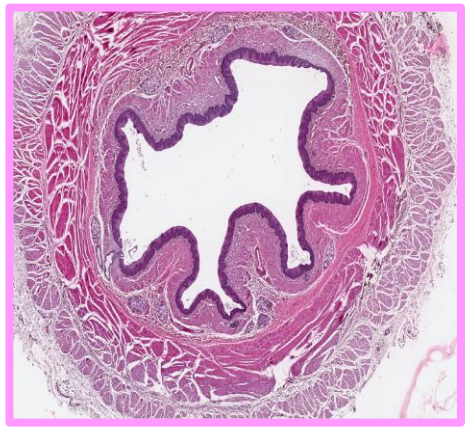
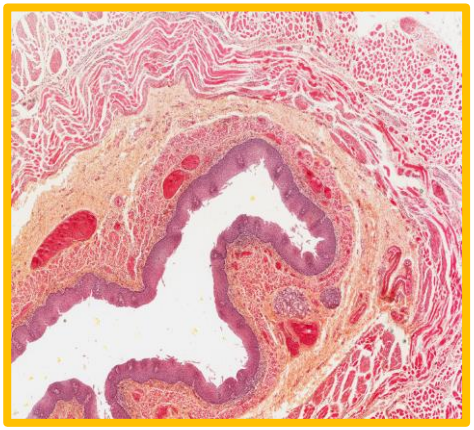
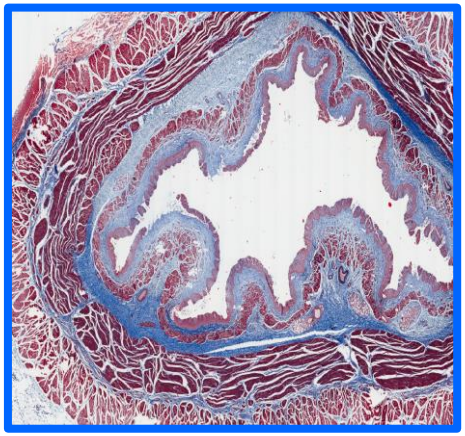
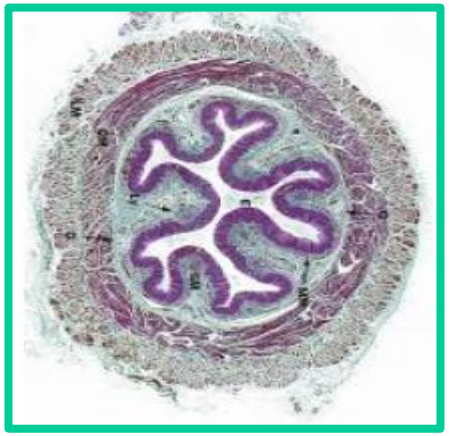
Basic staining methods in histology

How to use a light microscope

STAINING

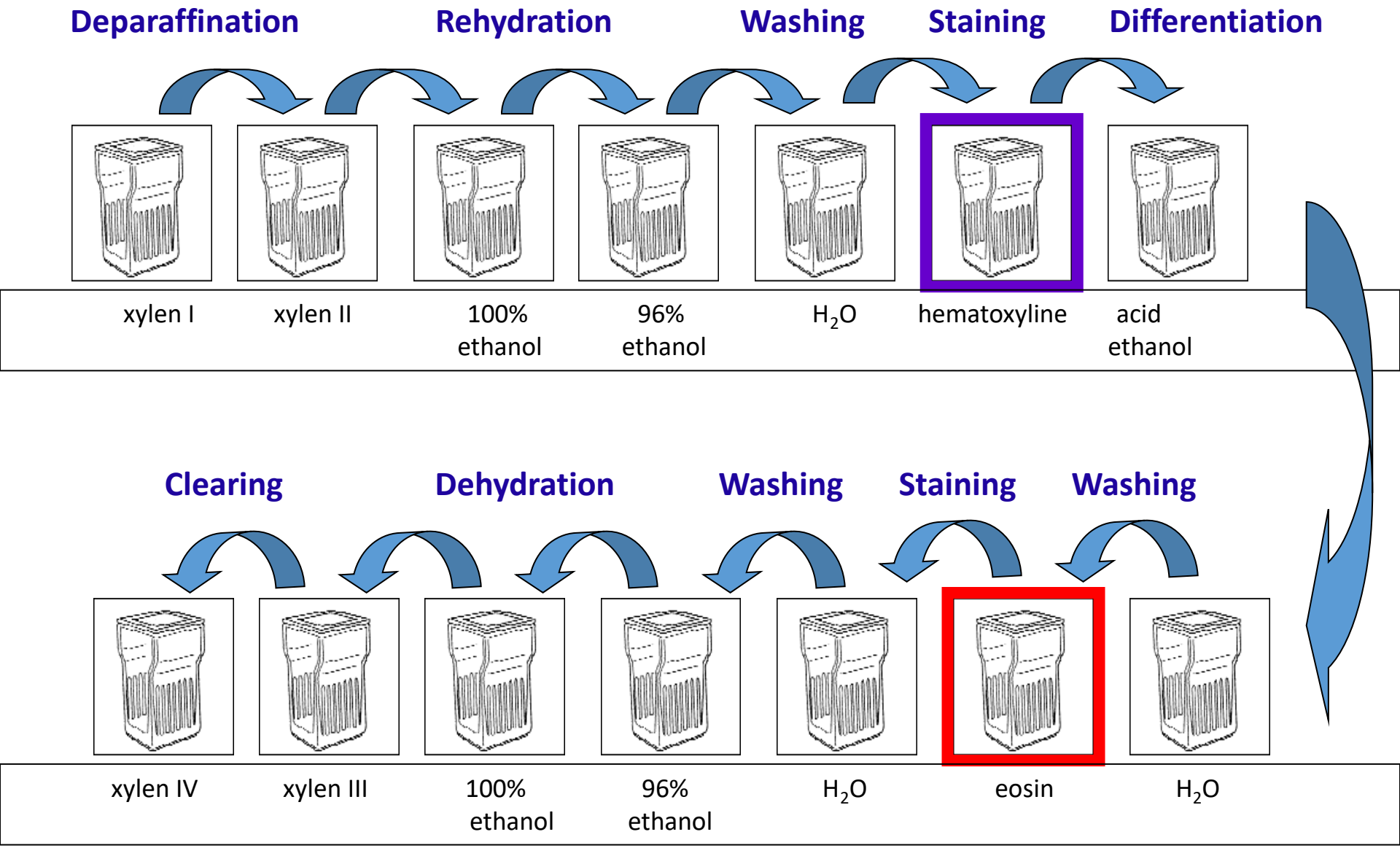
Different cell or tissue structures are not apparent without staining. Cellular structures exhibit different affinity to staining dyes:

alkaline dyes – react with anionic structures ➡ **BASOPHILIA**
(basophilic structures in the cell – nucleus, nucleolus, ribosomes, cytoplasm with rough ER)

acid dyes – react with cationic groups ➡ **ACIDOPHILIA** –
(acidophilic structures in the cell – cytoplasm, smooth ER)

no or weak reaction ➡ **NEUTROPHILIA**

HEMATOXYLINE – EOSIN (HE)



ROUTINE STAINING with 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

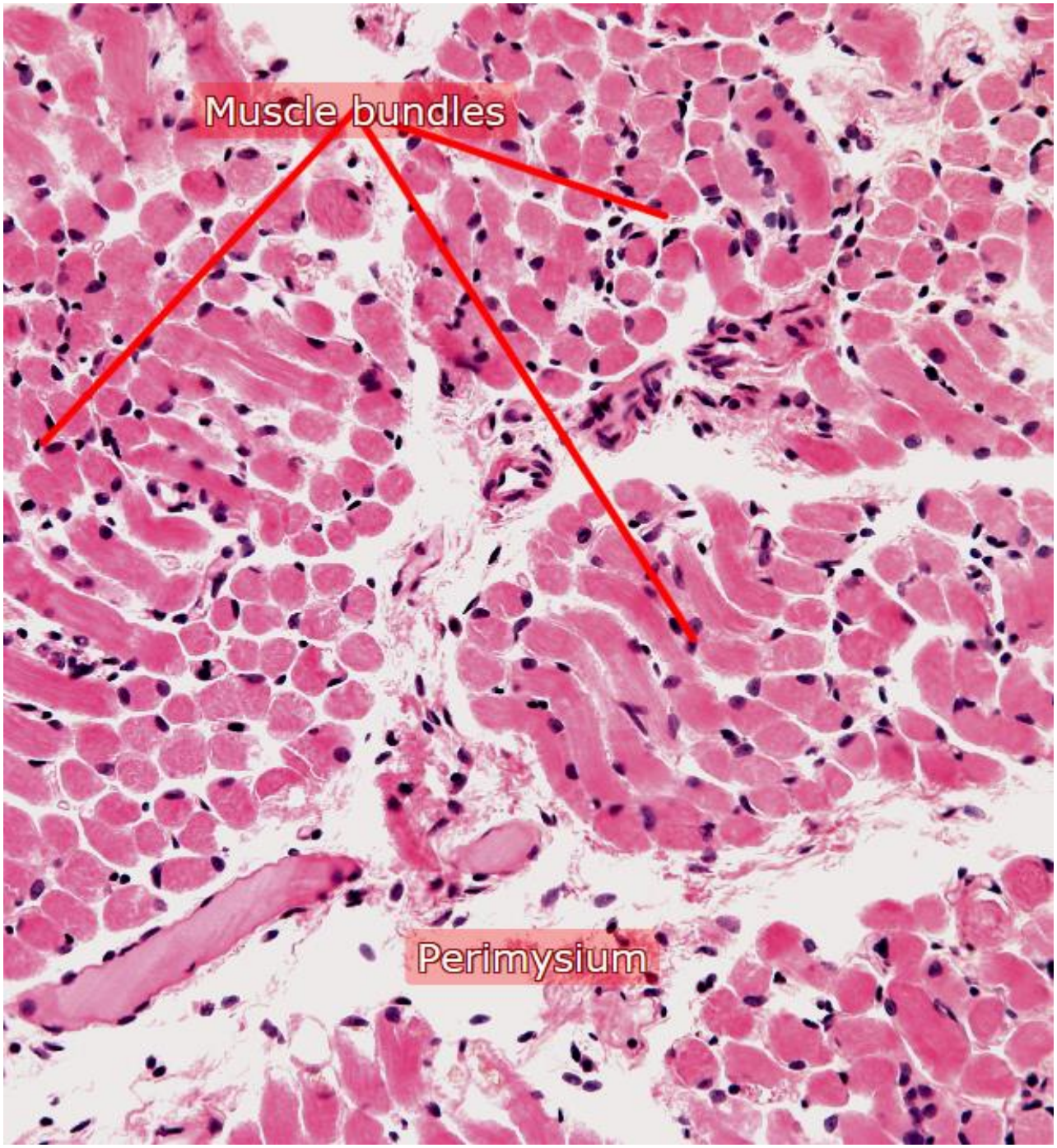
Automatic slide stainer

staining set of boxes with media

Hematoxyline
Eosin
(HE)

Results of staining:

cell nuclei – blue/violet
cytoplasm – pink
collagen fibers – pink
muscle cells – dark pink/red



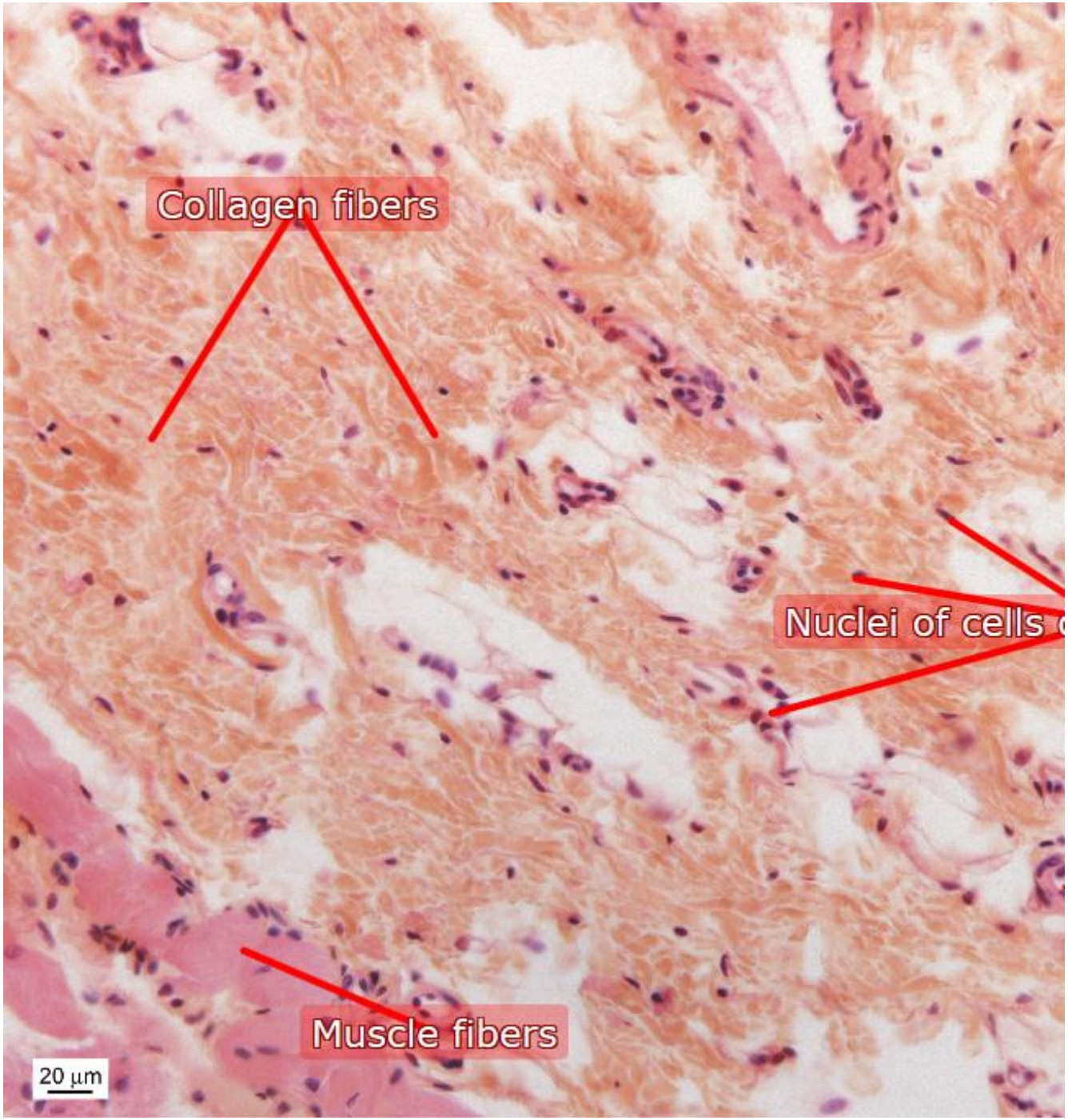
2 – Apex linguae (HE)

Hematoxyline
Eosin
Saffron
(HES)

Results of staining:

cell nuclei – blue/violet
cell cytoplasm – pink
collagen fibers – yellow

yellow Masson trichrom



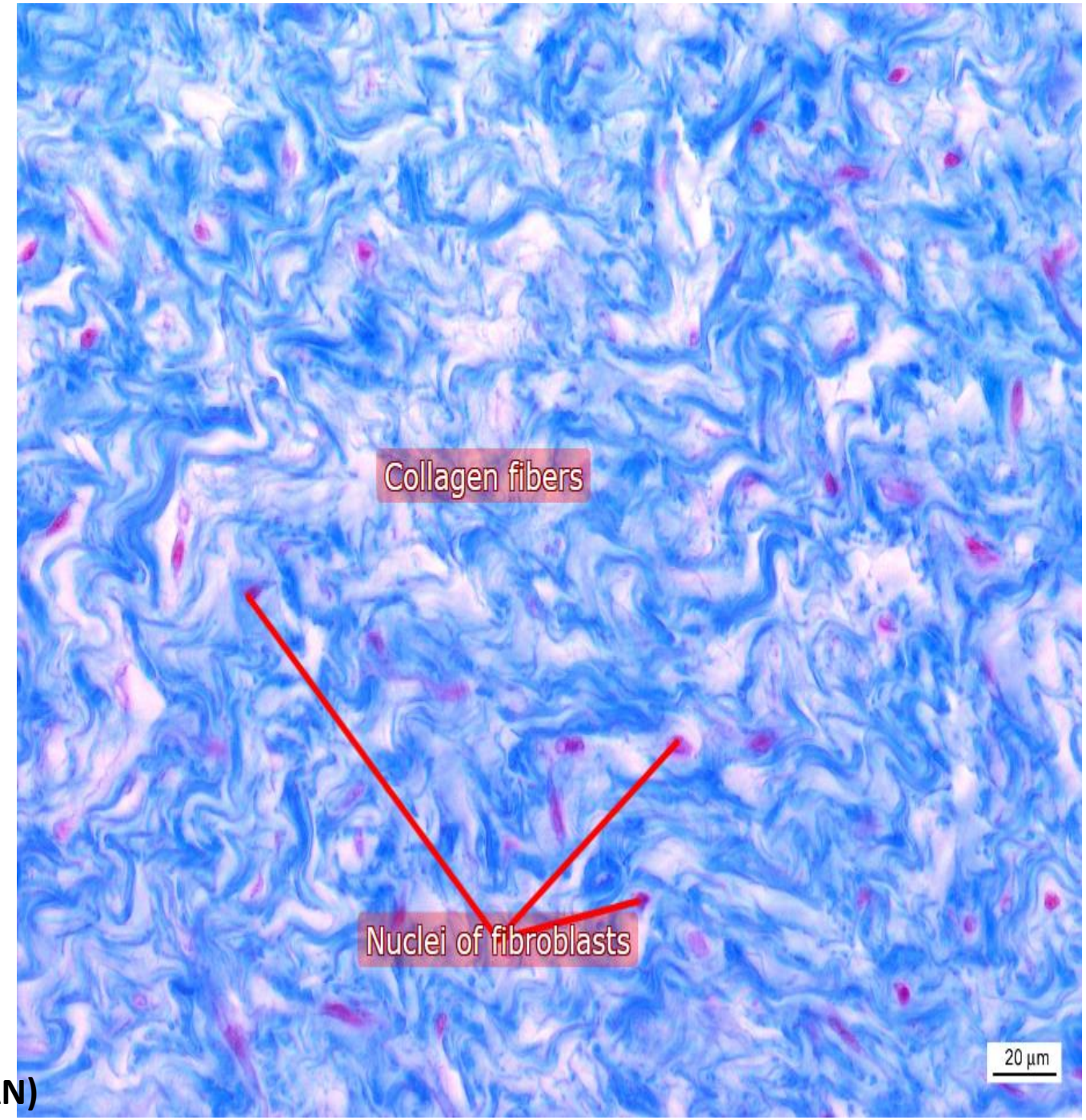
11 – Oesophagus (HES)

Azokarmin
Aniline blue
Orange G
(AZAN)

Results of staining:

cell nuclei – purple
cell cytoplasm – pink
collagen fibers – blue
erythrocytes – orange

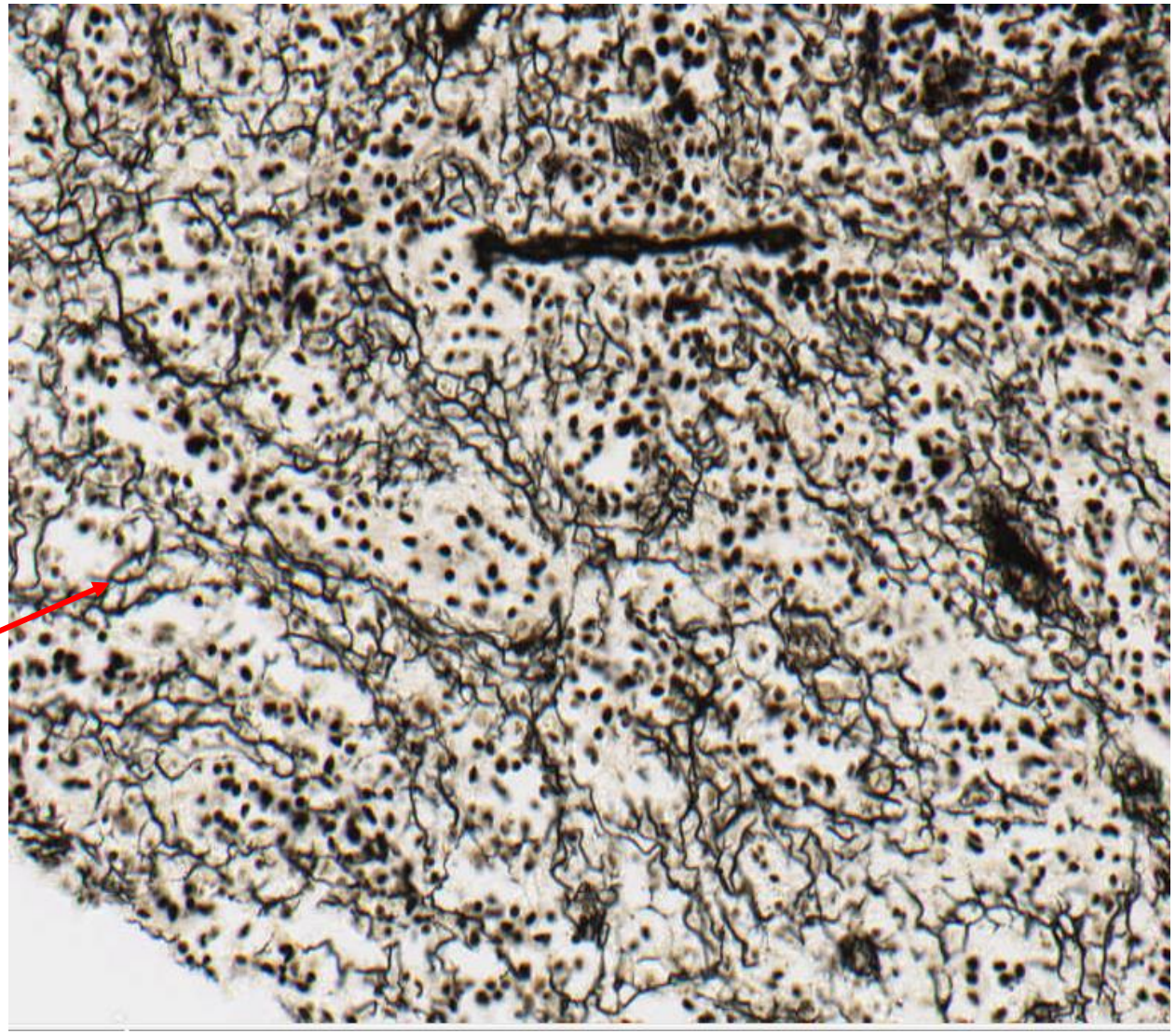
blue Masson trichrom



99 – umbilical cord (AZAN)

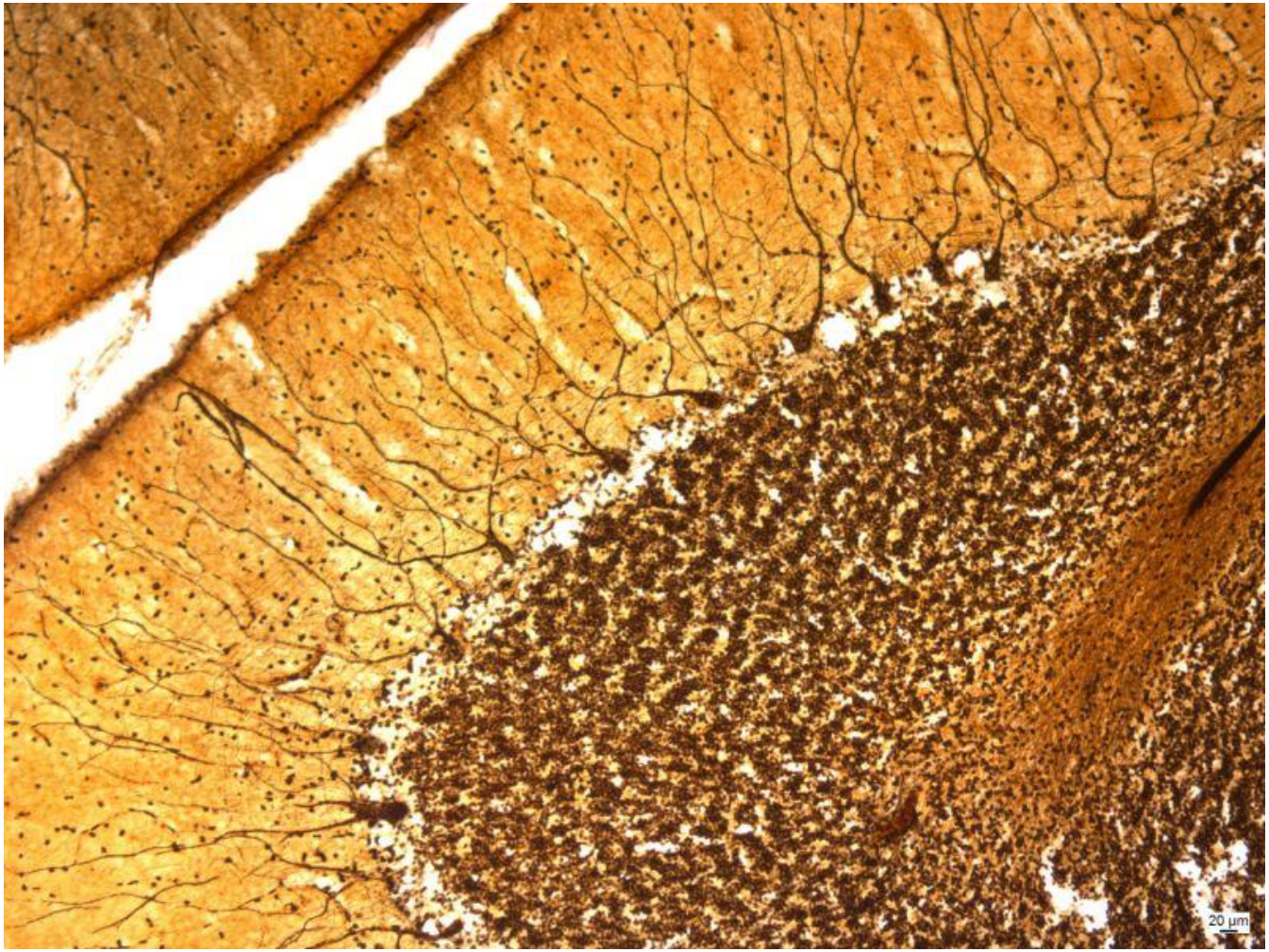
Impregnation with $AgNO_3$

Slide 68 – lien
Staining – **impregnation**
Result – **black reticular fibers**



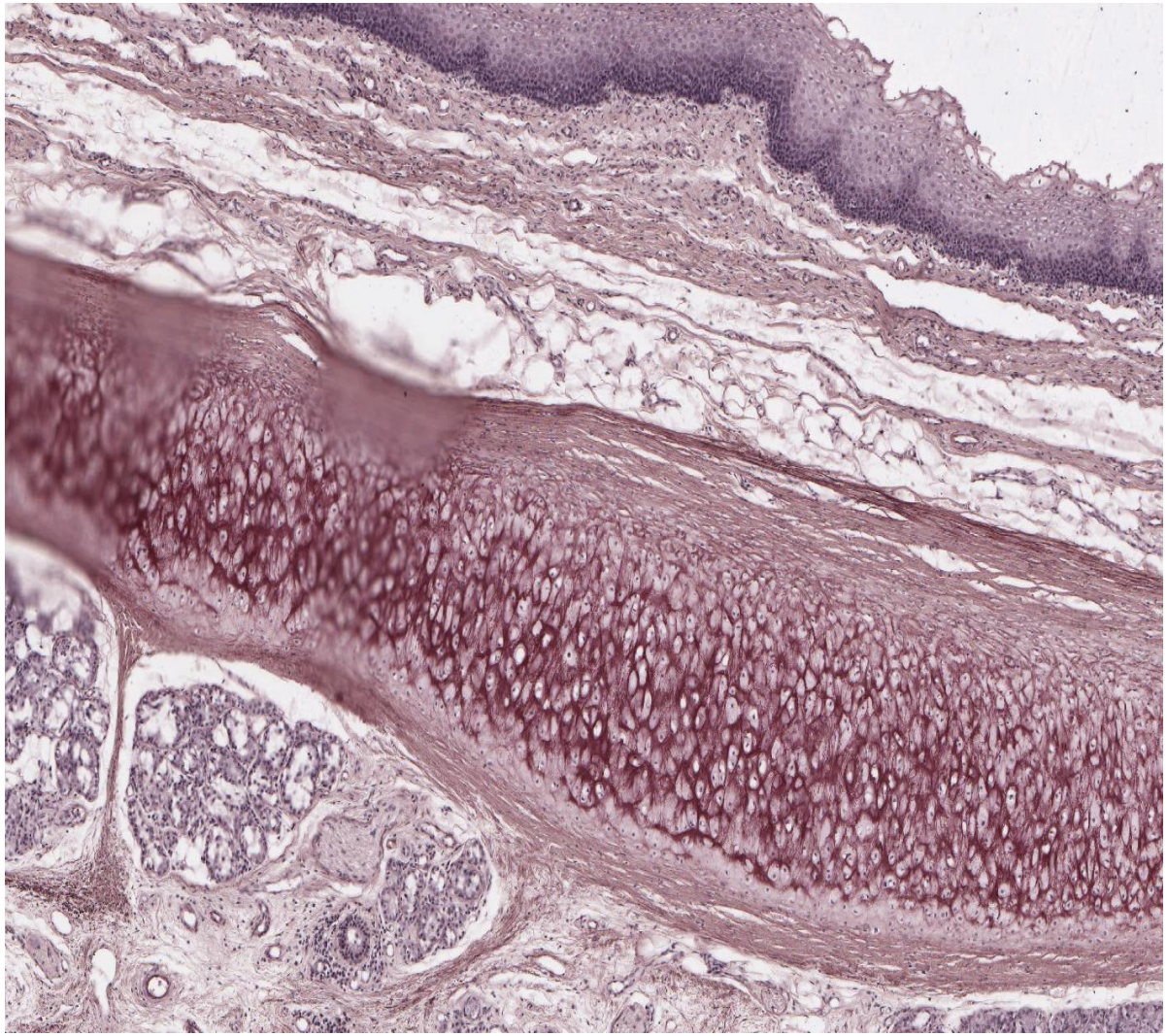
Impregnation with $AgNO_3$

Slide 77 – cerebellum
Staining – **impregnation**
Result – **black nerve processes**



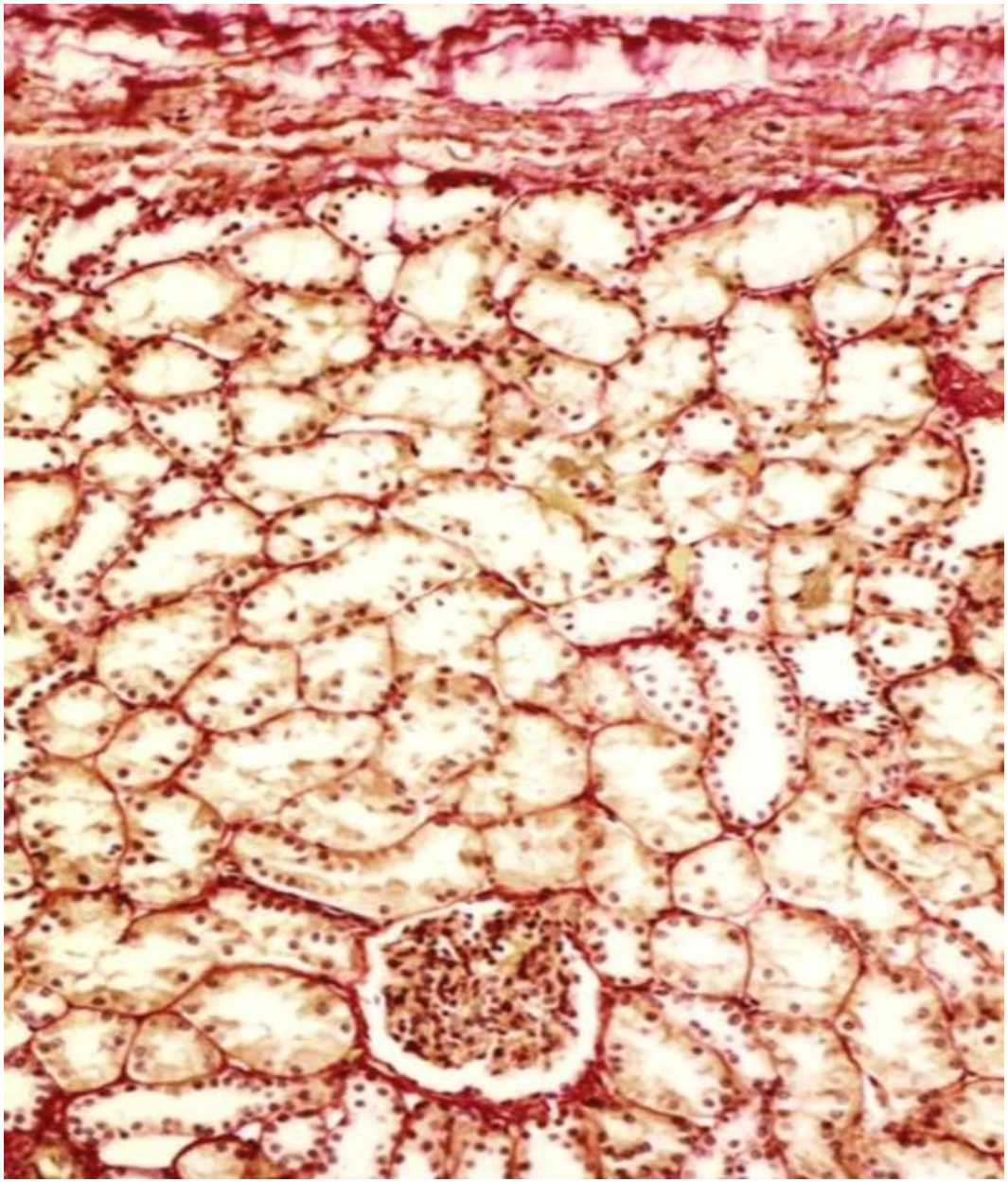
Orcein

Slide 28 – elastic cartilage
Staining – **orcein**
Result – **red-brown elastic fibers**



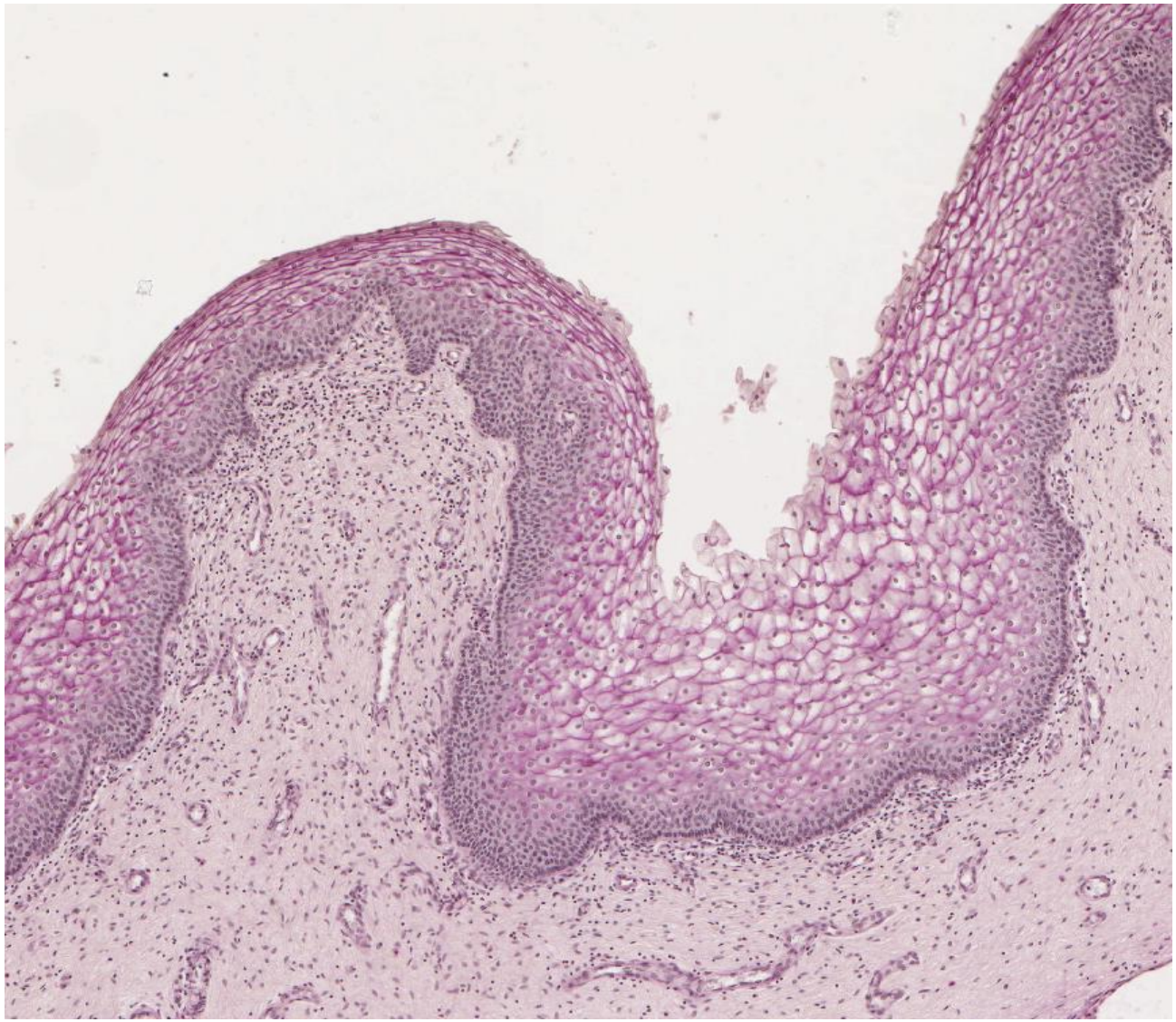
Weigert-van Giesson

Slide 31 – renal cortex
Staining – **Weigert-van Giesson**
Result – **cherry-red collagen fibers**



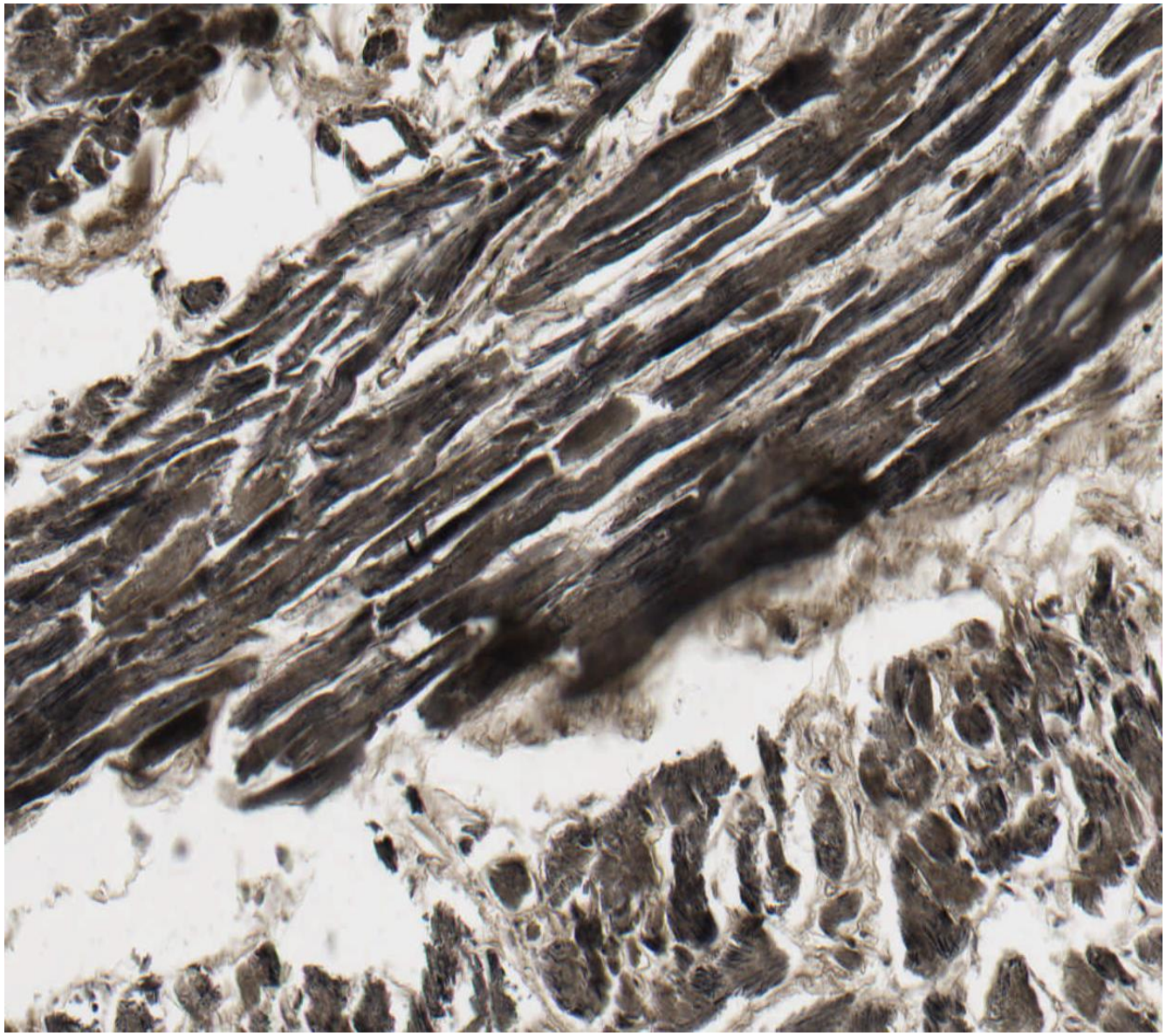
Best carmine

Slide 49 – vagina - glycogen
Staining – **Best carmine**
Result – **darc pink glycogen**



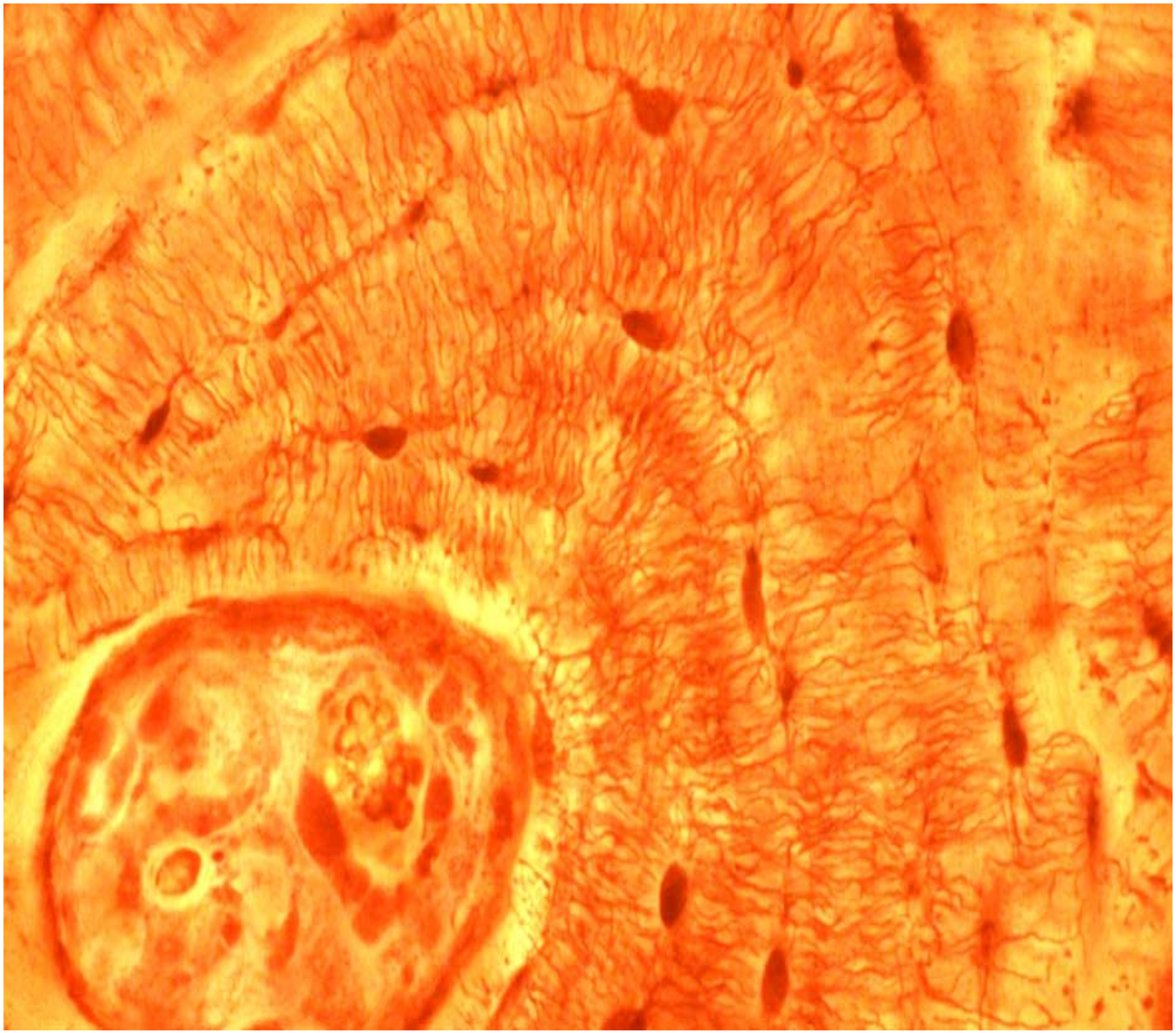
Heidenhain

Slide 65 – myocardium
Staining – **Heidenhain**
Result – **black cardiomyocytes** (cross-striation)

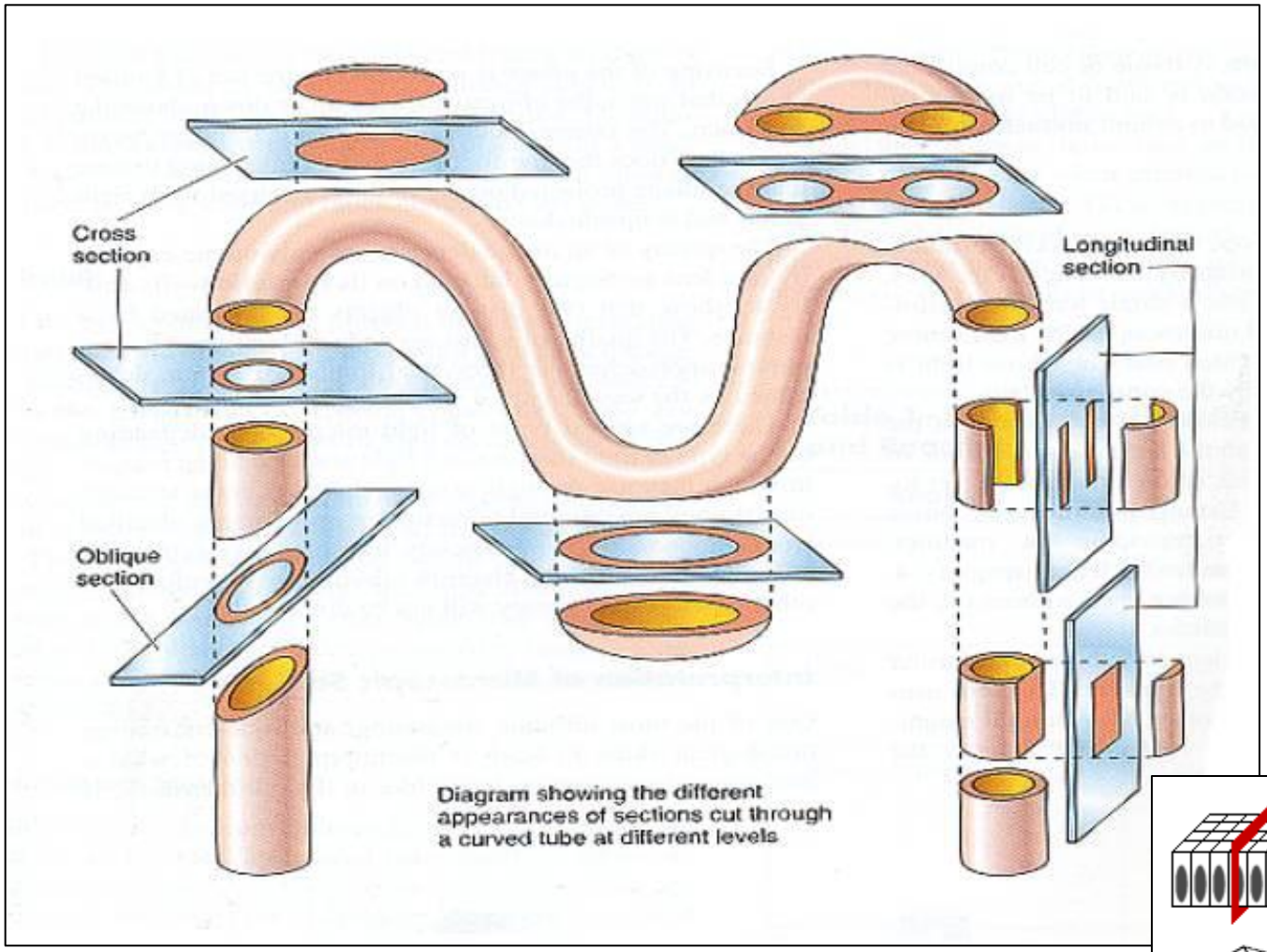


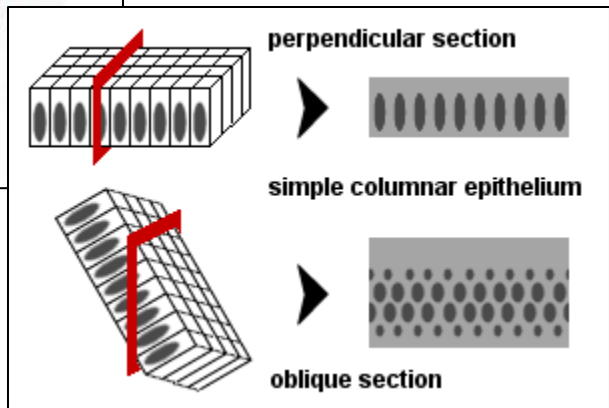
Schmorl

Slide 95 – bone
Staining – **Schmorl**
Result – rusty brown
bone tissue

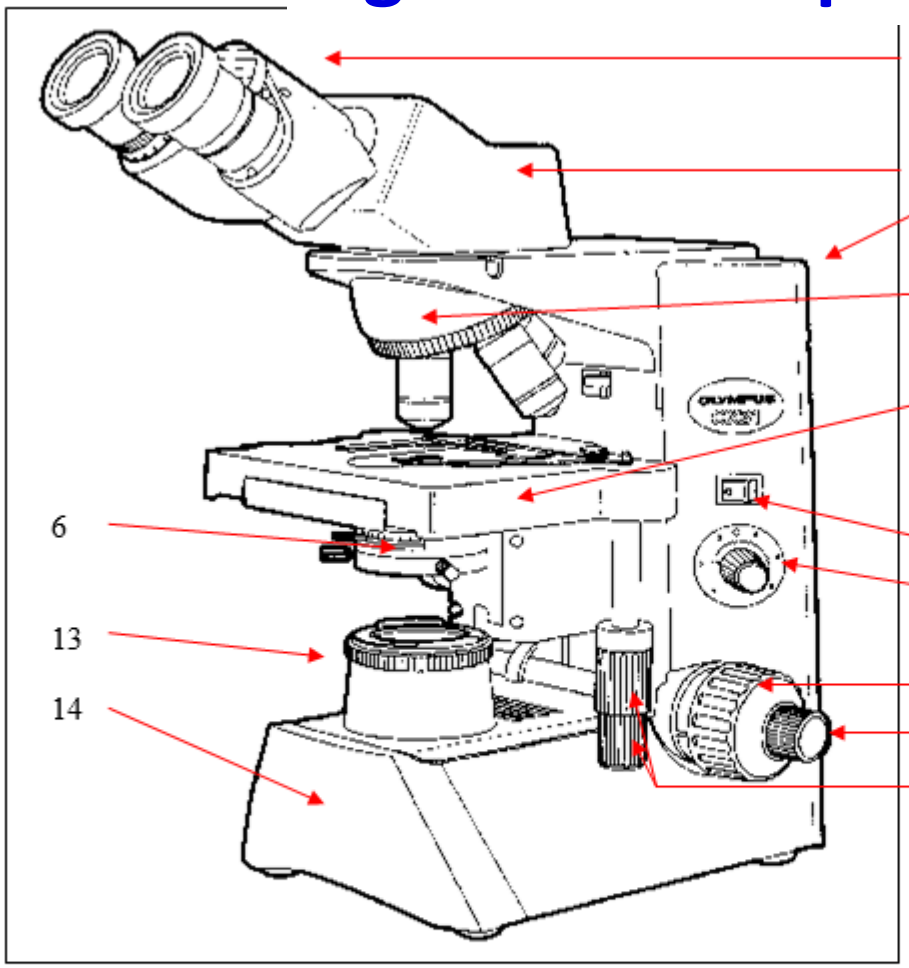


APPEARANCE OF 3D OBJECTS IN 2D SECTIONS





Light microscope



1 – eyepiece (ocular) (magnif. 10x)

pointer!

2 – revolving ocular tube

3 – arm of tube

4 – revolving head with objectives (magnif. 4x, 10x, 40x, 100x)

Obj. 100x / don't use it!

5 – stage with cross-shaped shift

stage clip holds the slide on stage

6 – condenser with iris diaphragm

7 – switch on/off knob

8 – light intensity control knob

FOCUSING KNOBS (9,10)

9 – coarse adjustment

9 – use only when using objective 4x

10 – fine adjustment

11, 12 – knobs for shift of slide

13 – light source

14 – base (stage)

The slide must lie on the stage (5) of LM so that the cover glass faces up to the objective lens.

Magnification: of eyepiece = 10x
of objectives = 4x, 10x, 40x, immersion objective = 100x

Total magnif.: ocular magnif. multiplied by objective magnif. = 40x, 100x, 400x, 1000x

Light microscope

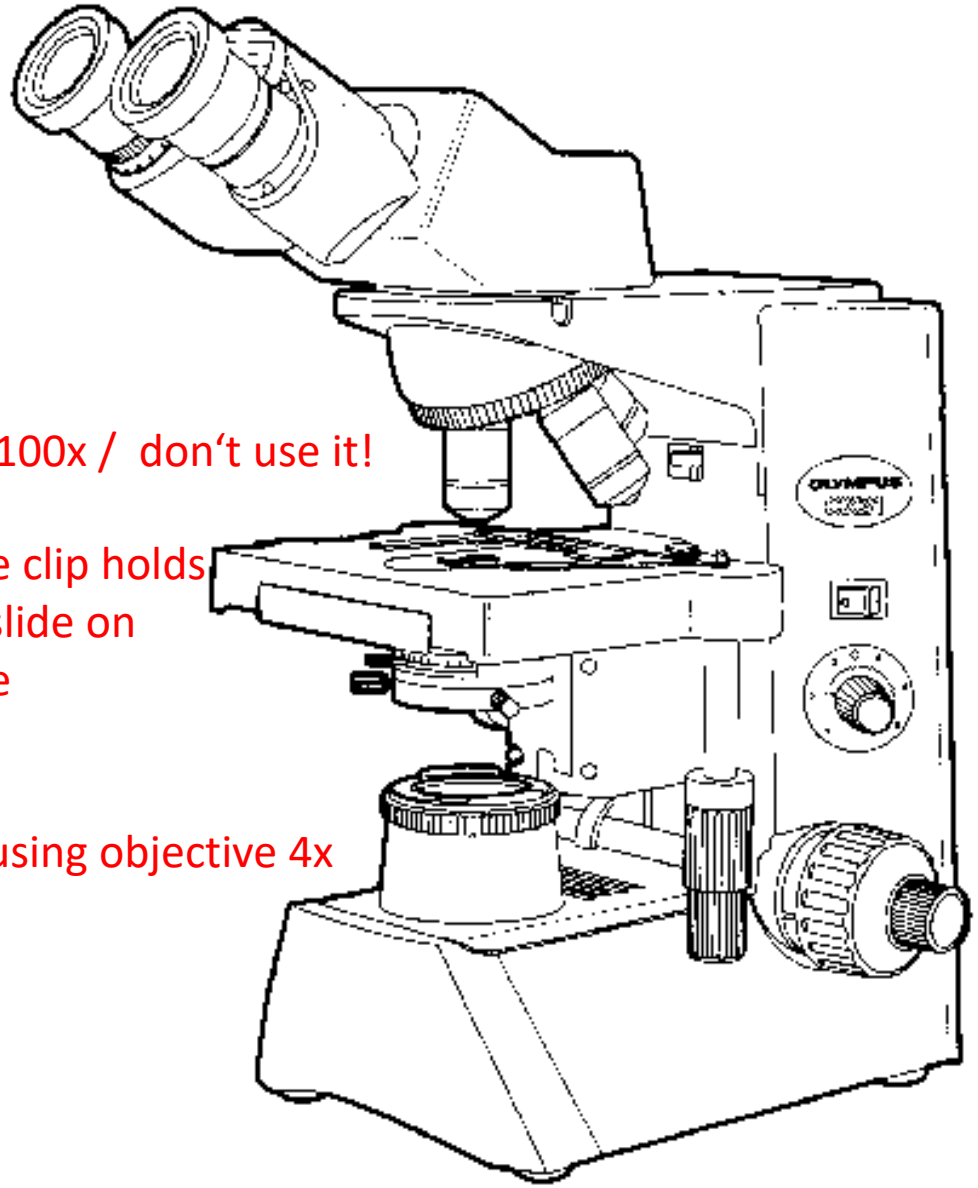
- Eyepieces
- Objective lens
- Stage with speciment holder
- On/Off
- Light control
- Condenser and Iris aperture
- Stage controls
- Coarse focus -
- Fine focus
- Light source with diaphragm

pointer!

obj. 100x / don‘t use it!

stage clip holds the slide on stage

use only when using objective 4x



HOW TO HANDLE A SET OF SLIDES

Rule 1: ***At the beginning*** of each practice, check the set of slides and any defects (missing or broken slide) report to teacher.

Rule 2: **Only one slide** can be taken out of the box and studied in LM.

Rule 3: The slide must lie on the stage of LM so that the cover glass faces up to the objective lens.

Rule 4: Treat the slides carefully; in case of damage of slide, inform the teacher.

Rule 5: ***At the end*** of each practice, the box with slides must be open for inspection and student must wait at workplace during inspection.

Instructions

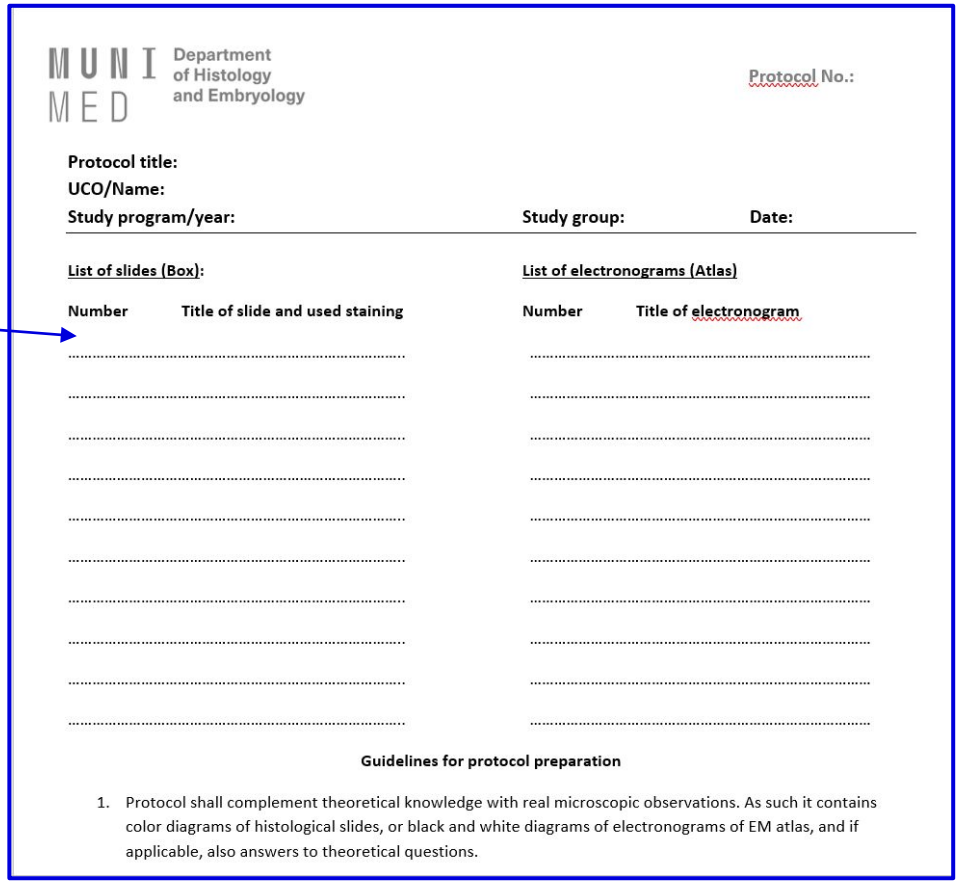
- Turn on the light.
- **Start with the 4x objective**.
- Put the slide on the stage – cover glass must be above
- Look through the scope and focus. Use the coarse focus knob at first, until the image is more or less in focus; then switch to the fine focus.
- Adjust the light. Not too bright, not too dim.
- Adjust the oculars.
- Switch to the 10x objective. A slight adjustment with the fine focus knob should get it just right. If you lose the focus and can't see your specimen at all, go back to the 4x and start again.
- Switch to the 40x objective if you want to see more detail. **Don't use the 100x!**
- When you want to look at a new slide, switch back to the 4x before changing slides.
- **Only one slide is out of the box at the moment!** Do not remove more!
- When you're done with the scope, switch to the 4x and turn the light all the way down before turning it off.
- At the end of lesson, the box with slides is checked in your presence before you leave your place

Basic staining methods in histology

Slides:

- HE staining: 2 – Apex linguae
- HES staining: 11 – Oesophagus (37. Epididymis)
- AZAN staining: 99 – Funiculus umbilicalis (21. Hepar)
- Impregnation: 77 – Cerebellum

- (Orcein: 28 – Epiglottis)



MUNI MED Department of Histology and Embryology

Protocol No.:

Protocol title:
UCO/Name:
Study program/year: **Study group:** **Date:**

List of slides (Box):

Number	Title of slide and used staining
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List of electronograms (Atlas)

Number	Title of electronogram
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Guidelines for protocol preparation

1. Protocol shall complement theoretical knowledge with real microscopic observations. As such it contains color diagrams of histological slides, or black and white diagrams of electronograms of EM atlas, and if applicable, also answers to theoretical questions.