# Practical training
# in Histology and Embryology

# Organization issues

- Beginning - **strictly on time**
- Change your shoes - you will not be allowed to enter the hall w/o indoor shoes
- Lockers – Jackets, coats, bags etc.
- Cell phone – switched off or in silent mode
- Microscopic hall = **laboratory**
  - eating, drinking, smoking not allowed
  - smoking strictly forbidden anywhere in LF
  - students have to follow the instructions
  - academic misconducts or inappropriate behavior result in excluding from the lesson or course

- **Follow safety rules**
- **You have dedicated working place**
- **You are responsible for microscope, slide set, EM atlas**

- **Practical lesson**
  - Introduction; the images free available through **Atlas of Histology**
  - your individual work = study of the slides, schematic but precise drawing of tissue architecture, careful description. You make your own „study atlas“
  - students come prepared for practices  - schedules and syllables – pin-boards or dpt. webpage
  - **your knowledge is verified during semester**
  - **break – 10 minutes**

- **Attendance**
  - 100% attendance
  - substitution only in exceptional cases, after permissions from both the teacher of your group and the lesson where you plan to substitute
  - sign in to the list
  - make a protocol, let it check and signed by the lecturer

# Registration of substitution:

| Datum<br>Date | Jméno<br>Name | Ročník<br>Year | Skupina<br>Group | Č. praktika<br>Nr. of practice | Č. místa<br>Nr. of place | Vyučující - podpis<br>Teacher- signature |
| --- | --- | --- | --- | --- | --- | --- |
| | | | | | | |

- **Protocols**

– you have to make **paper protocols** (no tablets, laptops)

– A4 size, blank, without lines, **according to the template** (can be downloaded from www.med.muni.cz/histology - Education)

– (color)pencil handdrawings (**no pen**)

– **complete set of signed protocols** is required for getting the credits

– **the quality of the protocol** is approved by your teacher's signature at the end of practical lesson

– **incomplete or low-quality protocols cannot be approved** and you have to substitute the respective practical lesson

**Protocol No: …………** **Name: ……………………………………………………………………**

**Date:……………………………………** **Year: ……………………** **Group: ……………………**

**TOPIC: ……………………………………………………………………………………………………………………………………………**

| **<u>List of slides for study</u>:** | | **<u>Atlas EM</u>: pictures for study** | |
|---|---|---|---|
| **Number** | **Designation (staining)** | **Page** | **Designation** |
| …………………………………………… | | …………………………………………… | |
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**Guidelines for the establishment protocol**

1. Student shall prepare color diagrams of histological slides (crayons) or black and white diagrams of pictures in EM atlas (pencils).
2. Each diagram must be provide with the following information:
   - **<u>designation of slide</u> and <u>staining method</u>** (see the list above), or designation of EM photograph,
   - **<u>magnification</u>:** 10 x 4 / 10 x 10 / 10 x20 / 10 x 40 or total magnification: 40x / 100x / 200x / 400x,
   - <u>description of the diagram</u>.

**Checking of protocol**

Practice: regular ☐ substitute ☐ date ……………………………………………

……………………………………………………………
Signature of teacher

- **Testing your knowledge**
  - **every student is examined 4× per semester**
  - testing the knowledge of **structures** of the **previous practical lesson**, including the **theory** (their English and Latin names, functions, development and biological context) **AND** the **theory** for the **curent practical lesson**
  - short written test with images or schemes, results: „**P**assed“ or „**N**ot passed“
  - all images and schemes are made public (Atlas of Histology, IS)
  - you have to successfully pass all 4 tests
  - if you fail in partial test, you can repeat it once per semester (resit test)
  - failing in the partial tests result in the overal Credit test at the end of semester
  - **no further resit**

| Test | Credit given | Credit given | Credit test | Semester must be repeated | Semester must be repeated |
|---|---|---|---|---|---|
| 1 | P | P | P | P | N |
| 2 | P | P | N → P (N) | N → P (N) | N |
| 3 | P | N→ P (N) | P | N | N |
| 4 | P | P | N | N | N |

- **Credits**
  - 100% attendance
  - complete set of signed protocols from all lessons
  - passed four tests

- **End of practical lesson:**
  - the practice is closed by the lecturer
  - you are allowed to leave your working place only after checking the microscope and slides
  - if you leave before the check you may be responsible for any damages/losses recognized later

**Department of Histology and Embryology**
**Faculty of Medicine MU**
Head: prof. Aleš Hampl

[http://www.med.muni.cz/histology](http://www.med.muni.cz/histology)

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DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY

EDUCATION | ABOUT OUR DEPARTMENT

Semester 1, 2013/2014

**Programme of lectures and practicals in histology and embryology for the 1st year of General medicine**

**Lectures** | **Practicals**

# RECOMMENDED LITERATURE

**Mescher, A.L. *Junqueira's basic histology :text and atlas*. 13th ed. New York: McGraw-Hill Medical, 2013. xi, 544. ISBN 9781259072321.**
**Moore, K.L., T.V.N. Persaud a M.G. Torchia. *The developing human: clinically oriented embryology*. 9th ed. Philadelphia, PA: Saunders/Elsevier, 2013. xix, 540. ISBN 9781437720020.**
Ovalle, W.K., P.C. Nahirney a F.H. Netter. ***Netter's essential histology***. 2nd ed. Philadelphia, PA: Elsevier/Saunders, 2013. xv, 517. ISBN 9781455706310.
Young, B. ***Wheater's functional histology :a text and colour atlas***. 5th ed. [Oxford]: Churchill Livingstone, 2006. x, 437. ISBN 044306850X.
Sadler, T.W. a J. Langman. ***Langman's medical embryology***. Illustrated by Jill Leland. 11th ed. Baltimore, Md.: Lippincott William & Wilkins, 2010. ix, 385. ISBN 9781605476568.
Lowe, J.S. a P.G. Anderson. ***Stevens and Lowe´s Human Histology***. 4th. : Elsevier, 2015. ISBN 978-0-7234-3502-0.

+ **Lectures**
**Protocols**

Department of Histology and Embryology

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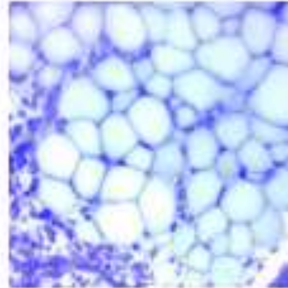
## Electronic textbooks and atlases

### Atlas of Histology

*recommended study tool*



Interactive Atlas of Human Embryology

http://www.med.muni.cz/histology/multimedia-and-textbooks/

# HISTOLOGY

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

- <u>relevance</u>: oncology, surgery, hematology, pathology, forensic,…

# EMBRYOLOGY

– prenatal (intra uterine) development

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

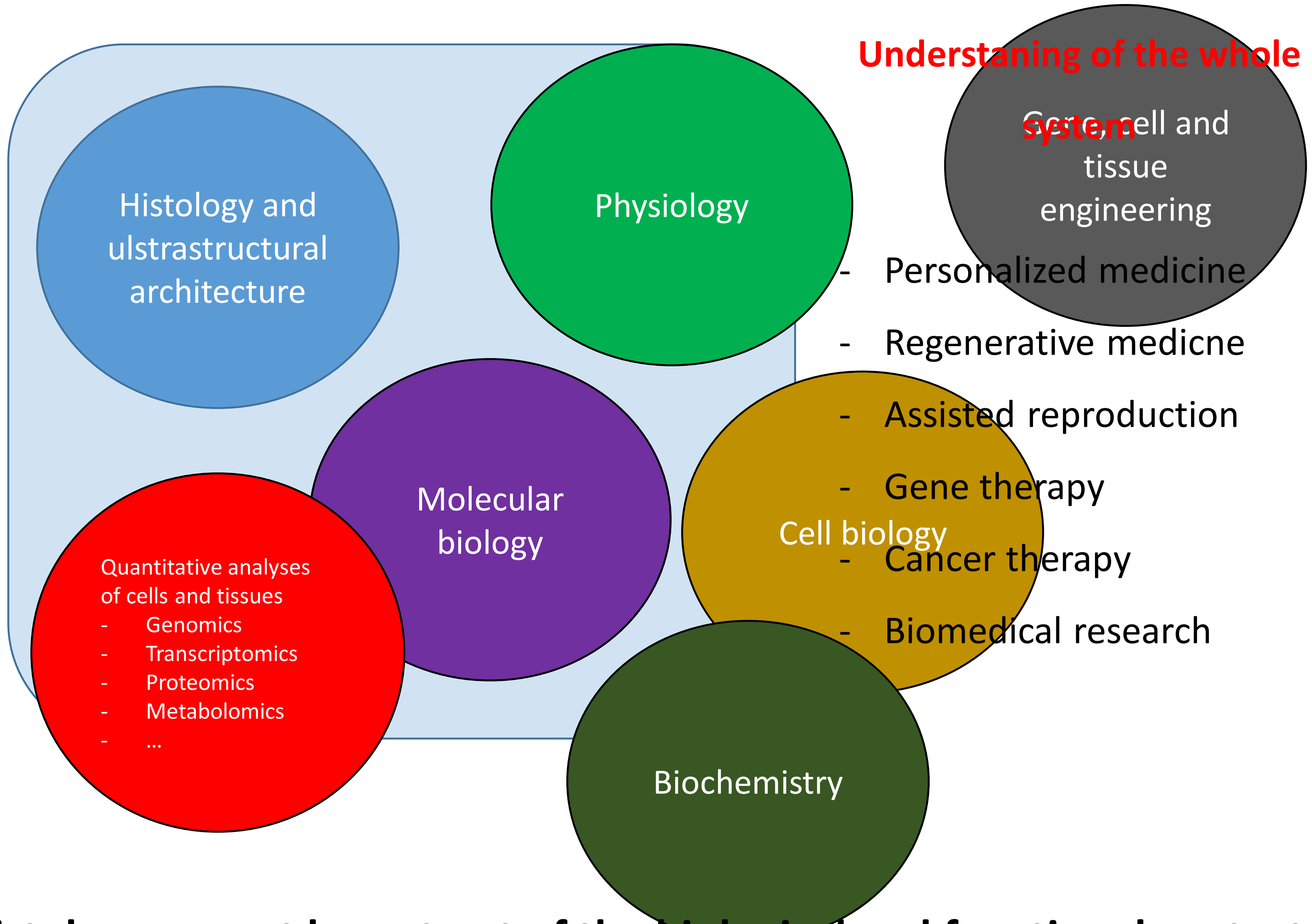
  gametogenesis and early embryonic development

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

  organogenesis

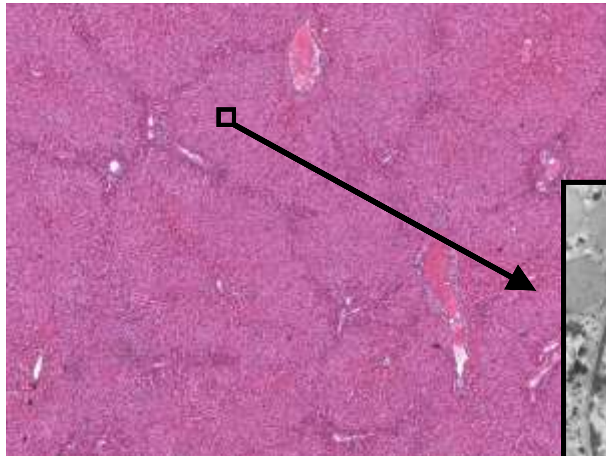
- **Teratology** – defects in organ development, malformations, anomalies; prenatal screening – ultrasonography, amniocentesis, genetic and karyotype screening

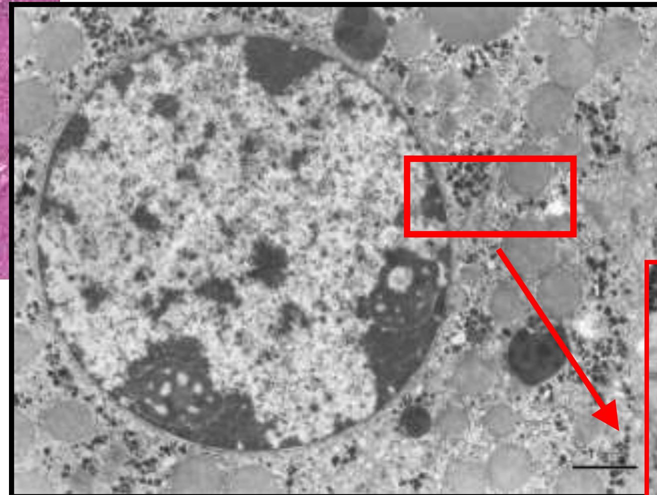
- Relevance: gynecology and obstetrics, pediatrics, assisted reproduction

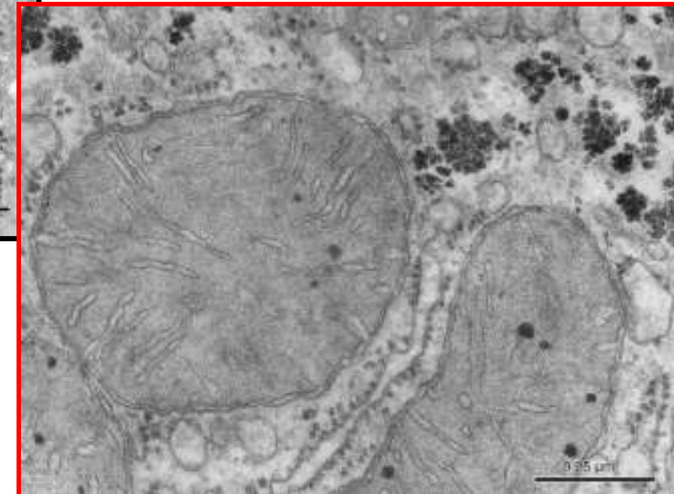

Understaning of the whole system
Histology and ulstrastructural architecture
Physiology
Gene, cell and tissue engineering
- Personalized medicine
- Regenerative medicne
- Assisted reproduction
- Gene therapy
- Cancer therapy
- Biomedical research
Molecular biology
Cell biology
Quantitative analyses of cells and tissues
- Genomics
- Transcriptomics
- Proteomics
- Metabolomics
- …
Biochemistry
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 purpose 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

- <u>Fixatives</u>: solutions of different chemicals

  - **organic fixatives** – <u>ALDEHYDES</u> – 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

Fixation is carried out at the room temperature, the time varies between **12 – 24 hours**, specimen must be overlayed 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.

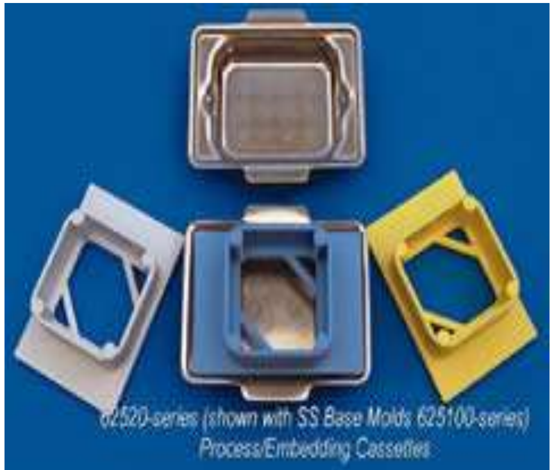
- The moulds are filled with melted paraffin, tissue samples are then placed inside and immediately immersed in cold water to cool paraffin quickly down.
- These paraffin blocks are ready for trimming

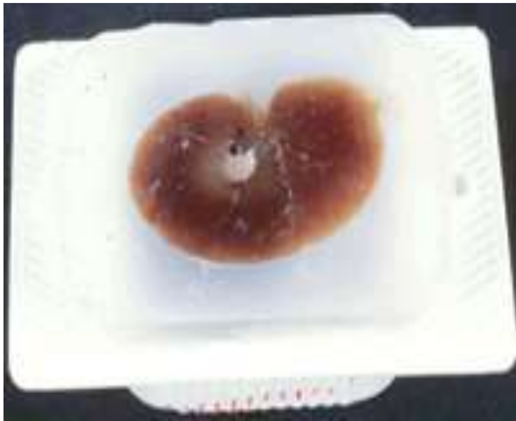
**Leica TP 1020**

Automated device for tissue dehydration

**Paper** chambers

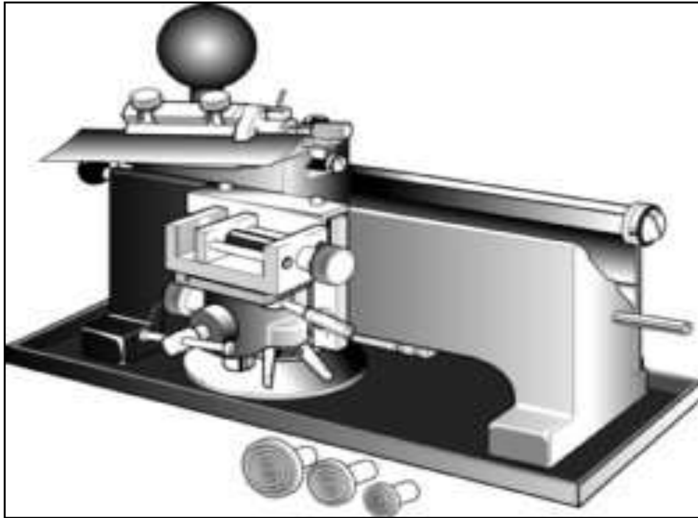
- **metal**



# CUTTING

- <u>Microtome</u> – 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

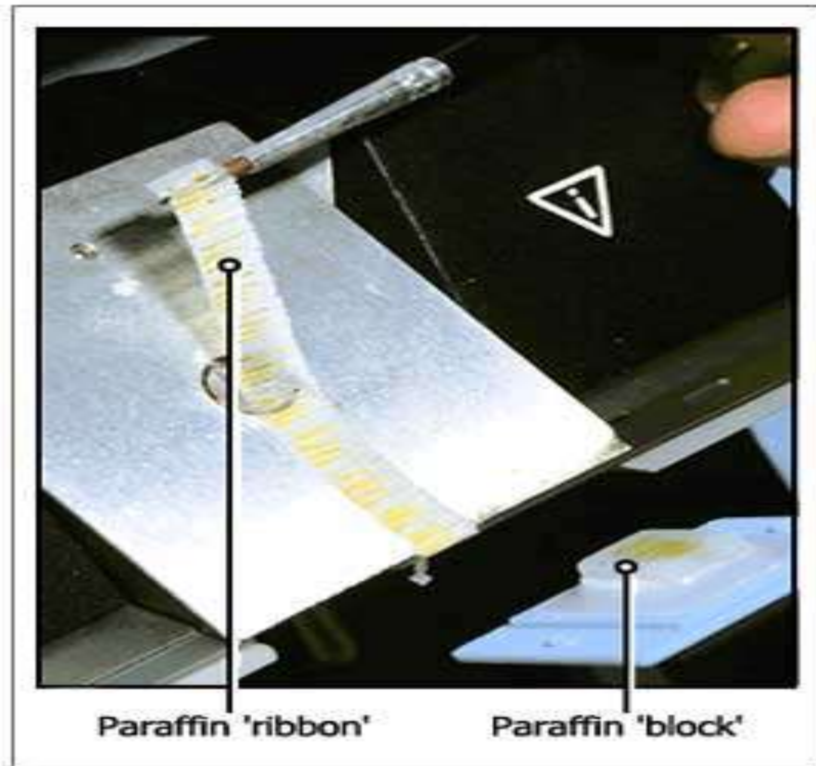
rotary microtome – knife is fixed, block holder moves vertically

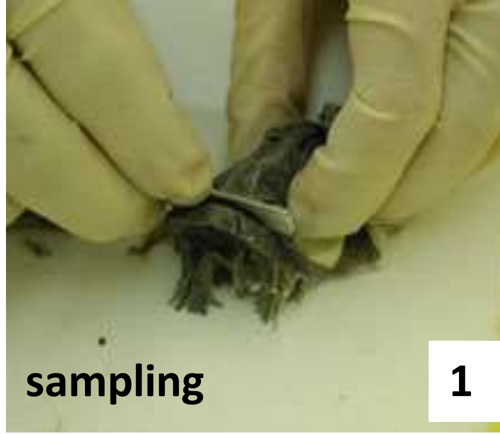
## Sliding microtome

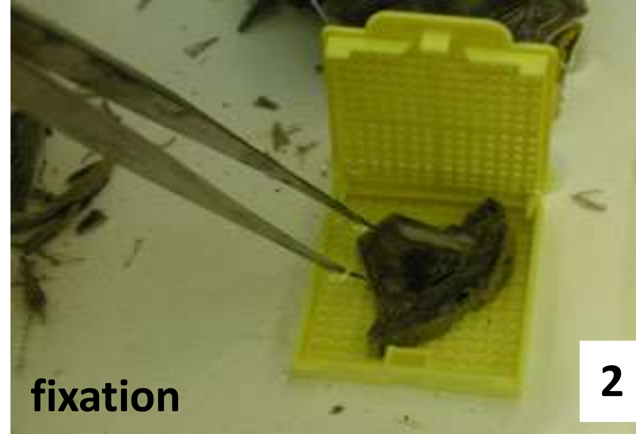
## Rotary microtome

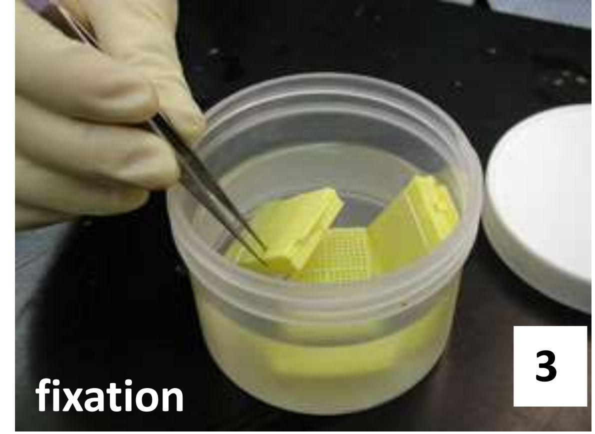
Freezing microtome (**cryostat**)
= rotary microtome housed in freezing box
(– 60º C)

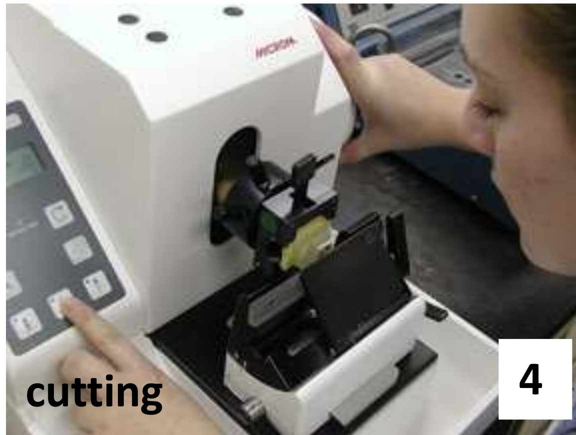
Cutting of frozen tissue without the embedding

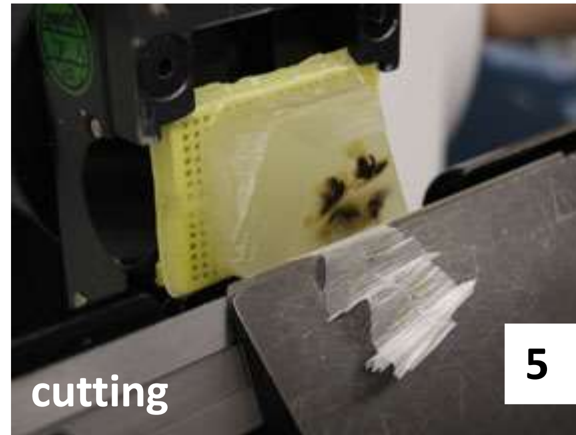



sampling
1

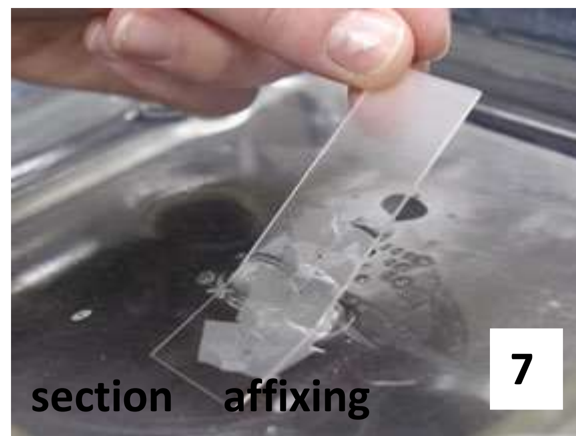

fixation
2

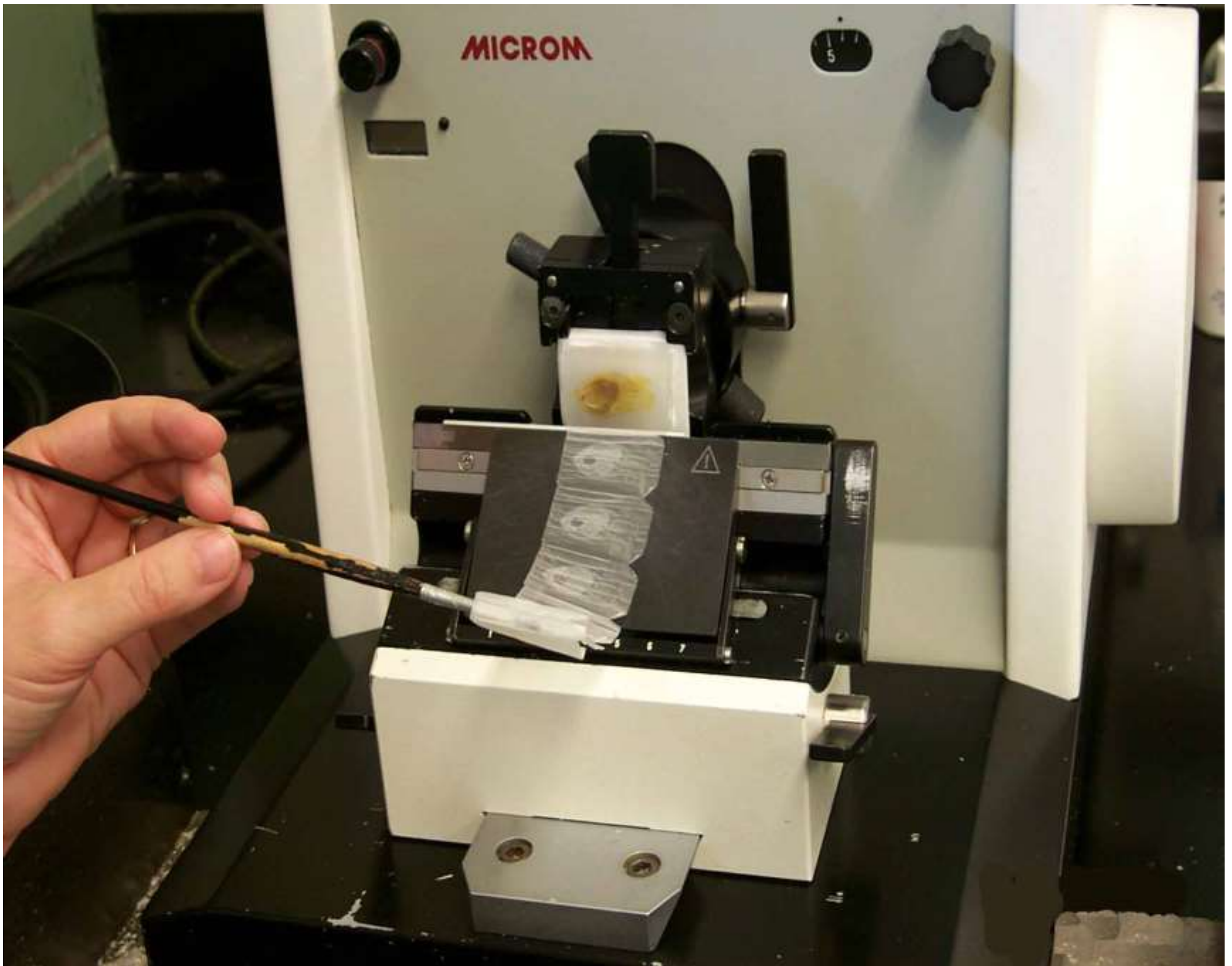

fixation
3

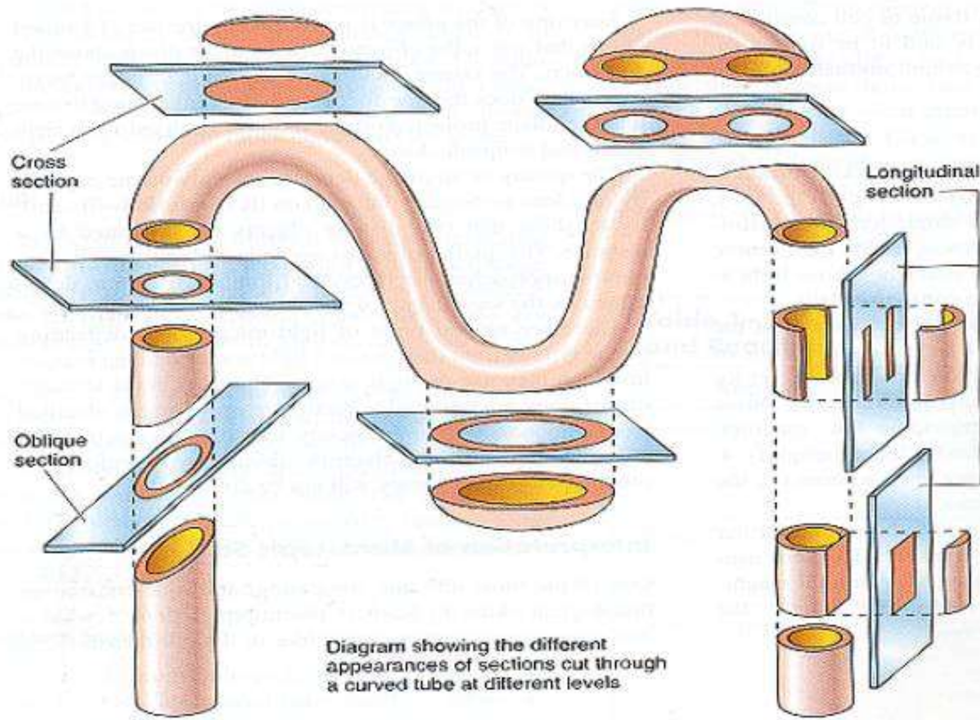

cutting
4

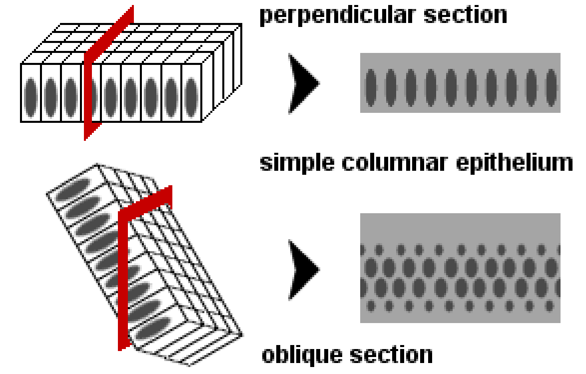

cutting
5

section affixing
6


section affixing
7


MICROM

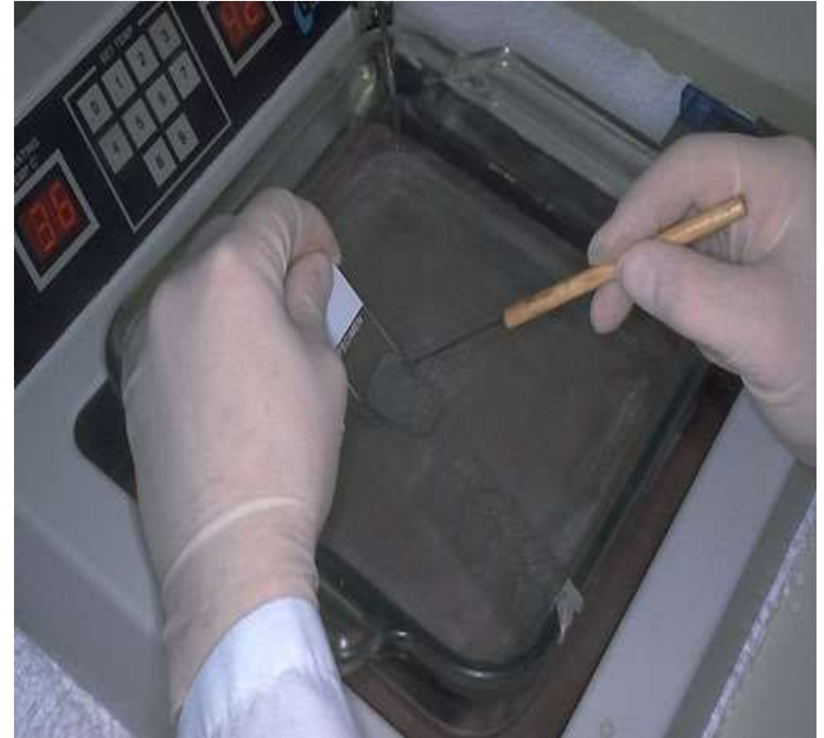

Cross
section
Longitudinal
section
Oblique
section
Diagram showing the different
appearances of sections cut through
a curved tube at different levels


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

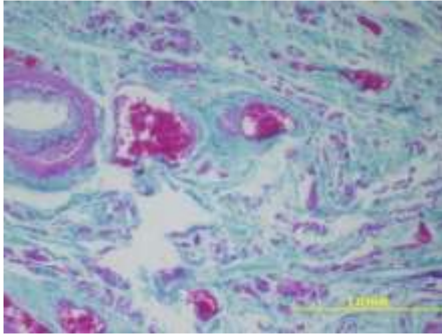
**acidophilia** – acidophilic structures in the cell

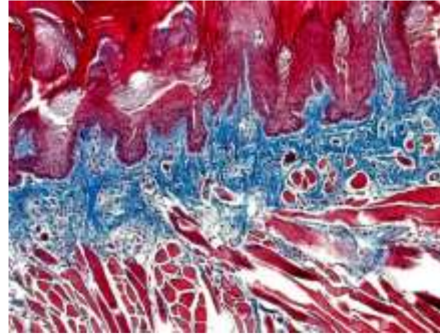
**neutrophilia** – no reaction

# Staining methods:

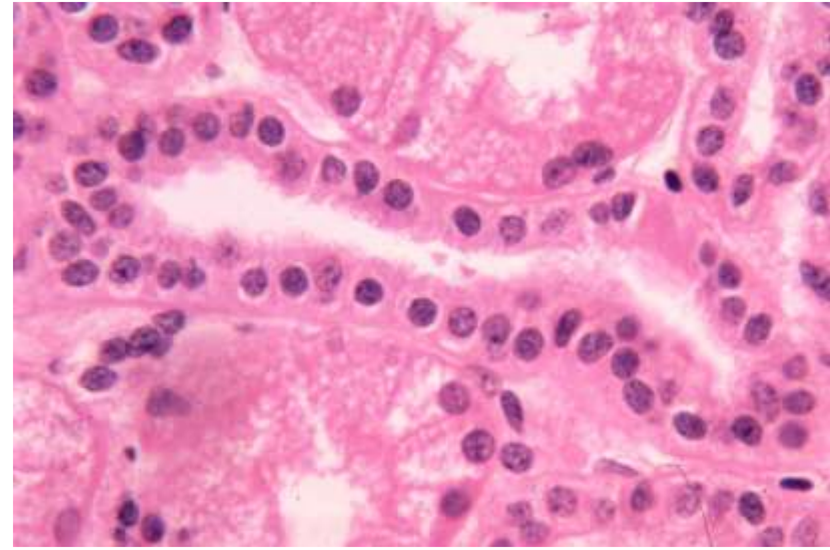
routine – HE, AZAN

(demonstrate all components of tissue)



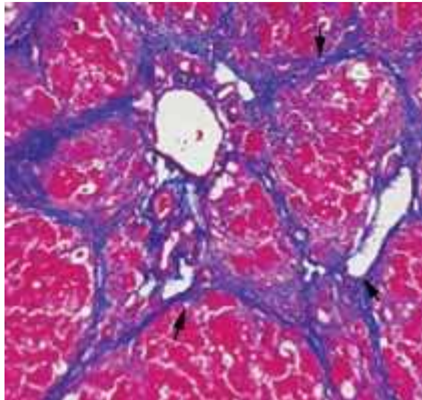


HE – the most frequent used method



special

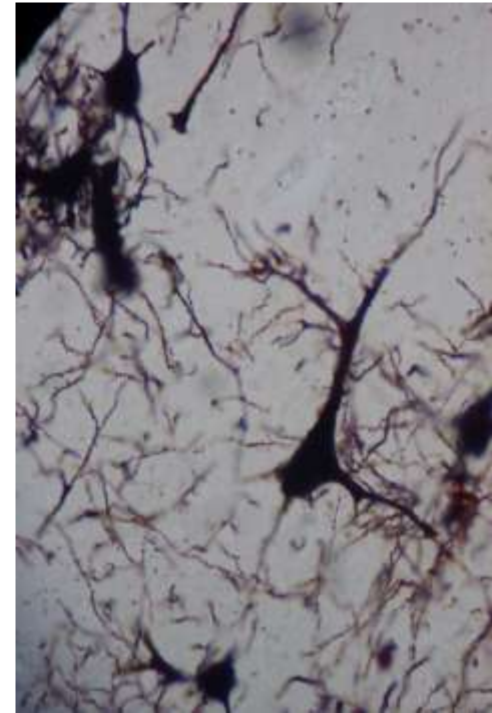
visualizes only special structures



*Lipid droplets detected by oil red*

impregnation

by silver salt for detection of nerve or reticular fibers



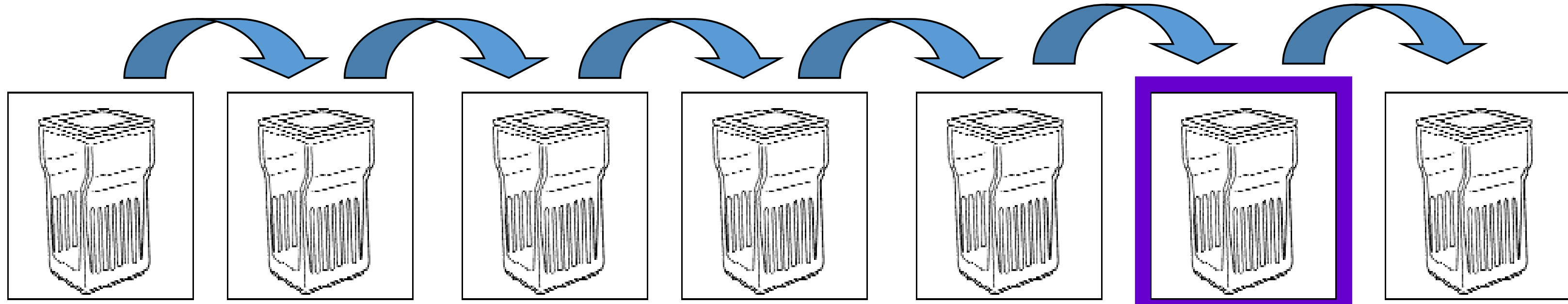
# ROUTINE STAINING with HEMATOXYLINE – EOSIN (HE)

**Hematoxyline – basic (nuclear) dye**

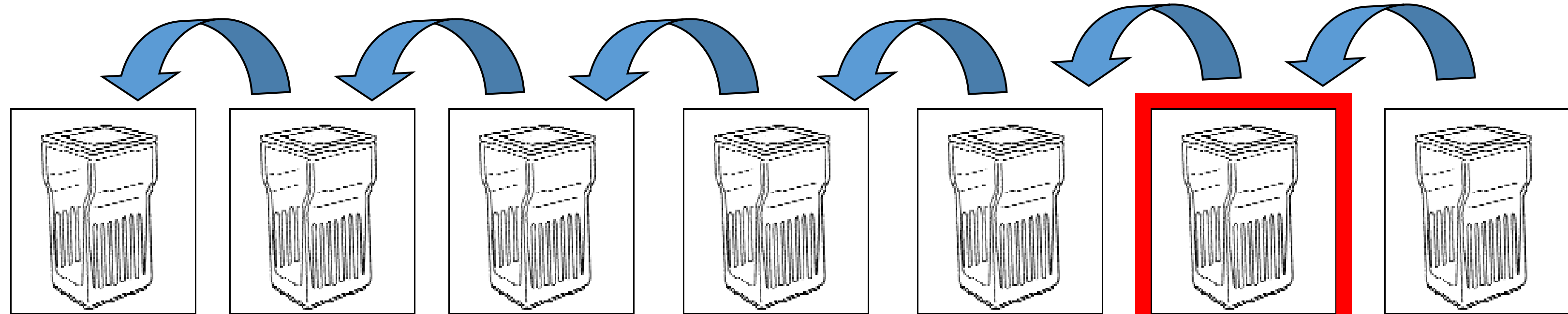
**Eosin – acid (cytoplasmic dye**

- 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)




Xylen I | XylenII | 100% ethanol | 96% ethanol | $H_2O$ | hematoxyline | acid ethanol




Xylen IV | xylen III | 100% ethanol | 96% ethanol | $H_2O$ | eosin | $H_2O$

# 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

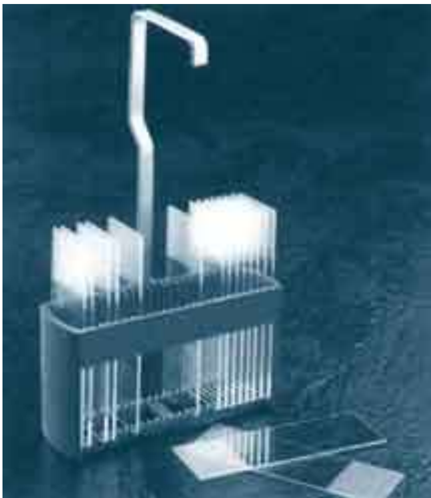
  muscle – red

  collagen fibers – blue

Staining tools:

cuvette

flask



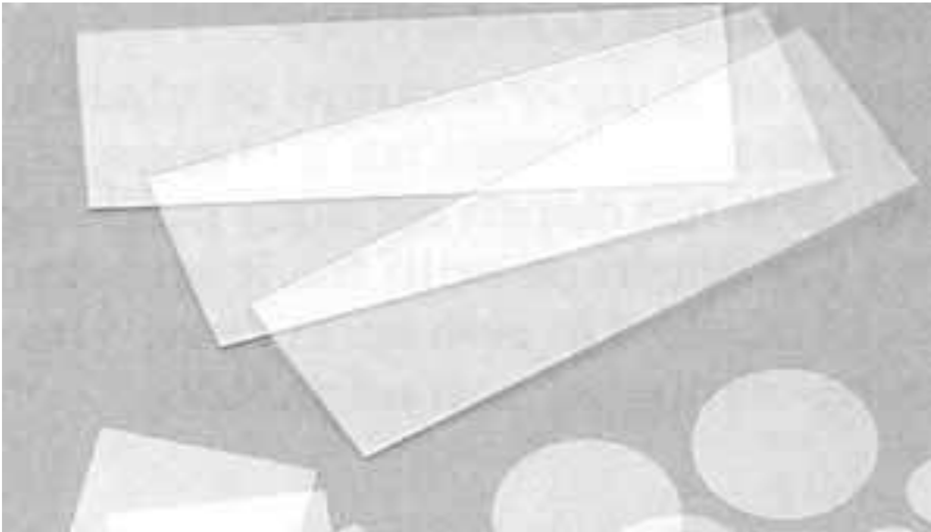
slides holder
(basket)

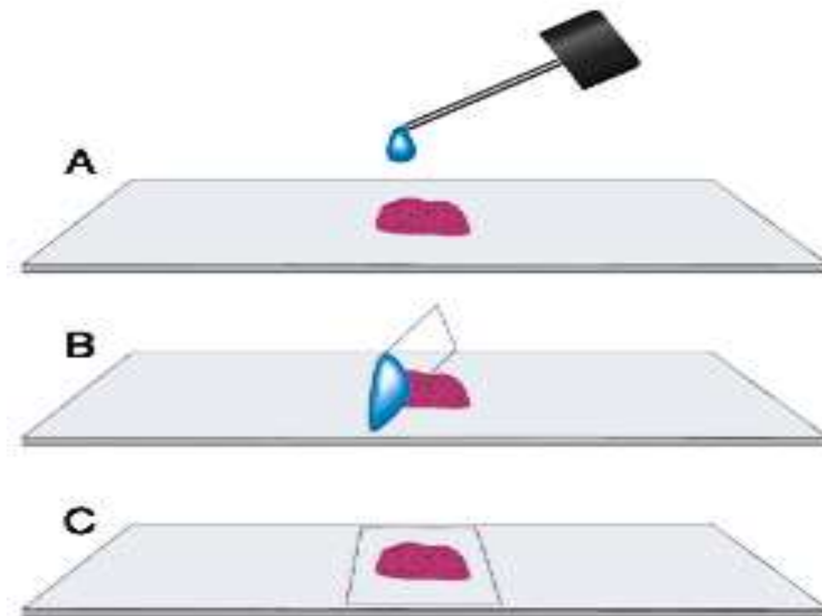
# Automatic slide stainer

**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.

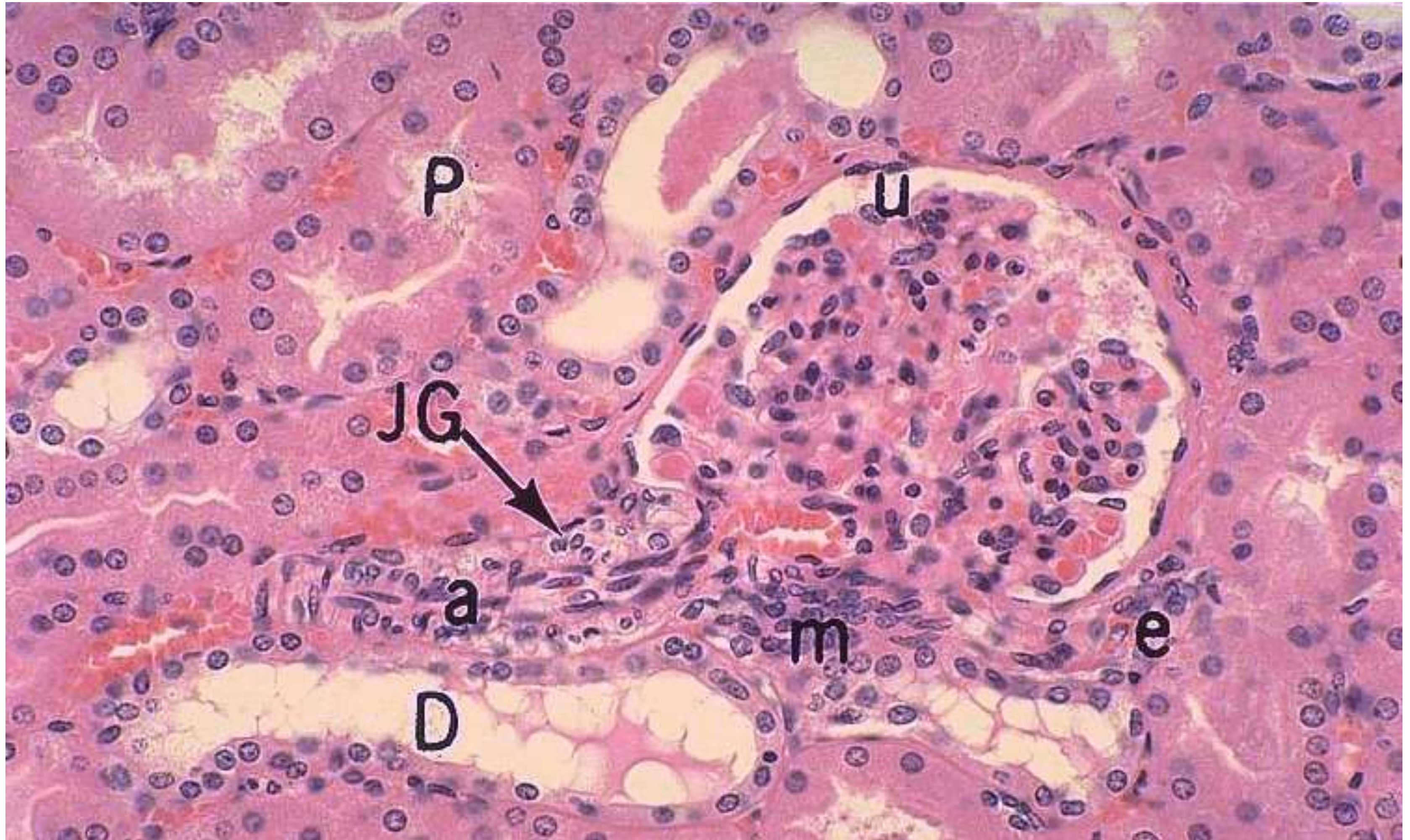




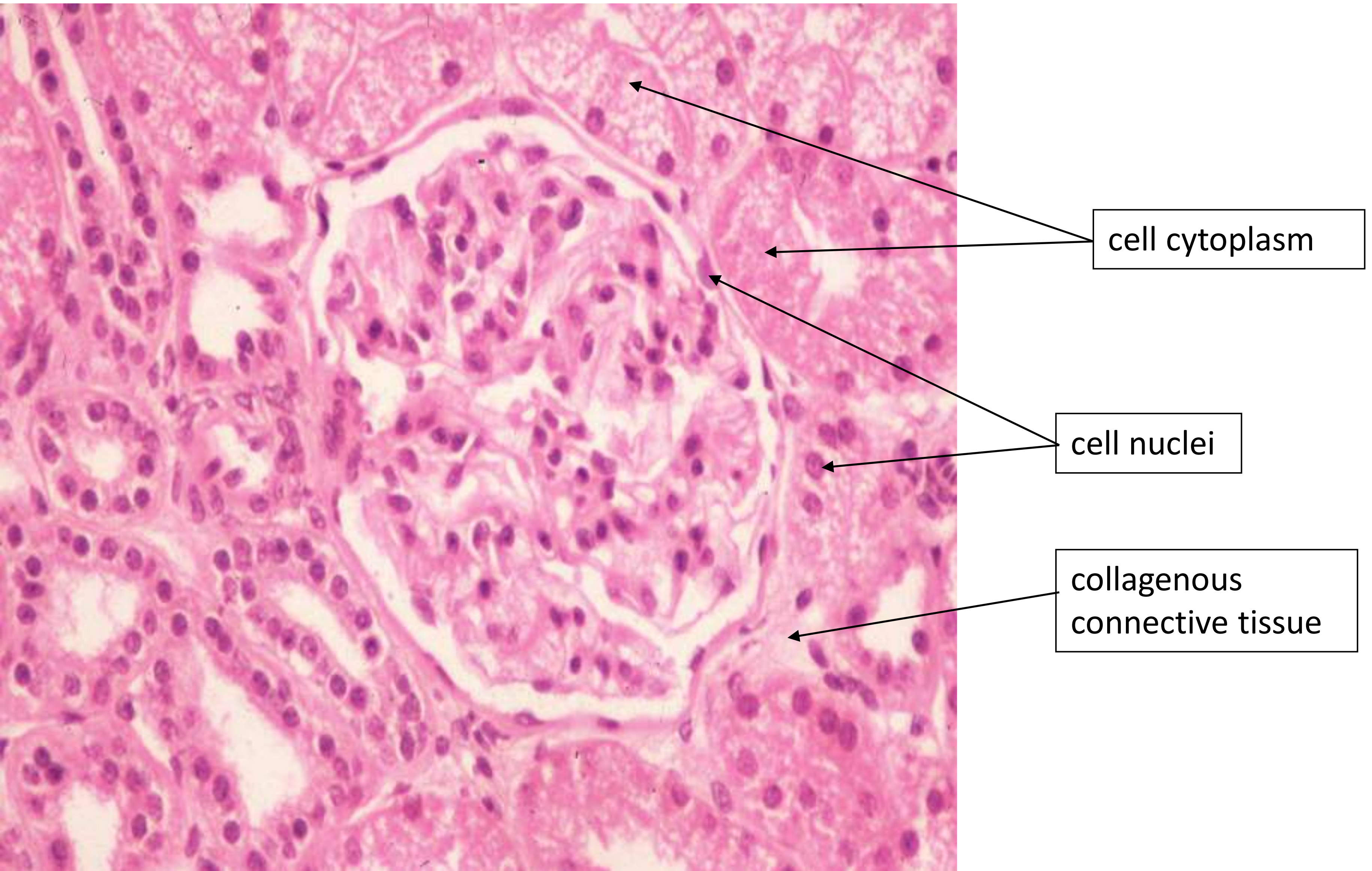

- **Mounting media**: soluble in xylene – **canada balsam**
  soluble in water – glycerin-gelatine, arabic gum

**Permanent histological slides for study in the light microscope**

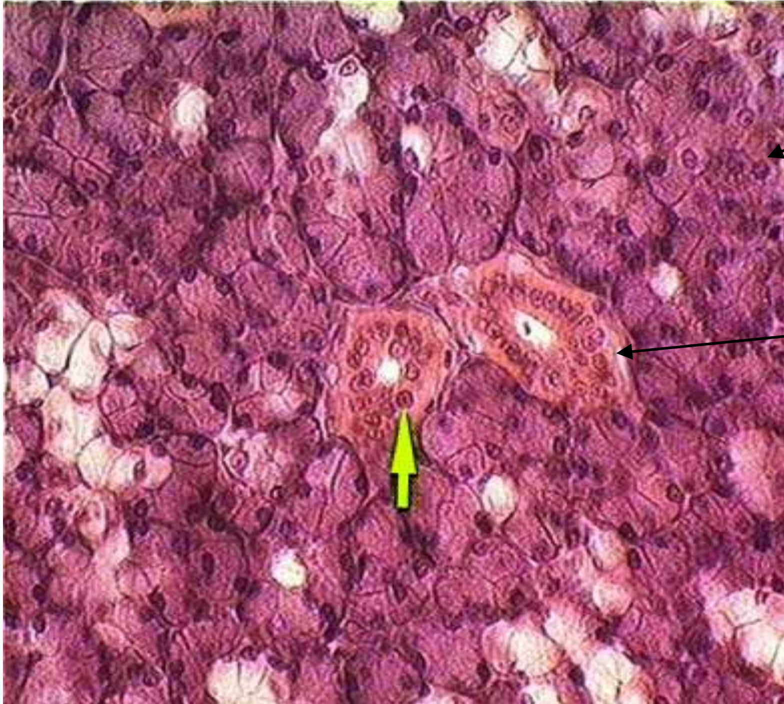
# Hematoxyline and eosin (HE)



# Hematoxyline and eosin (HE)



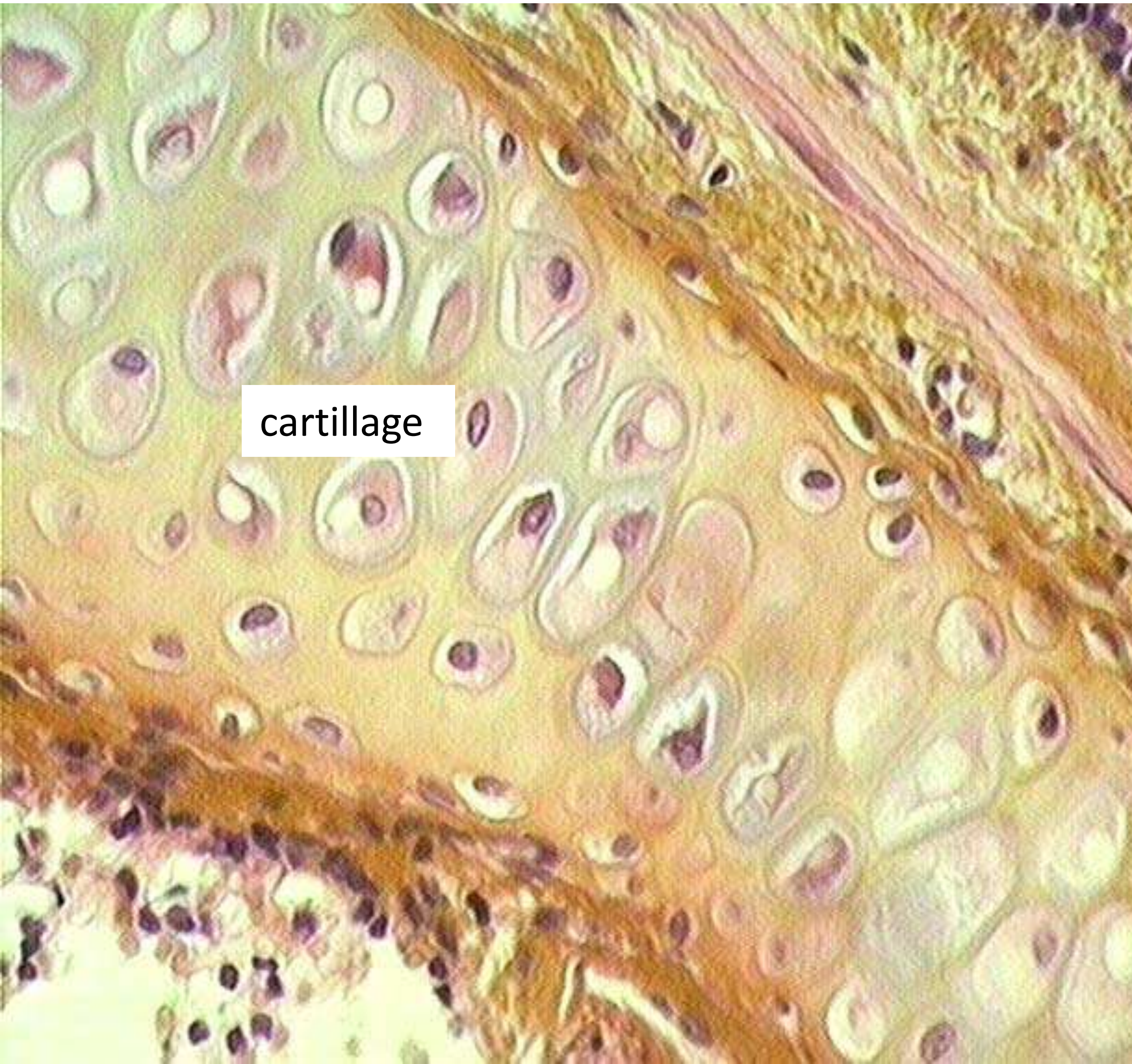
# Hematoxyline and eosin (HE)



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

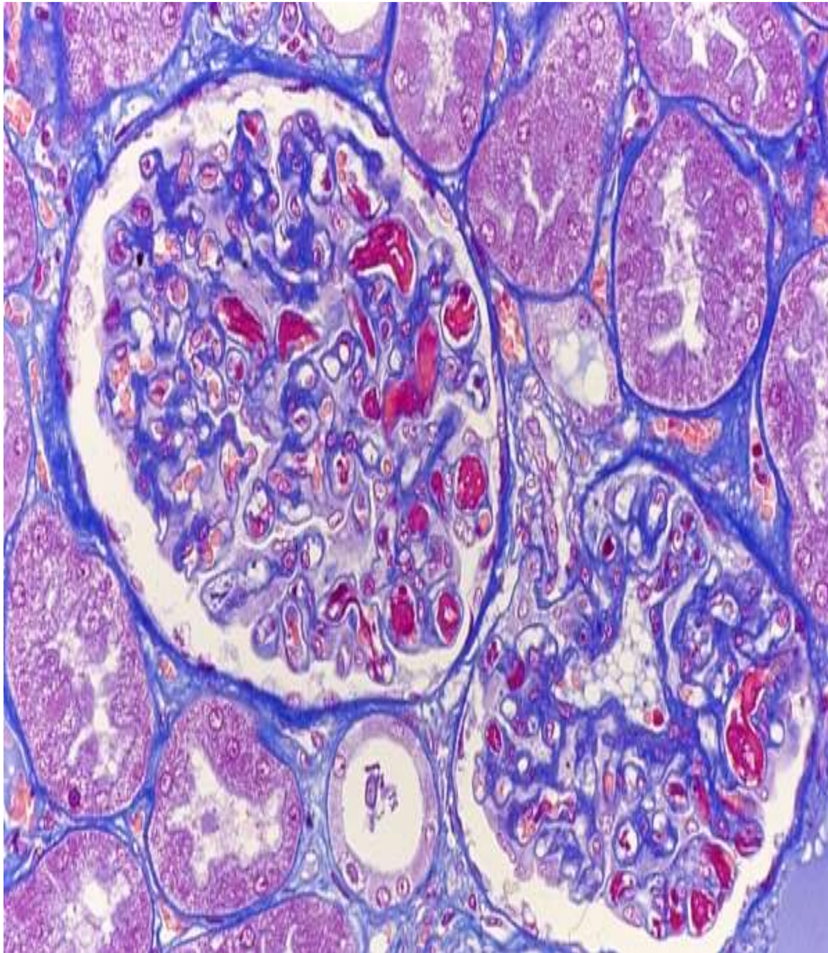
acidophilic cytoplasm of epithelial cells

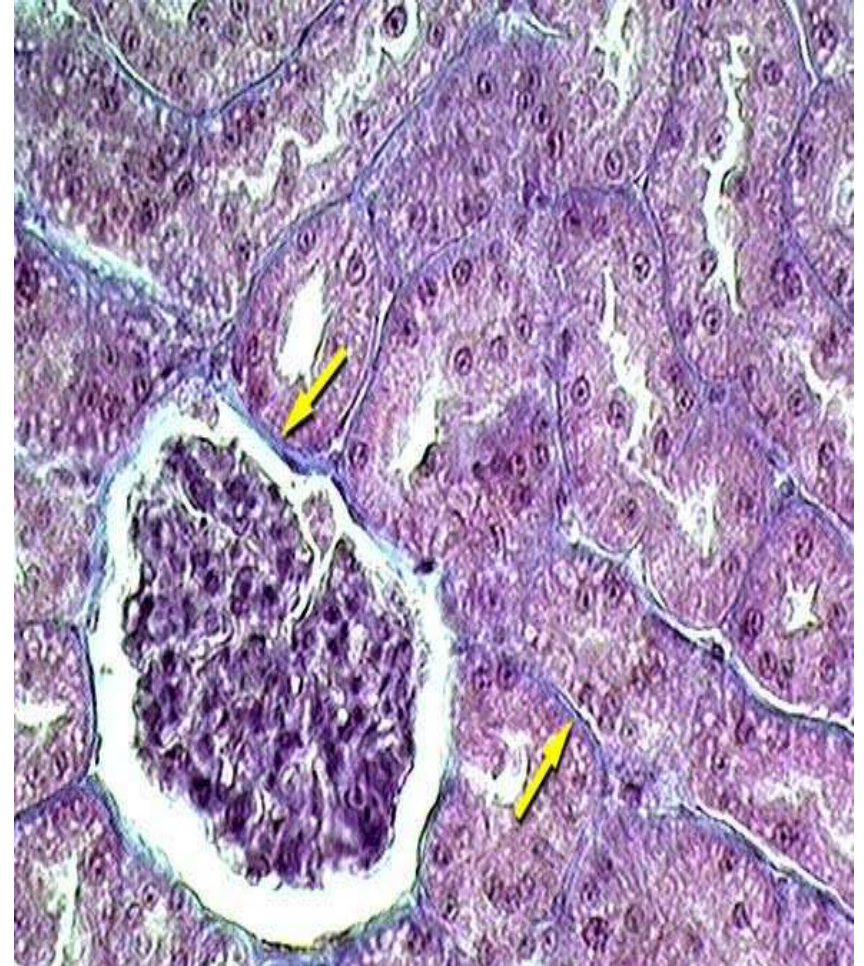
# Hematoxyline, eosin and saffron (HES)




Collagenous fibers
of connective tissue
are yellow after staining
with saffron

# Azocarmine and aniline blue (AZAN)





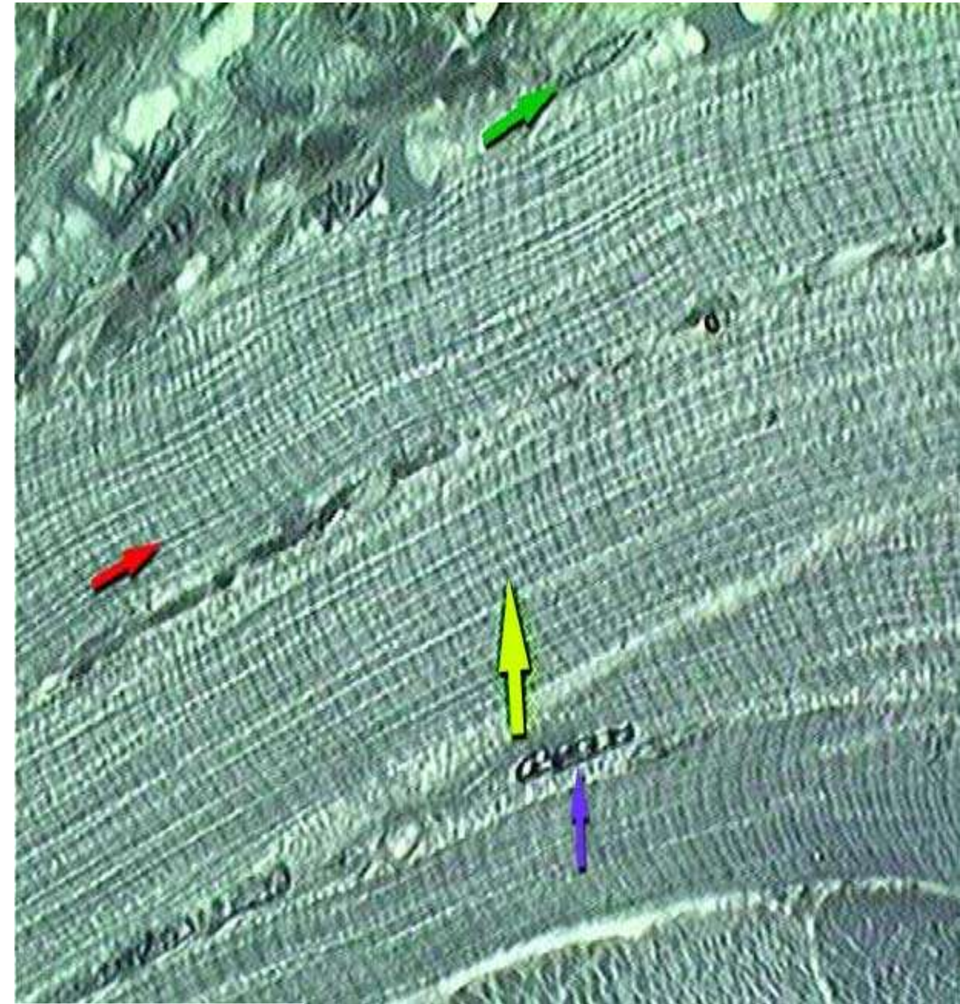
**Kidney – collagen connective tissue**

# Impregnation of tissue with silver

**Lien - reticular fibers**

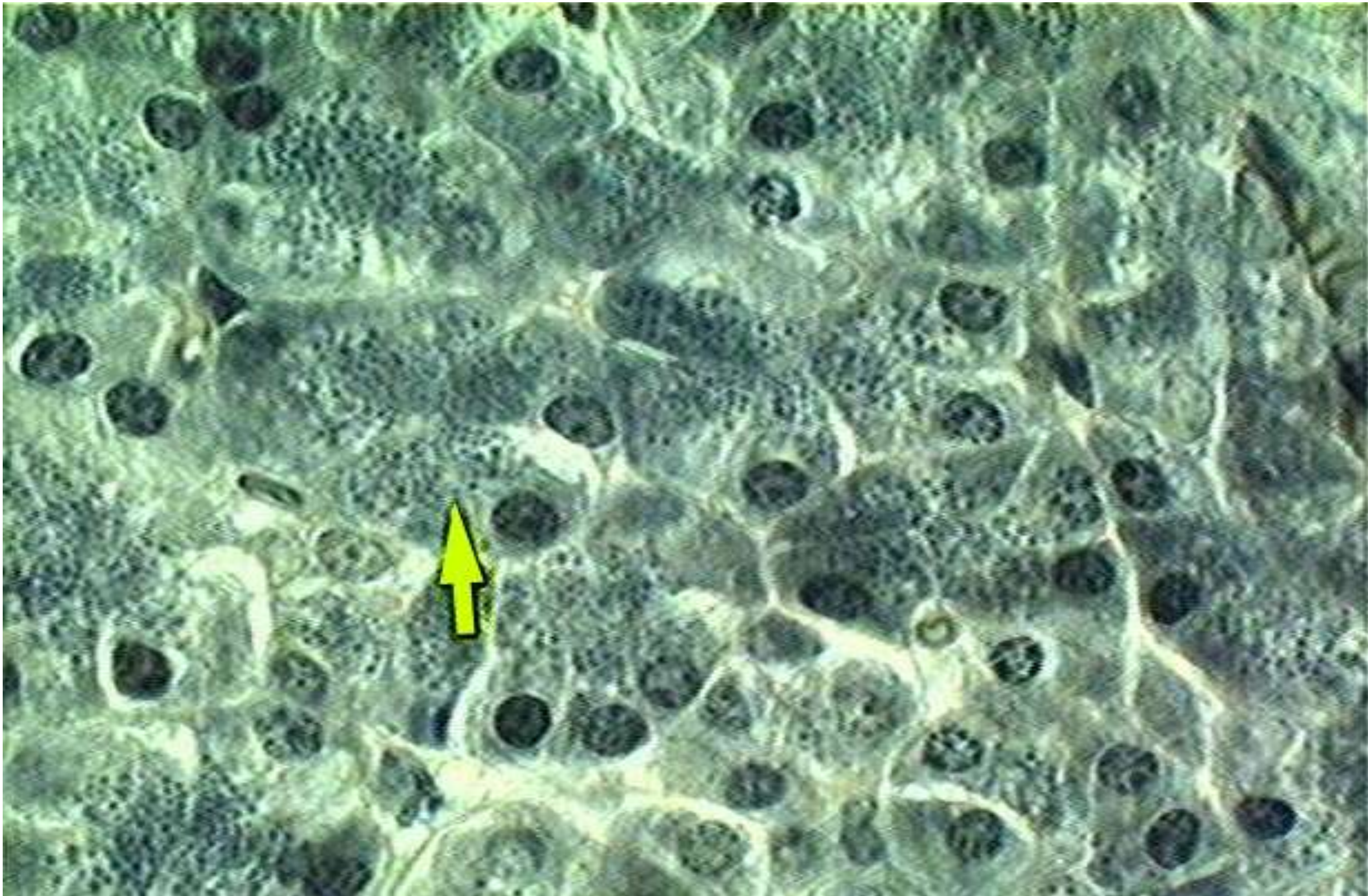
**Cerebellum – nerve fibers**

# Iron hematoxyline

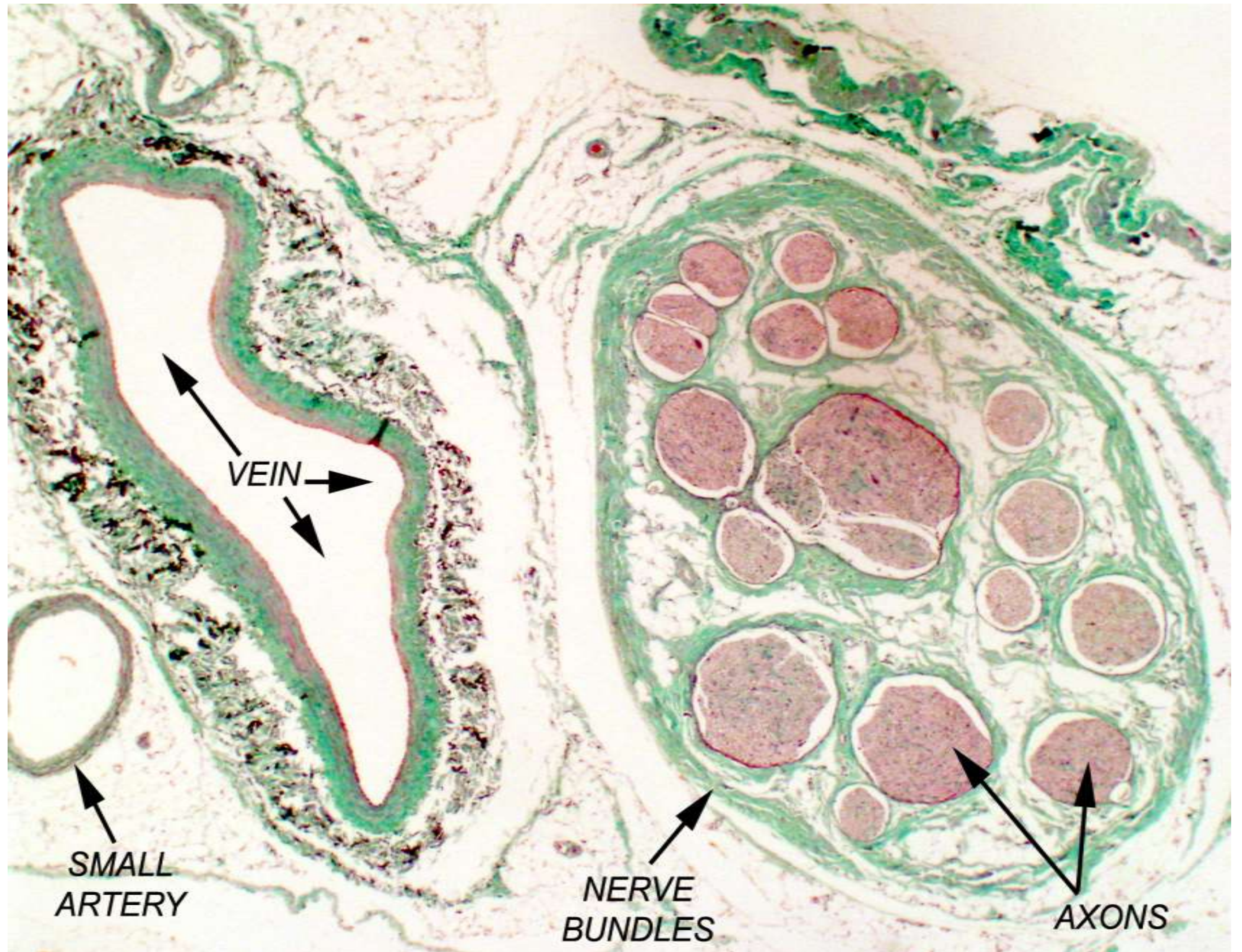


**Skeletal muscle cells (fibers)**

# Iron hematoxyline



**Mitochondria in hepatocytes**

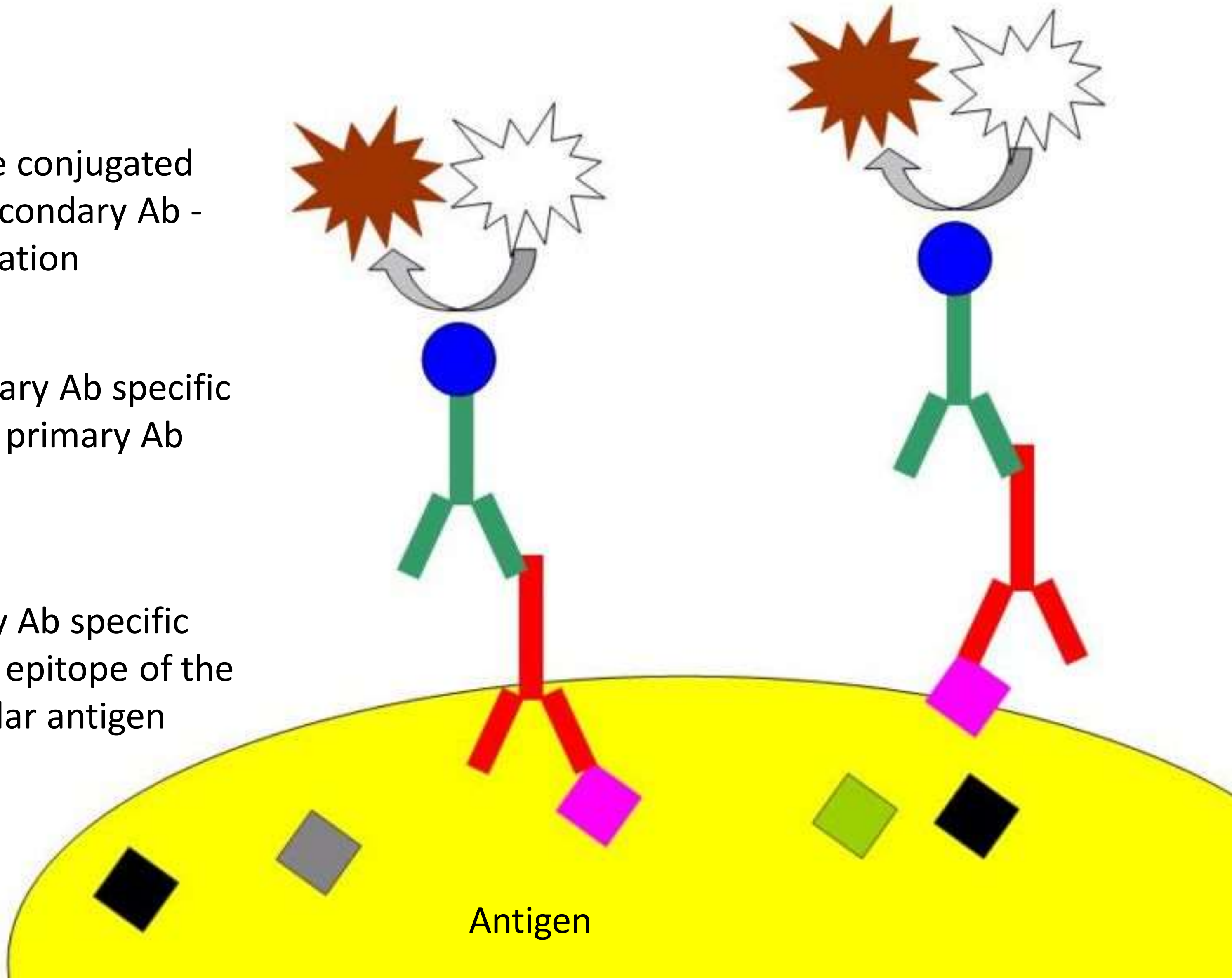

VEIN
SMALL ARTERY
NERVE BUNDLES
AXONS

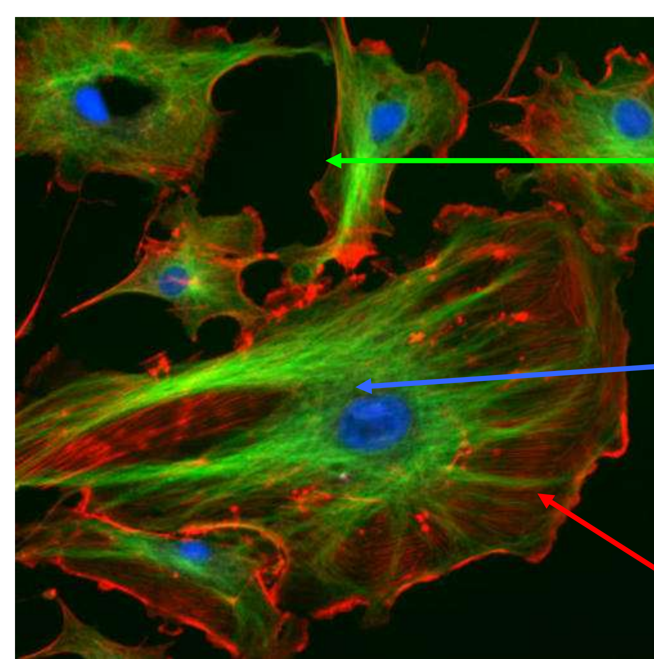
# Histochemistry and Immunohistochemistry

- Relevance:

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

Various epitopes detected by immunotechniques

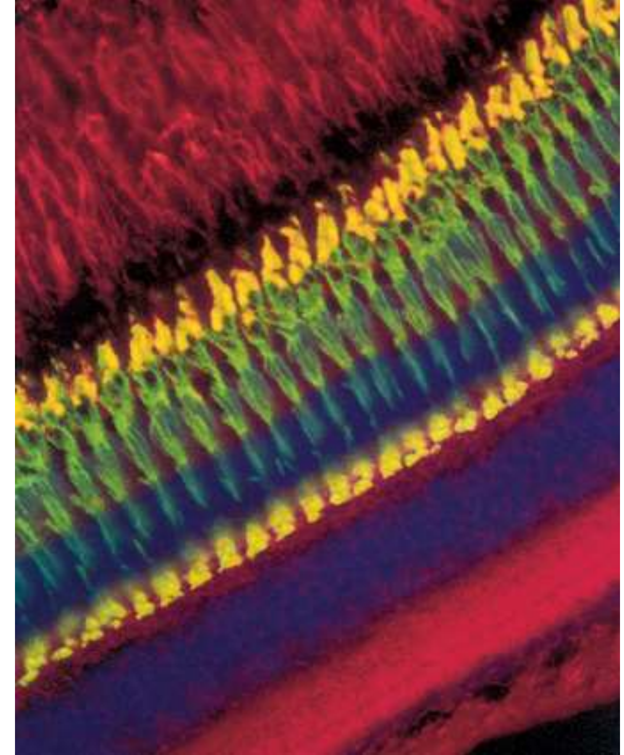

Enzyme conjugated
with secondary Ab -
visualization
Secondary Ab specific
against primary Ab
Primary Ab specific
against epitope of the
particular antigen
Antigen

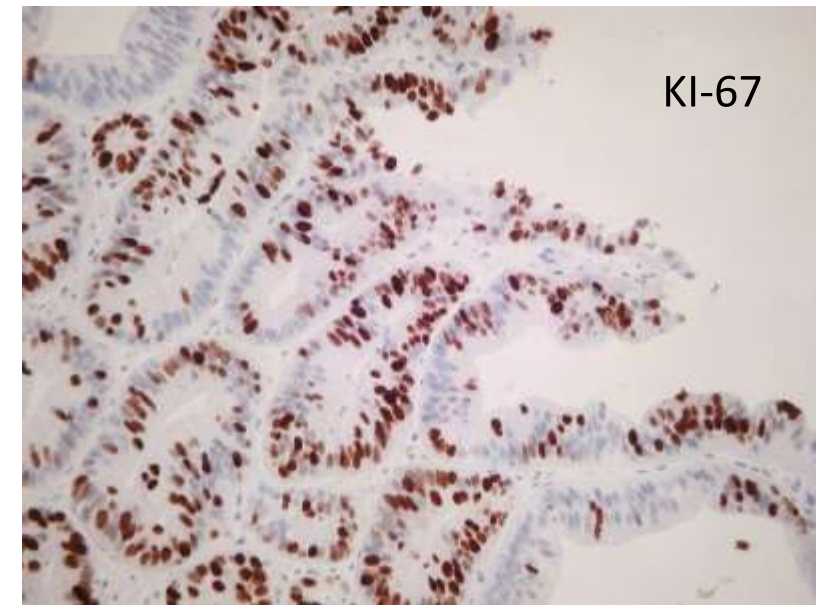


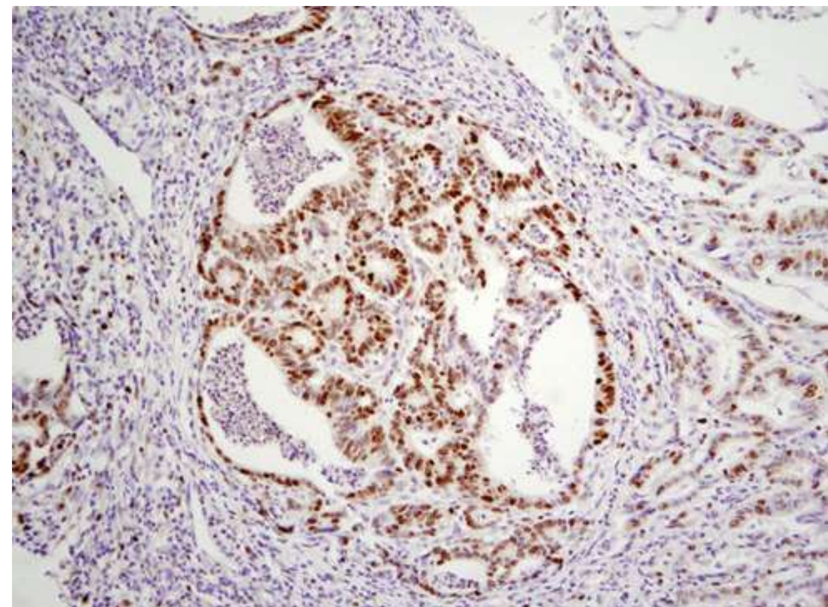
Actin (cytoskeleton)

DAPI (nucleus)

Microtubules (cytoskeleton)



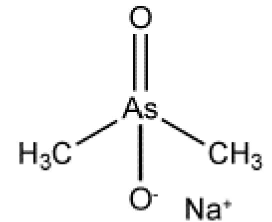


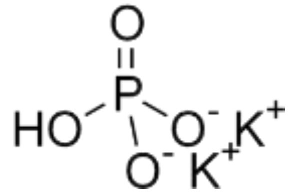
# Tissue processing for the EM

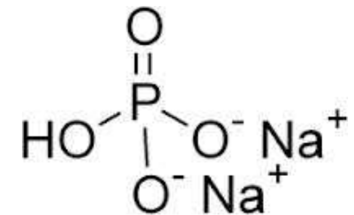
- pH of all solutions (media) must be buffered on **7.2 – 7.4**
  Cacodylate or phosphate buffer is frequently used.

- Absolutely dustfree environment




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



# Tissue processing for the EM

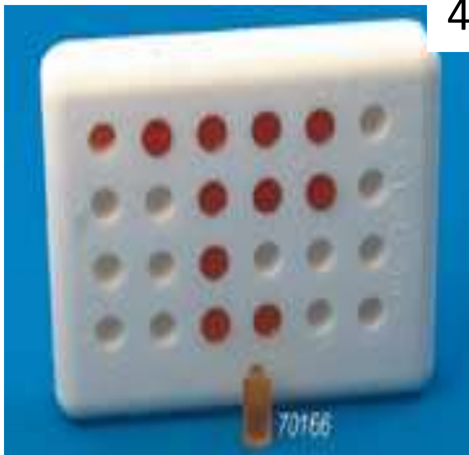
- **SAMPLING** – immediatelly after arresting of blood circulation, tissue block sized no more than **1mm$^3$**
- **FIXATION** – glutaraldehyde (binds amine groups) + $OsO_4$ (binds lipids) are used as double fixation
- **RINSING** – distilled water
- **DEHYDRATION -** ethanol
- **EMBEDDING** – gelatin 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

Embedding tools:

gelatin (1) or plastic (2) capsules

capsule holder (3)

embedding plates
(4, 5)

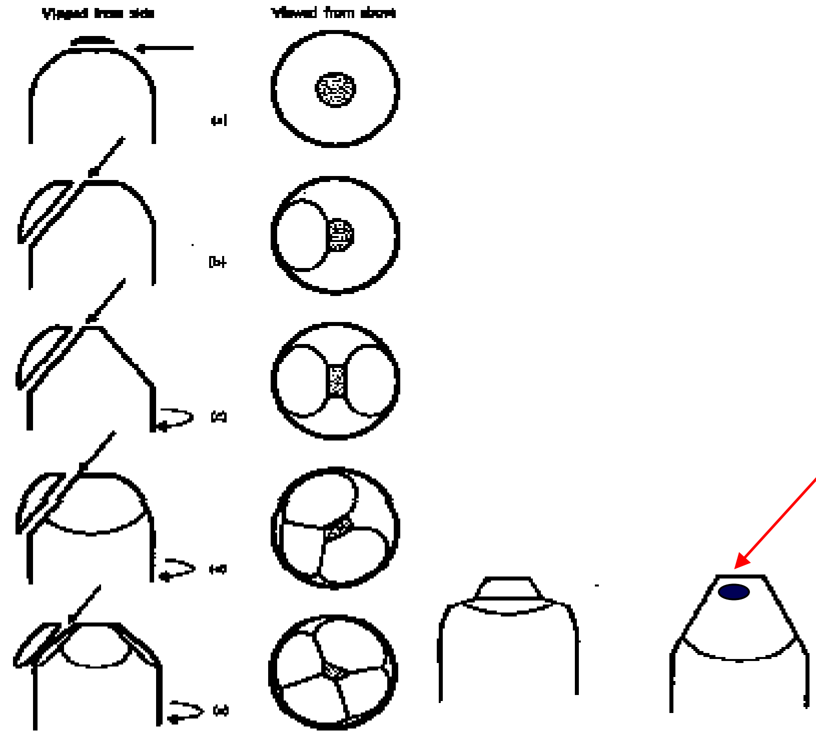


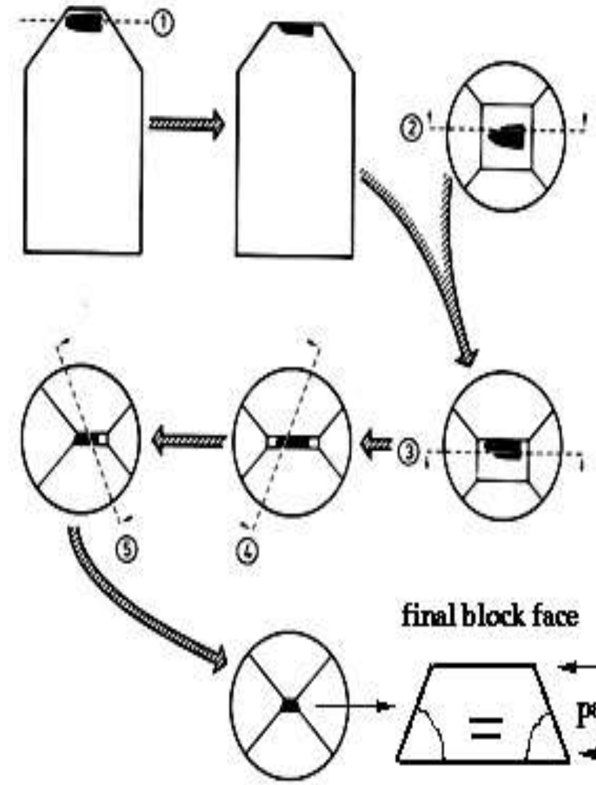

Embedded blocks
prepared for cutting

# 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*





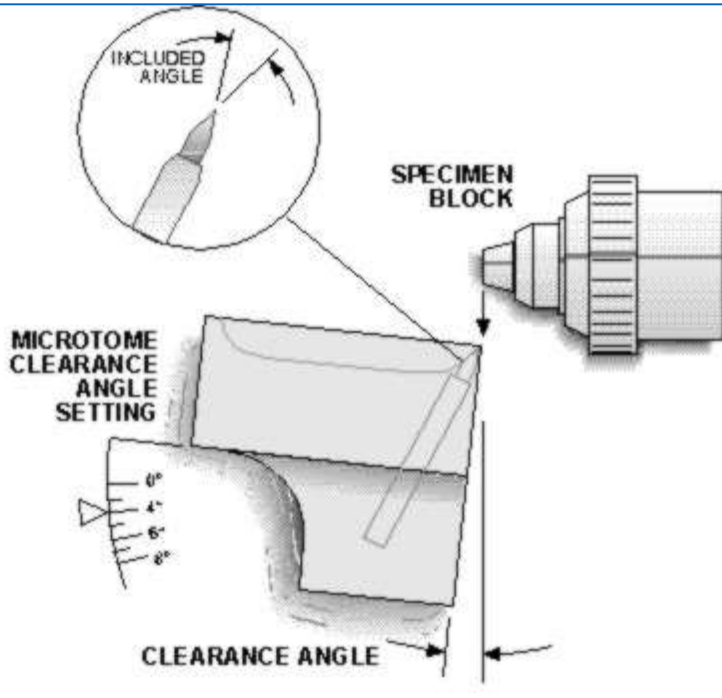

## Pyramid Side Profile

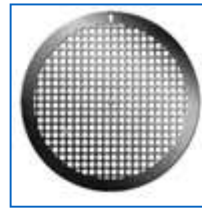
Too Steep-Trans Am Building (not rigid-vibrations)

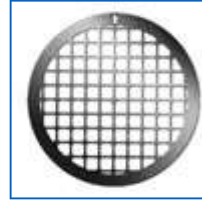
Too Flat-Pyramid of the Sun (section size changes rapidly)

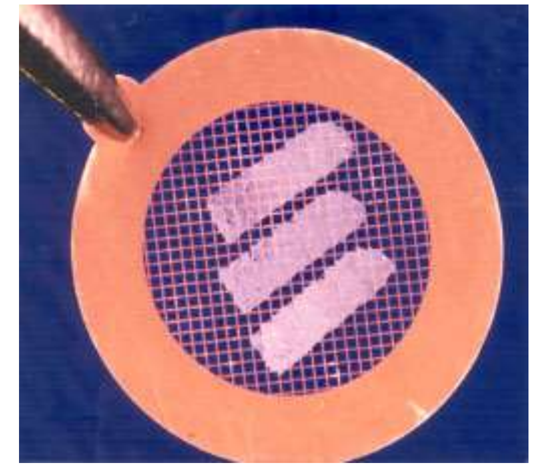
Just Right-Egyptian Pyramid with top cut off

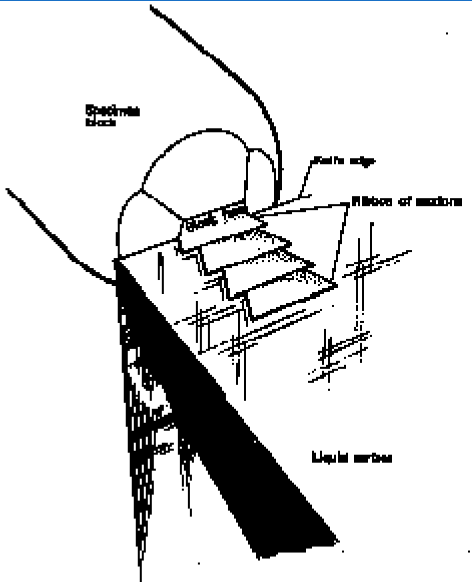
# Cutting

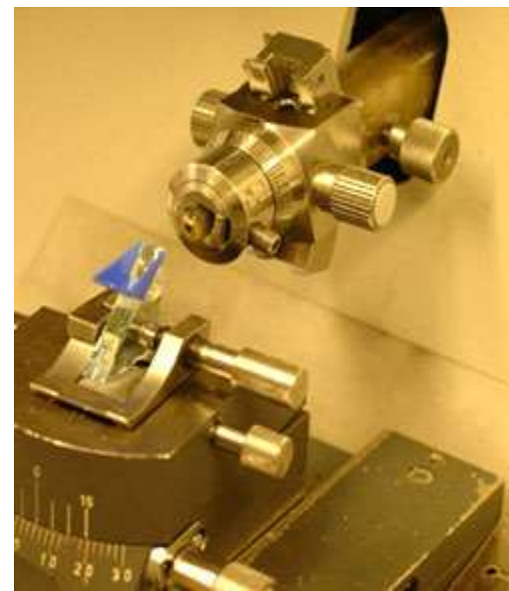


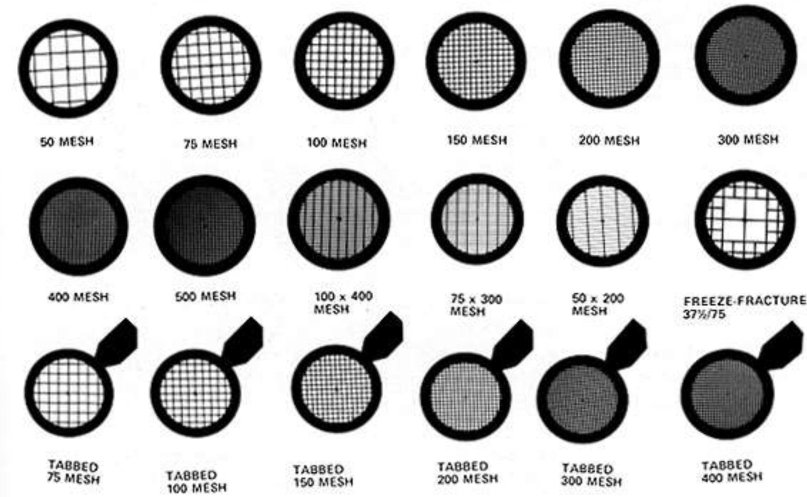








## Grid Types and Mesh Sizes



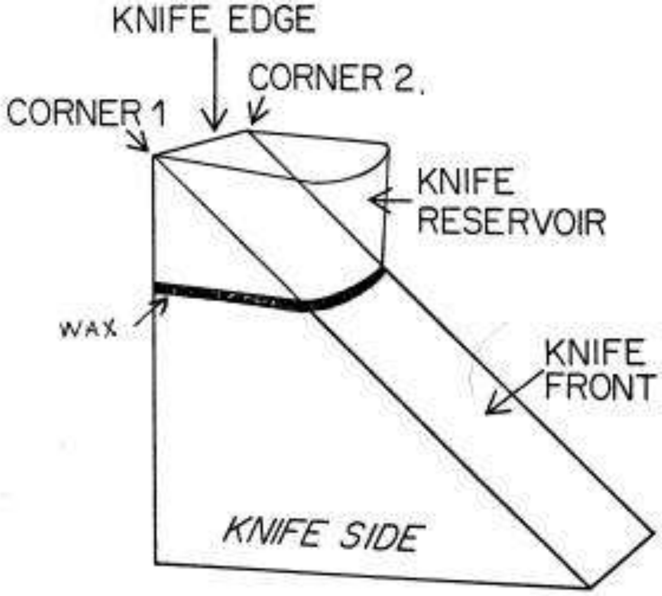
# Cutting

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

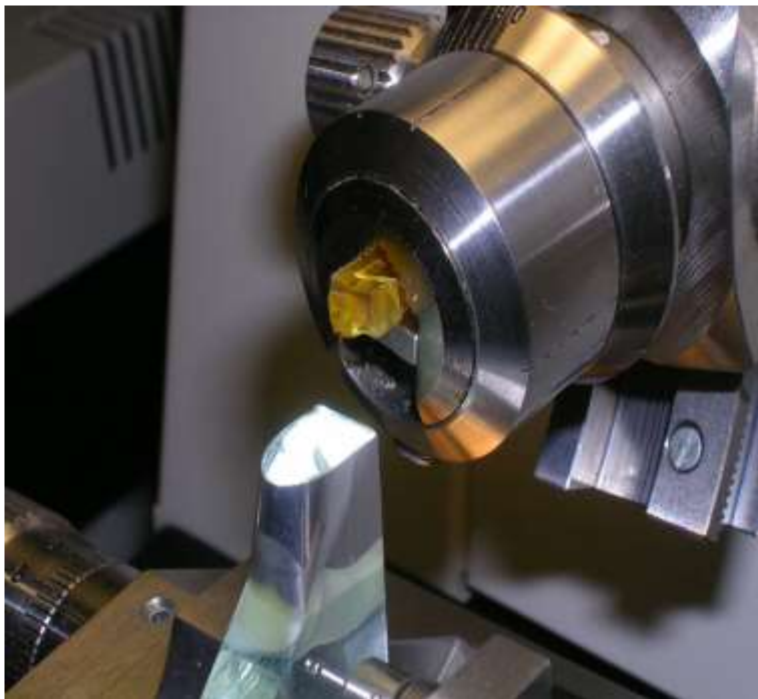
Supporting grids




# Ultramicrotom knives:

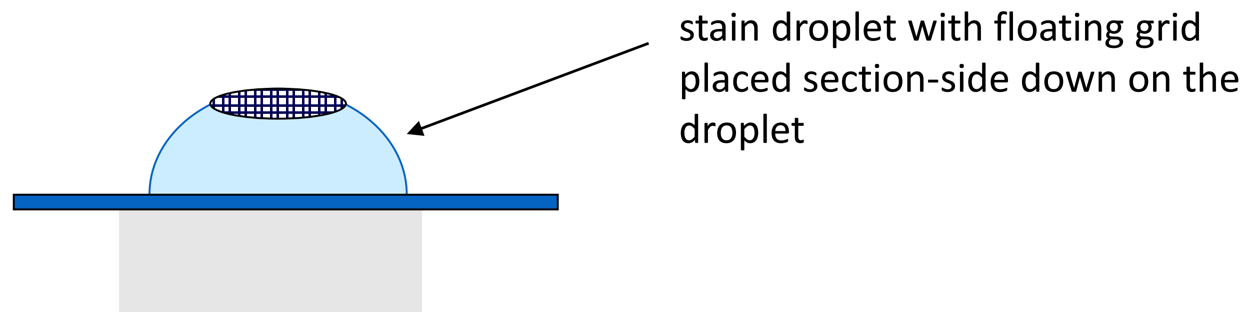
glass

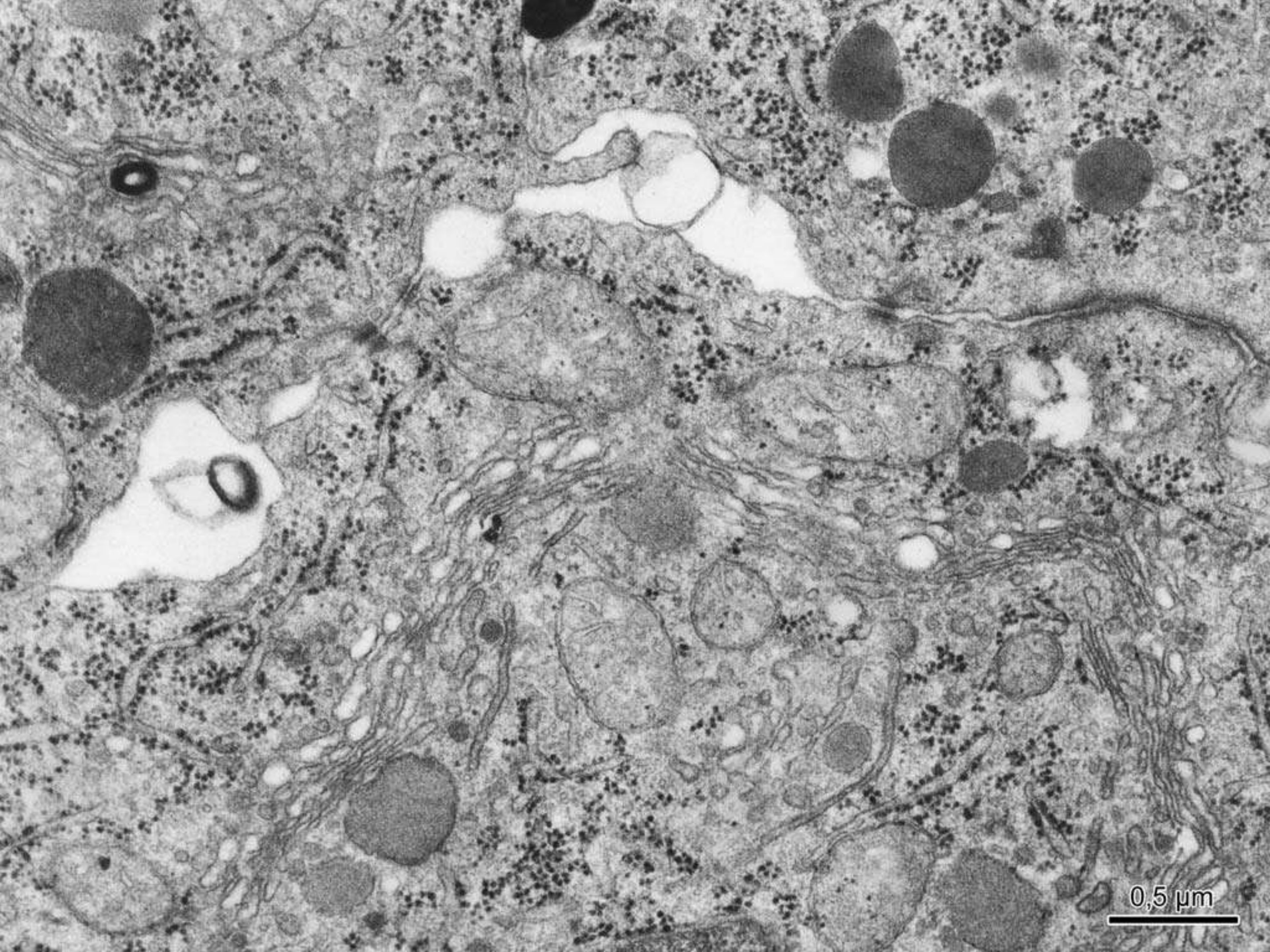
diamond

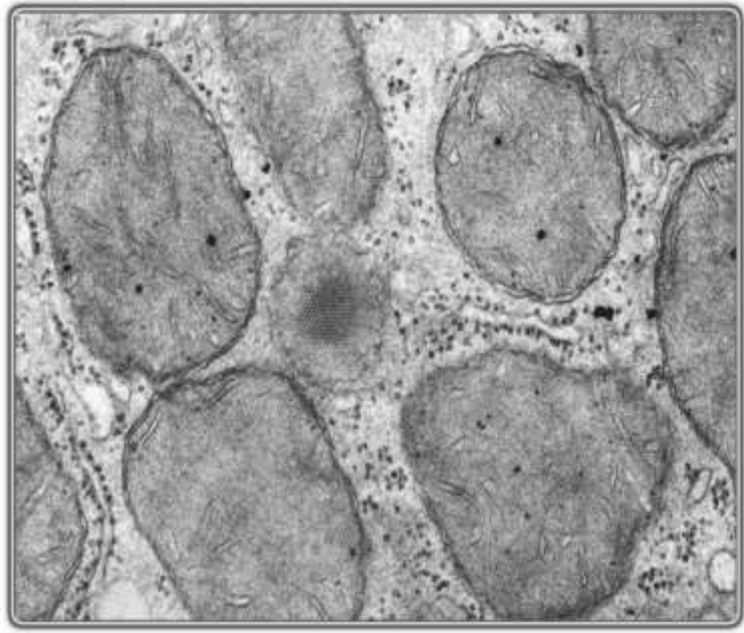


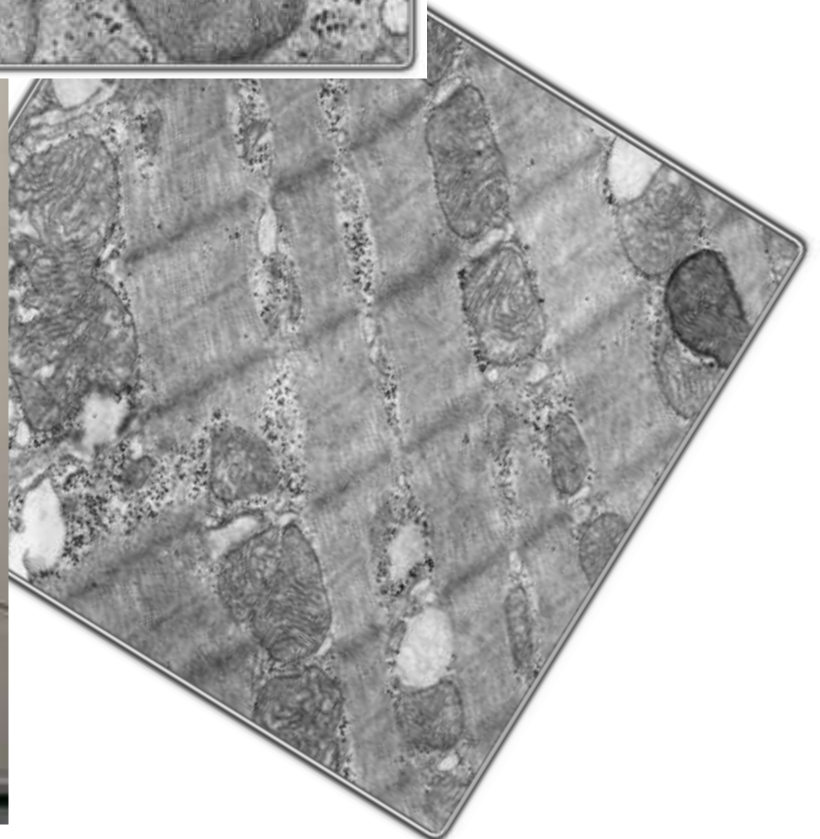
# CONTRASTING (=STAINING)

- 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




0,5 µm





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

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http://www.med.muni.cz/histology

Thank you for attention