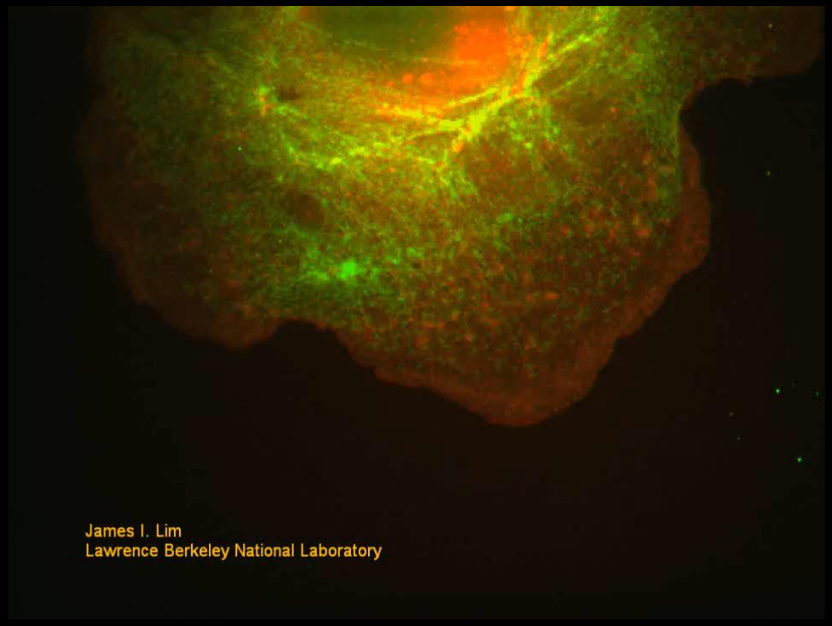




- Fluorescence labelled proteins





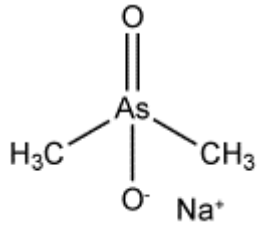


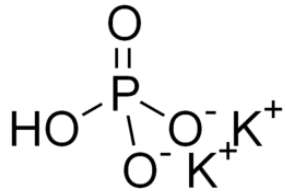
Tissue processing for the EM

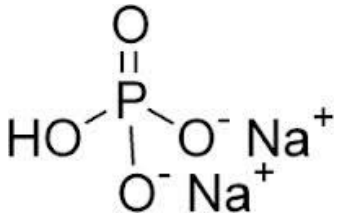
- pH of all solutions (media) must be buffered on **7.2 – 7.4**
  Cacodylate or phosphate buffer is frequently used.

- Absolutely dustfree environment

- Solutions (media) have to be precise (artifacts)







Tissue processing for the EM

- **SAMPLING** – immediatelly after arresting of blood circulation, tissue block sized no more than **1mm^3**
- **FIXATION** – glutaraldehyde (binds amine groups) + OsO_4 (binds lipids) are used as double fixation
- **RINSING** – distilled water
- **DEHYDRATION -** ethanol
- **EMBEDDING** – gelatin capsule or plastic forms are filled with some medium (which can be polymerized from liquid to solid form) and pieces of fixed tissue are placed into this medium. Epoxyd resins (Epon, Durcupan, Araldite) are usually used as in water insoluble media.
- **CUTTING** – ultrathin sections (in ultramictomes)
- **CONTRASTING** ≈ staining

<u>Embedding tools</u>:


1

2

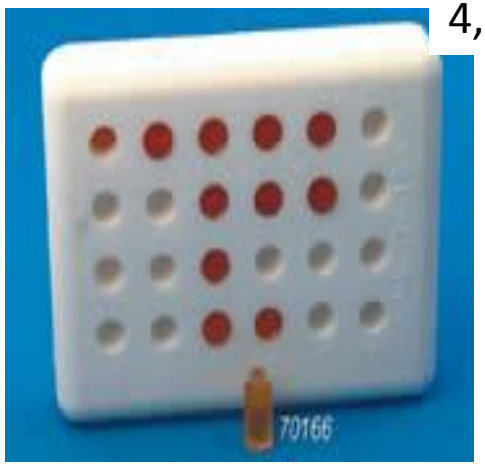
gelatin (1) or plastic (2) capsules

capsule holder (3)


3

embedding plates
(4, 5)

4,5

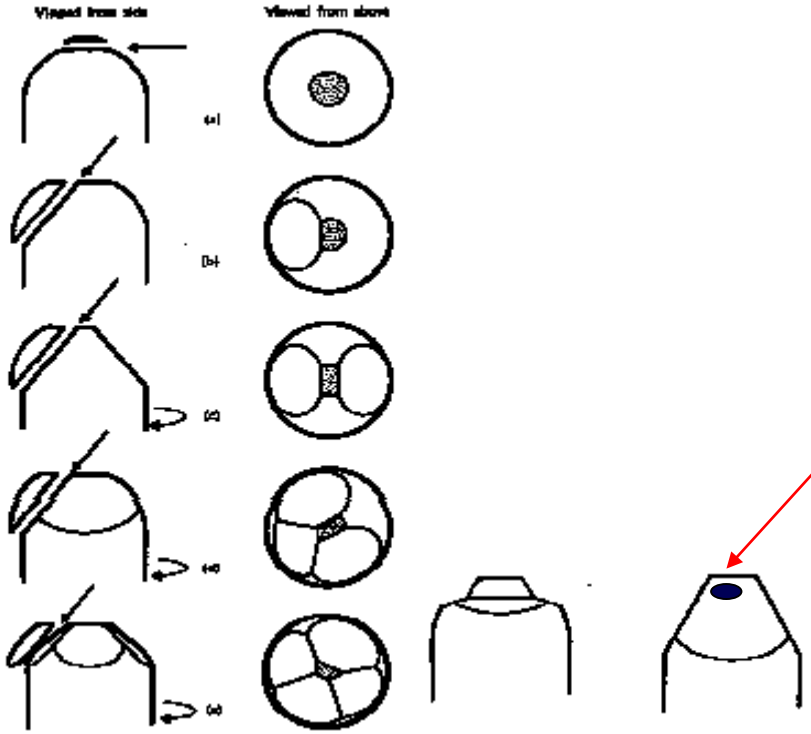


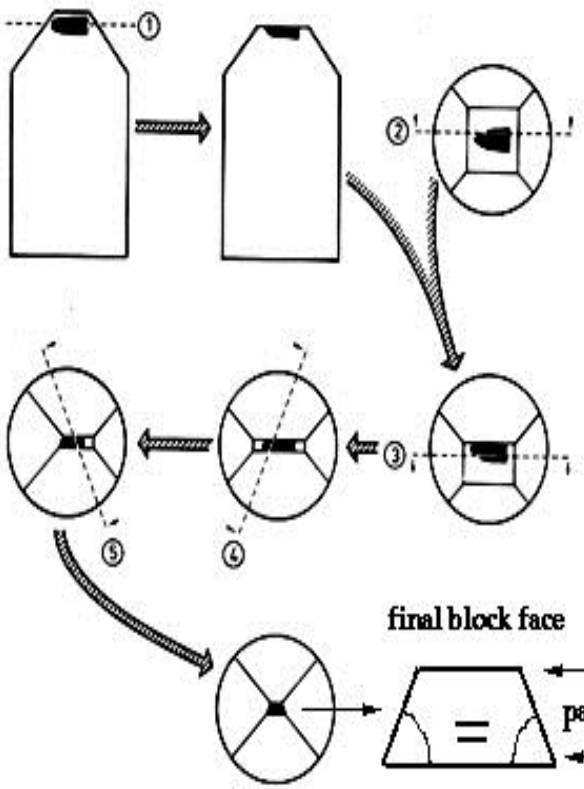
Embedded blocks
prepared for cutting

Trimming the Specimen Block

By trimming, using ultramicrotome, an excess of hard medium is removed and pyramide with minimal cut surface (0.1 mm2) is prepared.

Minimum of tissue (black) is in the top of pyramid





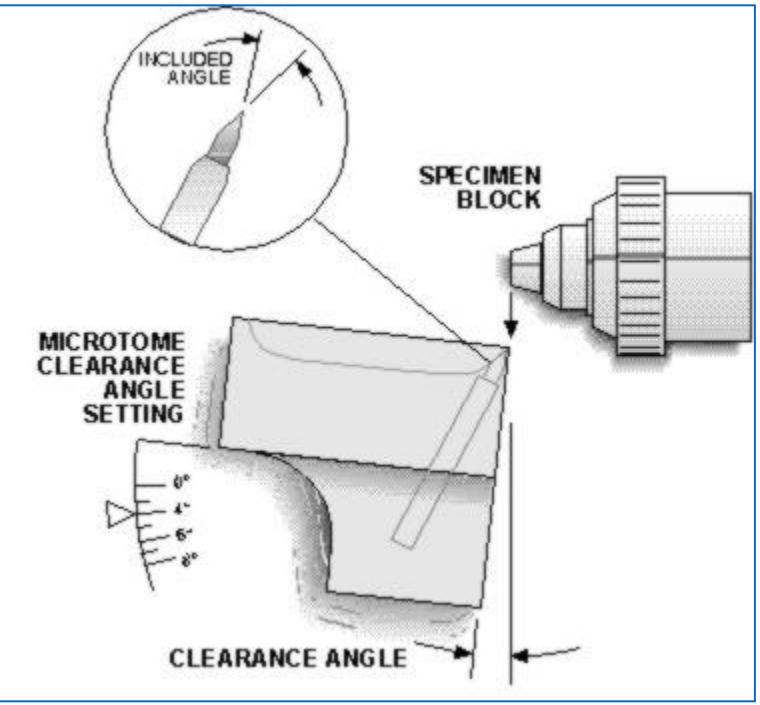
Pyramid Side Profile

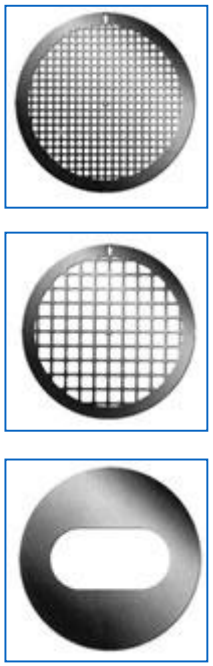
Too Steep-Trans Am Building
(not rigid-vibrations)

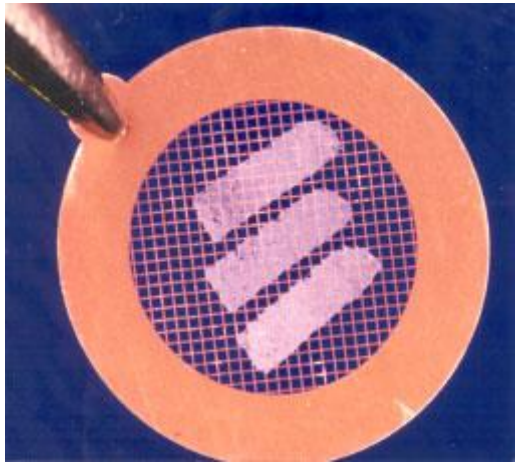
Too Flat-Pyramid of the Sun
(section size changes rapidly)

Just Right-Egyptian Pyramid
with top cut off

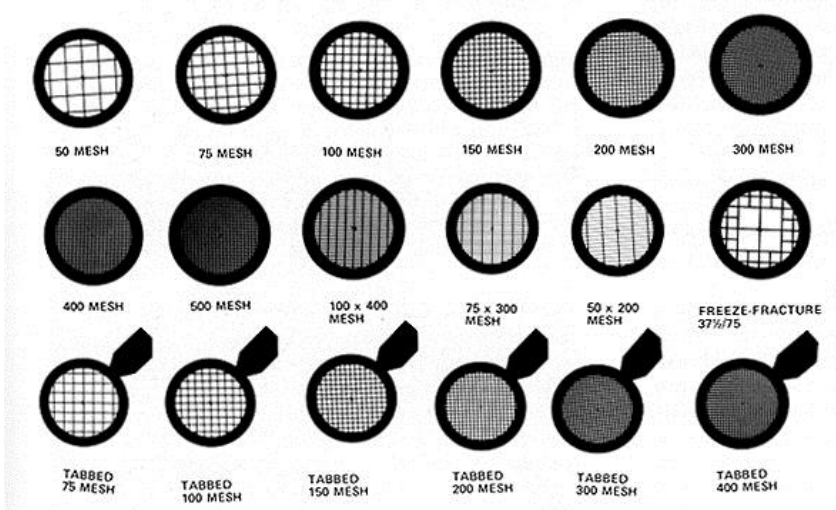
Cutting

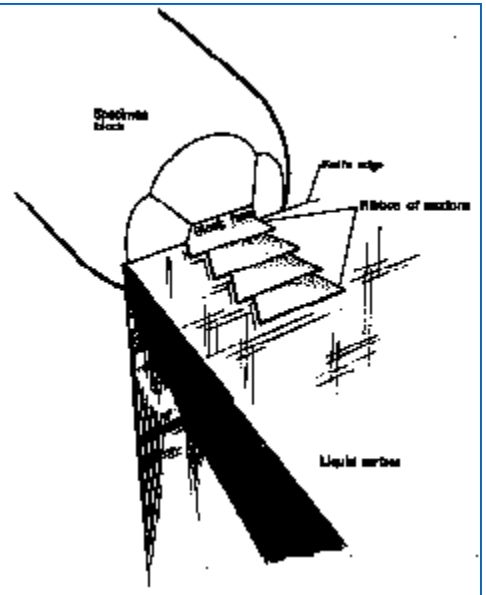


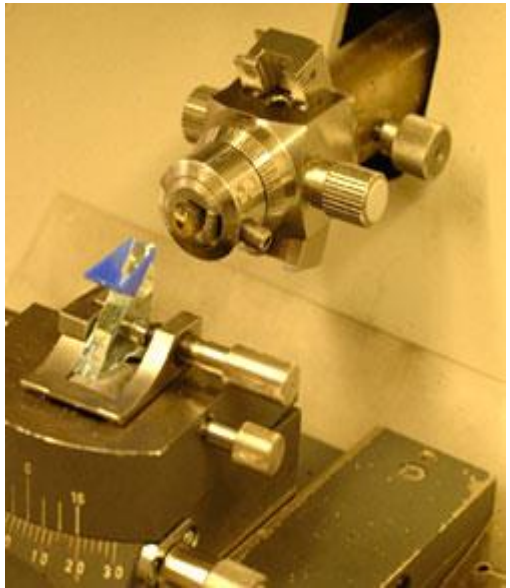




Grid Types and Mesh Sizes







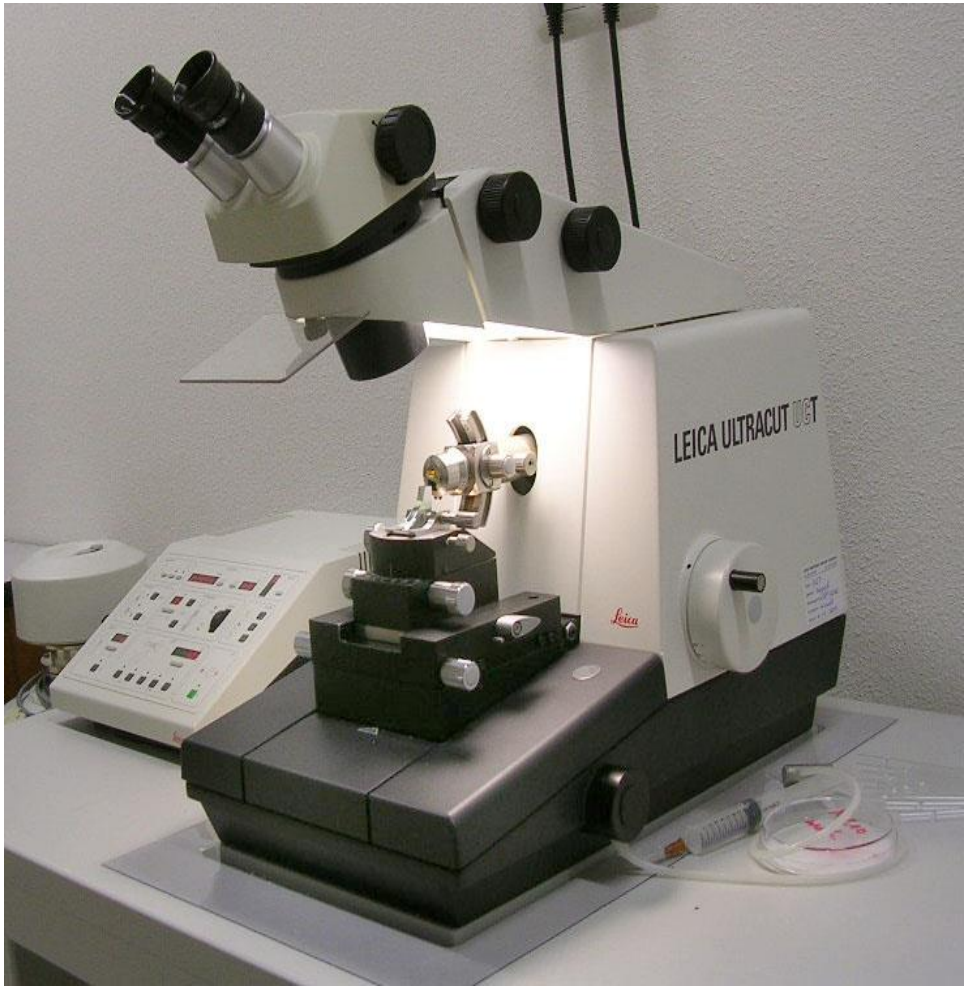
Cutting

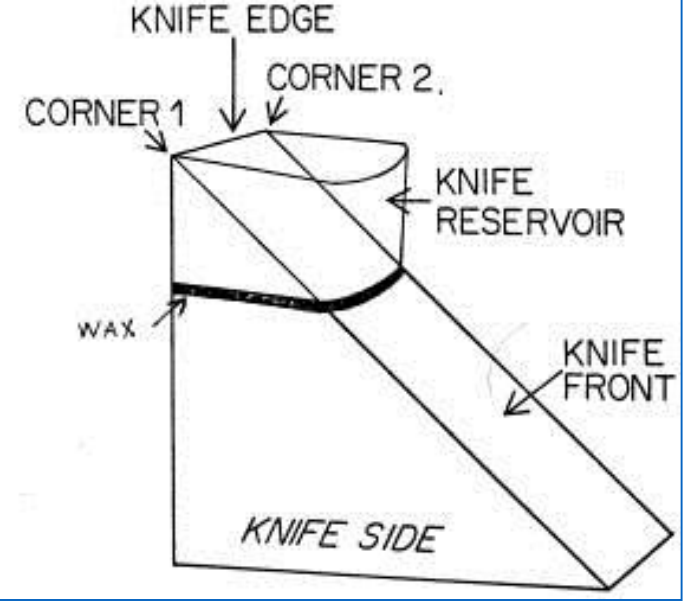
Ultrathin sections (70 – 100 nm) - ultramicrotomes.

Glass or diamond (b) knives with water reservoir are used

Sections slide flow on water in small container attached to the knive

Supporting grids

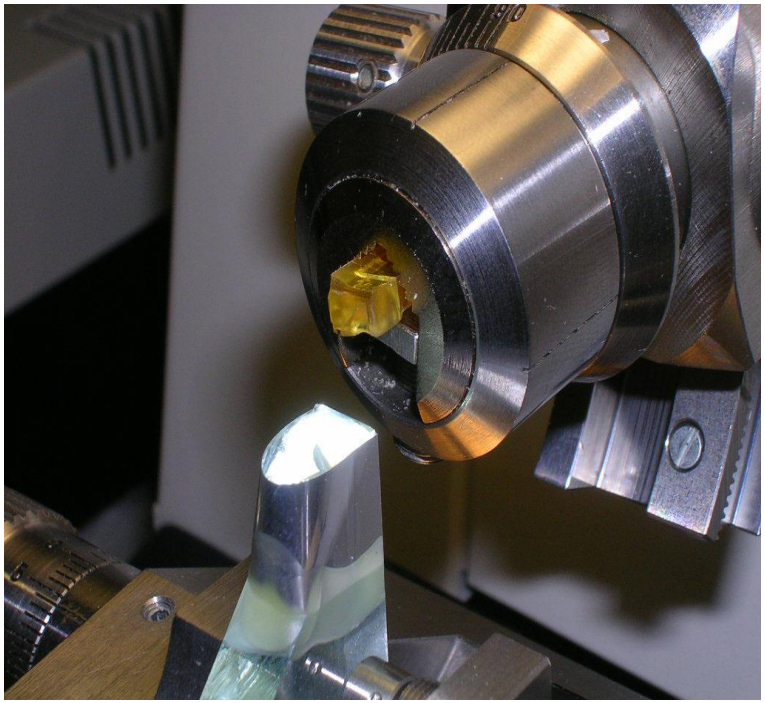


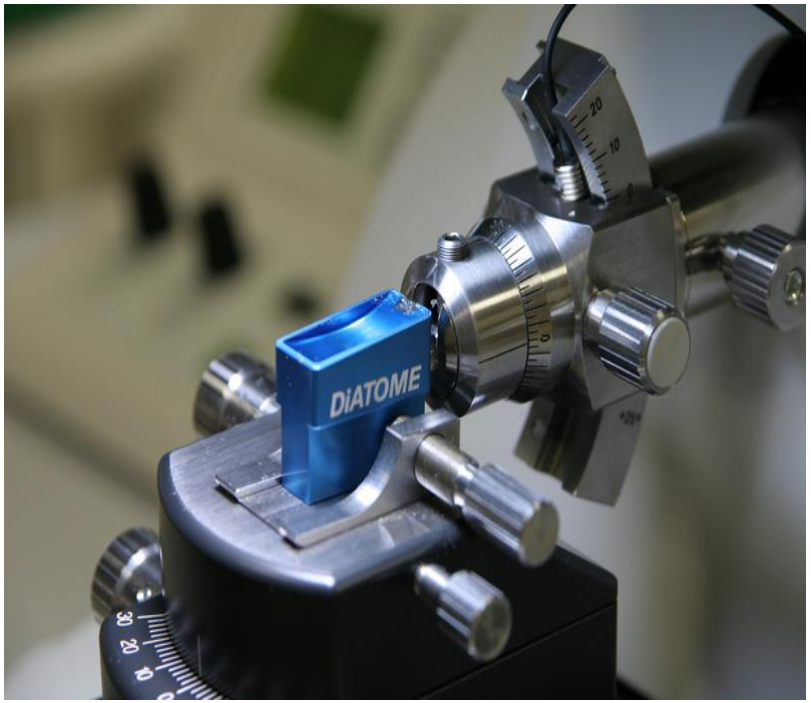


Ultramicrotom knives:

glass

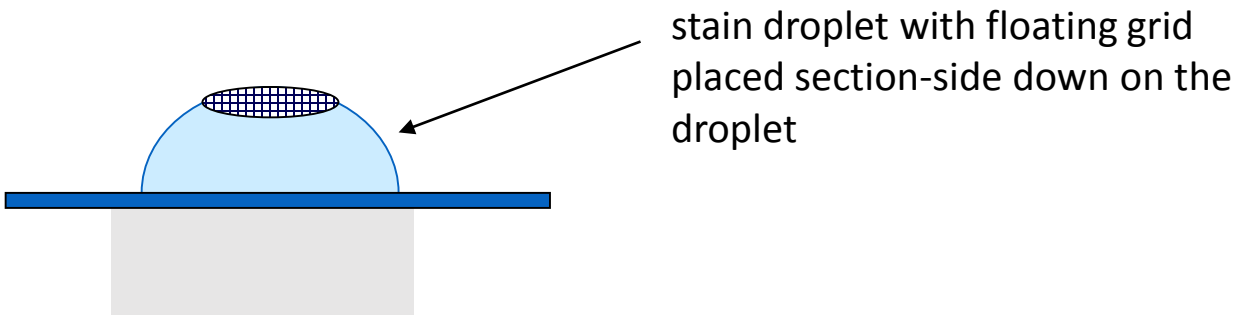
diamond



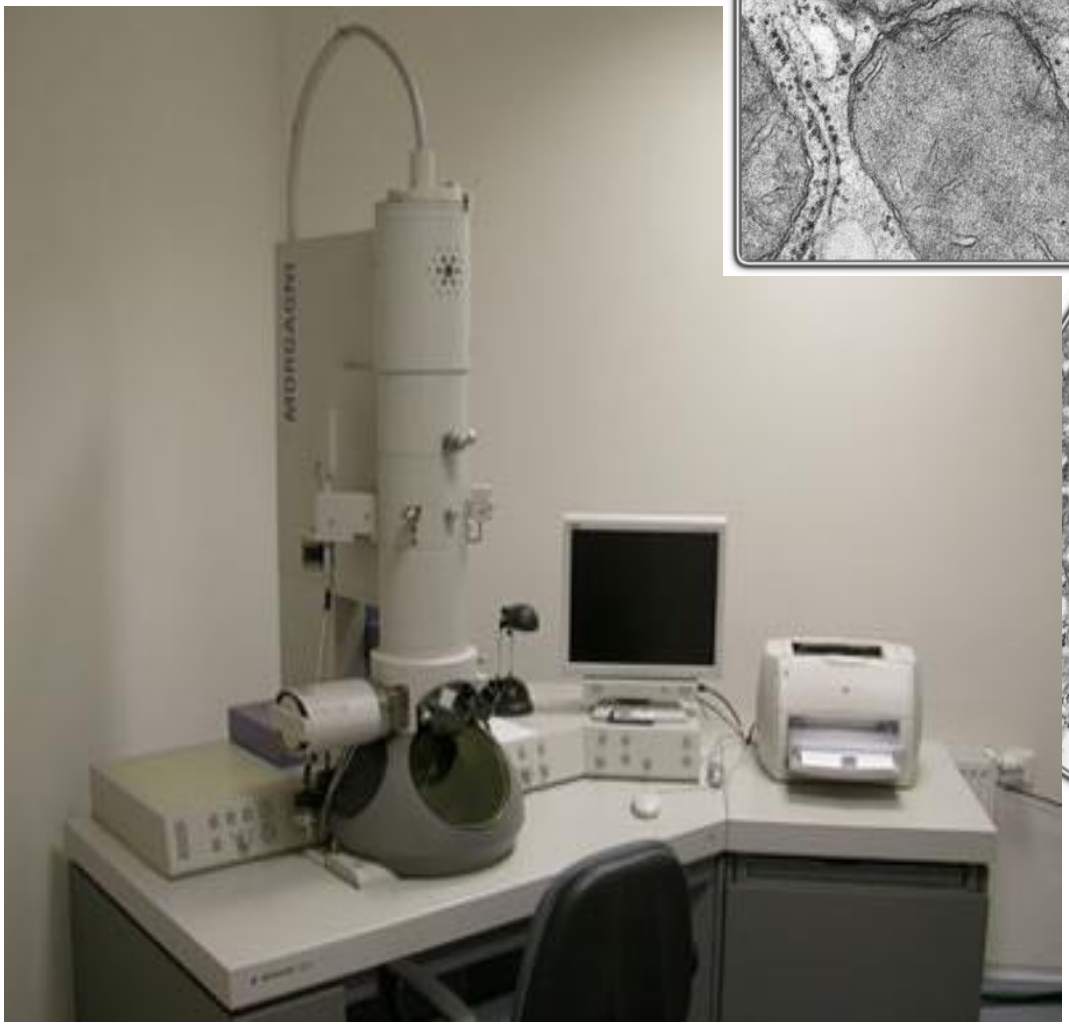


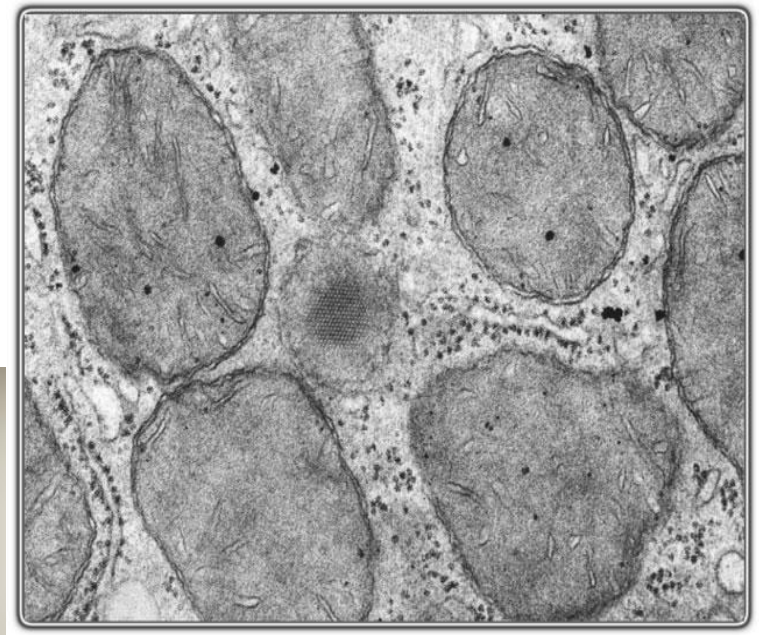
CONTRASTING (=STAINING)

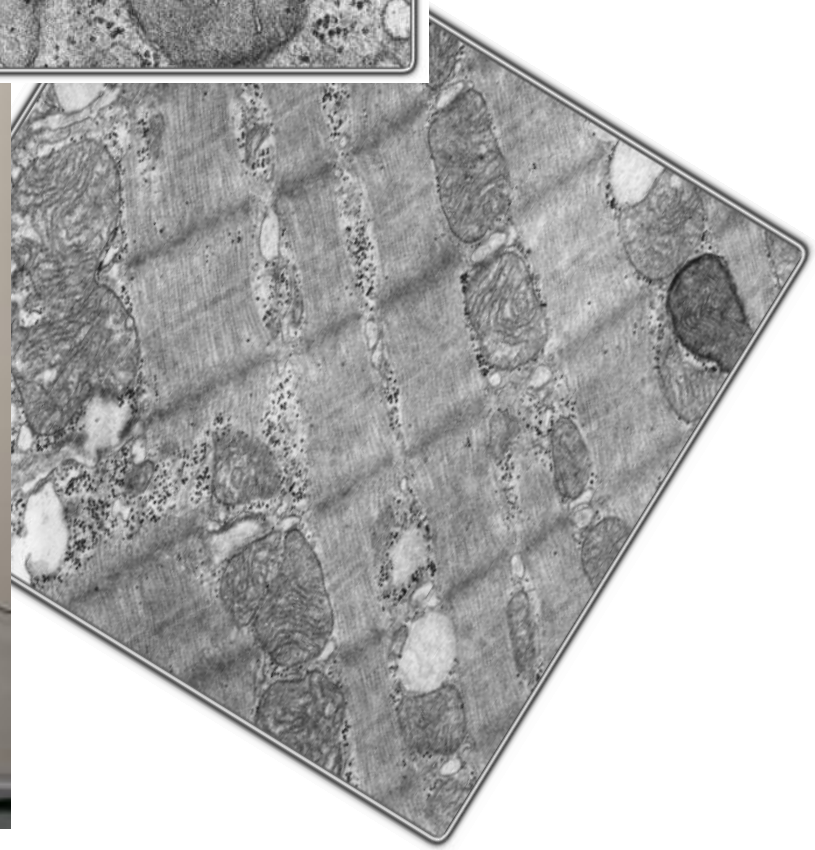
- principle of differentiation of structures – different dispersion of beam of electrons depending on atomic weight of elements.
  „electron dyes“ are thus mixtures of heavy metals: uranylacetate or lead citrate



Differences between LM and EM		
	LM	**EM**
Sampling	< 1 cm^3 minutes	< 1 mm^3 seconds
Fixation	formaldehyde 12 – 24 hours	glutaraldehyde 1 – 3 hours
Embedding	paraffin	epoxid resins (Durcupan)
Cutting Thickness of sections	microtome 5 – 10 µm	Ultramicrotomes 50 – 100 nm
Staining (LM) contrasting (EM)	dyes (*hematoxyline – eosin*)	heavy metals (*uranylacetate,lead citrate*)
Mounting (only LM)		---
Result	histological slide (preparate)	photograph of ultrathin section


MORGAGNI





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