Practical training in Histology

Physiotherapy (bc.)

Organization issues

- Beginning - **strictly**
- Change your shoes - you cannot enter the hall with outdoor shoes (slippers)
- Locker room – shoes, jackets, coats, bags etc. (use padlock)
- Cell phone – switched off or in silent mode
- Microscopic hall = laboratory
  - – eating, drinking, smoking not allowed
  - – 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, presentation (demonstrated topic)
- Your individual work = study of the slides, schematic but precise drawing of tissue structure, careful description. The result of your work is PROTOCOL in each practice.
- Students come prepared for practices - programmes on pin-boards or dpt. webpage

- **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
- Make a protocol during substitution, let it check and sign by the chief teacher

Registration of substitution:

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

Each absence must be excused via study department and in IS (medical report from doctor, Official invitation, etc. Is necessary).

- **Protocols**
- you have to make paper protocols (no tablets, laptops)
- A4 size, blank, without lines, according to the template
- pencil handdrawings (no pen)
- complete set of your 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
- Low-quality protocols cannot be approved and you have to substitute the respective practical lesson
- **Testing your knowledge**
- Credit test in the last practice of semester (20 questions – max. 20 points, result 12 – 20 points = P (passed), 0 – 11 N (not passed), one resit is possible.
- Exam in exam period: 2 questions from histology topics of lectures and practices (marks A – F).
- 3 terms: 1 regular and 2 resits.

Protokol č. ..... Jméno: .....

Datum:..... Ročník: ..... Skupina: .....

TÉMA: .....

Seznam preparátů ke studiu:
Číslo název (barvení)

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Atlas EM: doporučené obrázky ke studiu
str. název elektronogramu

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Pokyny pro vypracování protokolu

1. Student vyhotoví barevné nákresy histologických preparátů (pastelky) nebo černobílé nákresy obrázku z atlasu elektronogramů (obyčejná tužka).
2. Každý nákres musí být opatřen následujícími údaji:
   - **název preparátu** s uvedením **metody barvení** (viz Seznam výše), event. název elektronogramu
   - **zvětšení:** 10 x 4 / 10 x 10 / 10 x20 / 10 x 40 (tj. okulár x objektiv) nebo celk. zv.: 40x / 100x / 200x / 400x
   - popis obrázku.

Kontrola protokolu

Praktické cvičení: řádné ☐ náhradní ☐ datum .....

.....
podpis učitele

Protokol č. ..... Jméno: .....

Datum:..... Ročník: ..... Skupina: .....

The english form of protocol: on www of department

- **Credits**

  - 100% attendance

  - complete set of signed protocols from all lessons

  - Passed credit test (written in practice before the last)

- End of practice:

  - The practice is closed by teacher

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

Department of Histology and Embryology

Home Page Research Education

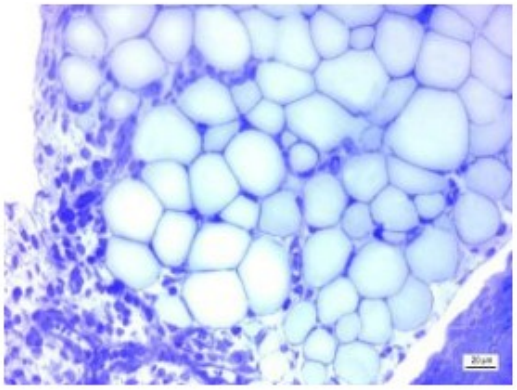
Education

- ✓ Bachelor's degree programmes
- Master's degree programmes
- ✓ Timetable
- ✓ Electronic textbooks and atlases

protocol

Physiotherapy

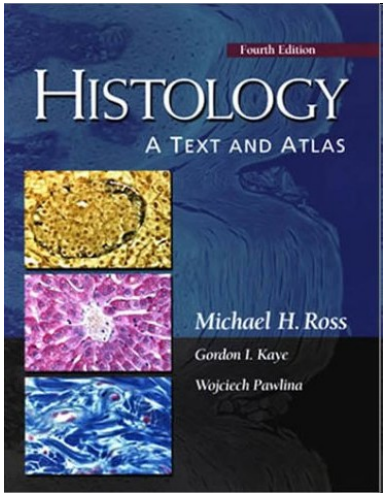
- spring semester
- Lectures & Practicals (CZ, EN)
- Required knowledge to exam (CZ) – Physiotherapy
- Required knowledge to exam (CZ) – Optometry

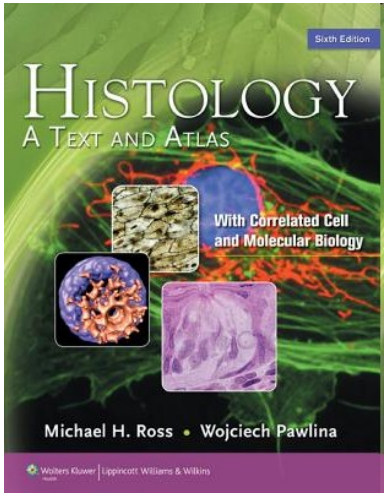


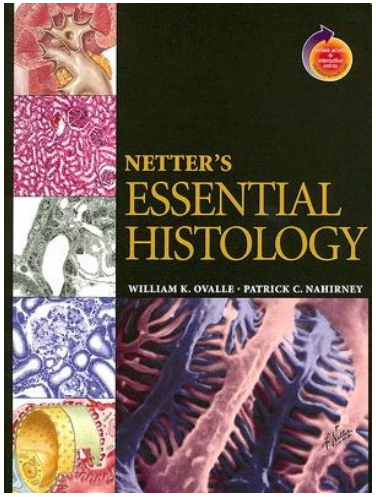
New Atlas of Histology

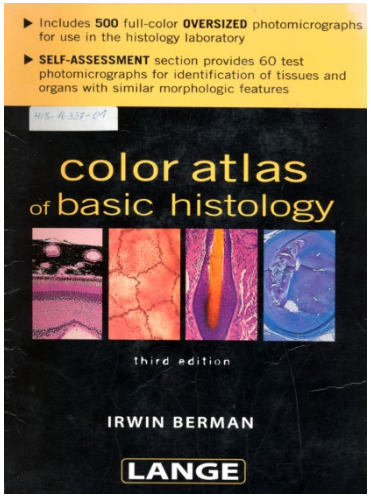
(beta version, January 2017)

Recommended literature









+
Lectures
Protocols

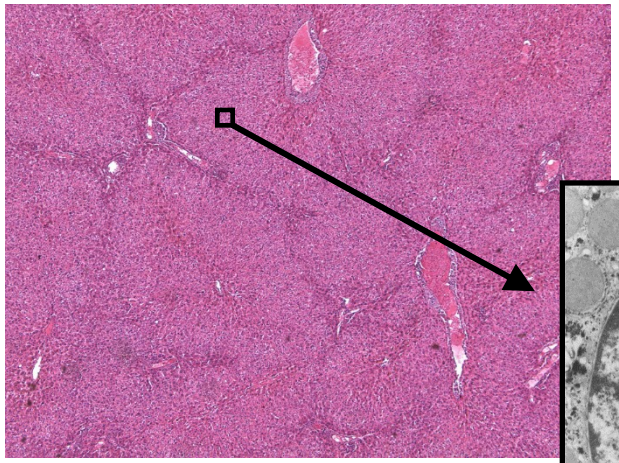
HISTOLOGY

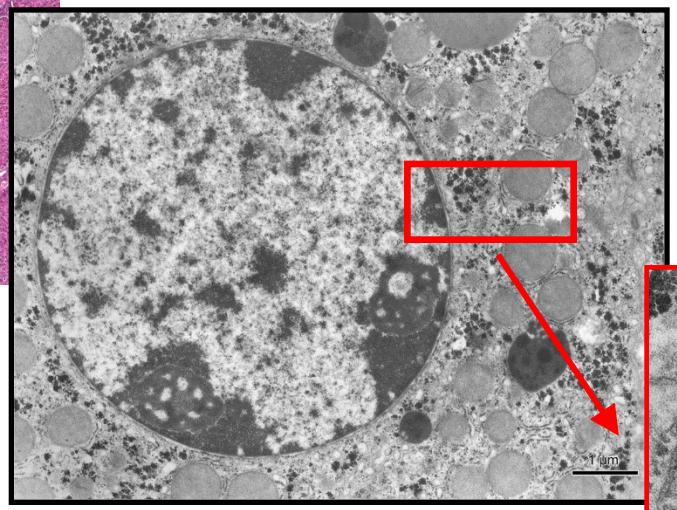
- structure and ultrastructure of normal cells and tissues,
- **cytology and general histology**
- **special histology** = microscopic anatomy of individual organs

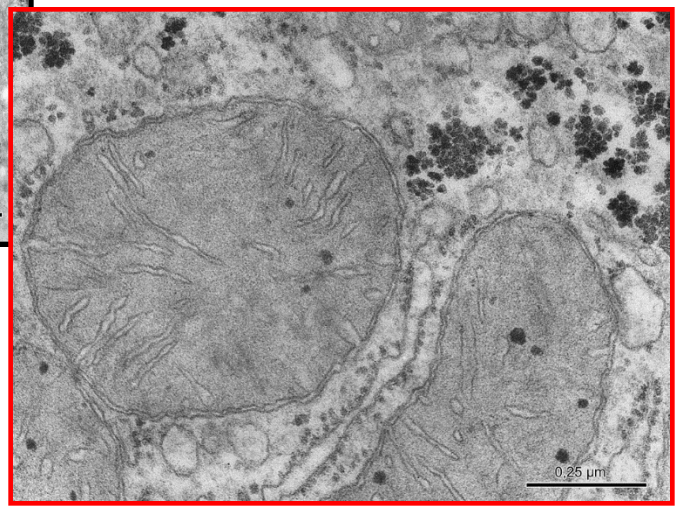
- relevance: oncology, surgery, hematology, pathology, forensic,…

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.

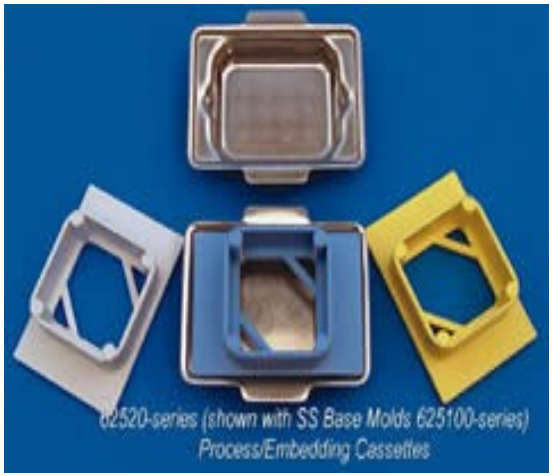
- The moulds are filled with melted paraffin, tissue samples are then placed inside and immediately immersed in cold water to cool paraffin quickly down.
- These paraffin blocks are ready for trimming

Leica TP 1020

Automated device for tissue dehydration

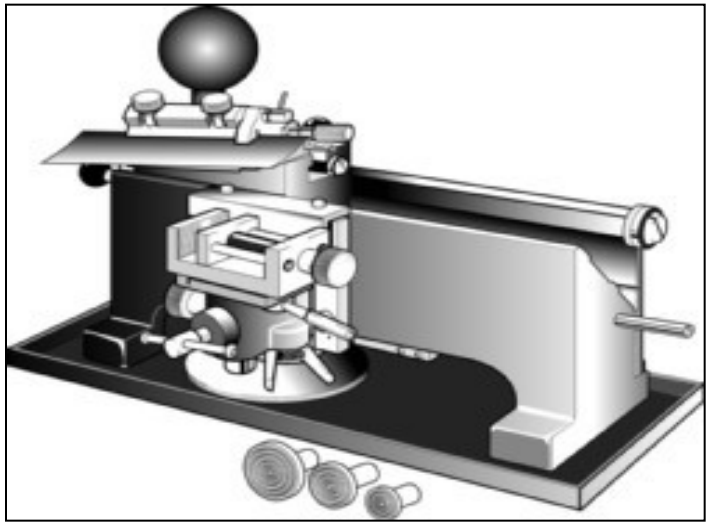
Paper chambers

- **metal**

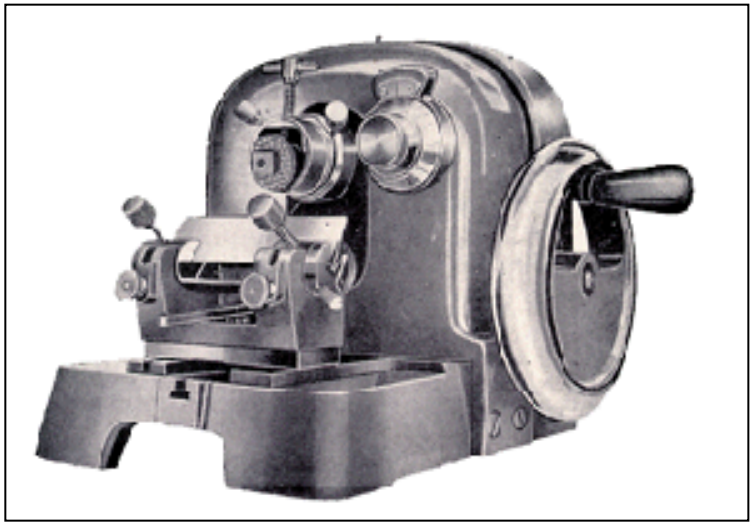


CUTTING

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

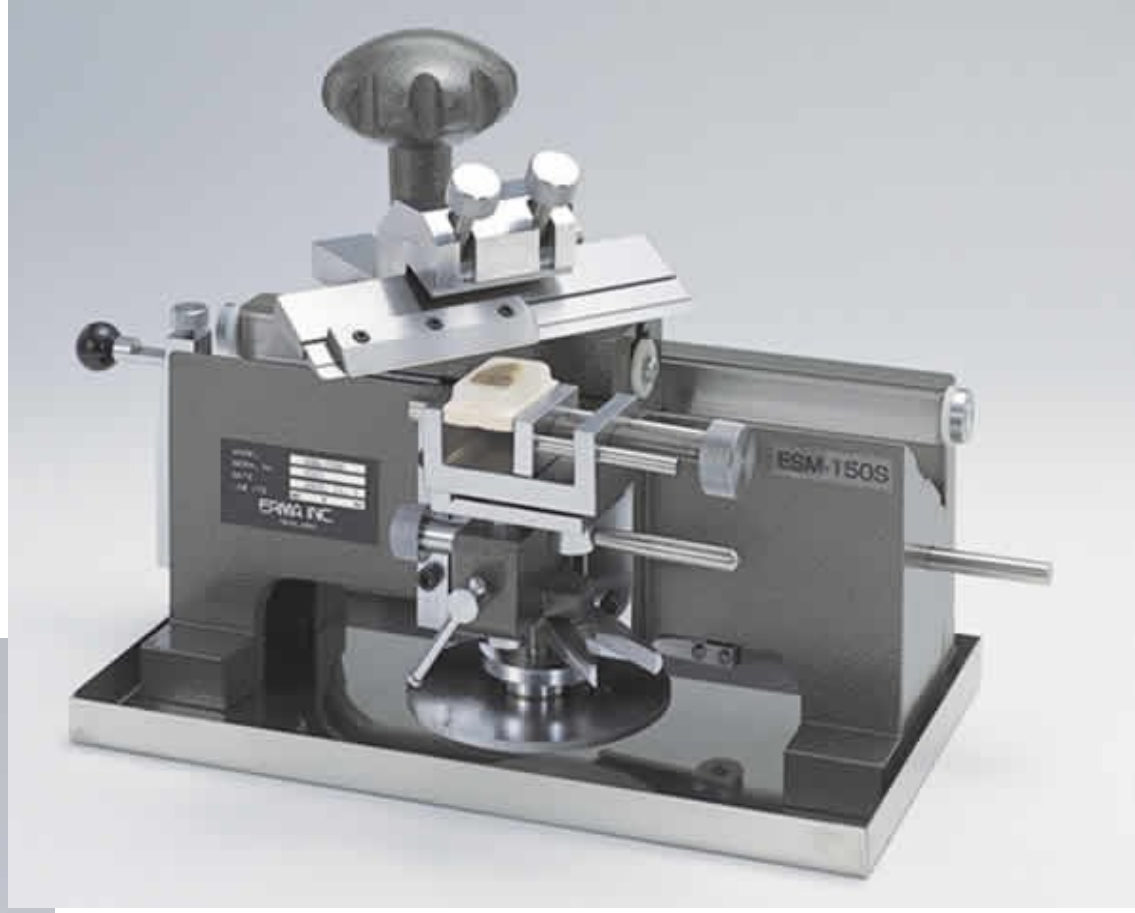


sliding microtome – block is fixed in holder, knife or razor moves horizontally



rotary microtome – knife is fixed, block holder moves vertically

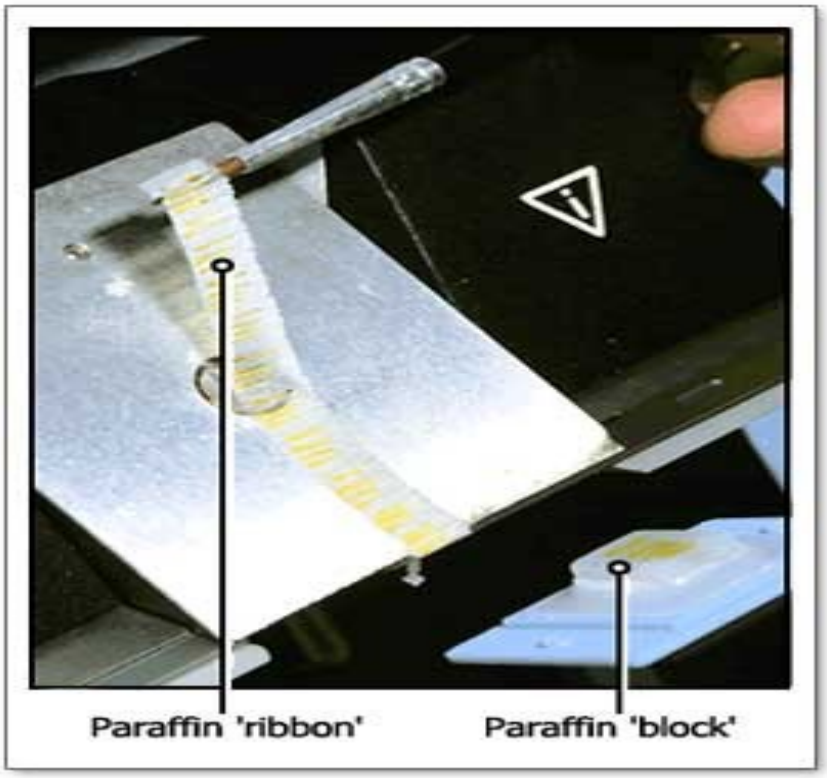
Sliding microtome

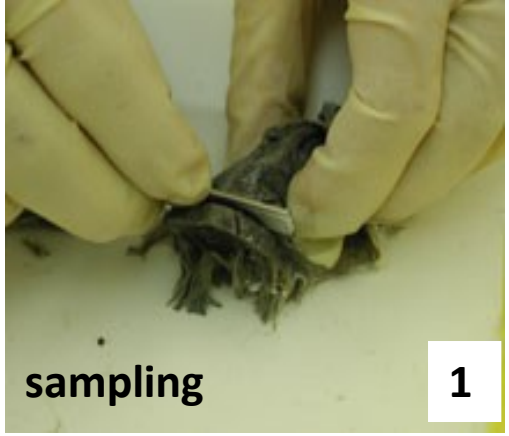


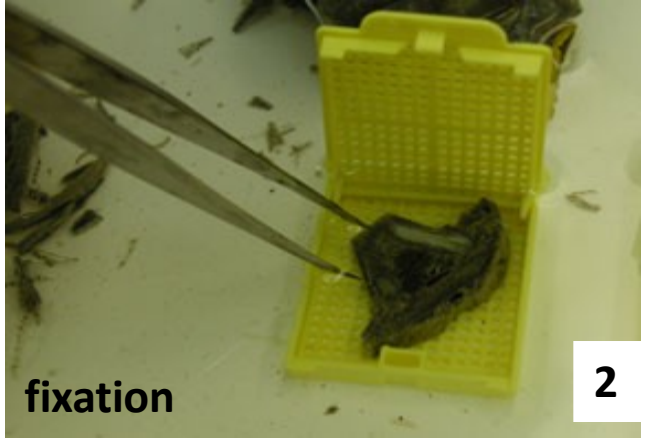
Rotary microtome

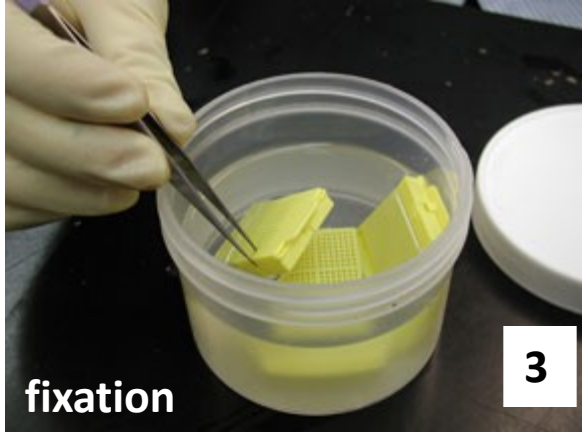
Freezing microtome (**cryostat**)
= rotary microtome housed in freezing box
(– 60º C)

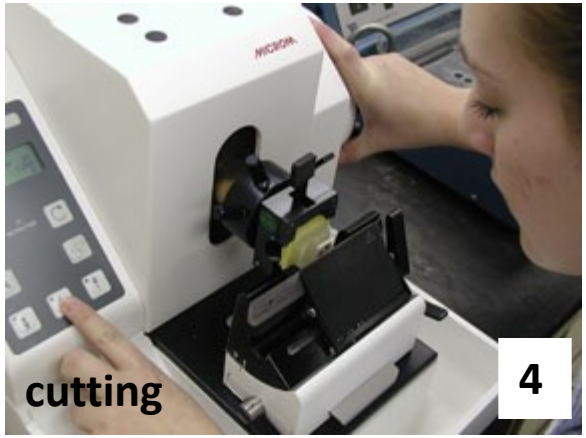
Cutting of frozen tissue without the embedding

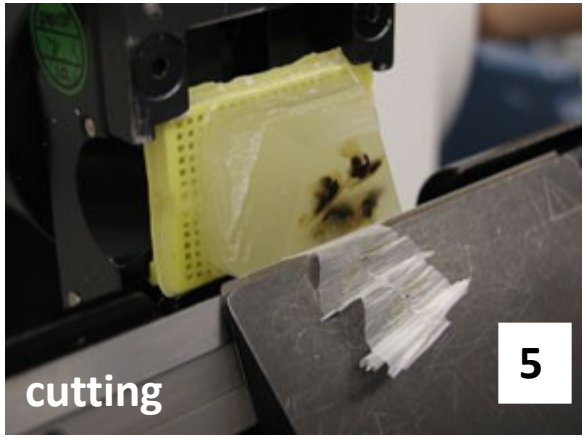


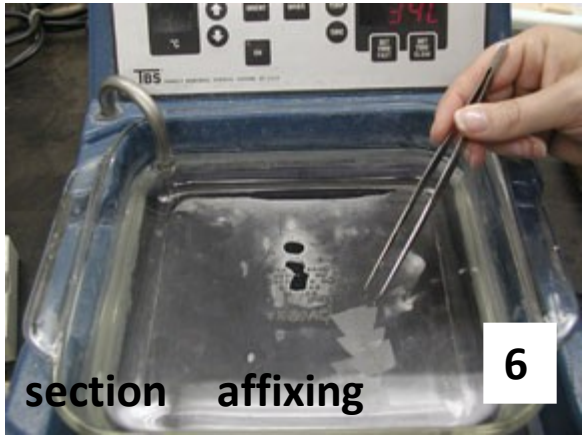

sampling
1

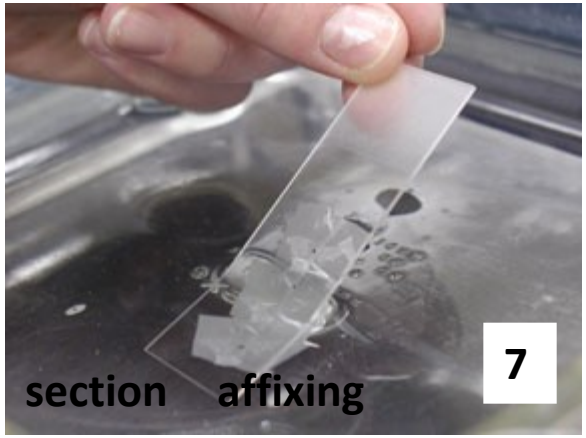

fixation
2

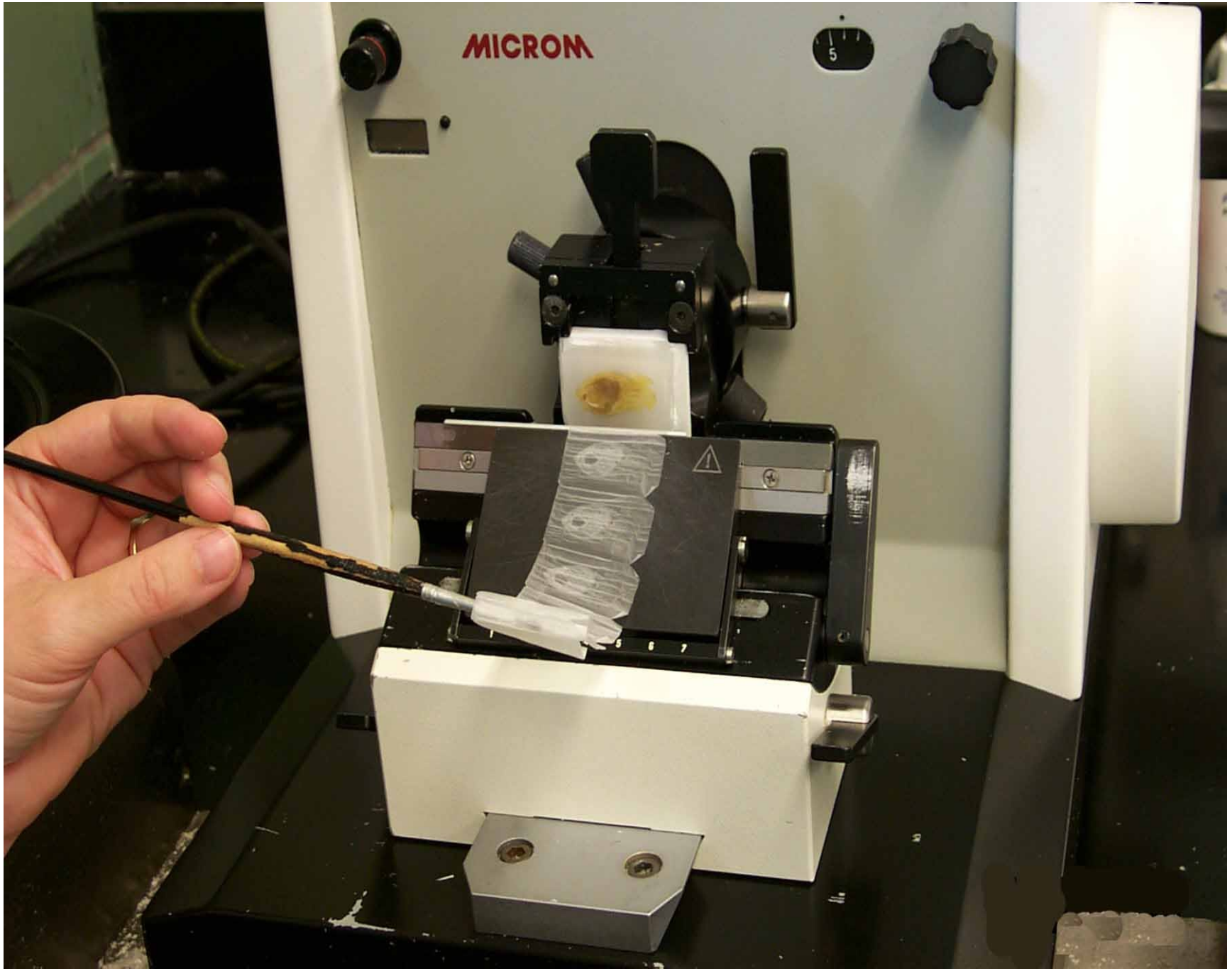

fixation
3


cutting
4


cutting
5


section affixing
6


section affixing
7


MICROM

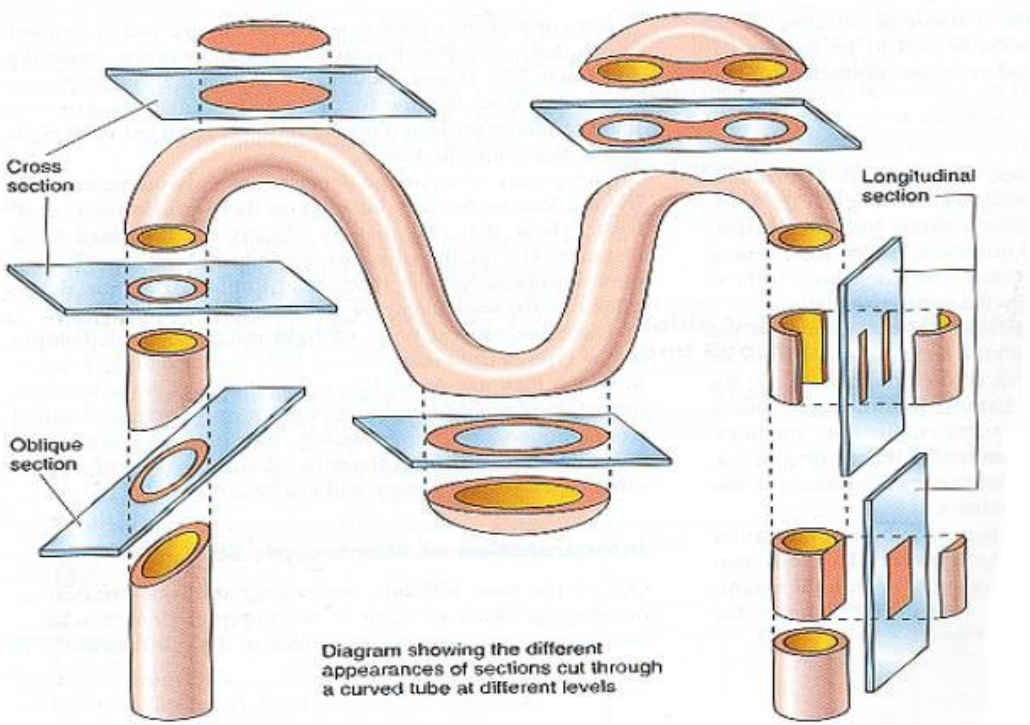
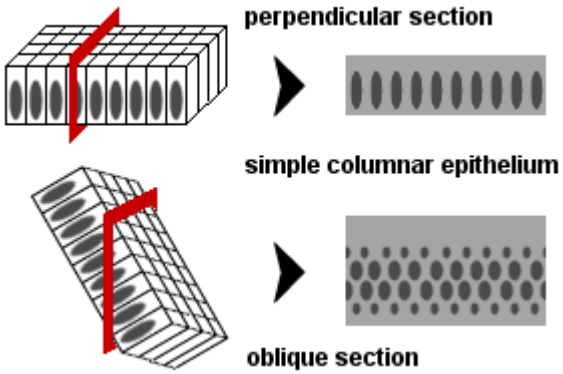


Diagram showing the different appearances of sections cut through a curved tube at different levels



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

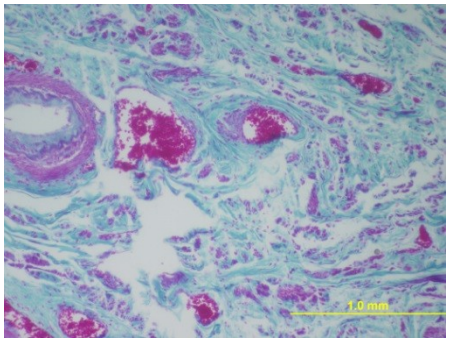
acidophilia – acidophilic structures in the cell

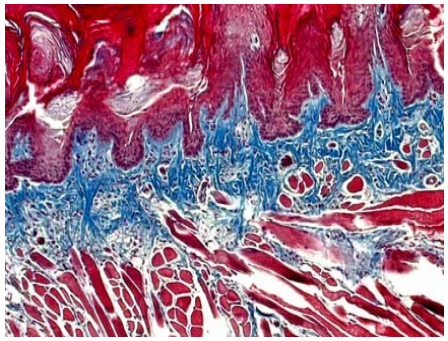
neutrophilia – no reaction

Staining methods:

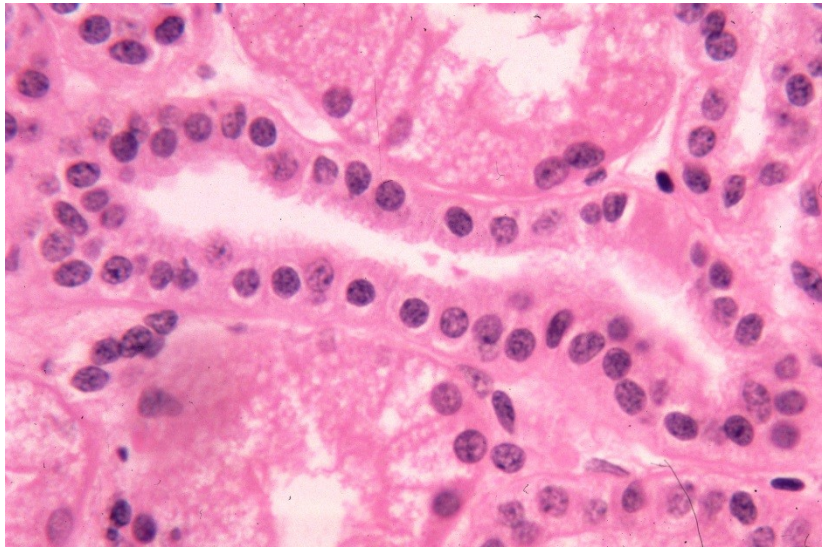
routine – HE, AZAN

(demonstrate all components of tissue)



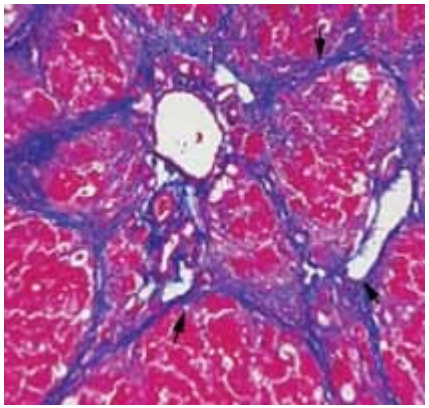


HE – the most frequent used method



special

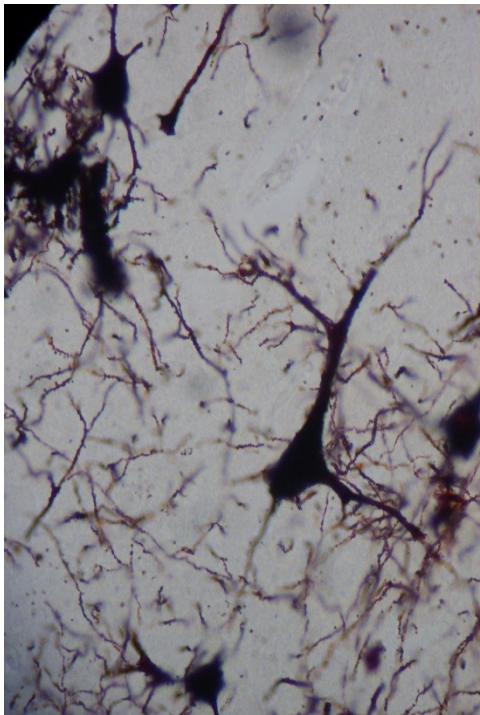
visualizes only special structures



Lipid droplets
detected by oil red

impregnation

by silver salt for detection
of nerve or reticular fibers



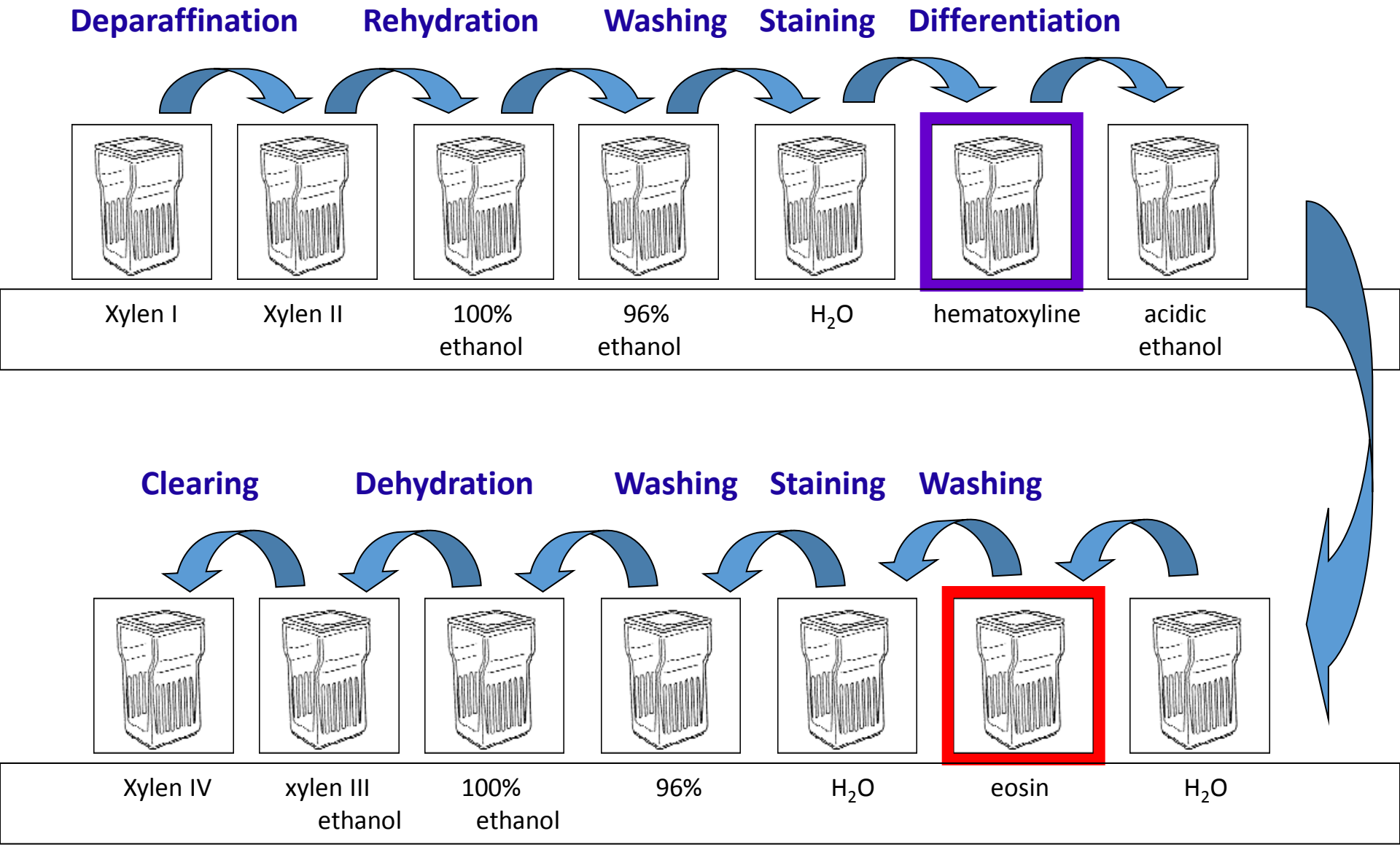
ROUTINE STAINING with HEMATOXYLINE – EOSIN (HE)

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)



Staining results:

- **HE** = ***Hematoxyline*** – ***Eosin***

  nuclei – bright clear blue or dark violet

  cytoplasm and collagen fibers – pink

  muscle tissue – red

- **HES** = ***Hematoxyline*** – ***Eosin*** – ***Safron***

  connective tissue – yellow

- **AZAN** = ***AZocarmin*** – ***ANiline blue*** – ***orange G***

  nuclei – red

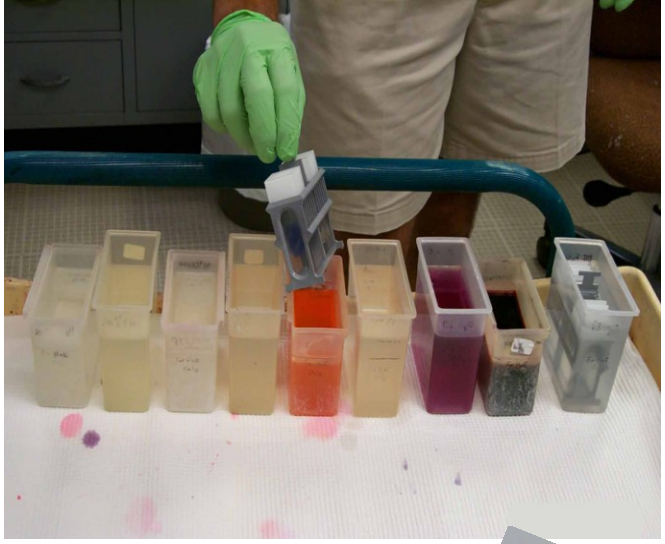
  erythrocytes – orange

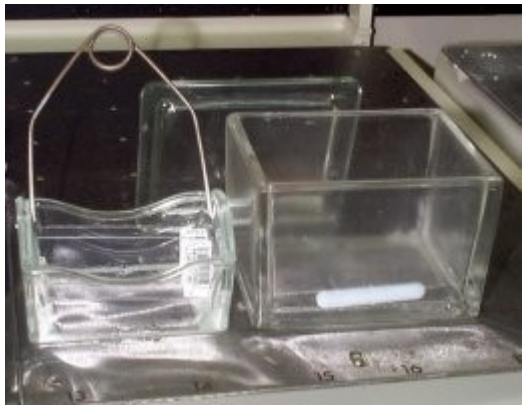
  muscle – red

  collagen fibers – blue

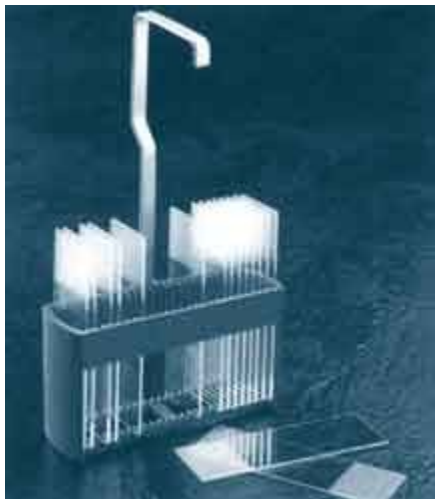
Staining tools:

cuvette





flask



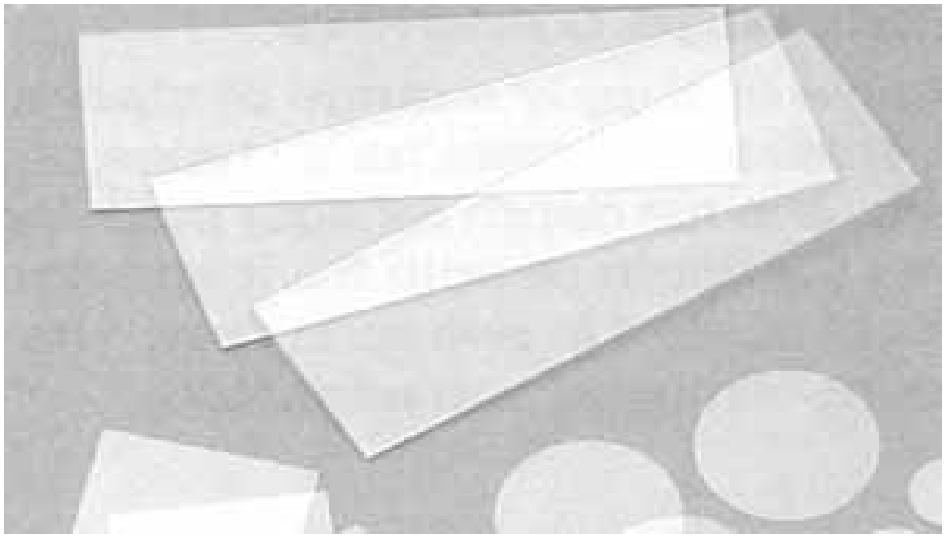
slides holder
(basket)

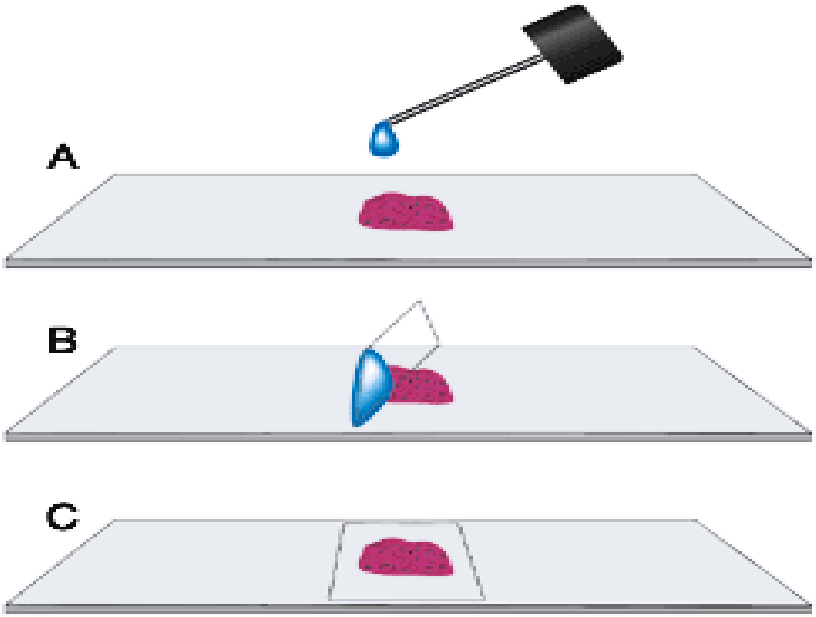
Automatic slide stainer

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.

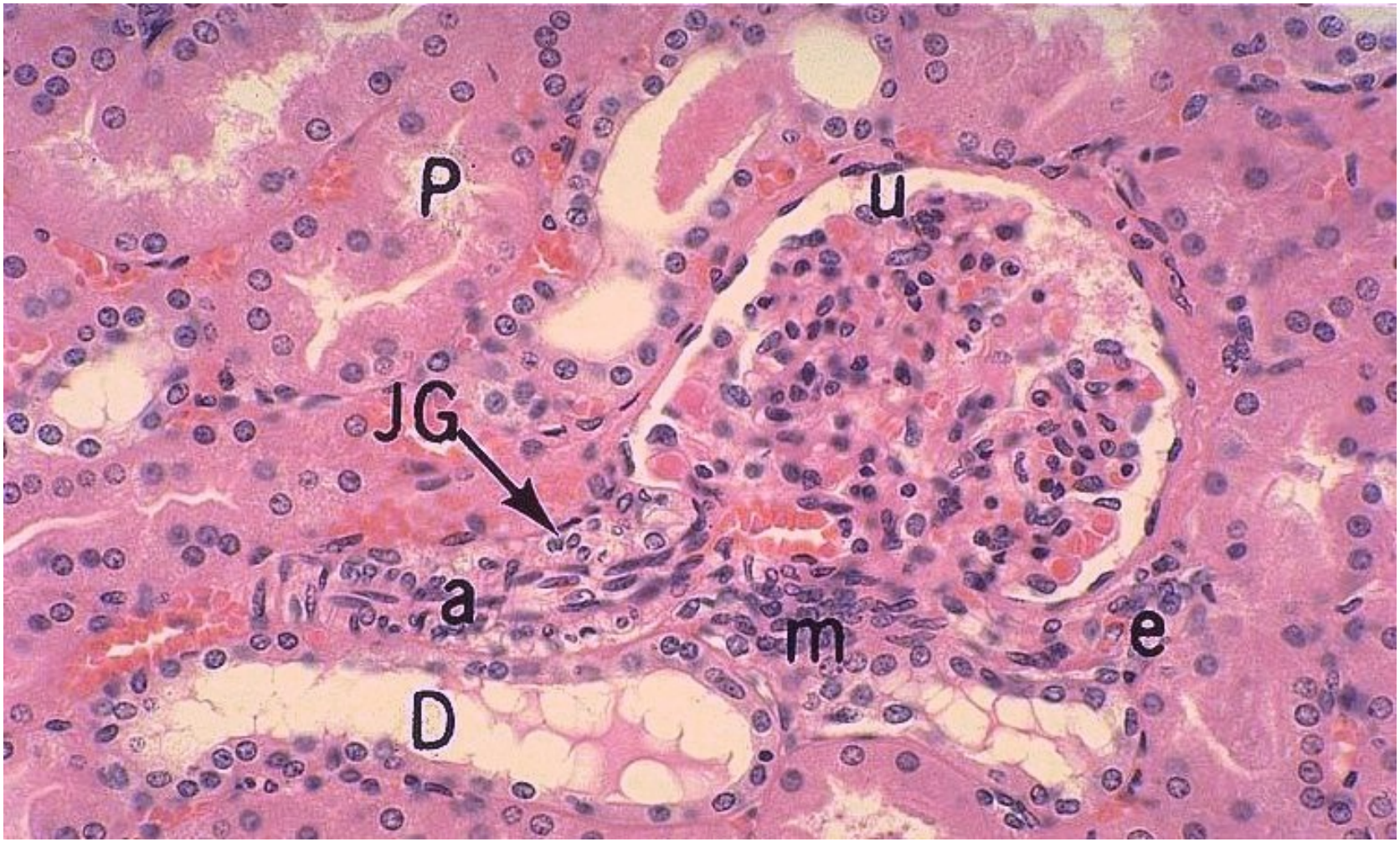




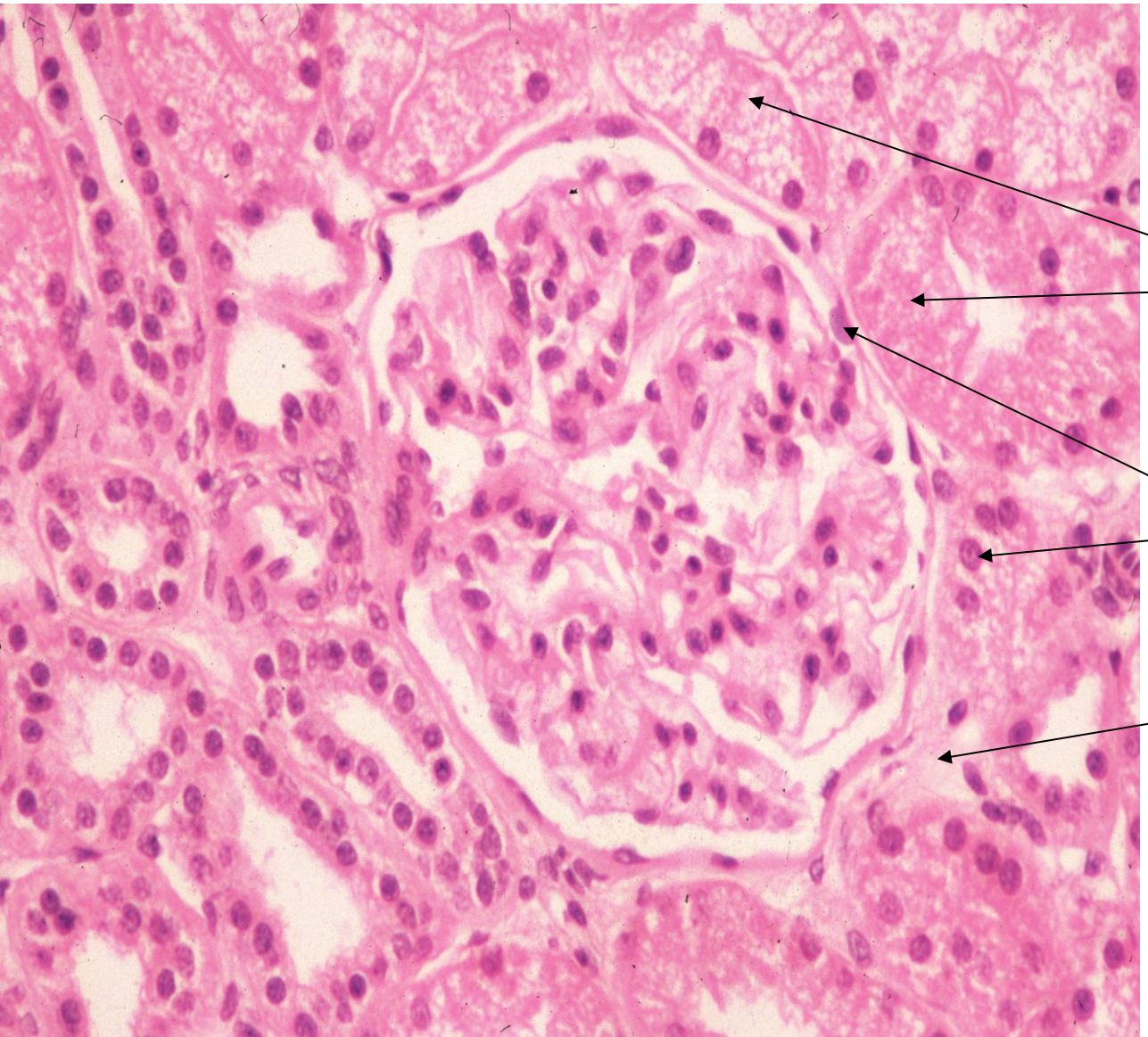
- **Mounting media**: soluble in xylene – **canada balsam**
  soluble in water – glycerin-gelatine, arabic gum

Permanent histological slides for study in the light microscope

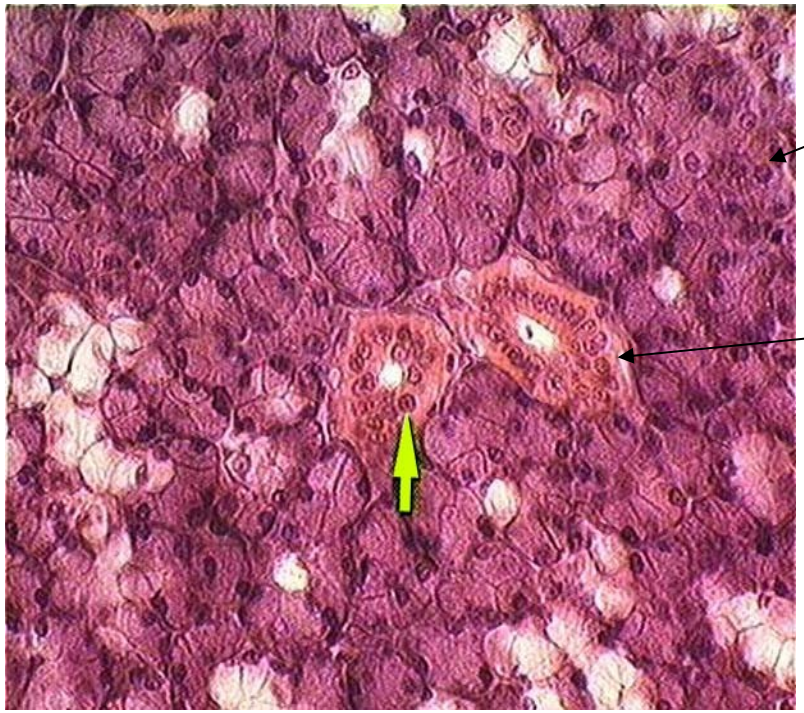
Hematoxyline and eosin (HE)



Hematoxyline and eosin (HE)



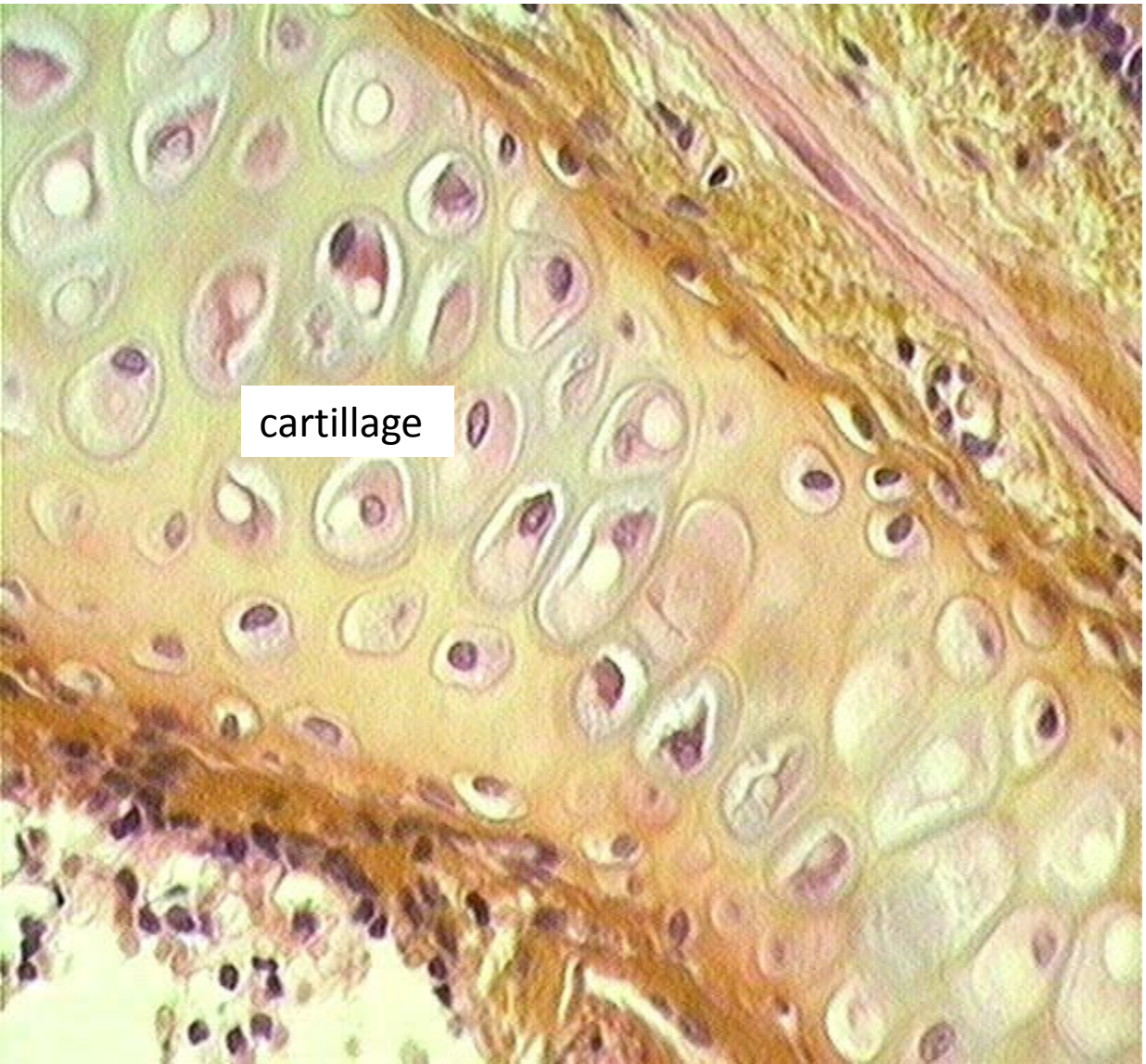
Hematoxyline and eosin (HE)



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

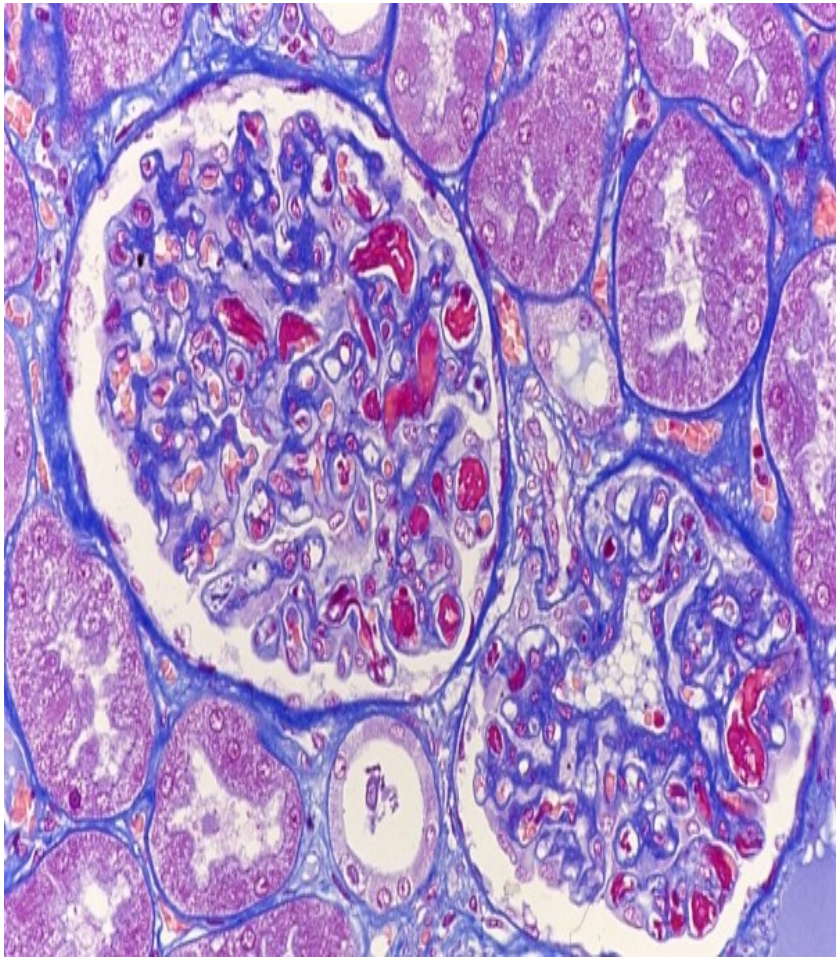
acidophilic cytoplasm of epithelial cells

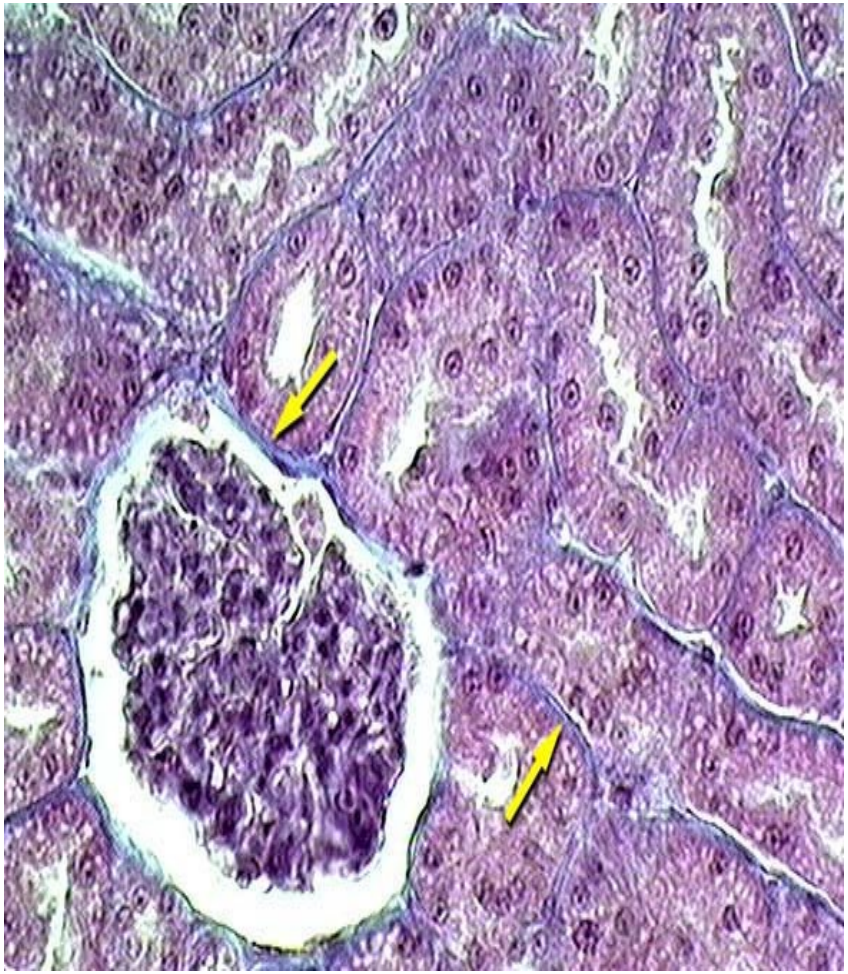
Hematoxyline, eosin and saffron (HES)



Collagenous fibers
of connective tissue
are yellow after staining
with saffron

Azocarmine and aniline blue (AZAN)

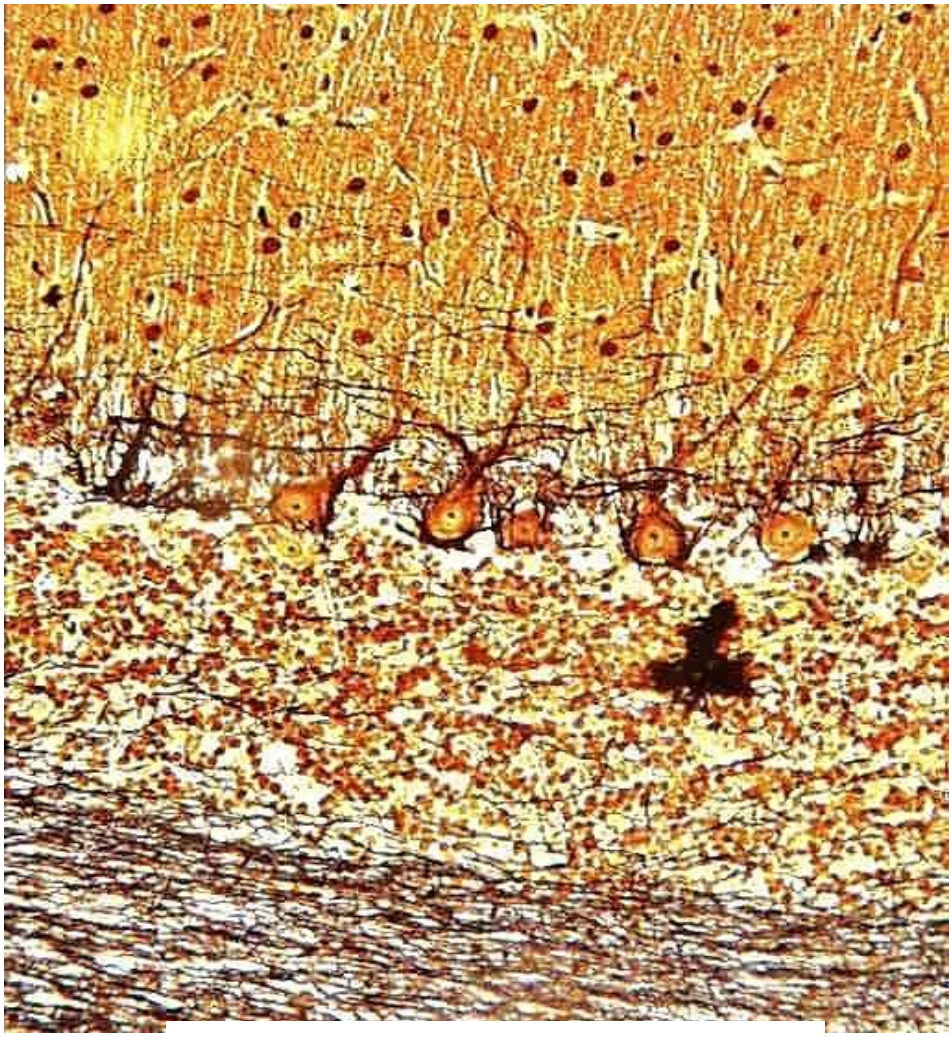




Kidney – collagen connective tissue

Impregnation of tissue with silver

Lien - reticular fibers



Cerebellum – nerve fibers

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