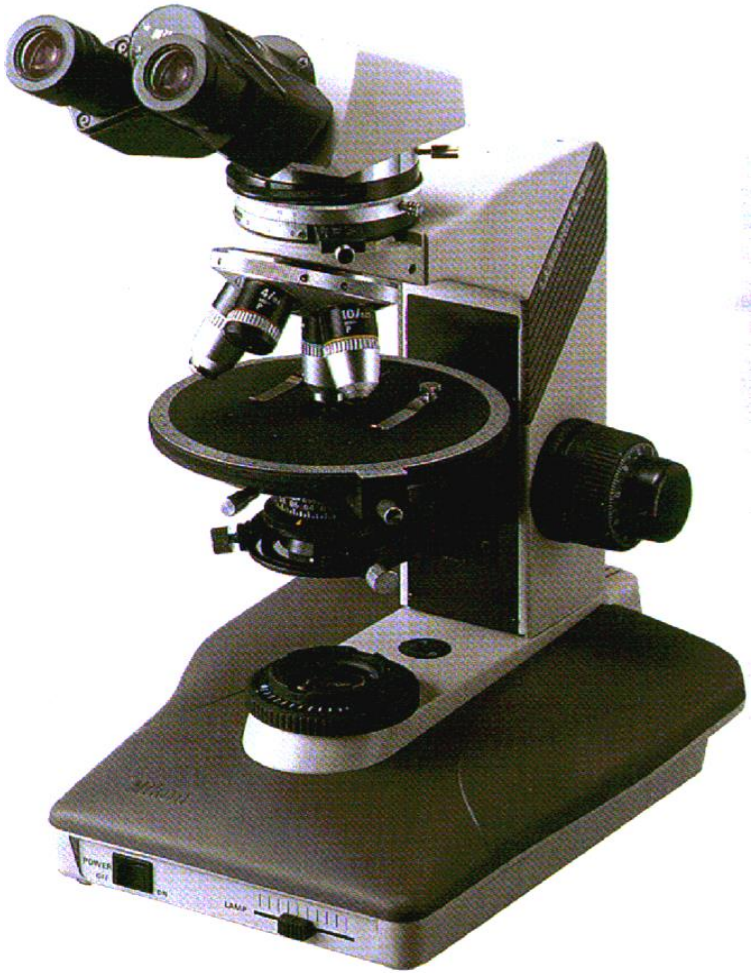


Histology seminars and practical classes

General regulations - Histology and Embryology for medical students 6ED 2023/2024

Organization of classes and seminars

1. Histology and Embryology is taught during lectures, seminars and practical classes.
2. Presence in lectures, seminars and practical classes is obligatory. Coming late to class by more than 15 minutes will be treated as an absence.
3. Classes begin with the seminar followed by a practical part.
4. Students have to be prepared for the class. Tutor will verify student's preparation to the class. Subject of seminars and classes are specified in the Topics of classes and lectures.
5. During the class, students discuss with their professor topics of the class and inspect microscopic slides,
6. schemes and electronograms. Images of tissues and organs inspected under the microscope should be drawn with color crayons in the notebook. All drawings have to be properly described (legend to the drawing).
7. Microscopes are provided for every student in the class. At the end of the class student should switch off the microscope and cover it. Microscopic slides, electronograms, microscopes or their parts must not be removed from the class.
8. During the period preceding intermediate or final examinations, every student group can borrow a set of demonstration slides for an at-home training. Sets can be exchanged any number of times. Before exchanging or returning the set, students have to put slides in order, according to the attached list. Students are financially liable for lost or damaged slides.

Presence in the classes and seminars

1. To get the credit for the semester Student must be present in lectures and seminars and get credit in all classes.
2. The prerequisite for getting a credit for the class is a positive note received on the knowledge of the discussed subject and properly done drawings of microscopic slides.
3. Days of classes, including days of intermediate examinations, are days of obligatory presence.
4. **It is permitted to be absent up to 2 times during lectures and 2 times during classes in each semester.** Absence must be justified with the tutor. **Absence on 3 or more classes, regardless of the reason, results in not getting a credit for the semester**, hence student will not be admitted to the intermediate examination.
5. **When students are absent, they are expected to negotiate with professors the form for make-up of lectures, seminars or classes missed.**
6. Student is obliged to make up for missed class.
7. Classes uncredited because of an absence or being unprepared must be passed in the form established by the Head of the Department. Head of the Department will appoint the date of this test.

Department. Head of the Department will appoint the date of this test.

Credit

13. Dates of the intermediate examinations are decided by the university Pedagogical Council and cannot be changed.
14. Only students who were present in lectures, seminars and got credit for all the classes are admitted to the intermediate examination.
15. Intermediate examination in general histology and in microscopic anatomy consist of two parts: practical (slide recognition) and theoretical.
16. Intermediate examination in embryology has no practical part.
17. Intermediate examinations on the first and the second date are MCQ tests. Third final attempt of the intermediate examination (commission) have the form that is determined by the Head of the Department and is set after the permission obtained from the Dean's office.
18. Electronic intermediate examination tests online consist of 50 single choice questions. The duration of intermediate examination is 50 minutes. Electronic test examinations are held in the building of Main Library in the computer room.
19. The criteria to pass the test are determined by the Head of the Department, after the test, and they are expected to be not less than:
    - 60% of all questions in the test.

Note	Criteria
2,0 (failed)	0 – 59 %
3,0 (satisfactory)	60 – 68 %
3,5 (rather good)	69 – 76 %
4,0 (good)	77 – 84 %
4,5 (better than good)	85 – 92 %
5,0 (very good)	93 – 100 %

20. **Any reservations and irregularities regarding the course of the test or the content of the questions should be reported by the student only via the Examination Portal platform to the members of the Examination Team only during or immediately after the end of the test, before leaving his/her position in the computer room ("Regulations of Written Examinations of the Medical University of Warsaw", point 16.). Students have access to the questions only through the Examination Portal platform immediately after the end of the test, before leaving their workstation in the computer room.**
21. Intermediate practical part must be passed before the date of the retake MCQ test. Students who failed practical part of any intermediate examination before the date of the retake examination will not qualify for the retake and last retake of MCQ test.

20. **Any reservations and irregularities regarding the course of the test or the content of the questions should be reported by the student only via the Examination Portal platform to the members of the Examination Team only during or immediately after the end of the test, before leaving his/her position in the computer room ("Regulations of Written Examinations of the Medical University of Warsaw", point 16.). Students have access to the questions only through the Examination Portal platform immediately after the end of the test, before leaving their workstation in the computer room.**
21. Intermediate practical part must be passed before the date of the retake MCQ test. Students who failed practical part of any intermediate examination before the date of the retake examination will not qualify for the retake and last retake of MCQ test.

Final examination

22. The final examination comprises topics discussed during classes, seminars and lectures.
23. Student must pass all intermediate examinations scheduled in the program of the course to be admitted to the final examination.
24. Dates of the final examinations are decided by the university Pedagogical Council and cannot be changed.
25. The final examination consists of two parts: practical and theoretical.
26. Failing practical or theoretical part results in failing the examination.
27. **Head of the Department can set an oral appointment of THEORETICAL final examination for students, who obtained at least 92% of all points received on intermediate examinations. For such appointment student needs to apply to the Head of the Department in writing (template of the application is available on the Department web site). Student IS NOT exempted from PRACTICAL examination.**
28. **PRACTICAL EXAMINATION must be taken BEFORE the appointment with the Head for the Department.**
29. In the case of an absence during the final examination caused by medical condition, should present doctor's leave during three working days from the date of examination, or will receive a failing mark.
30. Retake of the examination is held during the retake examination session. If the student fails this examination, he/she can apply to the Dean for the permission for the second retake of the examination.

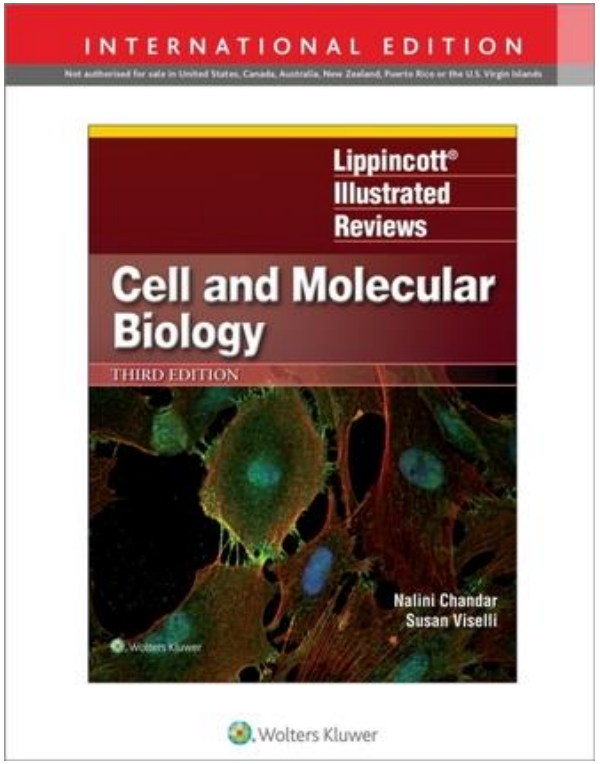
Position of the Chair regarding cheating during examinations

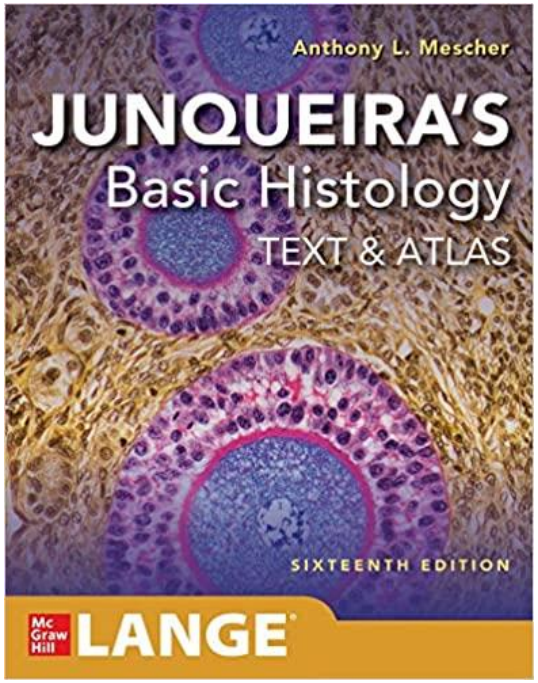
Cheating on examinations is a breach of ethics and Regulations of Studies at the Warsaw Medical University. Person actively or passively participating in cheating shall be punished by being expelled from the examination and receiving a failing mark. On the top of that, the Department shall institute disciplinary procedure against the cheating students.

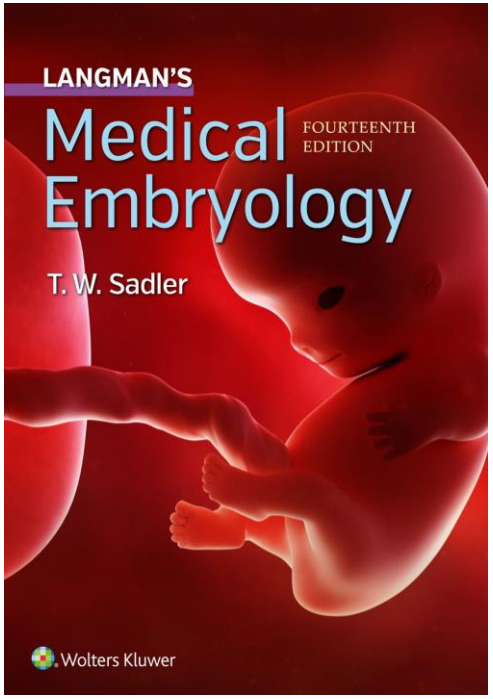
Person actively participating in cheating is the one, **who copies results from other students or uses illegal notes or electronic devices to communicate or store data. Bringing such devices to examinations is forbidden.** Passive participation in cheating means allowing other students copy one's own responses. Thus, a student is obliged to behave honestly, not to allow other students copy his/her own responses.

Head of the Department obliges students and examiners to strictly obey these regulations.

RECOMENDED HANDBOOKS







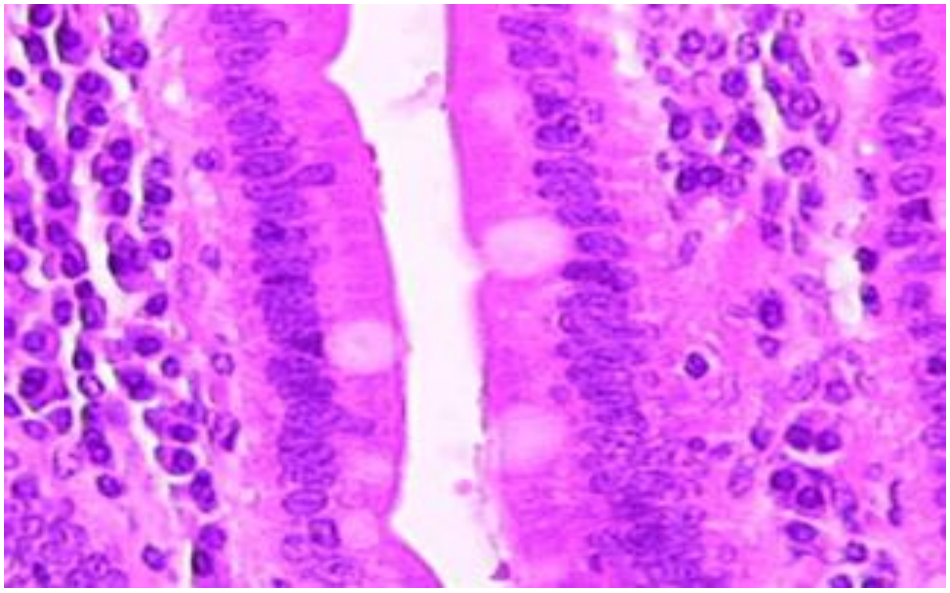
Histology

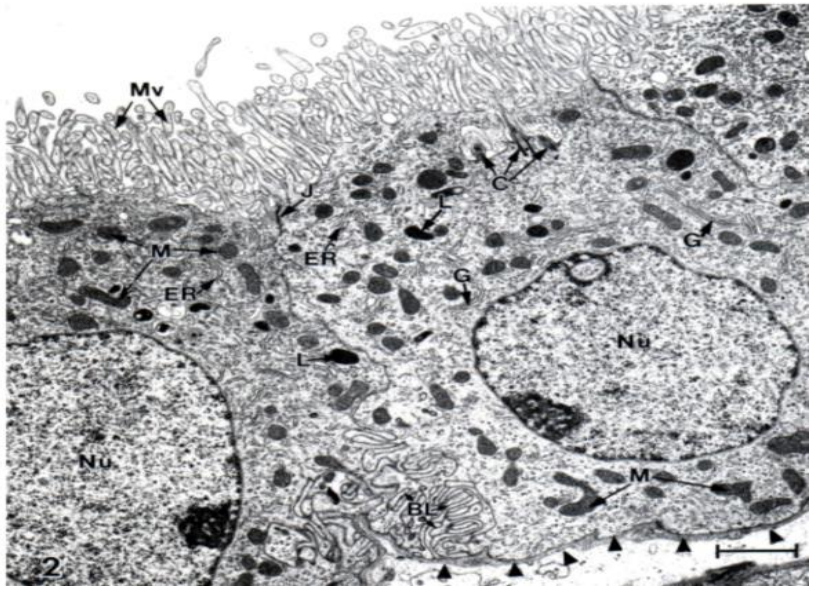
- the study of microscopic anatomy of cells, tissues and organs

- **performed by examining cells and tissues under a microscope**.

The fine structure can be distinguished at the level of light microscopy

Ultrastructure - the detailed structure of the cell cytoplasm, organelles and membranes is studied at the level of electron microscopy





The light source

Condenser lens

Condenser diaphragm

Objective lenses – usually magnify 4, 10, 40 times and oil magnification (100x)

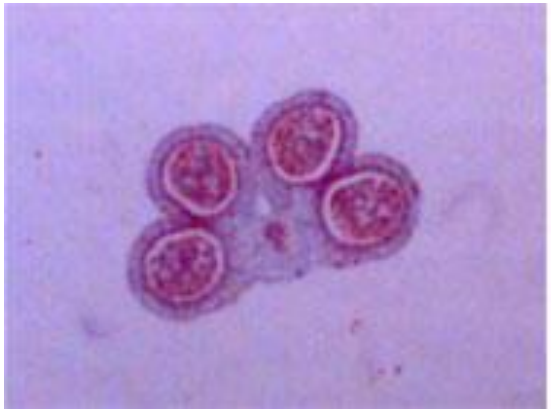
Ocular lenses - usually magnify 10 times

Magnification

$$M = M_{ob} \times M_{oc}$$

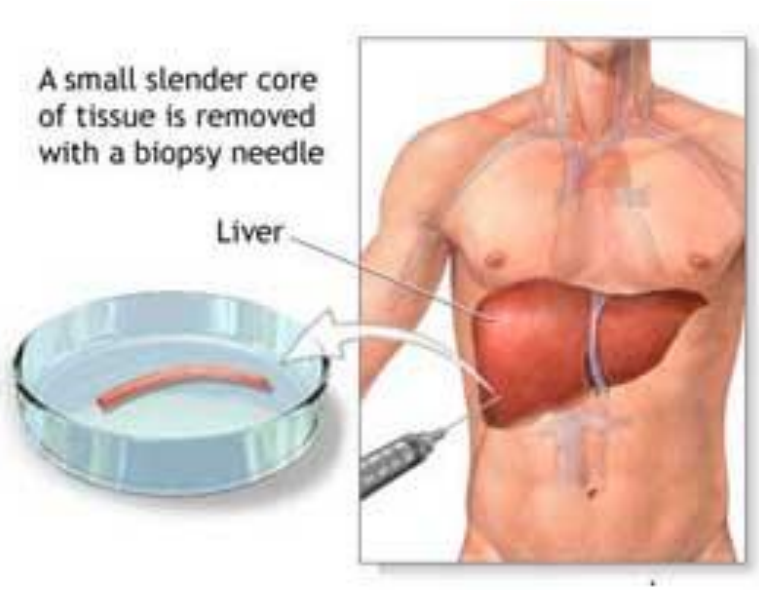
Knobs serve for focusing

Resolution - **the ability of the lens to show that two distinct objects are separated**



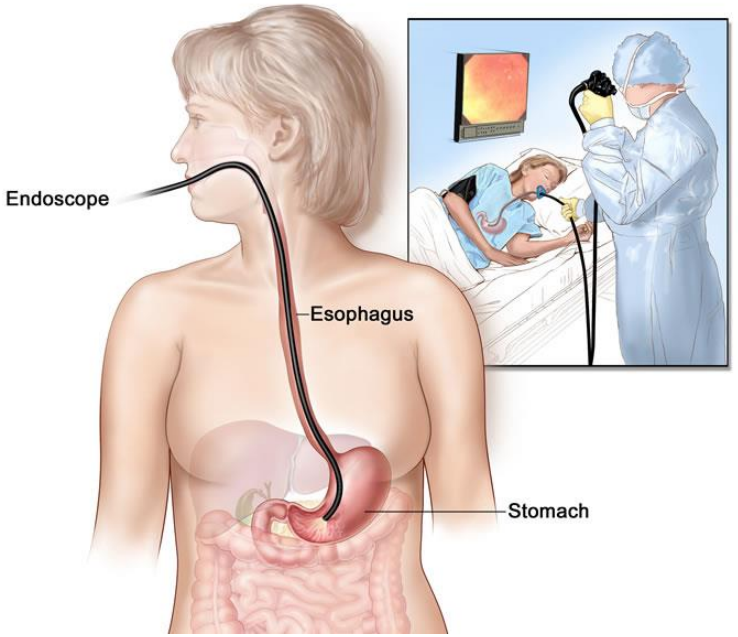
Examination of tissues starts with **surgery**, **biopsy**, or **autopsy**.

A biopsy is the medical removal of tissue from a living subject to determine the presence or extent of a disease:



-needle biopsy - tissue obtained by puncture of a tissue with the the needle, cells are removed without preserving the histological architecture of the tissue

-endoscopic biopsy - removal of tissue by appropriate instruments through an endoscope, a sample of tissue is removed with preservation of the histological architecture of the tissue's cells



STEPS IN PREPARING SECTIONS FOR LIGHT MICROSCOPY

1. FIXATION

- stabilizes the tissues to prevent decay
- preserves in a state as nearly as possible like the living condition
- prevent autolysis

Fixative - neutral buffered formalin

2. DEHYDRATION

- removes water

A grade series of alcohol baths (from 50% to 100%)

3. CLEARING

- the tissue becomes transparent

Xylene

4. EMBEDDING

- to allow sectioning the tissue.

Medium: paraffin

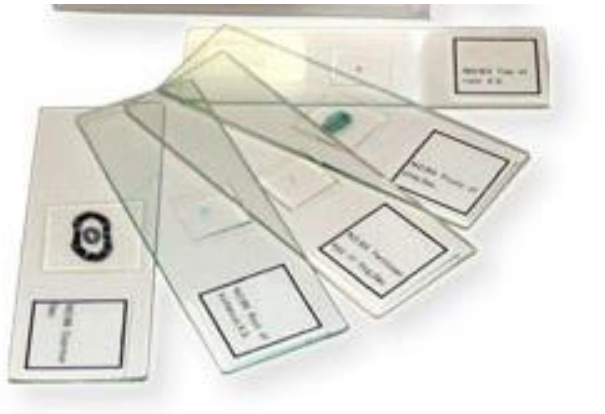
5. SECTIONING

- to obtain the thin slices of the tissue

Microtome - drive a knife across the surface of the paraffin cube and produce a series of sections of very precise thickness

A thickness between **5 – 10 µm** is optimal for viewing with a light microscope

6. MOUNTING
The sections are mounted on individual microscope glass slides.



7. REMOVAL OF PARAFFIN – xylene

8. REHYDRATION
Because the tissue will be stained by water soluble dyes
a grade series of alcohol baths (from 100% alcohol to water)

9. STAINING
Various types of stains

10. DEHYDRATION
- coverslip must be affixed by the use of medium which does not mix with water.
a grade series of alcohol baths (from 50% to 100%) and xylene

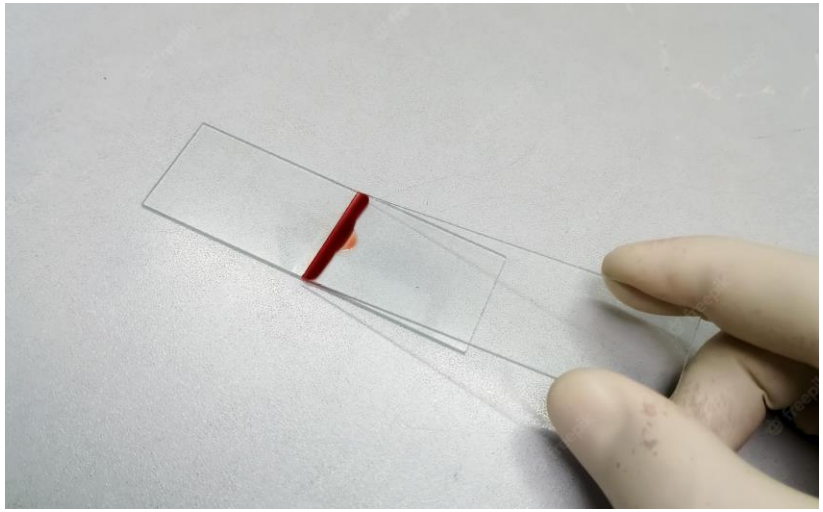
11. PREPARING PERMANENTLY MOUNTED SECTIONS UNDER THE COVERSLIP

The ideal mounting medium should not distort the stain color and preserve the slide

Canada balsam - the resin of the balsam fir tree

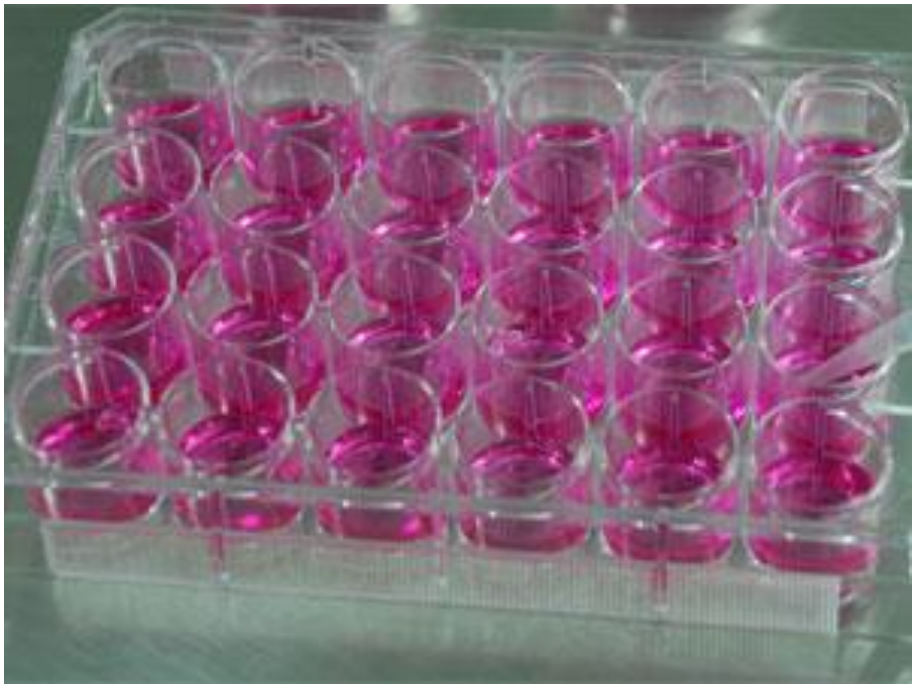
PREPARATION OF THE SLIDE SMEARS

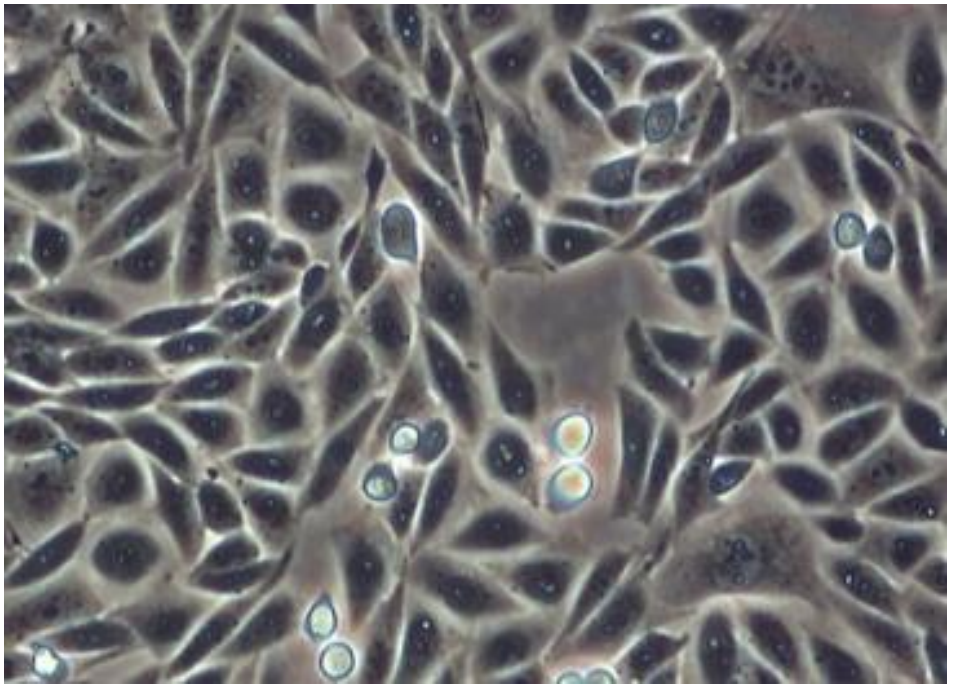
The drop of separated cells suspended in proper medium has to be placed on the microscopic slide, smeared and fixed



PREPARATION OF CULTURED CELLS

1. Isolation the cells from the tissue by enzymatic digestion.
2.Culturing cells in proper medium on the plates with coverslips until cultured cells adhere to coverslips.
3. Fixation.
4. Staining
5. Dehydration
6. Preparing permanently mounted slides



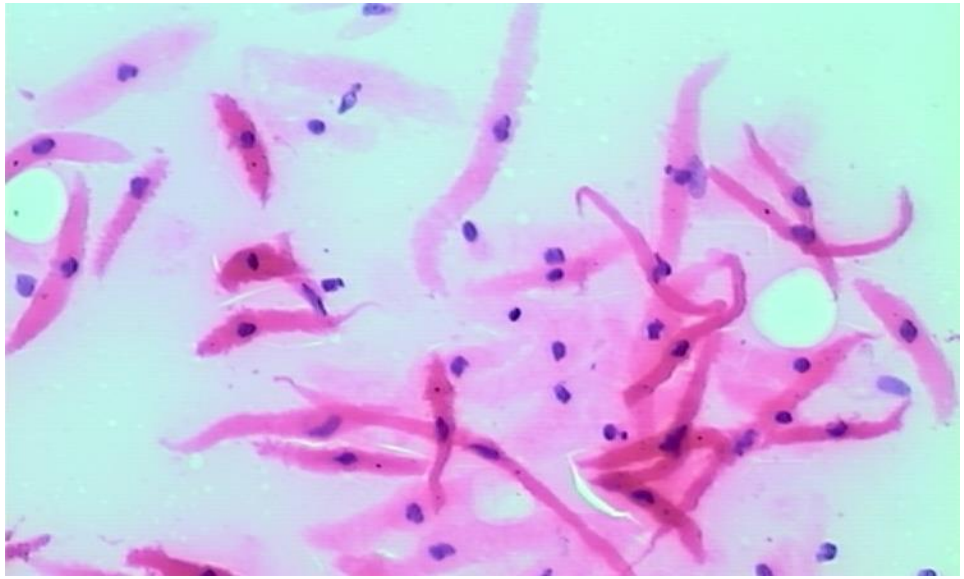


STAINING

HEMATOXYLIN - basic dye. Forms salts with tissue anions -the phosphate groups of nucleic acids and the sulfate groups of the glycosaminoglycans.

Hematoxylin stains basophilic structures dark blue color

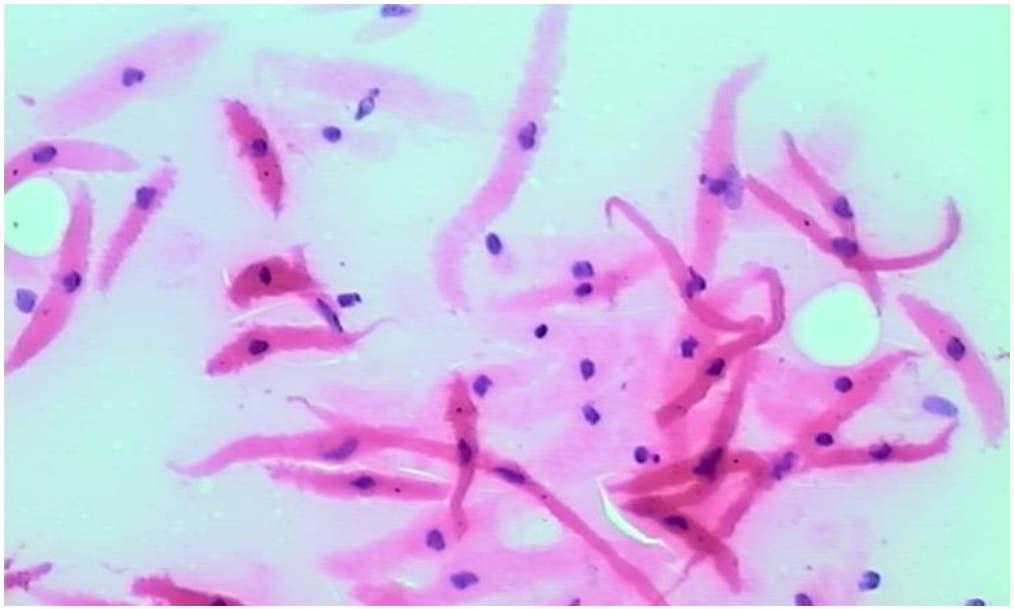
Basophilic is the term used to designate the components of a cell or tissue, which take up the basic dye. Nuclei are basophilic, because they contain the acids; DNA and RNA



EOSINE- acid dye. Forms salts with cationic groups in cells and tissues, particularly the amino groups of proteins

Eosin stains the acidophilic components of the tissue a pink color

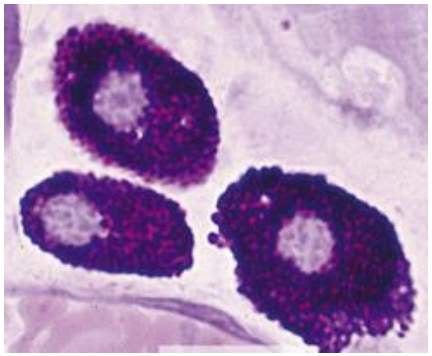
Acidophilic or oxyphilic is applied to parts, which show a greater affinity for acid dyes. The cytoplasm is usually acidophilic**.**



METACHROMASIA

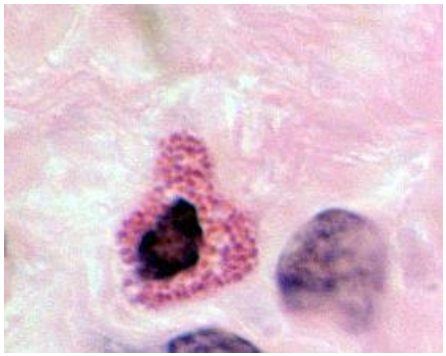
Characteristic change in the color of staining carried out in biological tissues, exhibited by e. g. toluidine blue when it binds to particular substances.

Toluidine blue



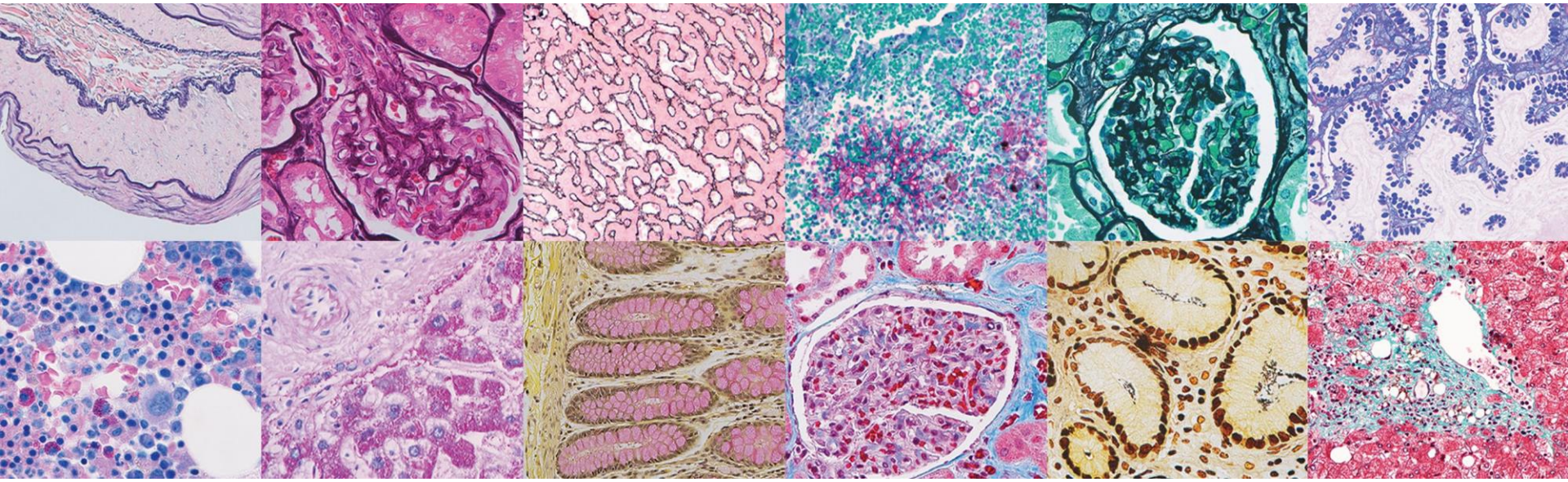
Mast cells

H&E



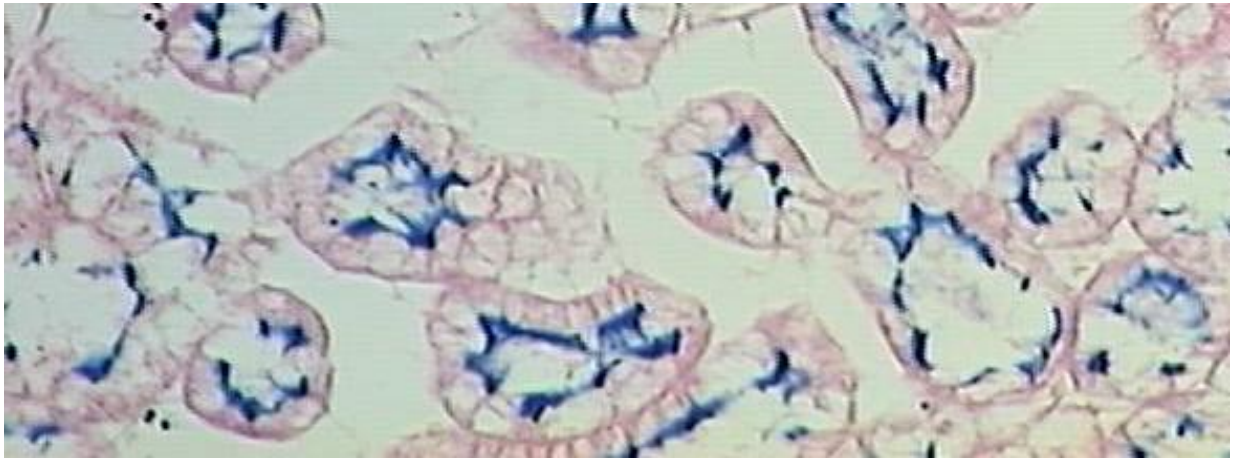
Other dyes:

- Giemsa
- Alcian blue
- Sirius red
- Oil red
- Orcein
- Rezorcin
- ……



DETECTION OF ENZYMES

	EXEMPLE: alkaline phosphatase in proximal tubules of kidney
ENZYME PRESENT IN TISSUE	
SUBSTRATUM	beta-naphthyl phosphate
PRODUCT	beta-naphthyl
INTERMEDIATE CHEMICAL	diazo dye
PRECIPITATE (colored, insoluble)	PRECIPITATE (colored , insoluble)



IMMUNOHISTOCHEMISTRY

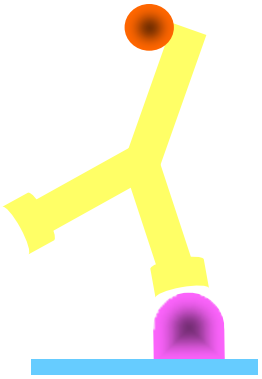
DIRECT METHOD

The antibody (primary Ab)is directed against the particular protein and is labeled.

Primary antibody

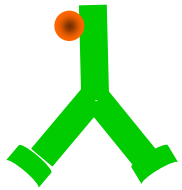
Antigen

Marker

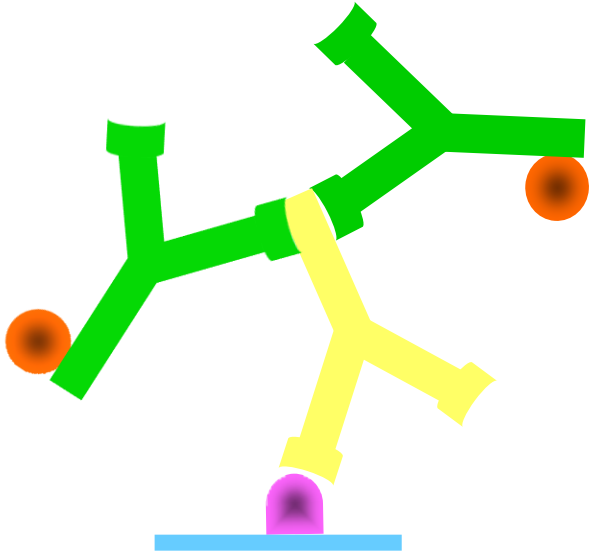


INDIRECT METHOD

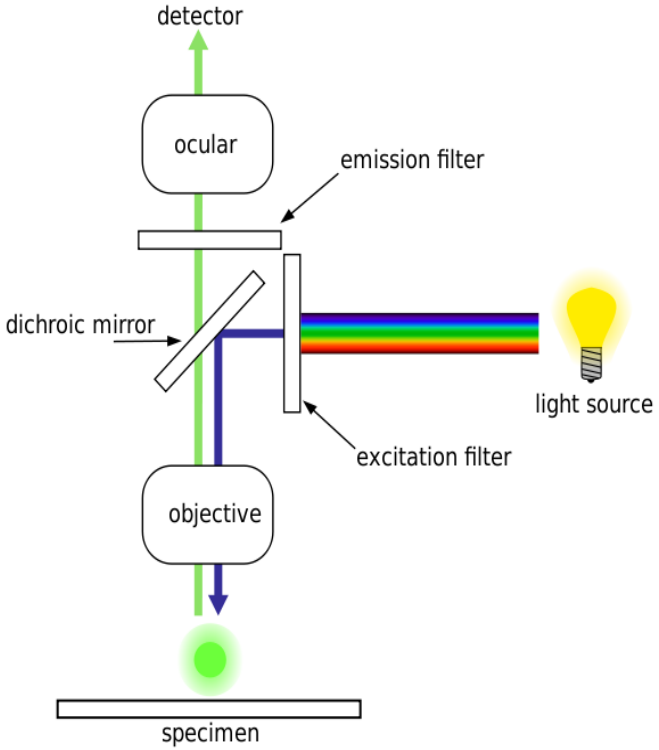
The labeled antibody (secondary Ab) is directed against Fc of primary antibody

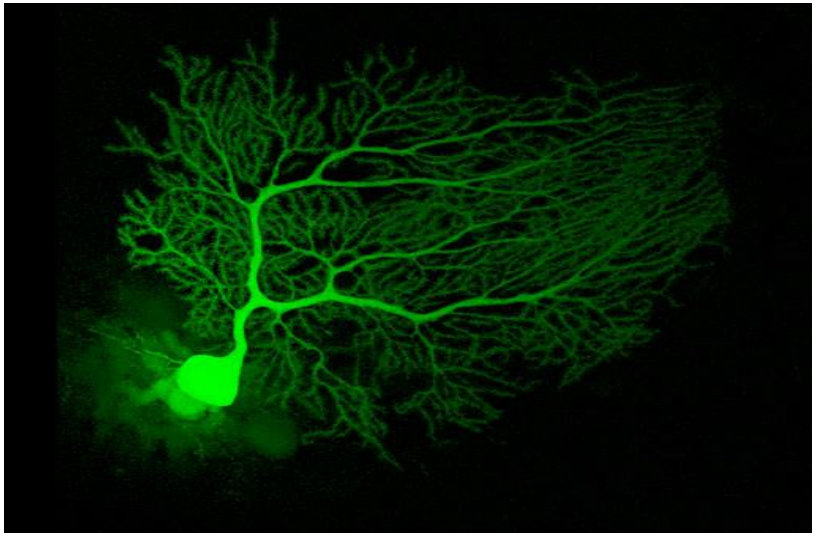


Secondary antibody

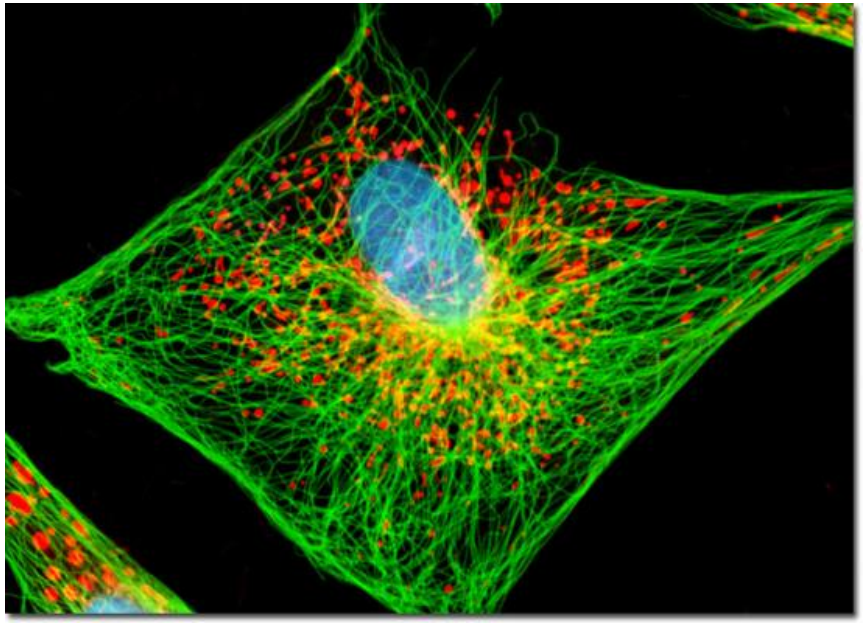


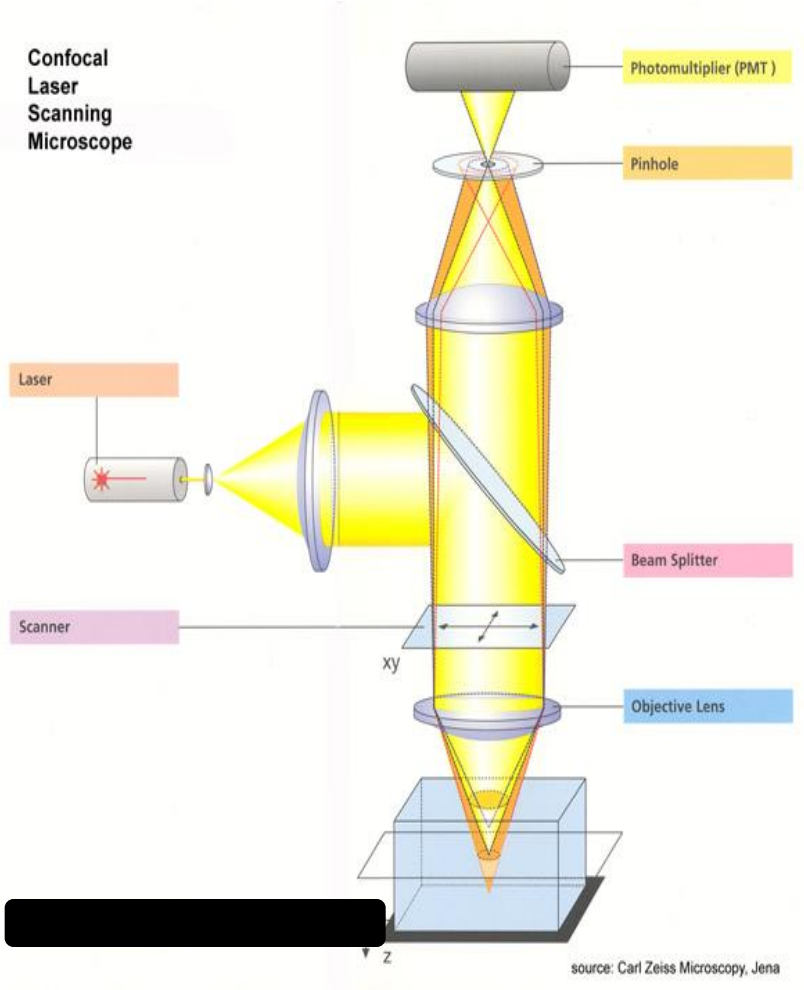
Fluorescent microscope



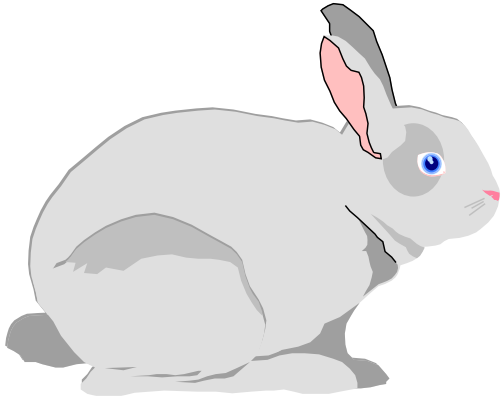


Confocal laser scannig microscope

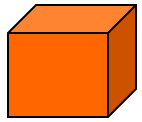




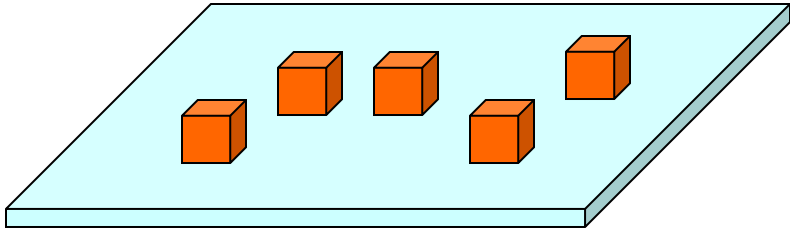
Transmission electron microscope TEM tissue preparation



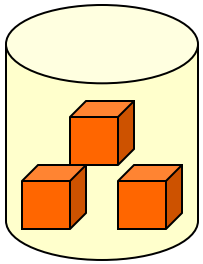
Tissue collection



Tissue trimming

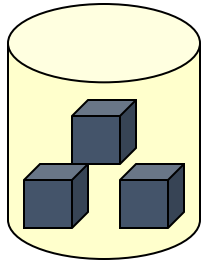


Fixation - glutaraldehyde

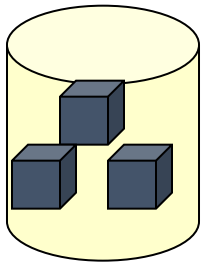


Washing in buffer

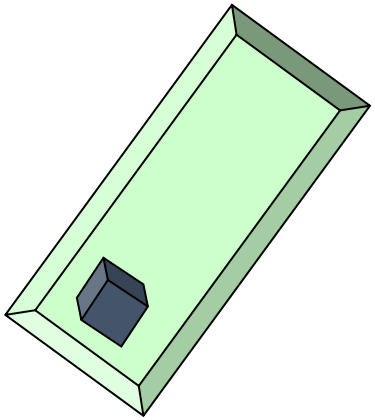
Post-fix in osmium tetroxide

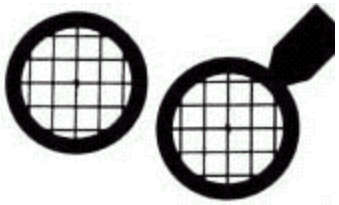


Dehydration in alcohol



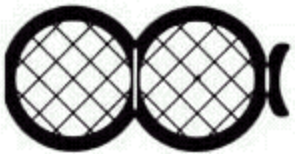
Embedding in Epoxy resins in 60 – 70 ºC



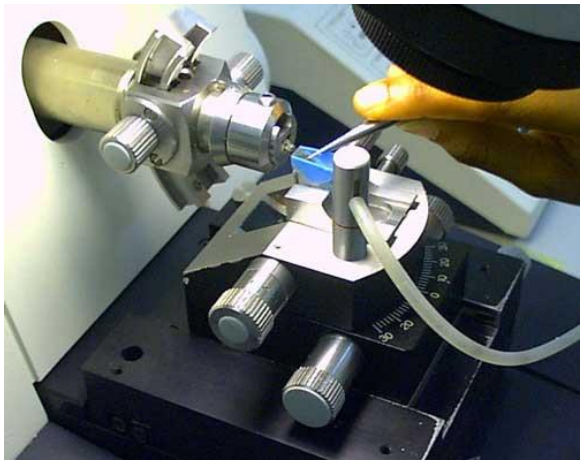


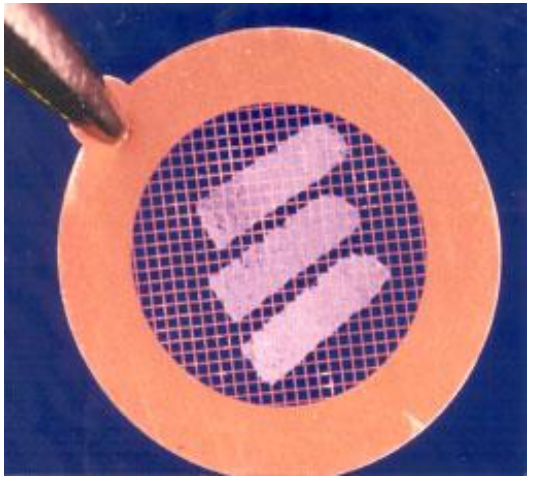
Ultrathin sections at 60-90 nm thick

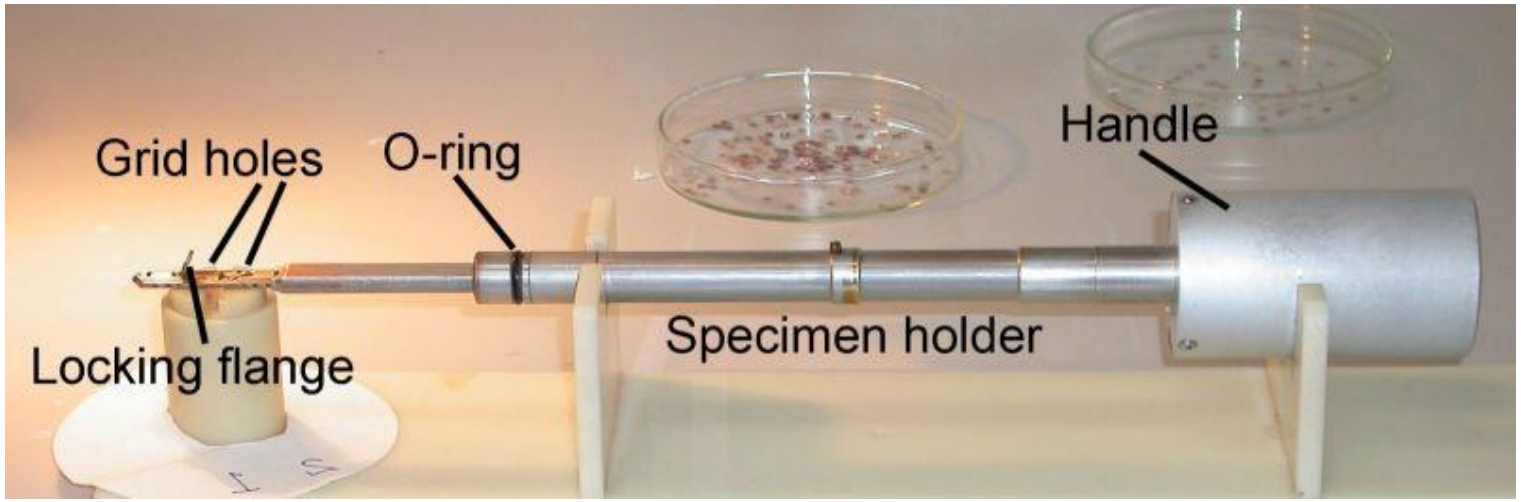
Collecting sections onto grids



Staining grids with uranyl acetate and lead citrate





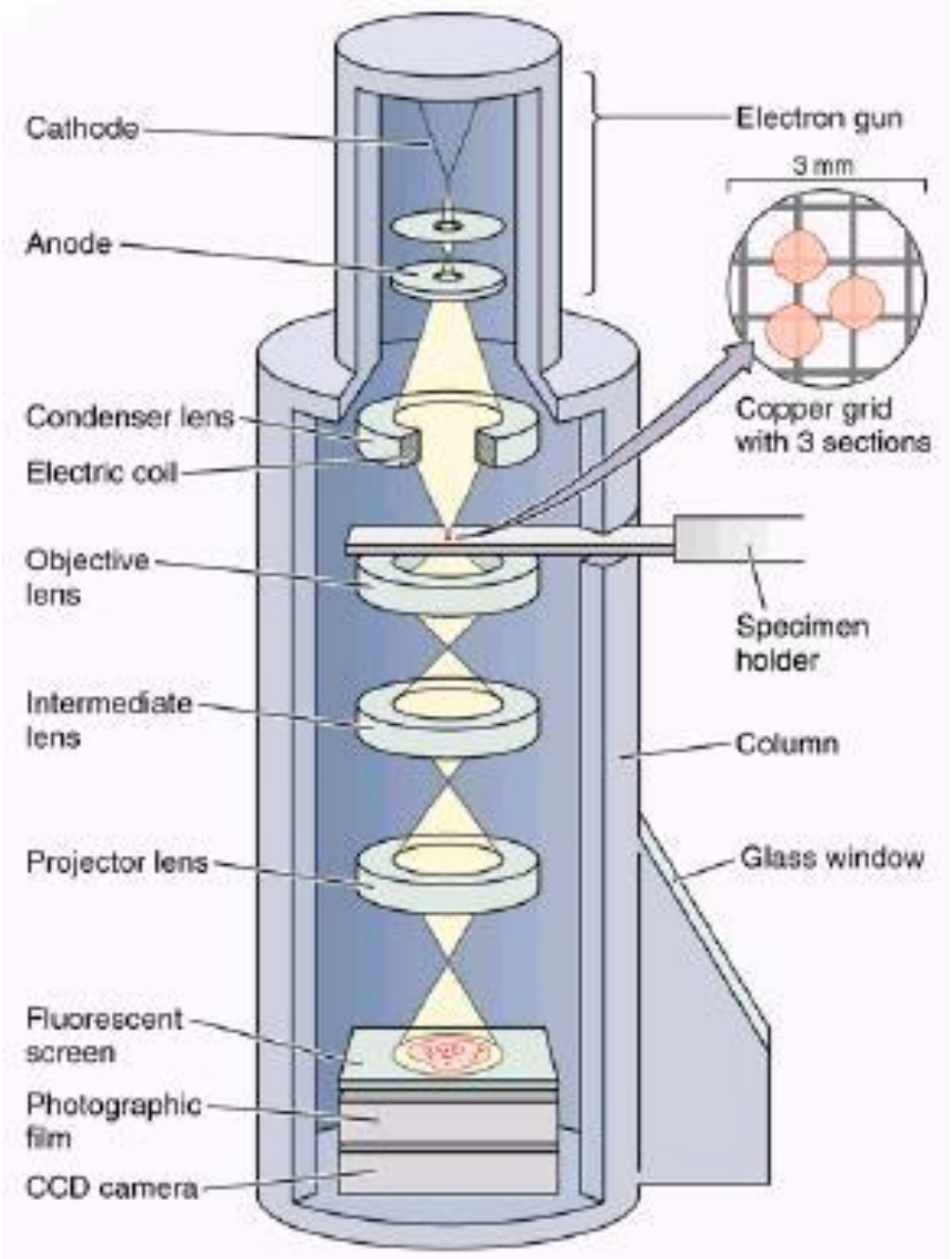

Grid holes
O-ring
Handle
Specimen holder
Locking flange

TEM stains

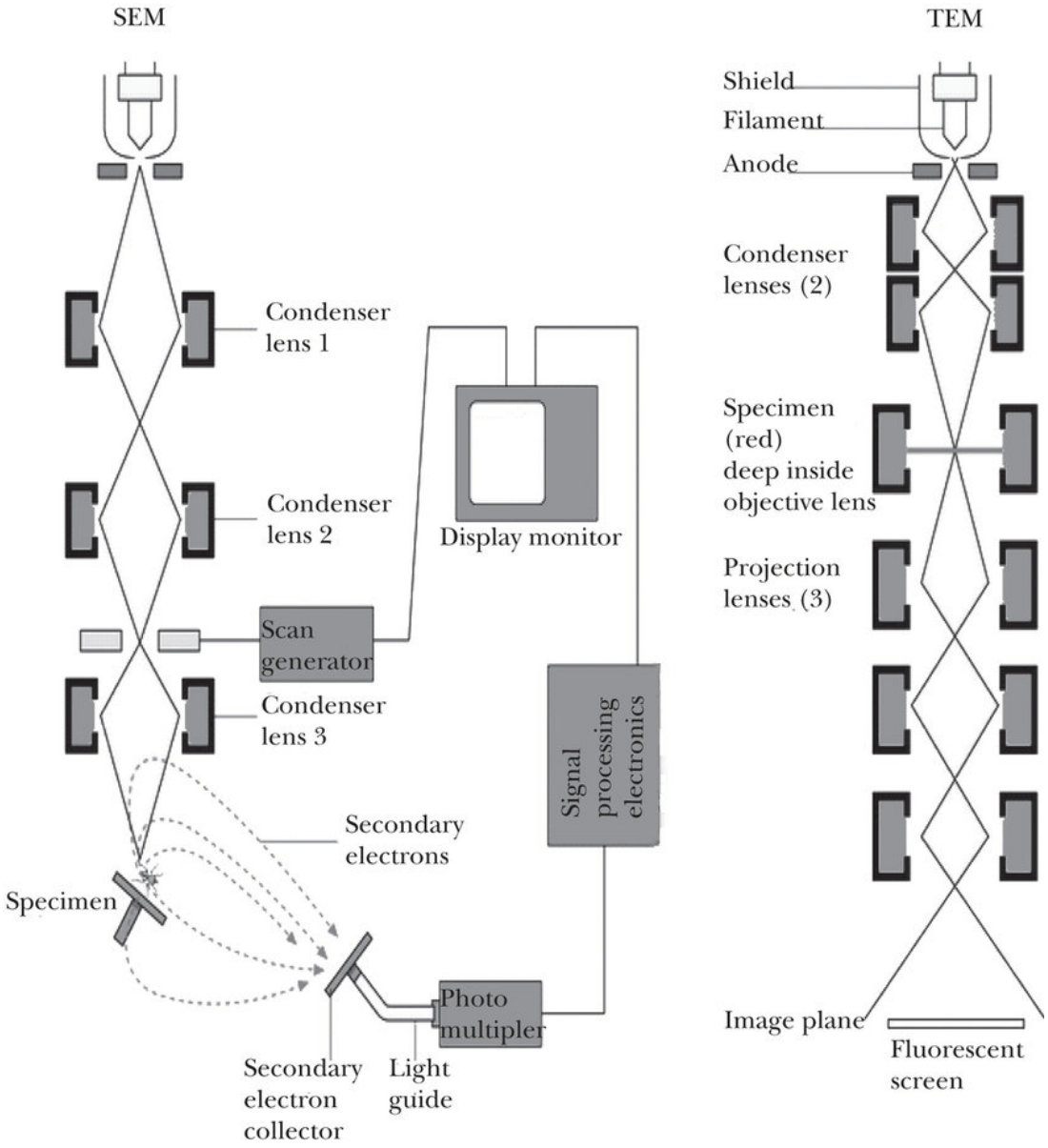
	Nucleic acids	Proteins	Phospholipids	Polysaccharides	Fat
OsO_4	○	◐	●	○	● Nienasycone +
aldehyde	○	●	○	○	○
Uranyl acetate	●	●	○	○	○
Lead citrate	●	●	●	○ Glikogen +	◐

● Strong ◐ Medium ○ Weak

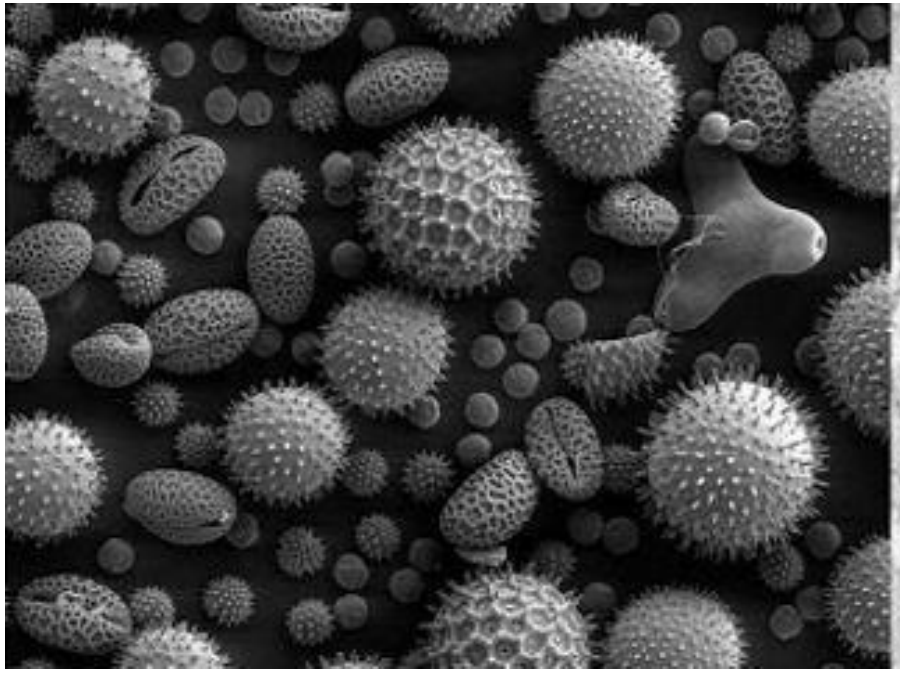
TEM

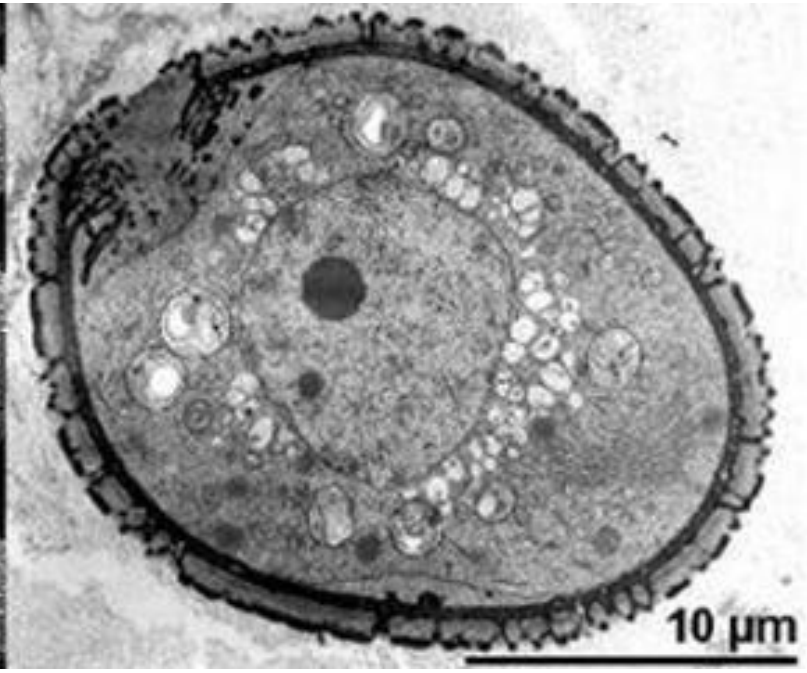

Cathode
Anode
Condenser lens
Electric coil
Objective lens
Intermediate lens
Projector lens
Fluorescent screen
Photographic film
CCD camera
Electron gun
3 mm
Copper grid with 3 sections
Specimen holder
Column
Glass window

TEM vs SEM

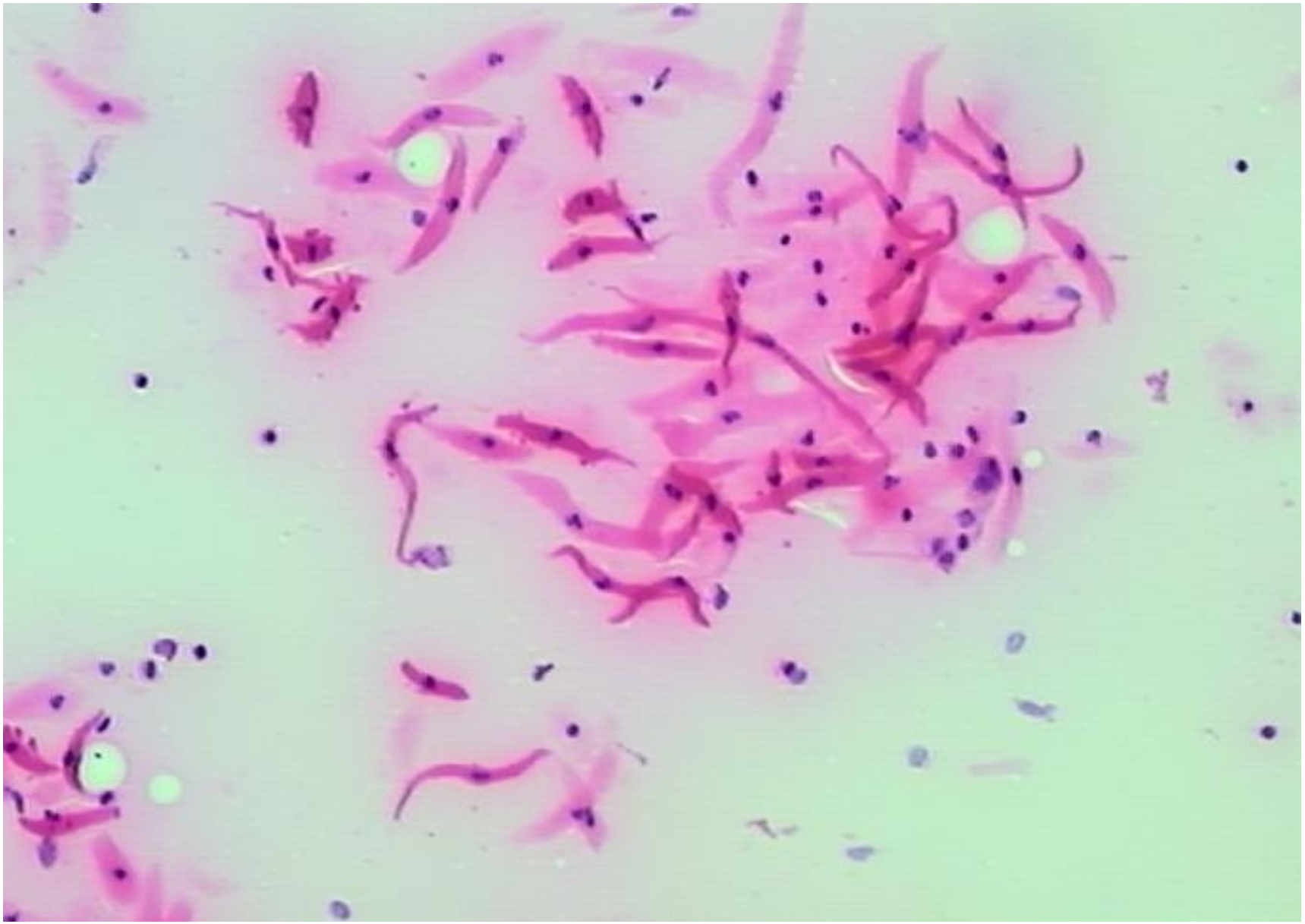

SEM
Condenser lens 1
Condenser lens 2
Display monitor
Scan generator
Condenser lens 3
Signal processing electronics
Secondary electrons
Specimen
Photo multipler
Secondary electron collector
Light guide
TEM
Shield
Filament
Anode
Condenser lenses (2)
Specimen (red) deep inside objective lens
Projection lenses (3)
Image plane
Fluorescent screen

TEM vs SEM

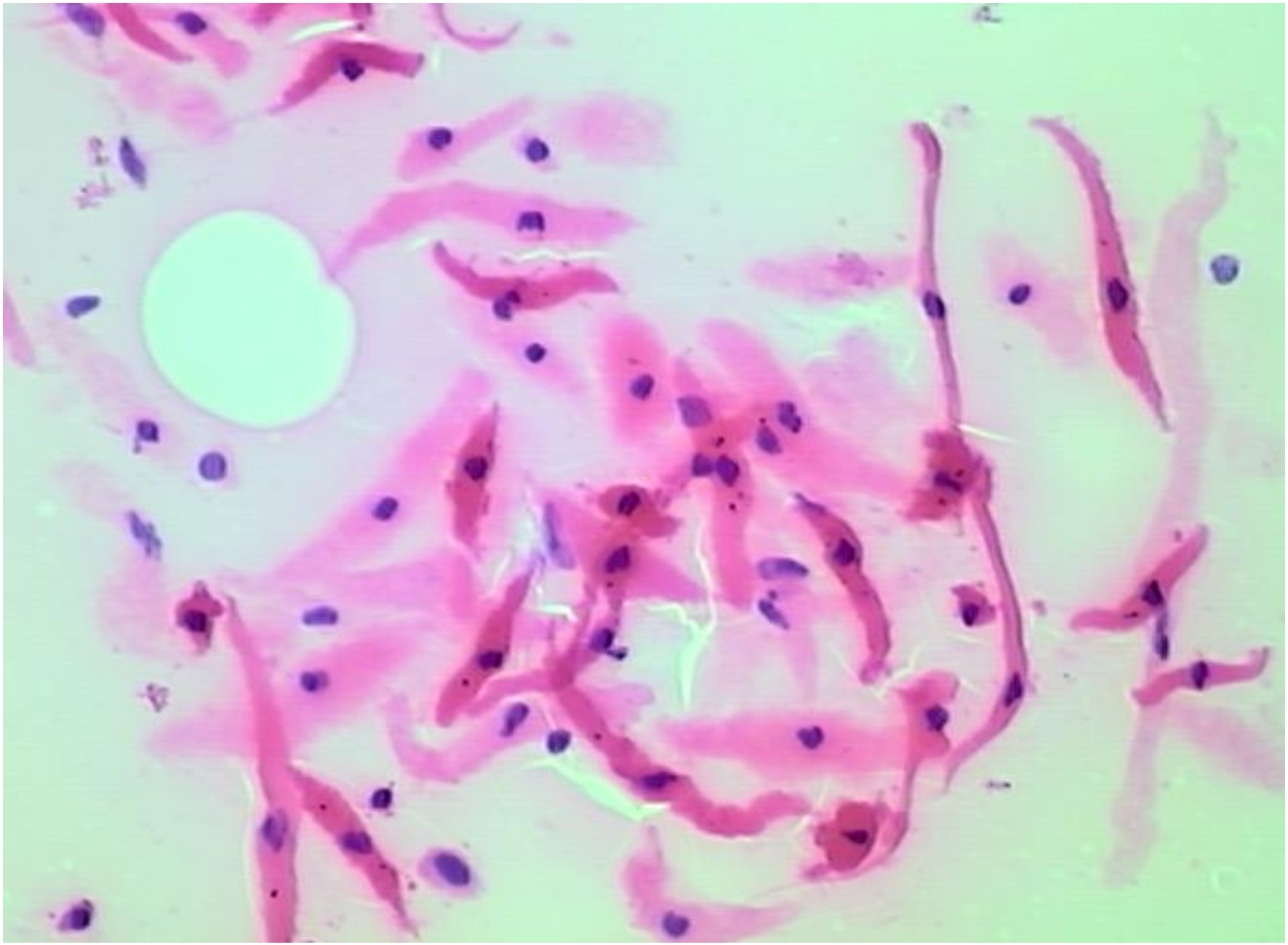




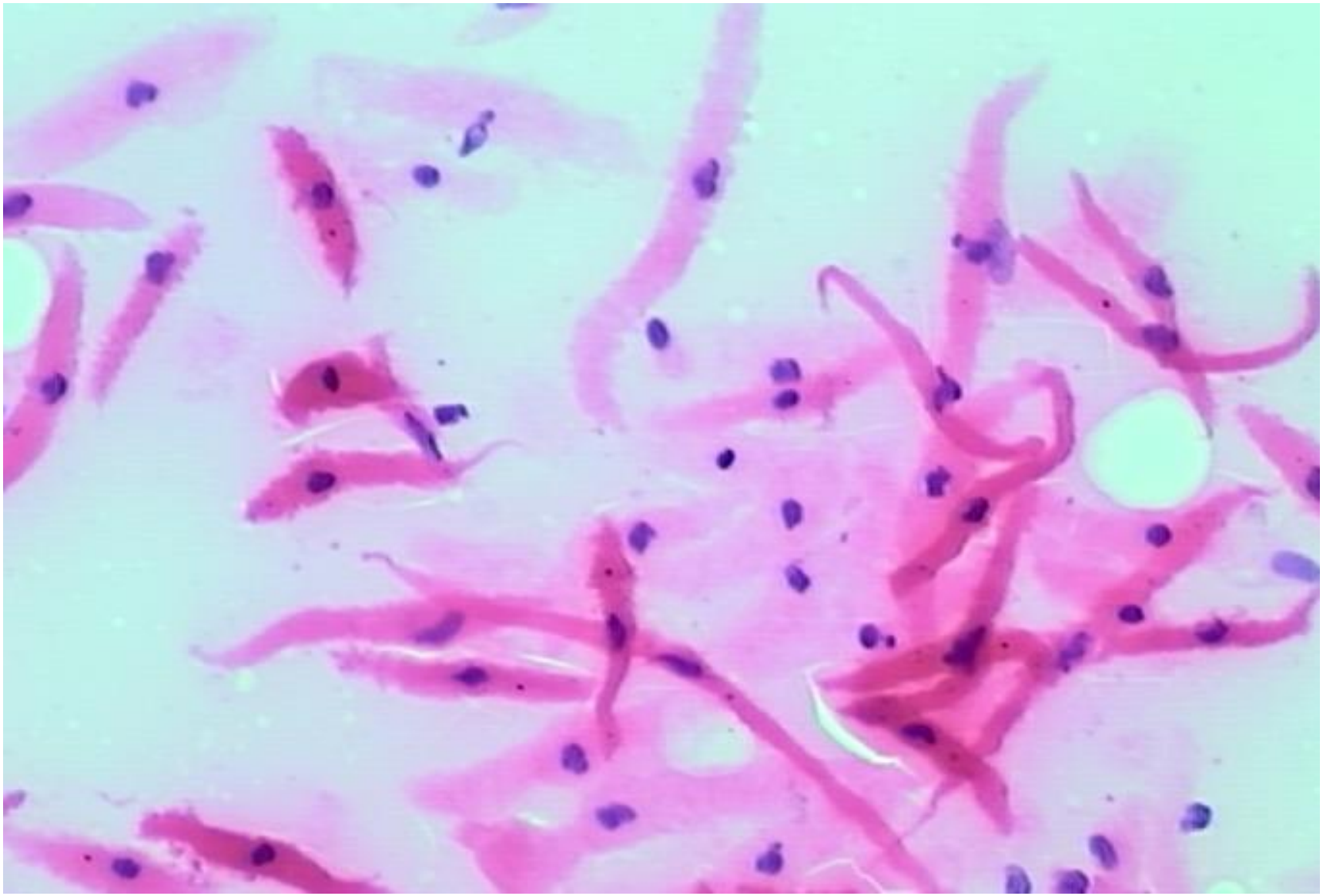
19. Isolated smooth muscle cells x10



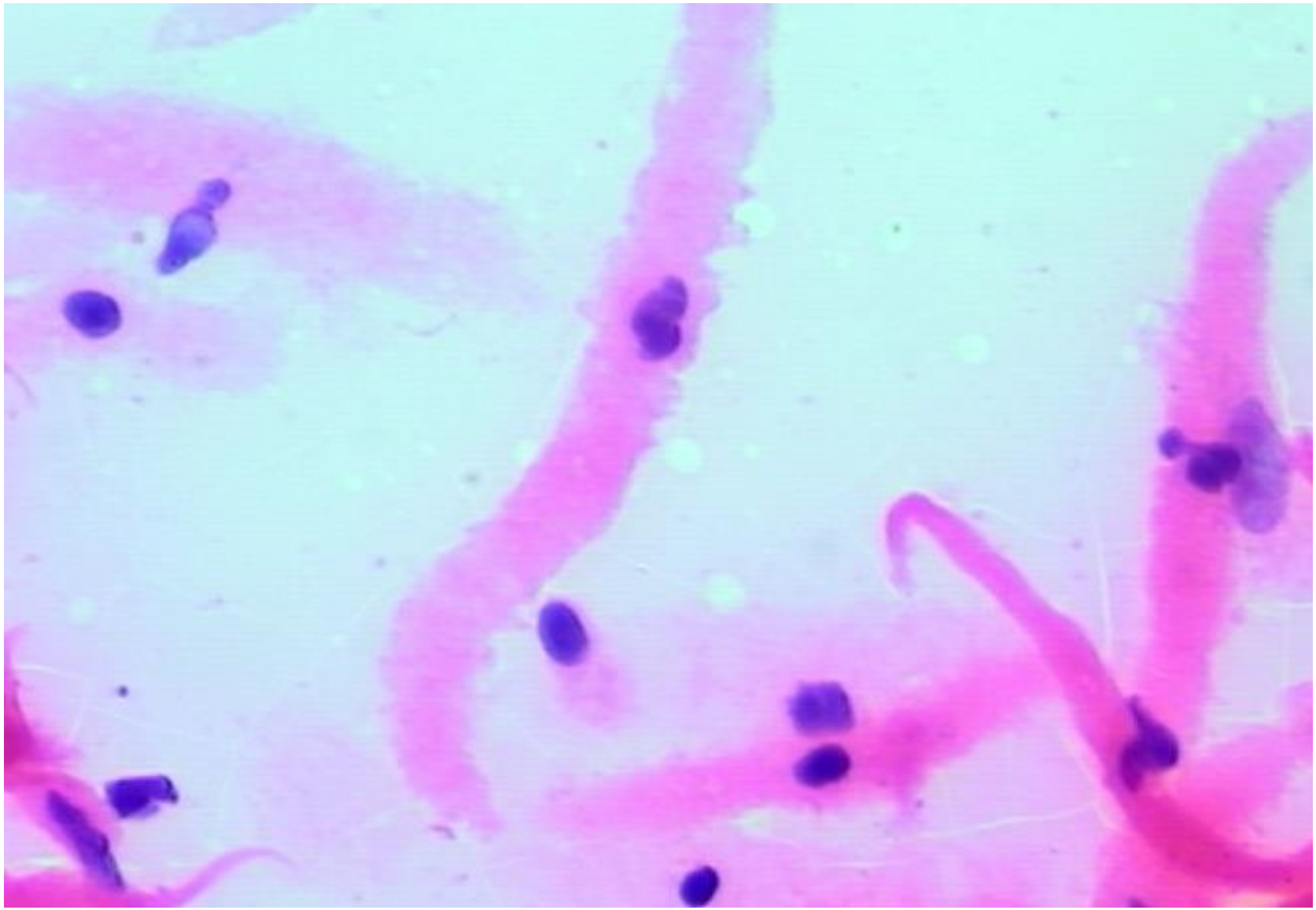
19. Isolated smooth muscle cells x20



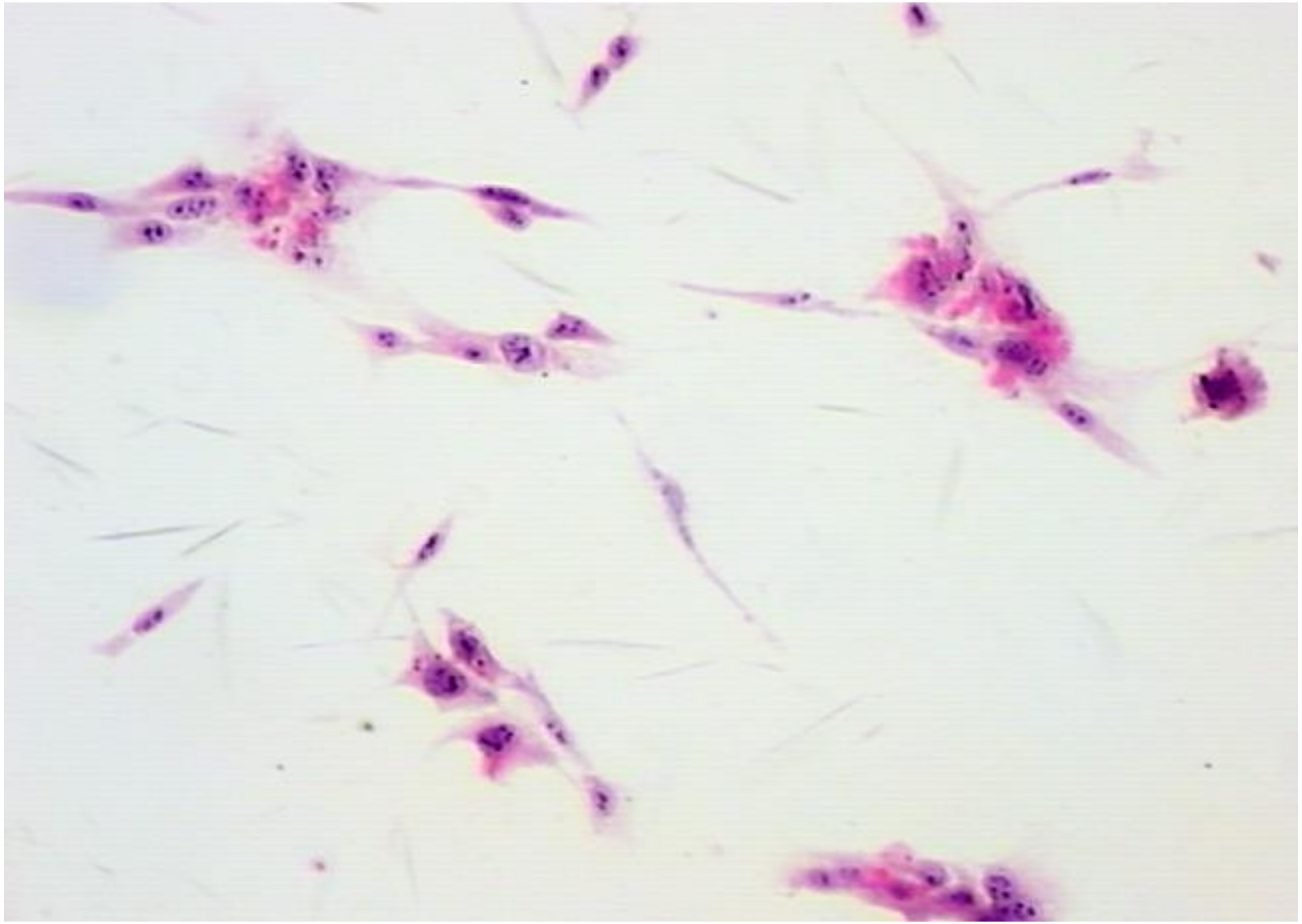
19. Isolated smooth muscle cells x20



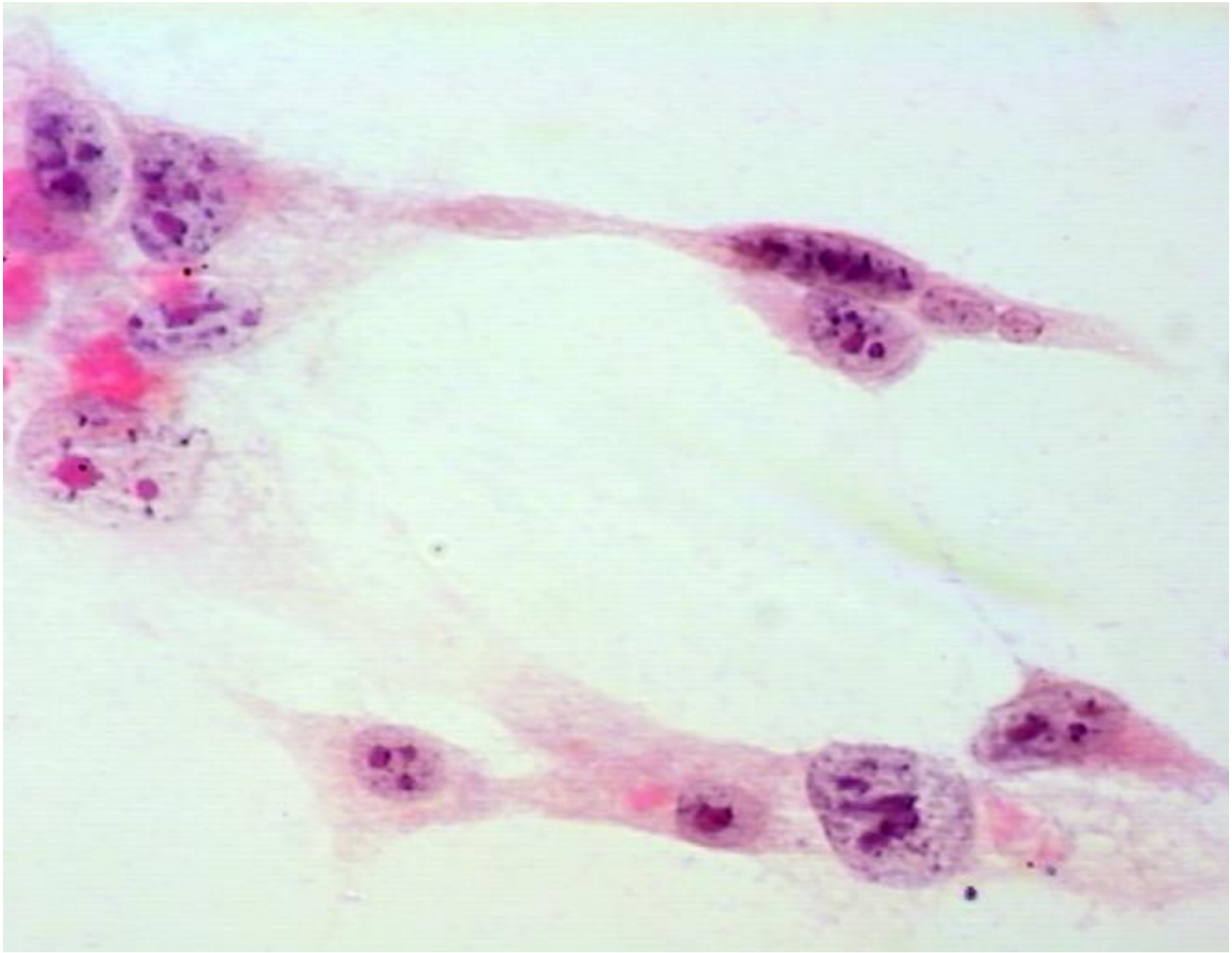
19. Isolated smooth muscle cells x40



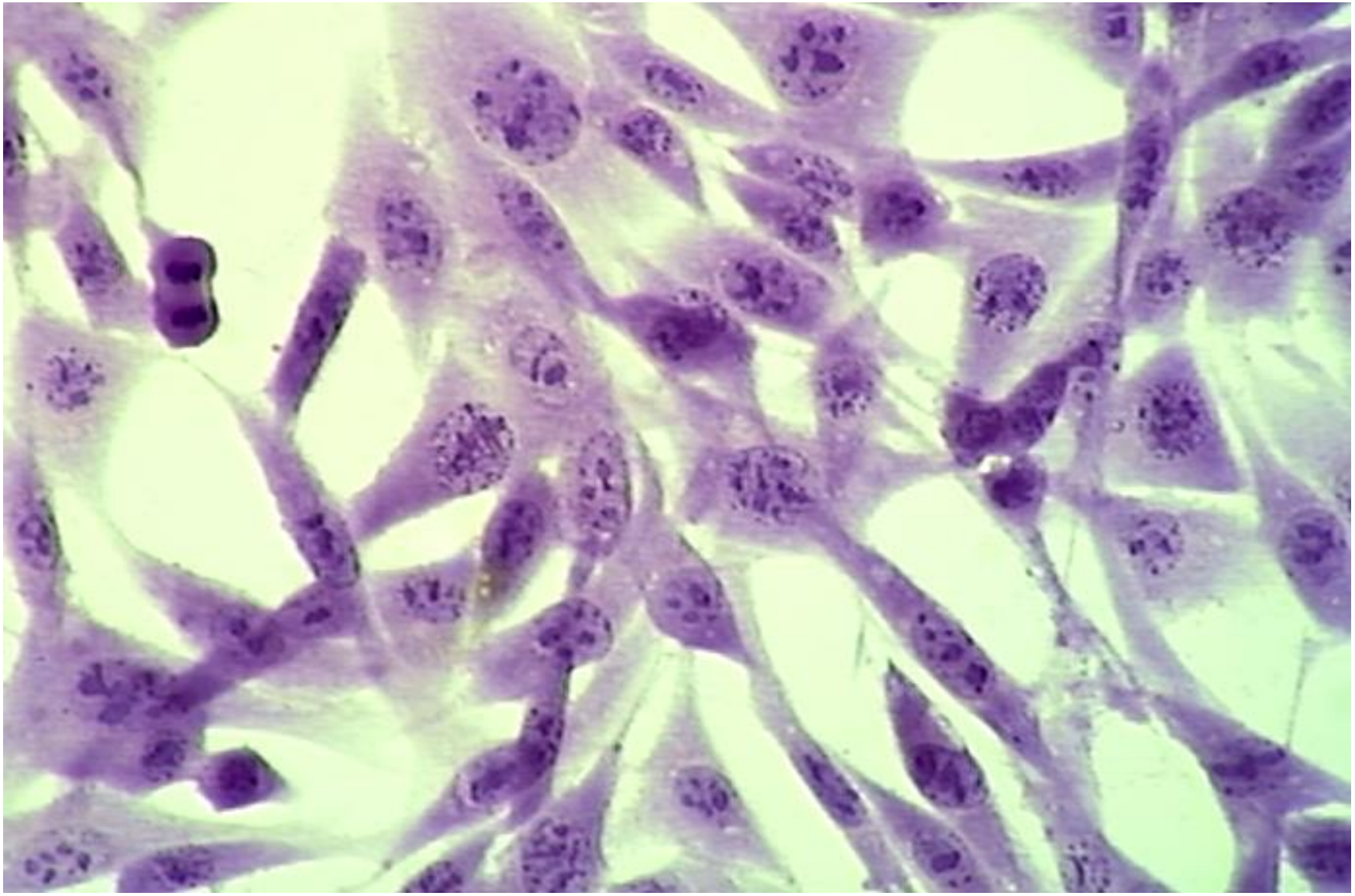
97. Fibroblasts x10



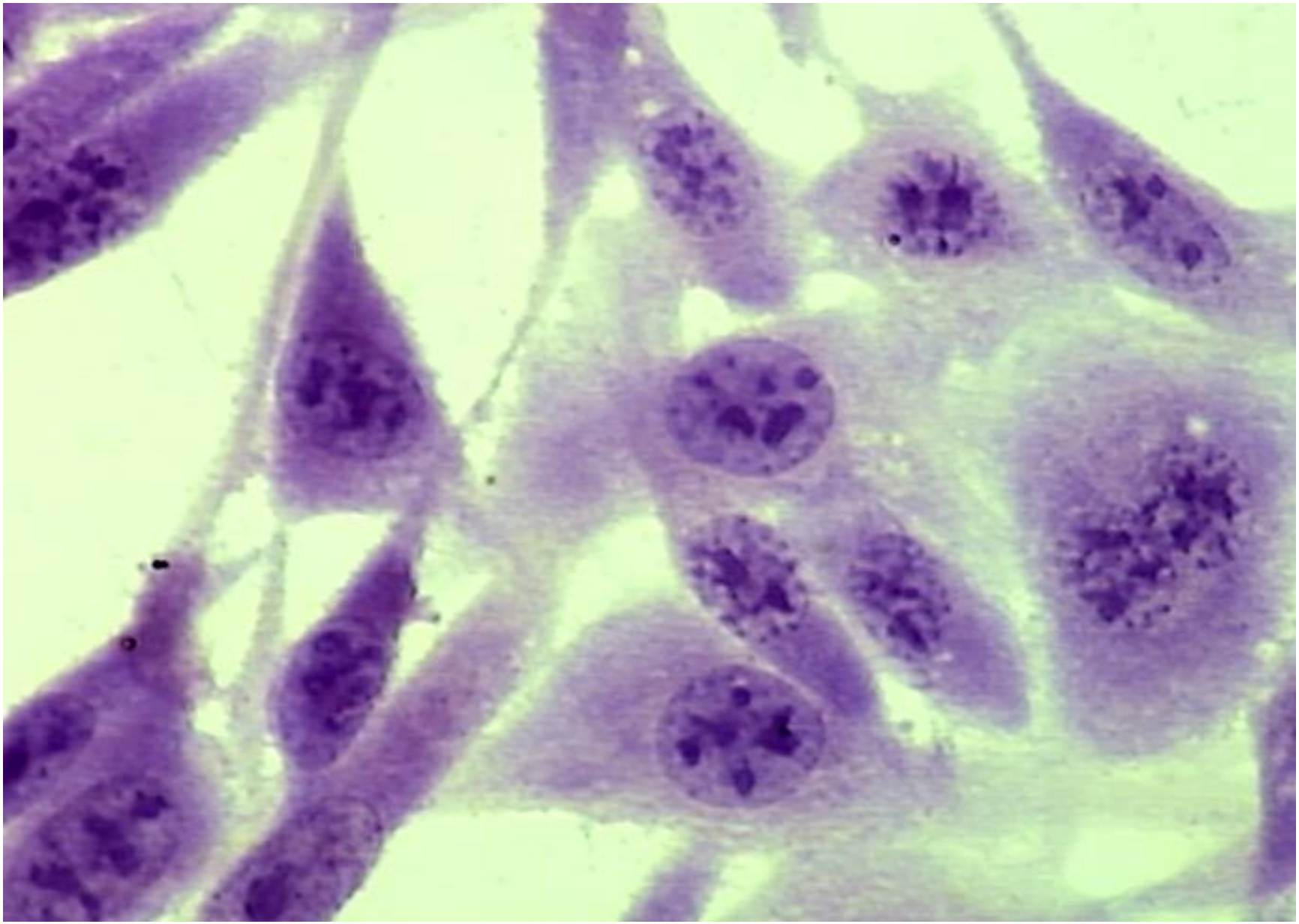
97. Fibroblasts x40



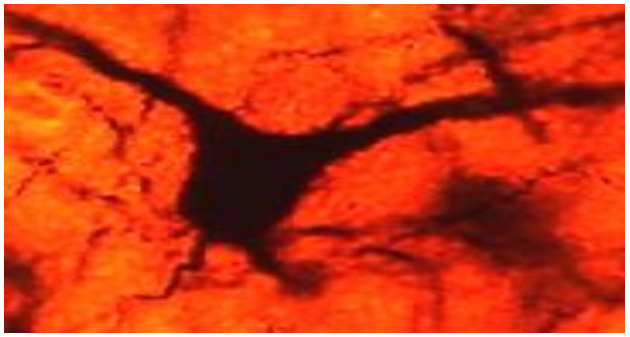
97. Fibroblasts x20

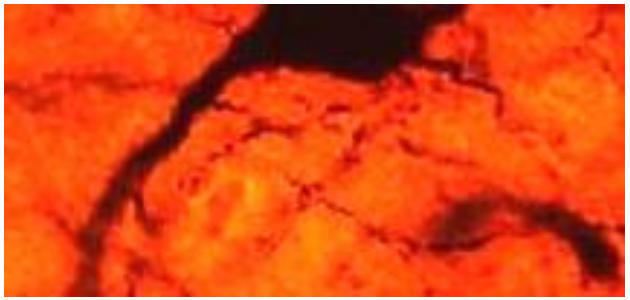


97. Fibroblasts x40



112. Nerve cells impregnated with silver nitrate



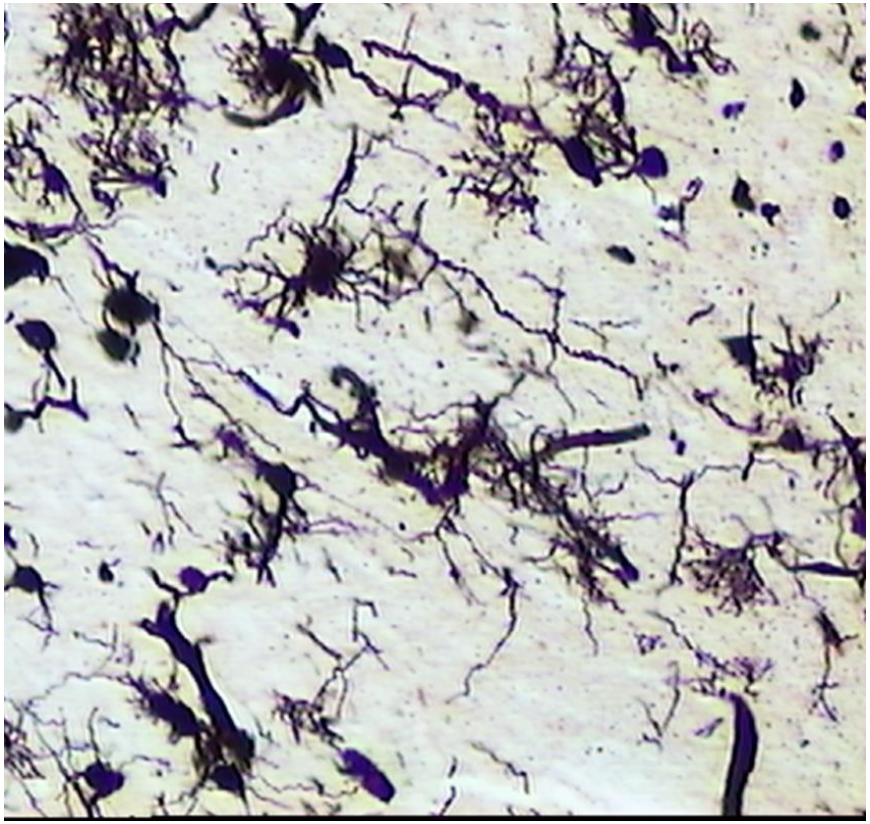


the image you see depends on section

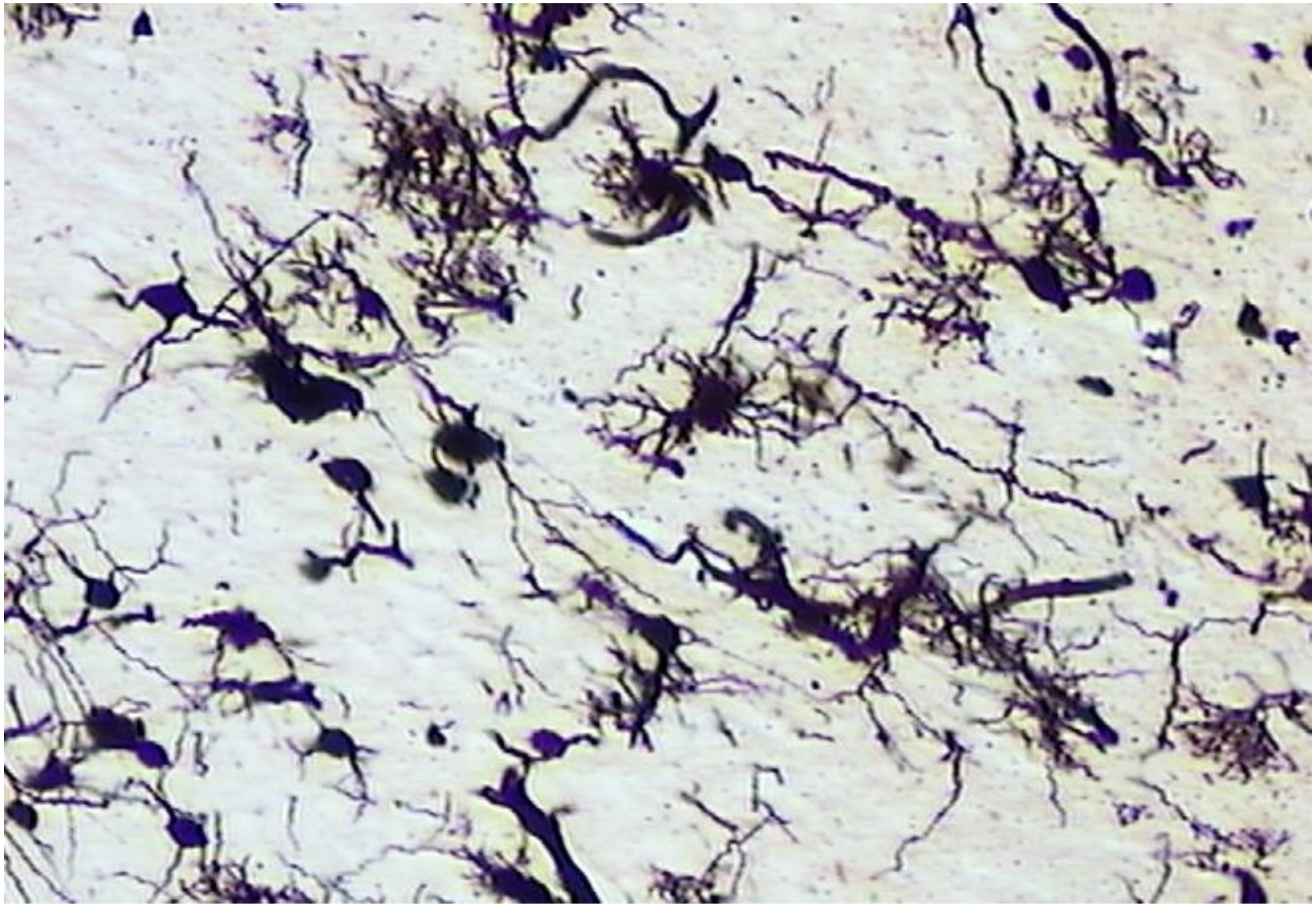
Impregnation with silver nitrate

Because the thin filamentary processes of nerve cells (the axon and the dendrites) are too slender and transparent to be seen with normal staining techniques - the impregnation method is better to visualize the whole cells. In this procedure the processes and cell body, are clearly stained in brown and black

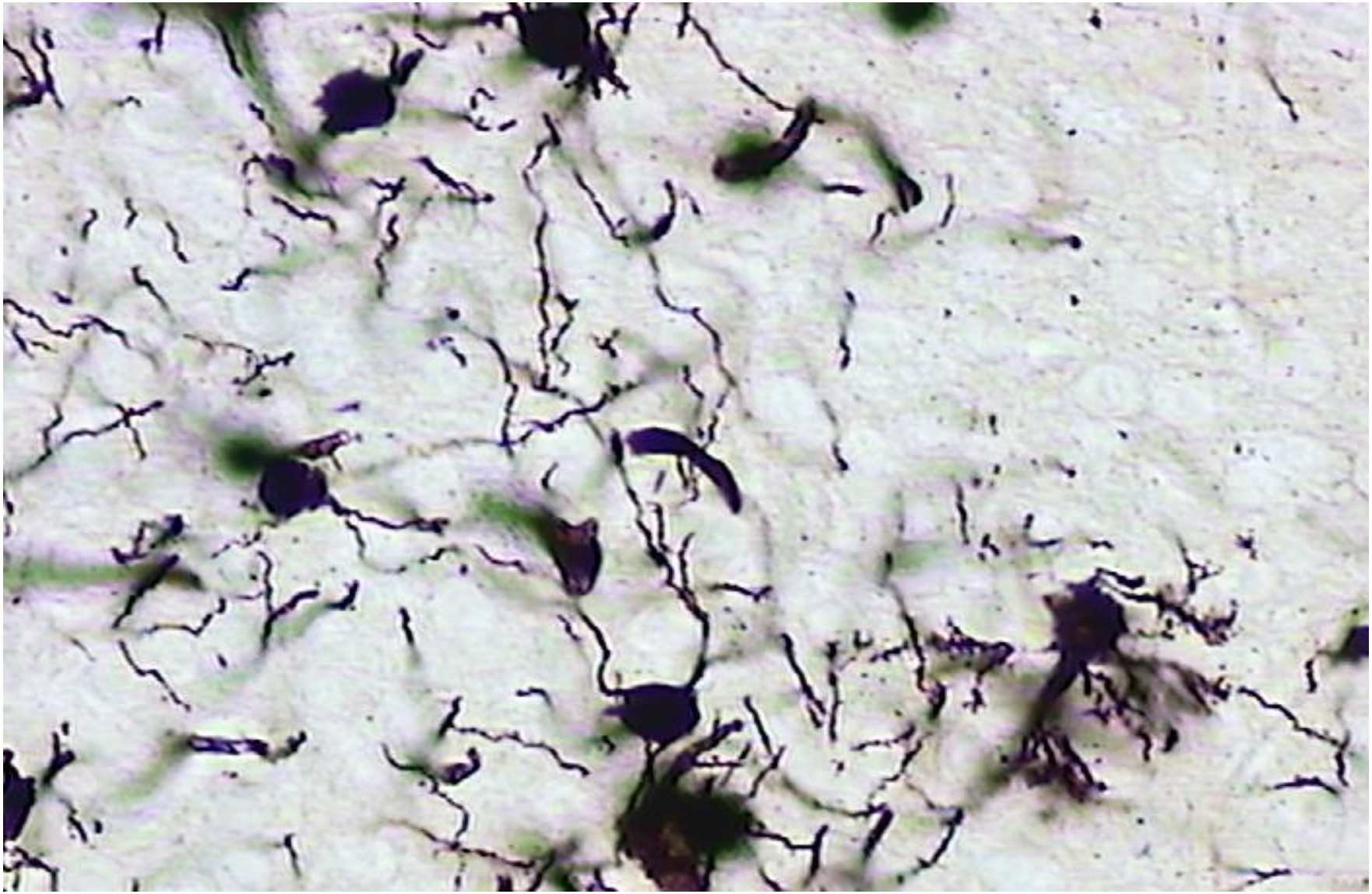
Impregnating fixed nervous tissue with silver nitrate. Cells are filled by microcrystallization of silver chromate.



112. Nerve cells impregnated with silver nitrate x10



112. Nerve cells impregnated with silver nitrate x20



112. Nerve cells impregnated with silver nitrate x40