



Introduction

Histological techniques

Methods in pathology

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What is pathology?



Clinical Pathologists



What our colleagues think
we do.



What our moms think we
do.

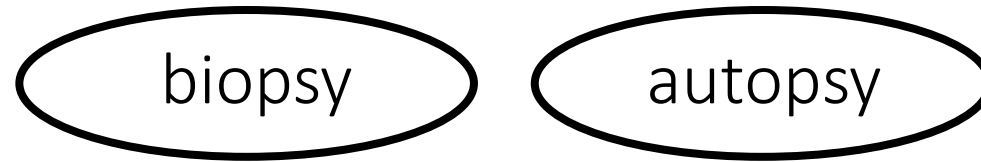


What med students think
we do.



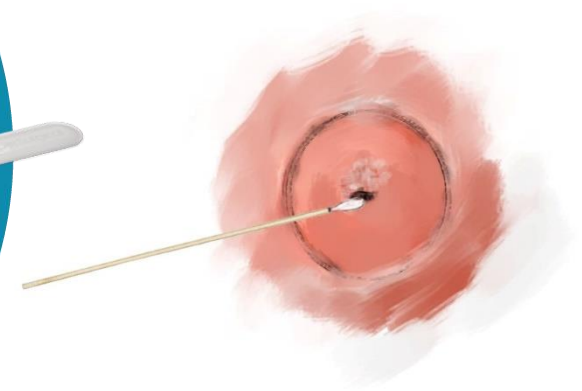
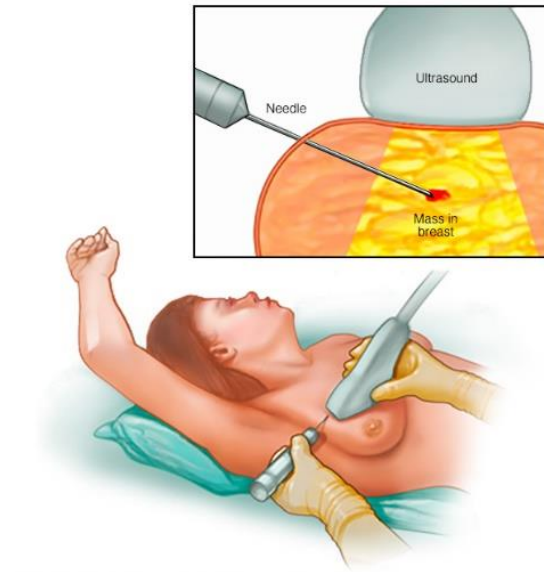
What we really do.

- Processing of biological material or any tissue removed from the living or dead body

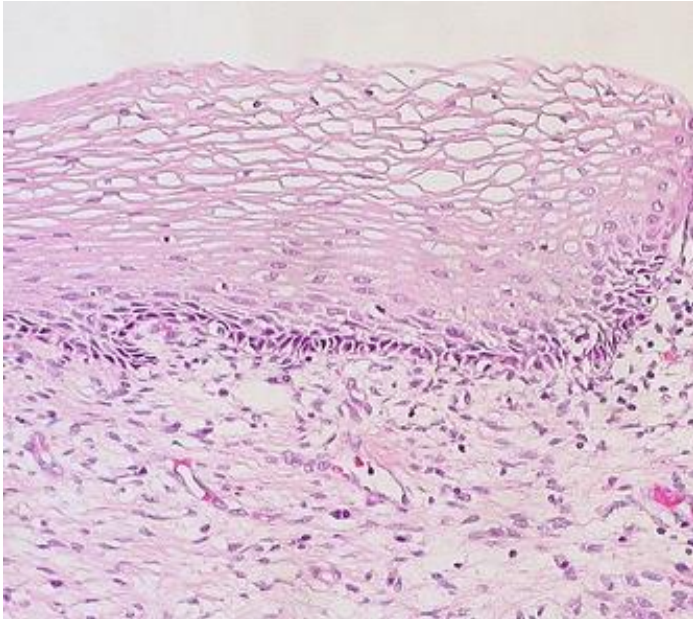


- Determining the diagnosis and disease / causes of death
- Determining the nature and causes of the disease
- The effect on the choice of an adequate therapeutic approach

Collection of biological material

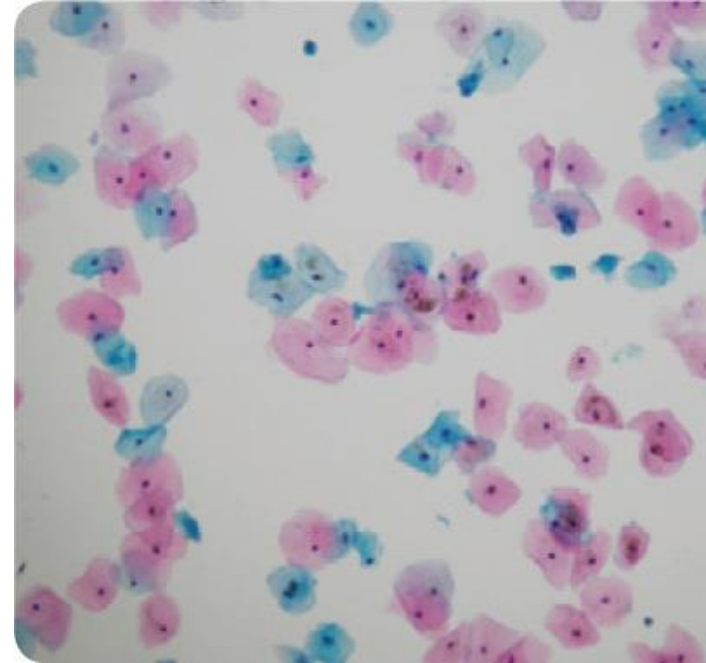


Biopsy



- Organs and tissues
- Excision
- Core-cut

Cytology



- exfoliative
- intervention

Biopsy

- Invasive method
- Representative (larger sample of tissue, we see relationships between cells)
- Financially more expensive (surgery, anesthesia, ...)
- Hospitalization of the patient (often needed)

Cytology

- Non-invasive (minimally invasive) method
- Screening, not always representative (dependence on collection, fewer tissues, missing cell relationships)
- Cheap and affordable method
- Hospitalization is not necessary

Biological material

- Processing and subsequent material evaluation
 - with the least possible damage to the structure of the cells and tissues
 - to prevent cellular degradation and autolytic process
- Immediately after the collection of tissue samples it is necessary **to fix** them
 - kill microorganisms and fix the cells
 - avoid autolysis and microbial damage
- Accurate labeling
- Fast delivery to the laboratory for further processing



- source of important information

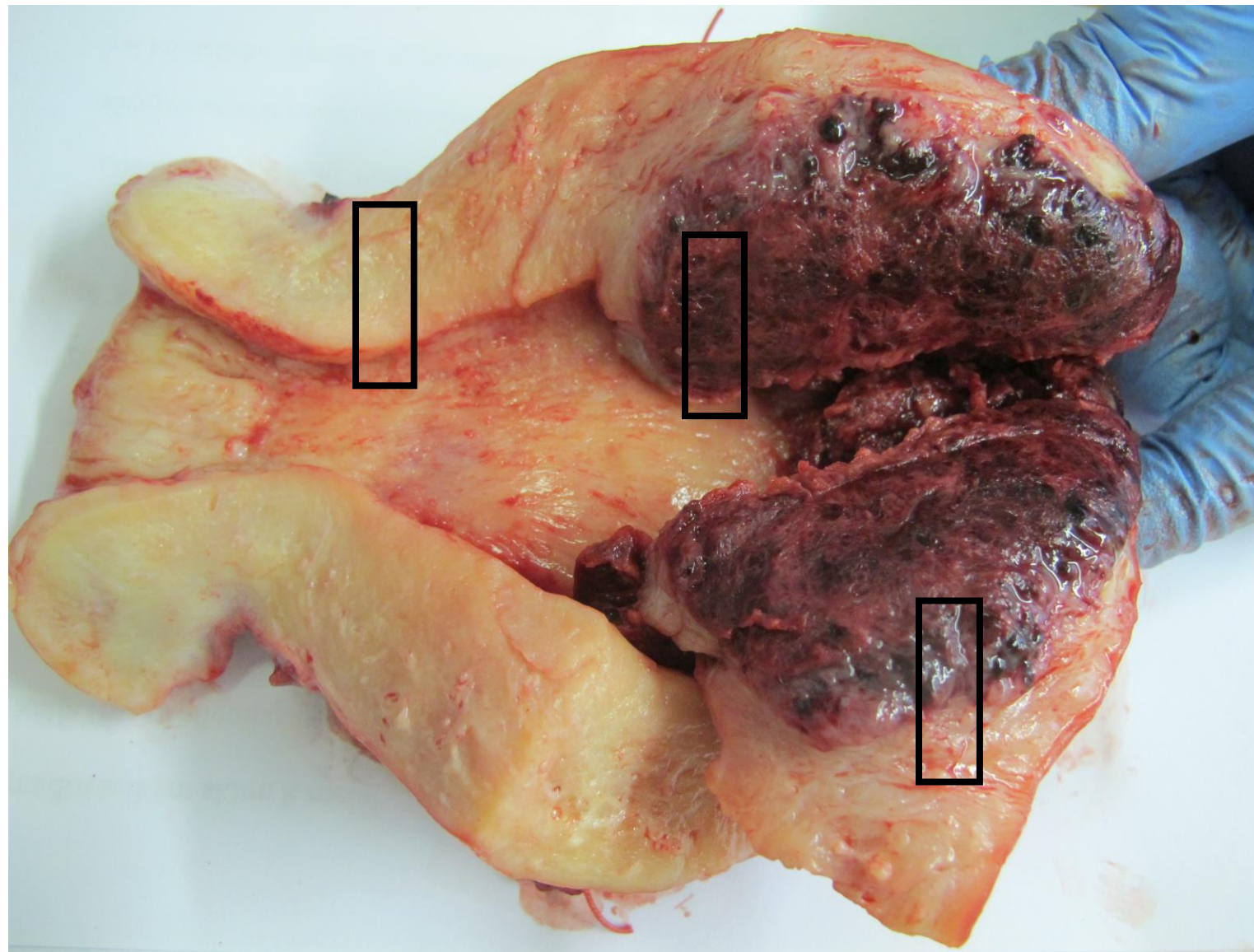
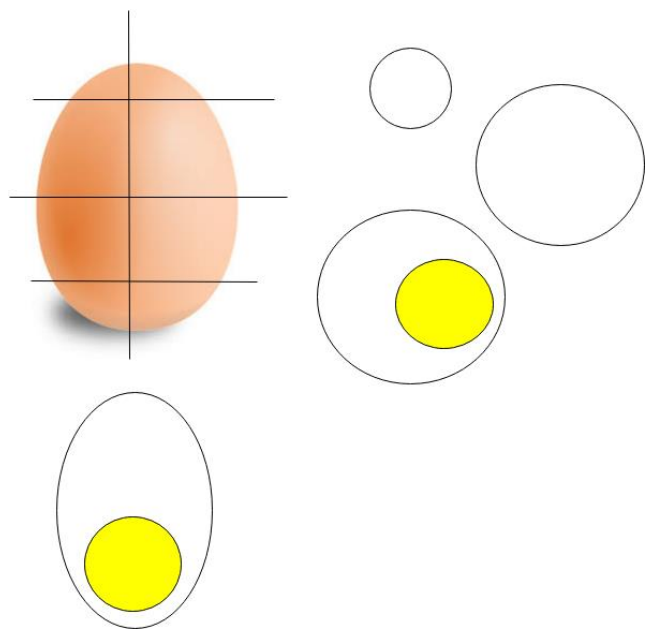
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Biopsy workflow

- Laboratory processing
- Excidation
- Fixation
- Impregnation with embedding medium
- Cutting the samples
- Results

Excidation





Fixation

- Quick process of protein denaturation in cells and preventing tissue decay – autolysis
1. Fixation with loss of biological function and structure
 - chemical and physical methods
 2. Fixation without loss of biological function and structure
 - quick freeze

Fixation agents

- Aldehydes (formaldehyde, glutaraldehyde, ...)
- Oxidising agents (osmium tetroxide)
- Protein-denaturing agents (ethanol, methanol, acetic acid, ...)
- Physical methods (heat, microwaves, ...)

- **MECHANISM OF FIXATION**

- cross-linking
- change of tertiary structure
- without changing of the
- antigenicity
- without changes in
- spatial relationships

Fixation factors

- Formalin 10%, glutaraldehyde 3%
- pH (physiological, pH 5.5-gastro-mucosa, pH 6.5-catecholamines)
 - standard is a phosphate buffered formaldehyde fixation solution
 - physiological pH important for eventual genetic analysis
 - if no buffered solution is available, dilute formaldehyde with tap water (not distilled) - formaldehyde spontaneously decomposes to formic acid
- temperature (laboratory, 0-4 ° C, higher temperature)
 - the standard is laboratory temperature, do not store the samples in the refrigerator unless it is required by the laboratory
- penetration of the fixing agent (material size)
- concentration (3% glutaraldehyde, 10% formalin)
- duration of fixation (formalin 24 hours)
- If the fixation is prolonged, there is a risk of tissue damage, wrinkling, and damage enzymatic and antigenic reactivity !!

Tissue processing

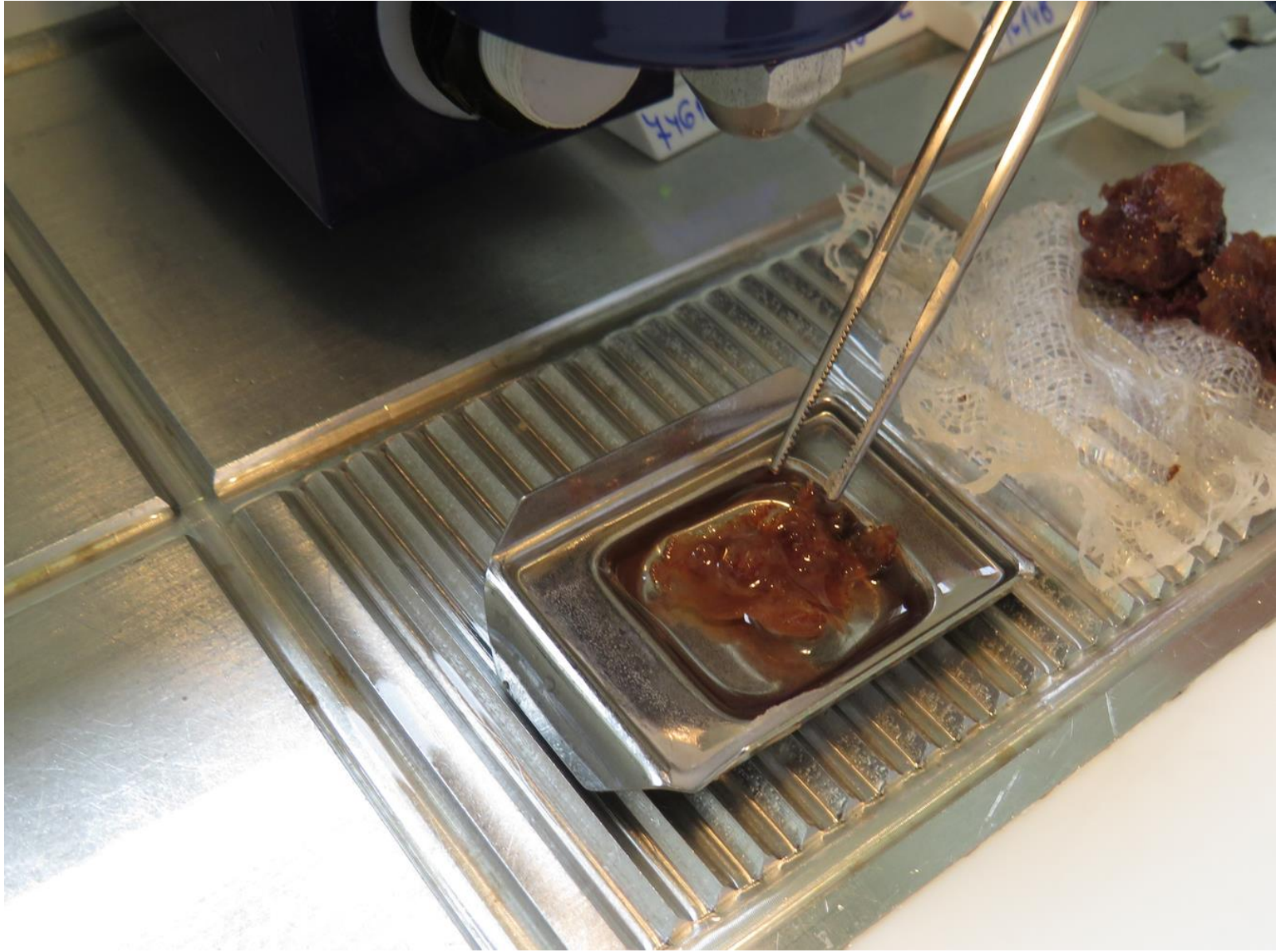
- Aim: it is necessary to put the tissue into a solid medium which allows its subsequent cutting
- After fixation, the sample must be dehydrated to remove the fixing solution
- Next we remove the dehydration solution and clarify the tissue (xylene, toluene, chloroform...)
- The last step is impregnation when we pour the sample into the medium (paraffin, resin, agar, gelatin)

Dehydration and cleaning



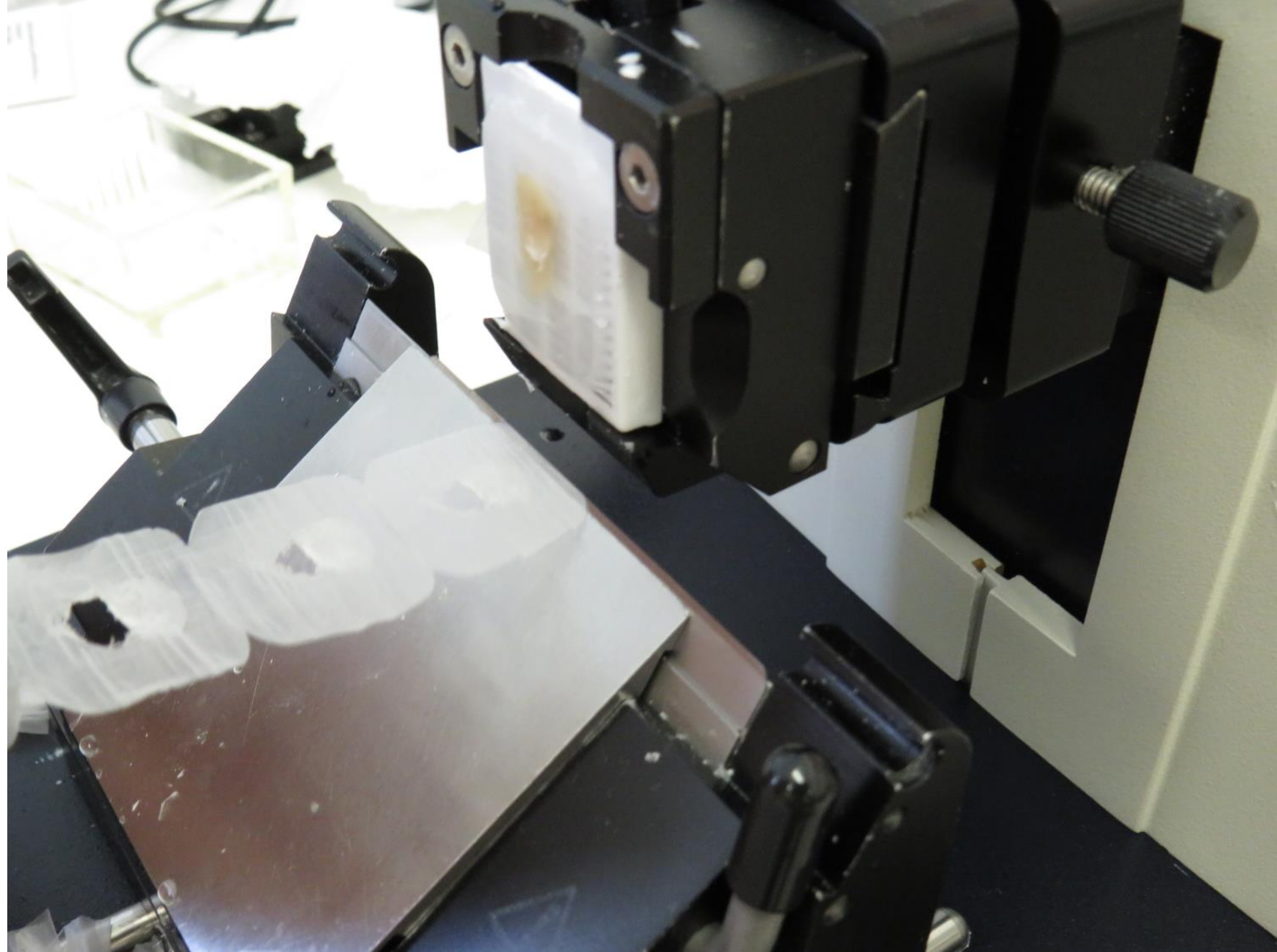
- 10% formalin
- 70% alcohol
- 95% alcohol
- 100% alcohol
- 100% alcohol
- 100% alcohol
- 100% alcohol +Xylén
 - Xylén
 - Xylén
 - Parafín
 - Parafín

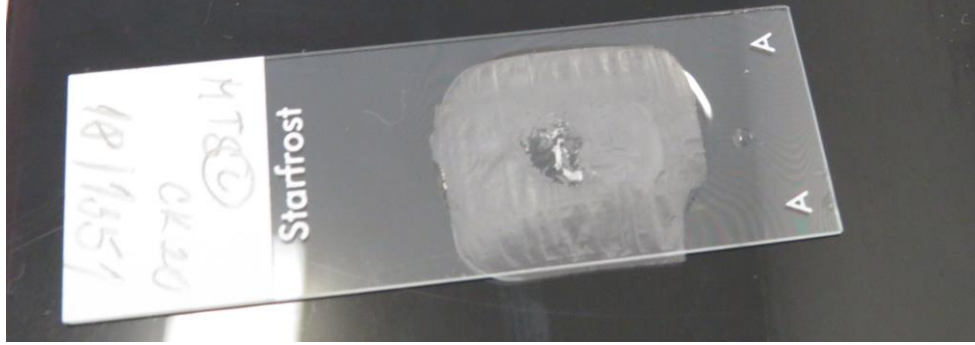












Processing of frozen sections

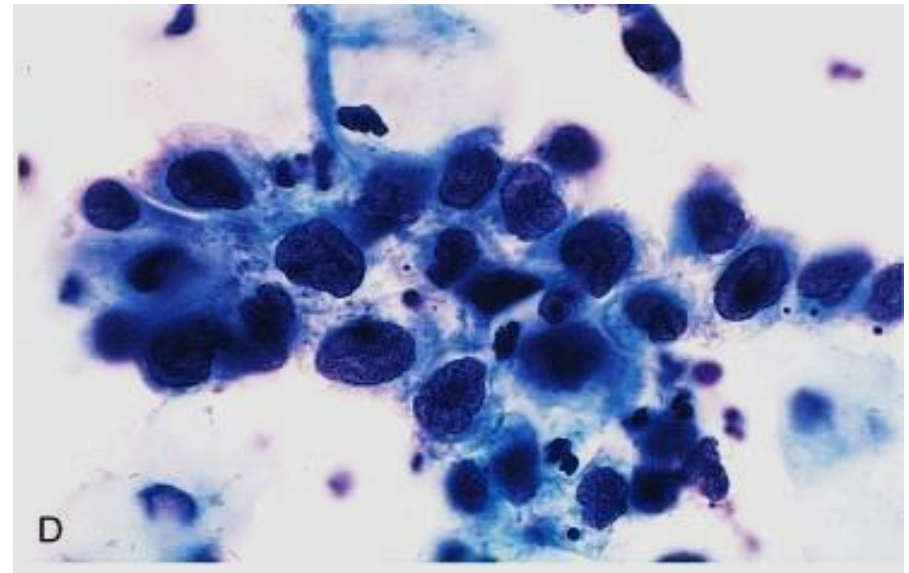
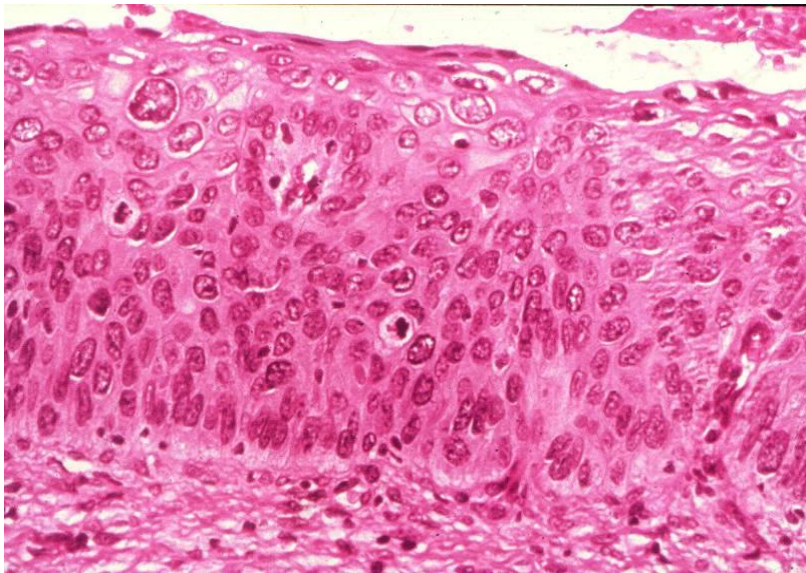
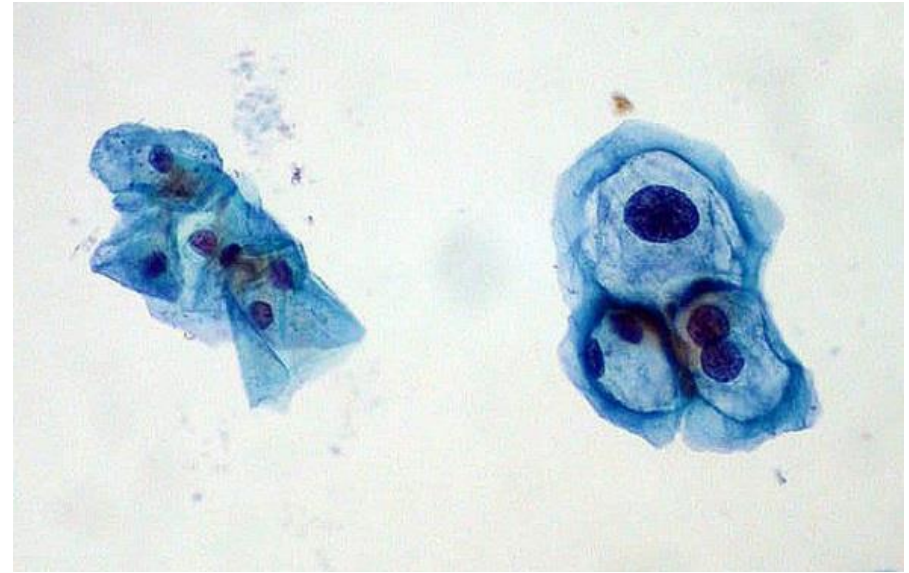
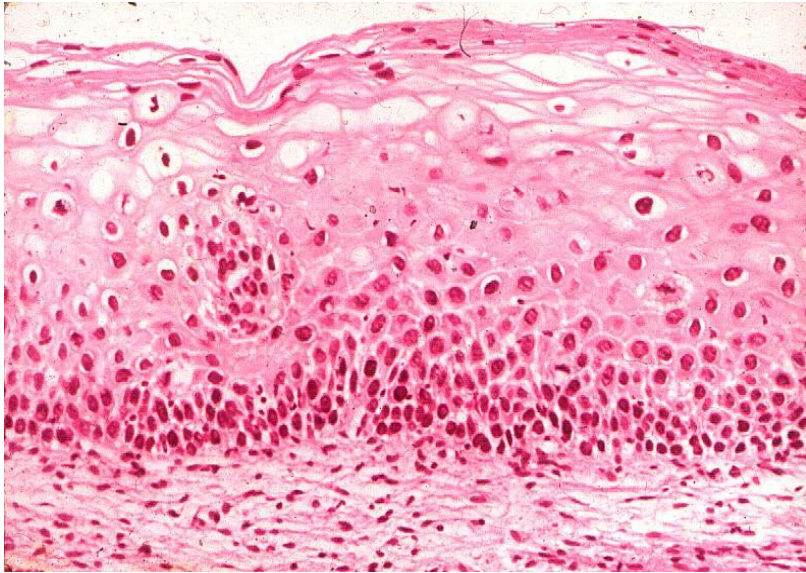
- Freezing as a form of "fixation" of fresh tissue
- Condition: speed (slow freezing - formation of i.c. ice crystals and destruction of cells)
- Quick diagnosis (lack of time for other fixation)
- Enzyme histochemistry when enzymes are labile
- Some immunofluorescence methods
- Indication for processing by freezing ? / Advantages and disadvantages?

CYTOLOGY

- The advantages of cytology are:
 - Speed of diagnostics
 - Opportunity to take and evaluate samples from hard-to-reach locations
 - Economic aspect
- The disadvantages of cytology are:
 - The impossibility of accurate classification and typing of tumors
 - Impossible to consider an invasion
 - Possibility of false negativity and false positivity

Today, cytology is used to diagnose tumors of all organ systems.

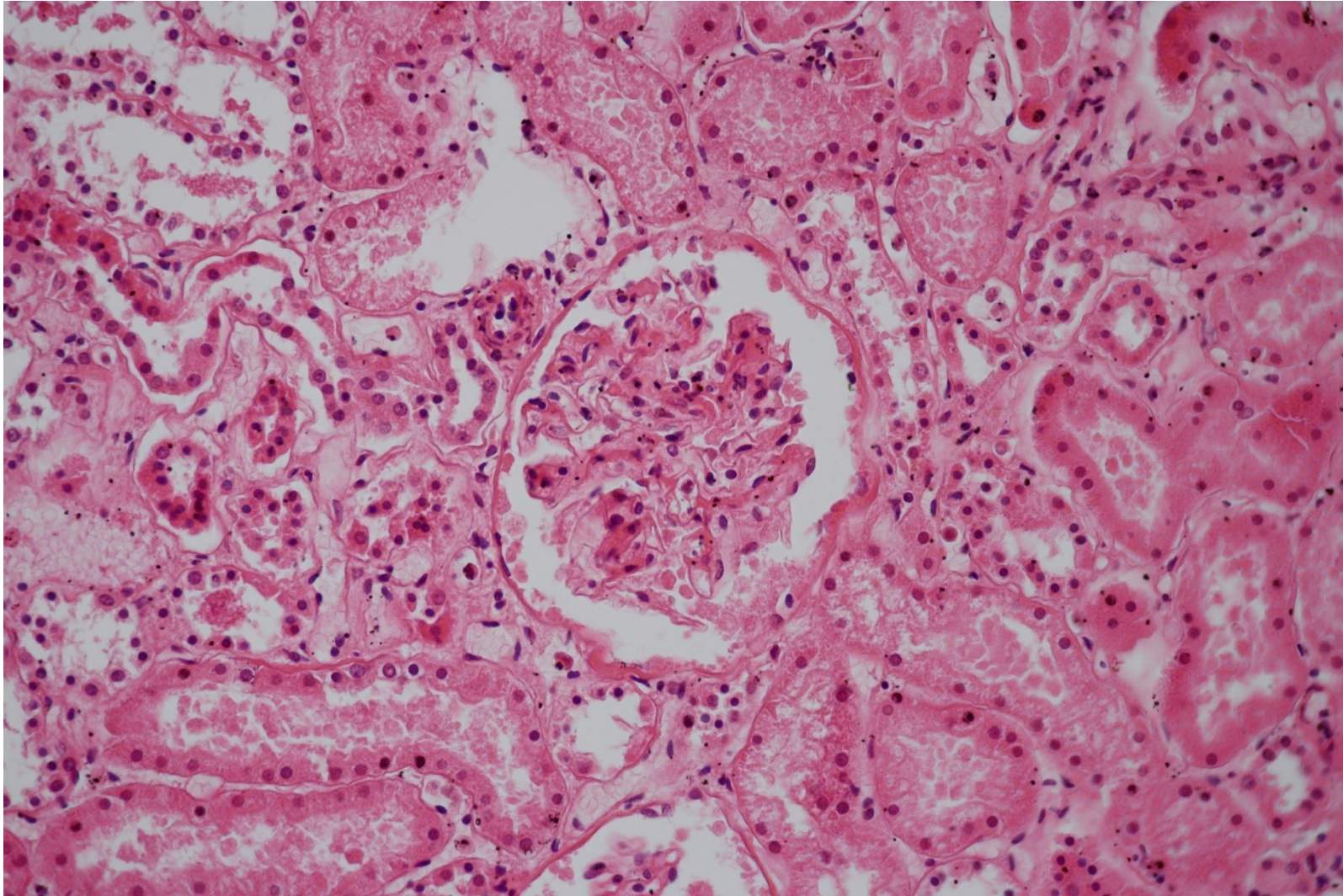
Cytology makes it possible to carry out **screening tests** at vulnerable and risky populations with low financial costs.



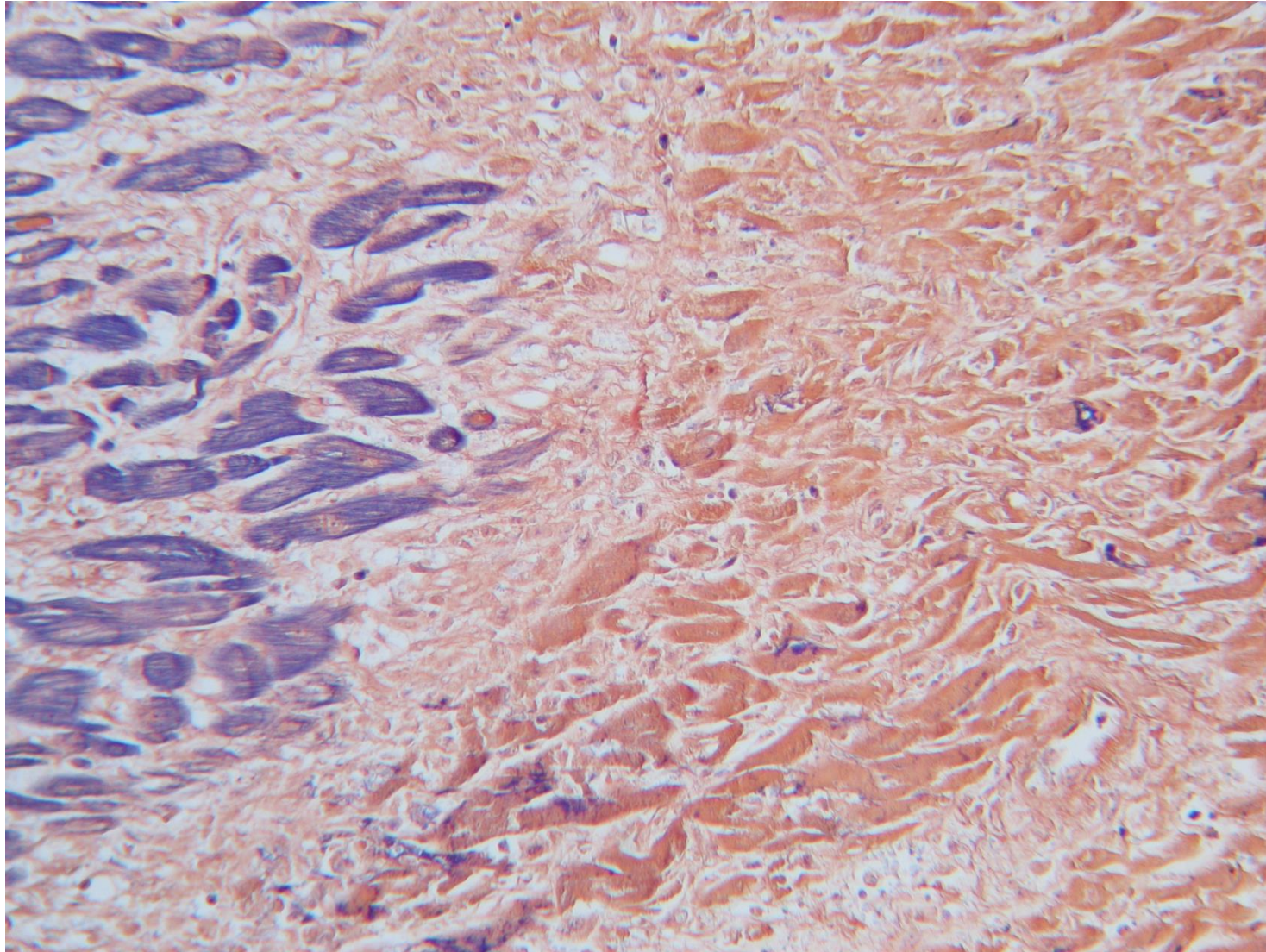
BASIC STAINING TECHNIQUES

- Acidofilia:
 - Staining with acidic dyes
 - Eosin, Azocarmine, Aniline Blue, Carbofucin
 - Substrates: Cytoplasm, intercellular substances, ...
- Basophilia:
 - Staining with alkaline dyes:
 - Methyl blue, Toluidine, Hematoxylin
 - Substrates: Chromatin, Ribozymes, ...
- Metal impregnation
 - Methods by Golgi and Cajal
 - Silver and Gold Salts
 - Neurons and glial cells

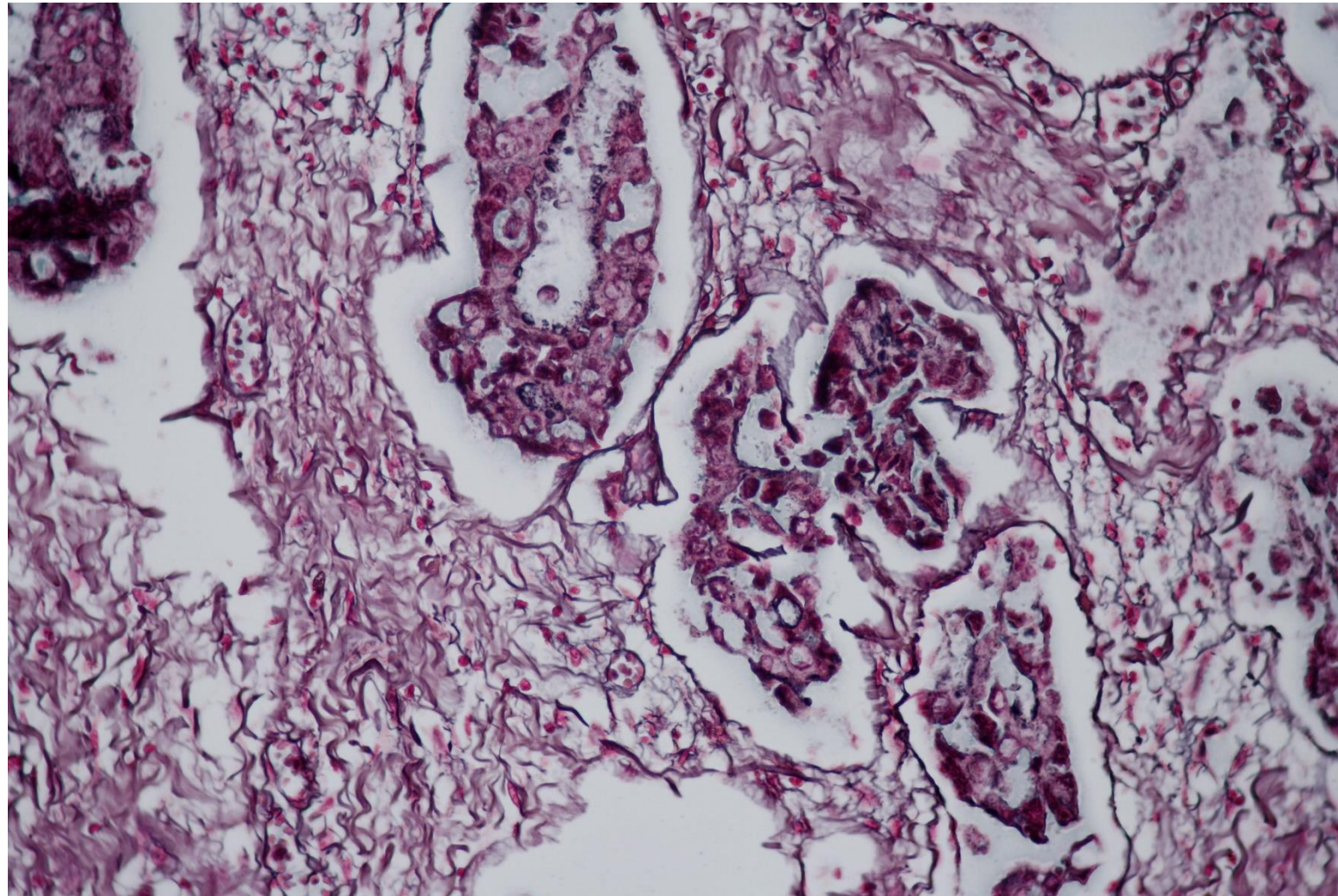
HEMATOXYLIN AND EOSIN



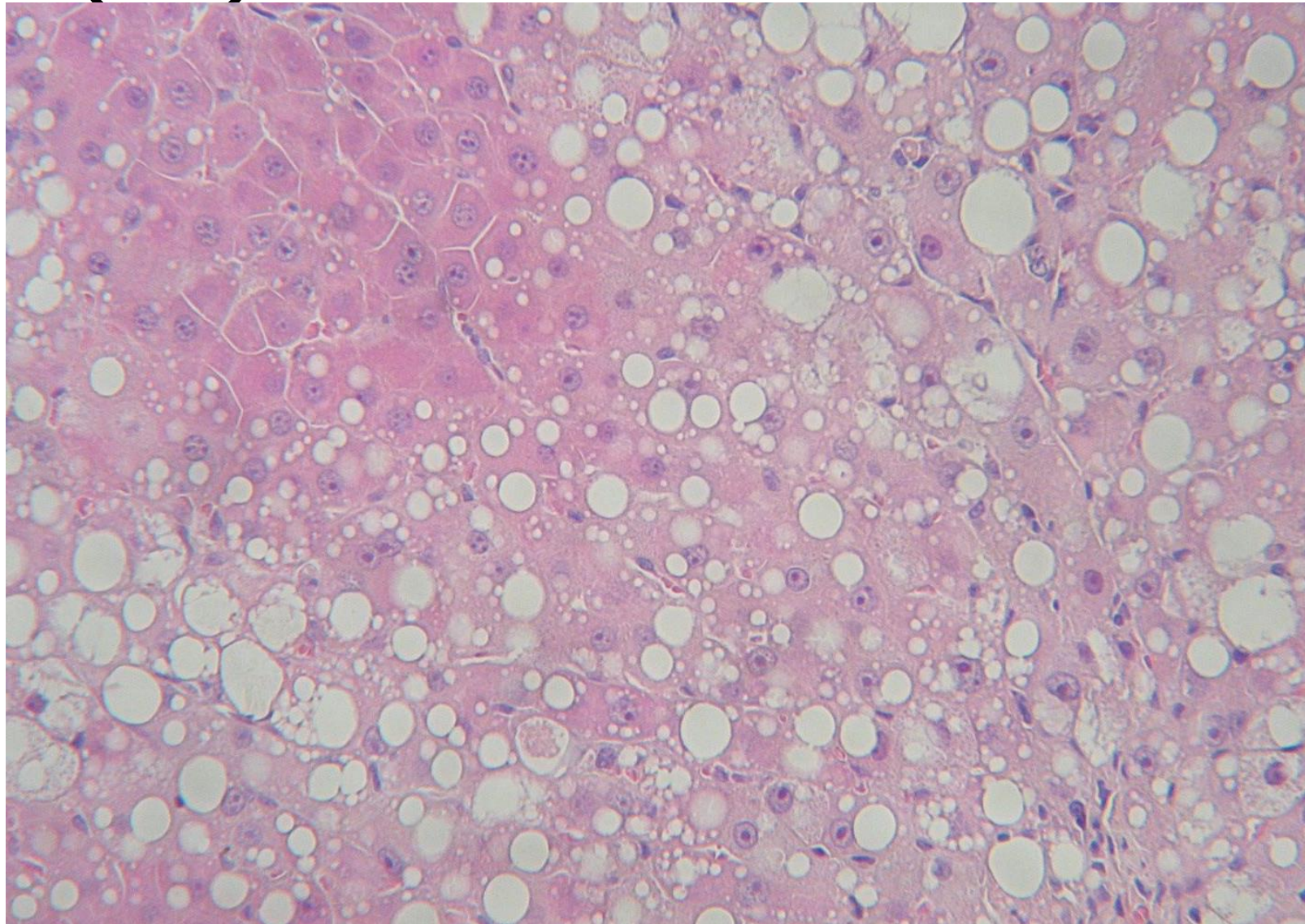
MALLORY HEMATOXYLIN



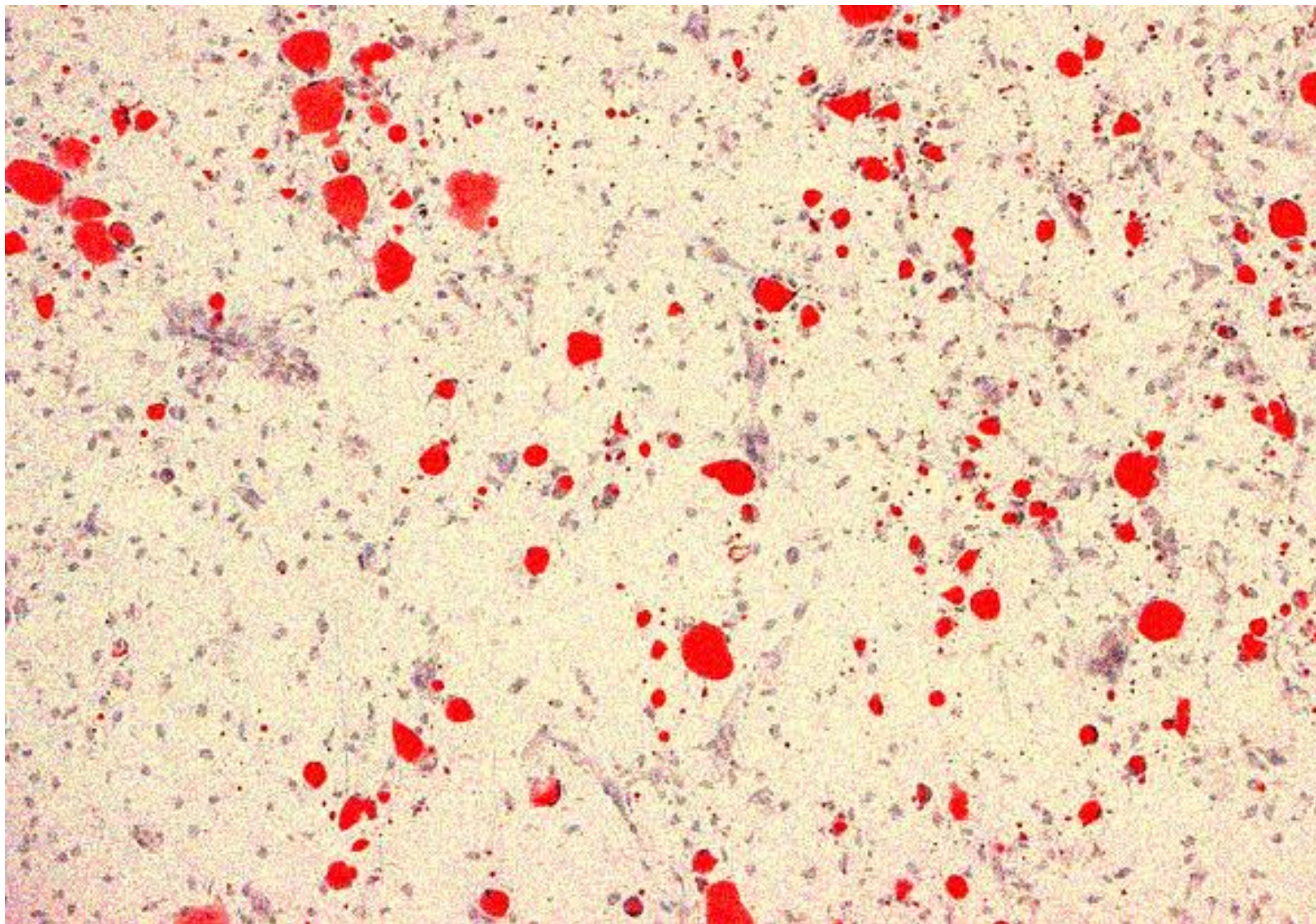
RETICULIN



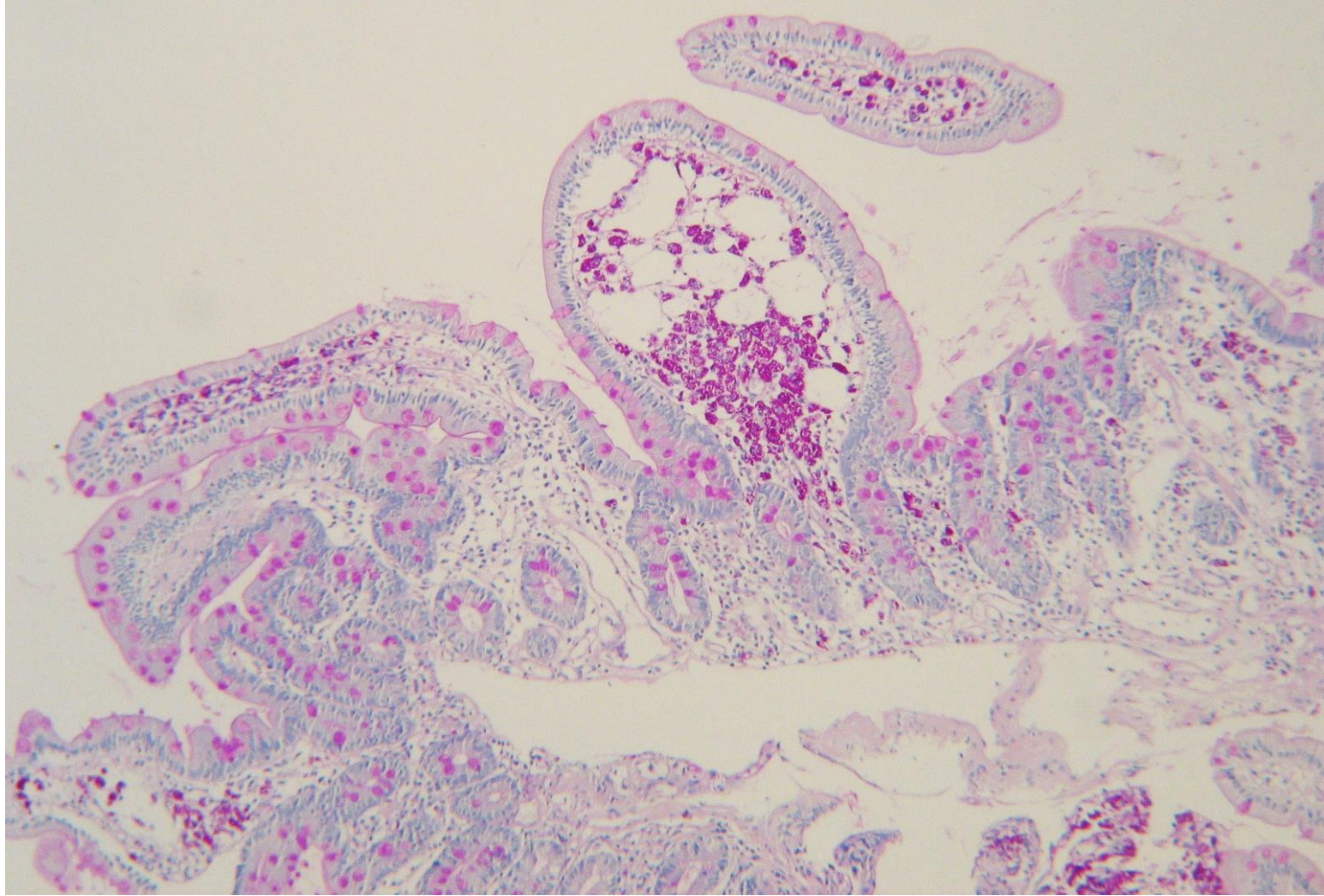
LIPIDS (HE)



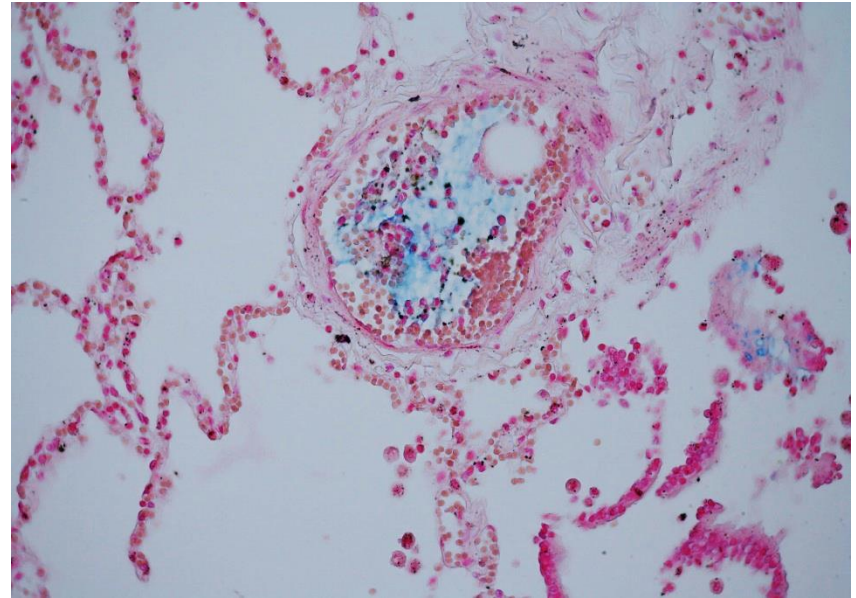
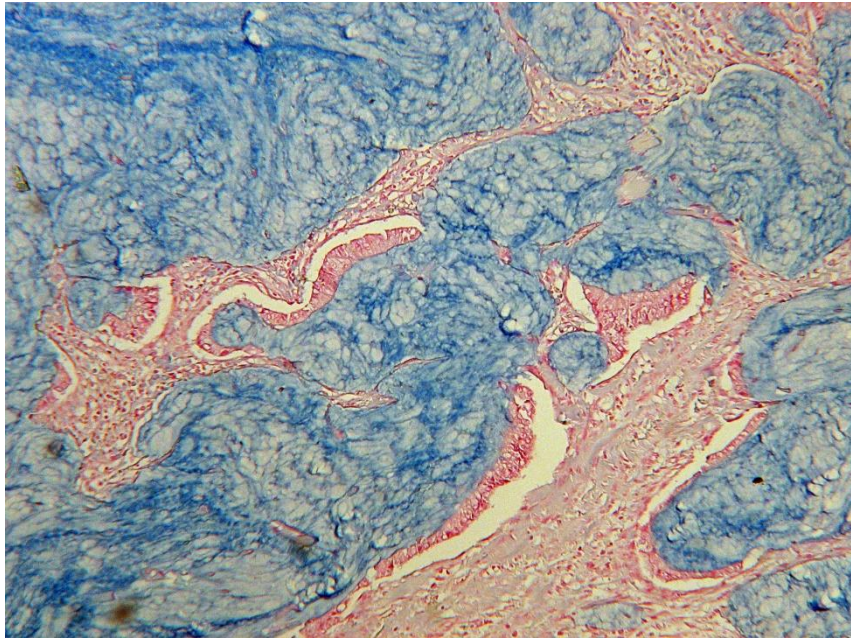
OIL RED



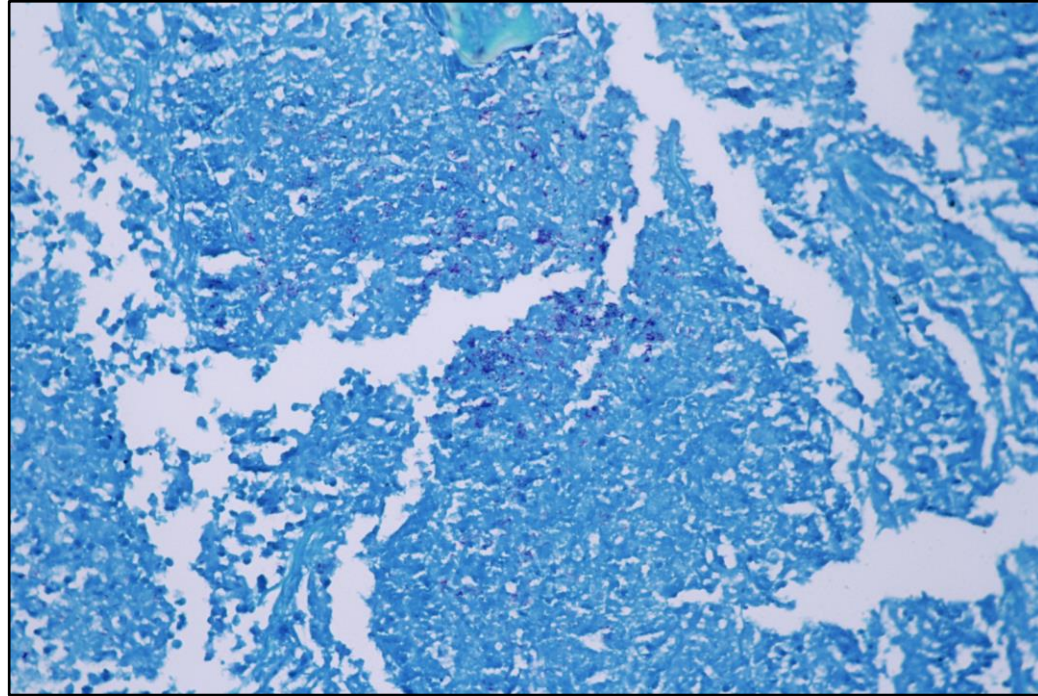
Periodic Acid Schiff



ACIAN BLUE



Staining BK (Ziehl–Neelsen)

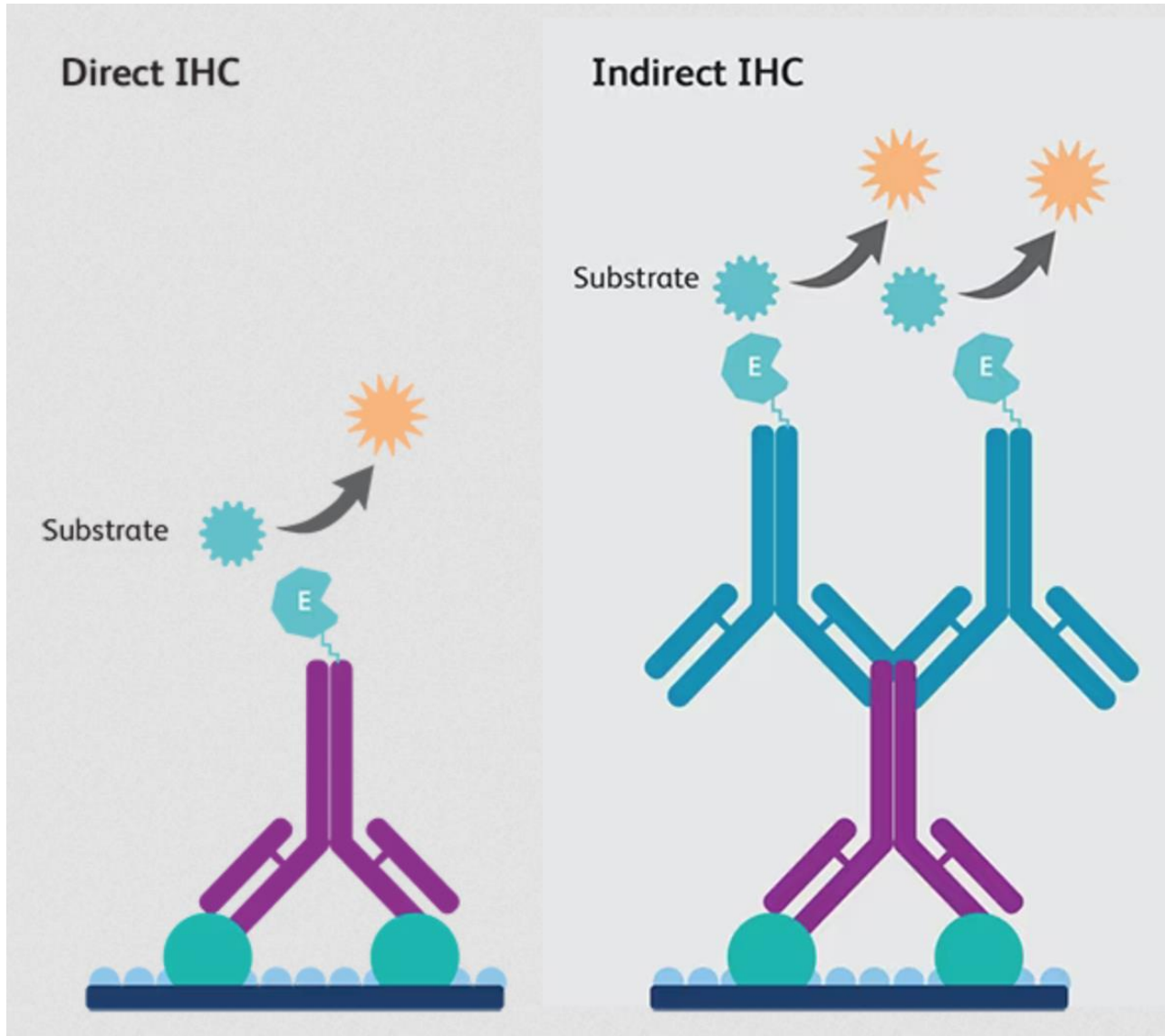


IMUNOHISTOCHEMISTRY

- The use of immunological methods in the diagnosis of diseases allows, in addition to the morphological evaluation of tissues, to demonstrate the production of various proteins
- Purpose
 - Diagnosis (ence or absence of specific markers)
 - Prognosis (prognostic markers)
 - Therapy (evidence of markers that are related to drug efficacy, influence treatment resistance, appropriateness of the treatment used)
 - Method
 - reliable
 - Low financial and staff requirements
- Standard diagnostic method

Examples of antibodies used for detecting tumors

Squamous cell carcinoma of the oesophagus	CK7- , CD20-, CK5/6+, CK10+, CK14+; p63 positivity in almost all cases; most cases are also positive for p53 and p16,
Adenocarcinoma of the oesophagus	commonly expresses CK7, variable expression of CK20, AMACAR and weak focal CDX-2 positivity, p16 negativity,
Adenocarcinoma of the stomach	a) intestinal type - variably expresses CK7, CK20, CDX-2, MUC1 and MUC5AC b) diffuse type - more than 70% are positive for CDX2, CK7, HepPar-1, variably express CK20, MUC2 and MUC5AC; these cases are negative for MUC1 and E-cadherin,
Neuroendocrine tumours	Immunopositivity to markers of neurocrine differentiation, such as chromogranin A, synaptophysin and NSE.

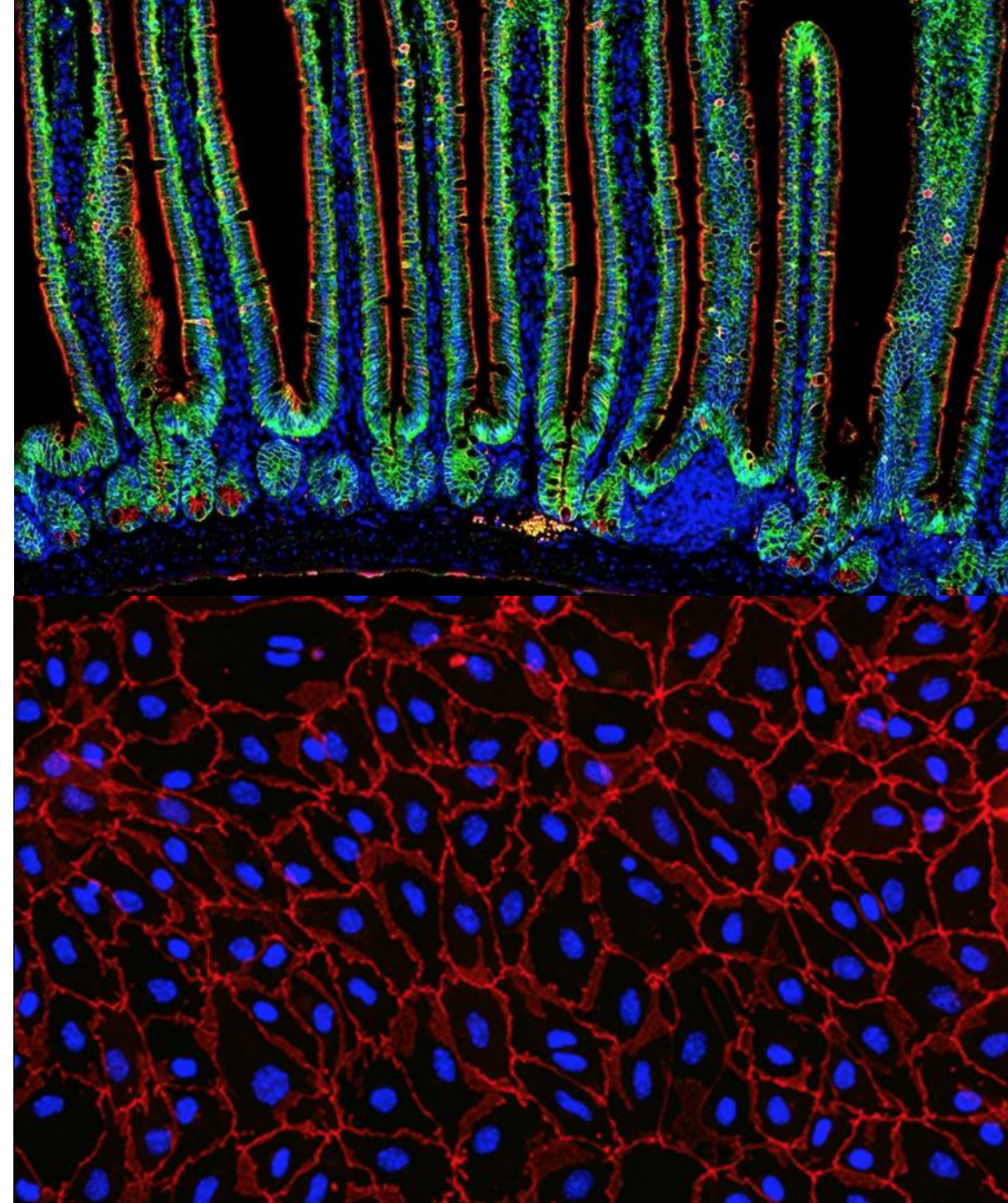


Direct and indirect IHC

- Direct- antibody binds directly to labelled antigen
- Indirect- primary antibody binds to antigen, and secondary labeled antibody binds to primary antibody

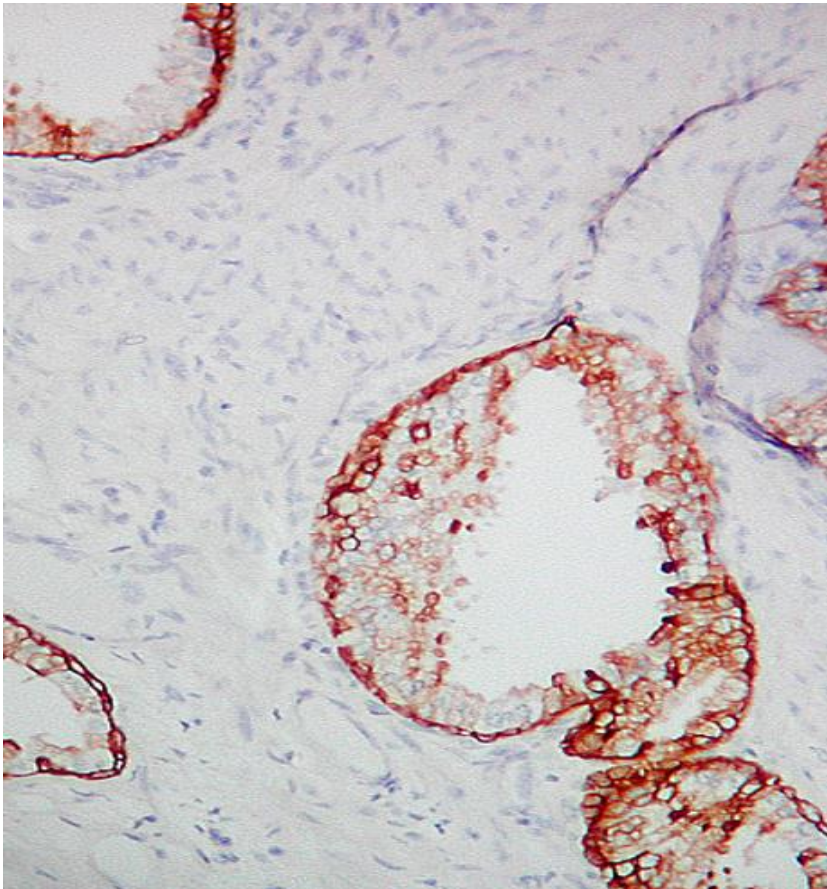
IMUNOFLUORESCENCE

- Use of fluorescently labelled secondary antibody



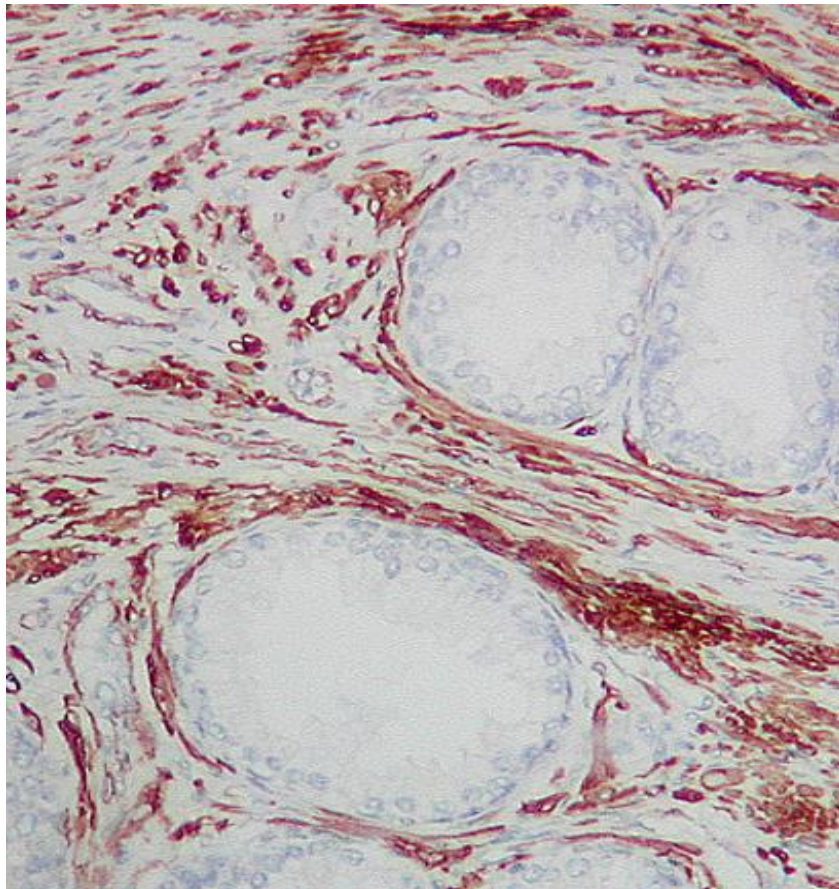
Cytokeratíny

- Proteínové filamenty prítomné v epitelových bunkách
- I. Kyslé cytokeratíny, 9-20
- II: Zásadité cytokeratíny, 1-8



Vimentín

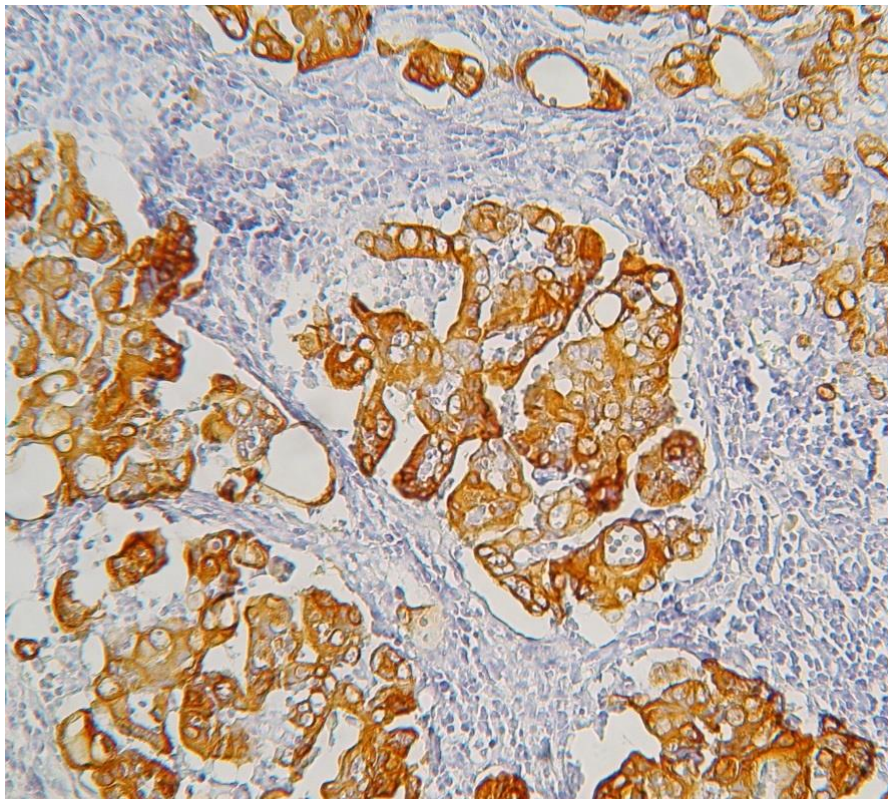
- Intermediárne filamenty (57 kD) prítomné v mezenchymálnych bunkách



Neuroendokrinné markery:

Chromograín, Synaptofyzín

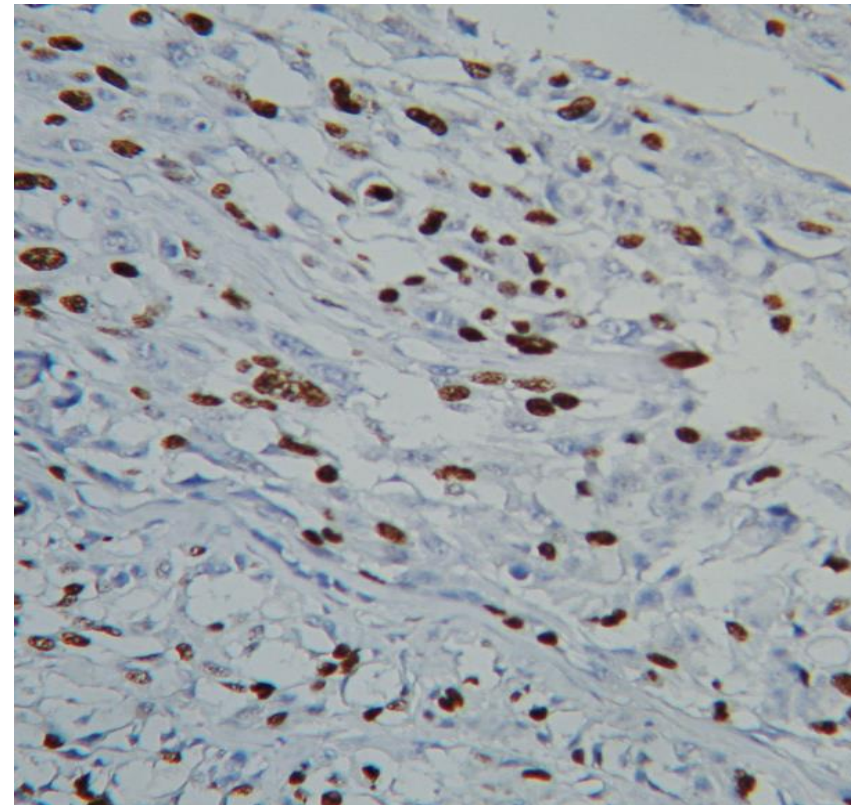
- Skupina kyslých glykoproteínov lokalizovaných v neurosekrečných granulách
- Prítomné vo všetkých neuroendokrinných tumoroch



Markery proliferácie:

Ki67

- Proteín exprimovaný bunkami v neskorej G1, S, G2 a M fáze. Nie je prítomný v G0 fáze
- Znak bunkového rastu a delenia



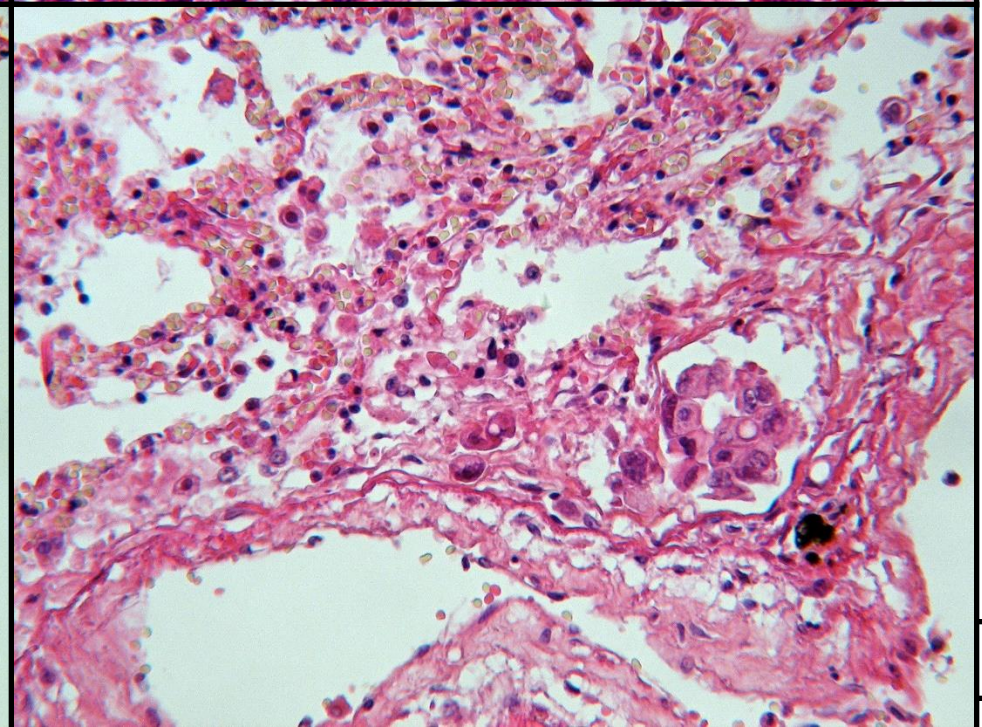
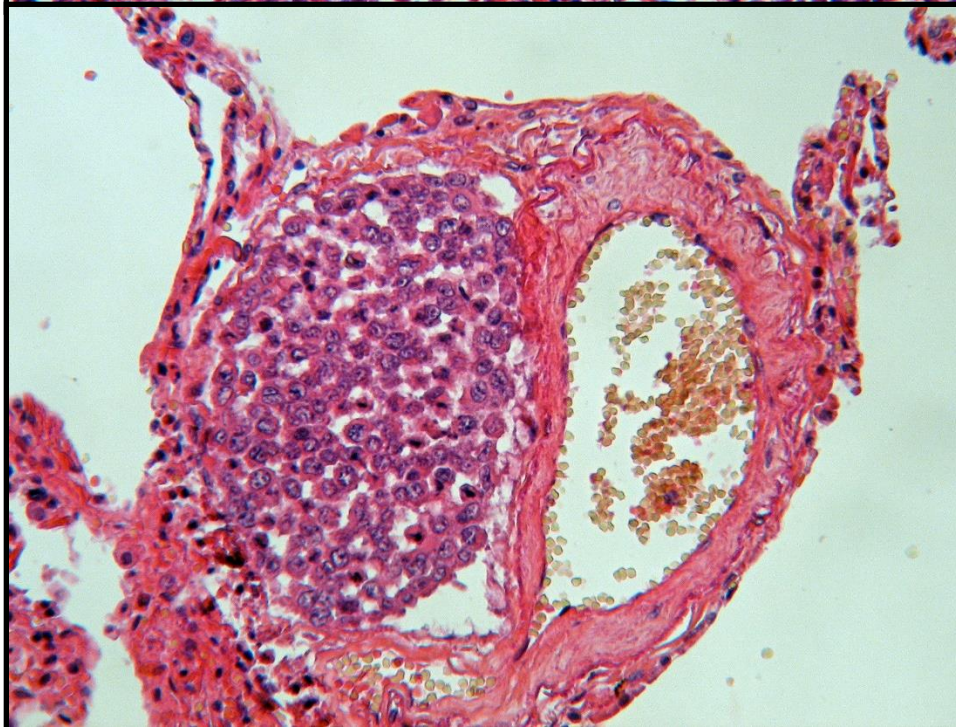
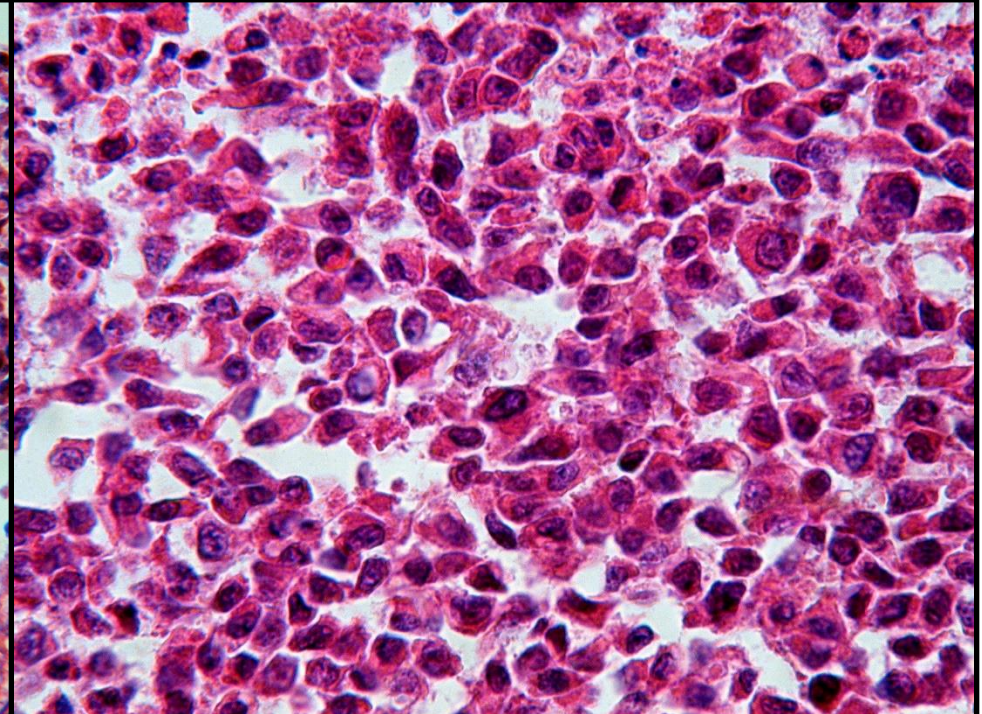
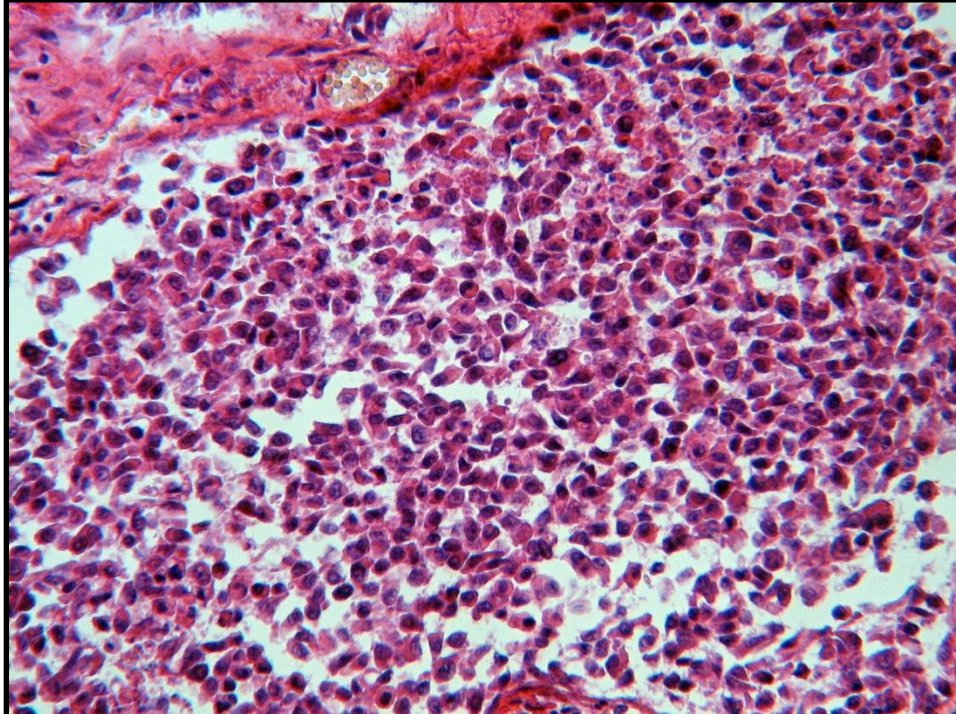
Case report

70 year old woman

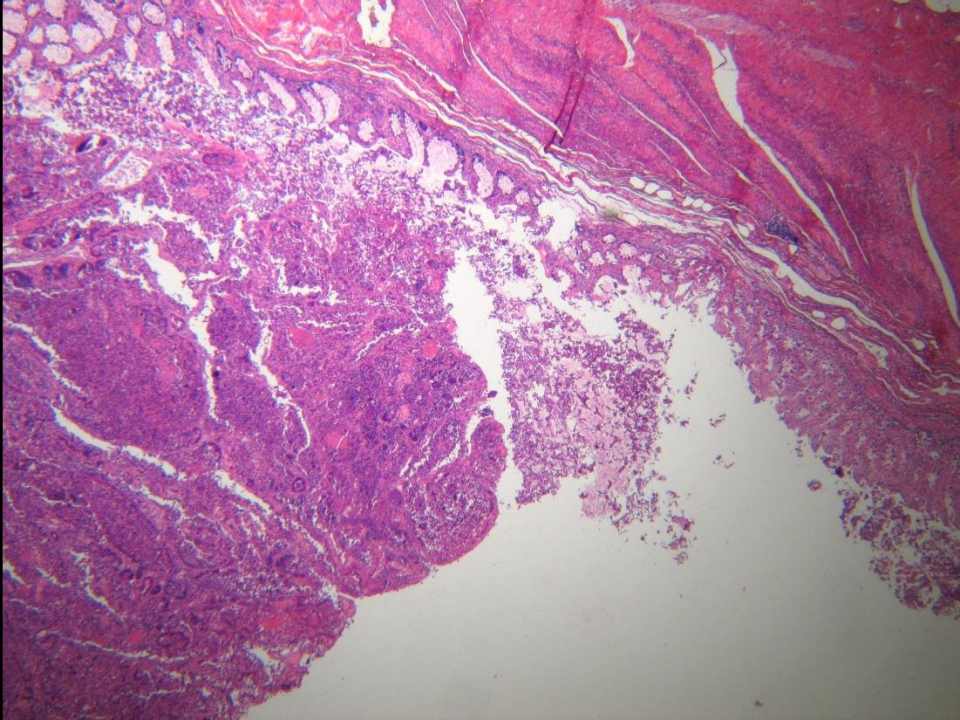
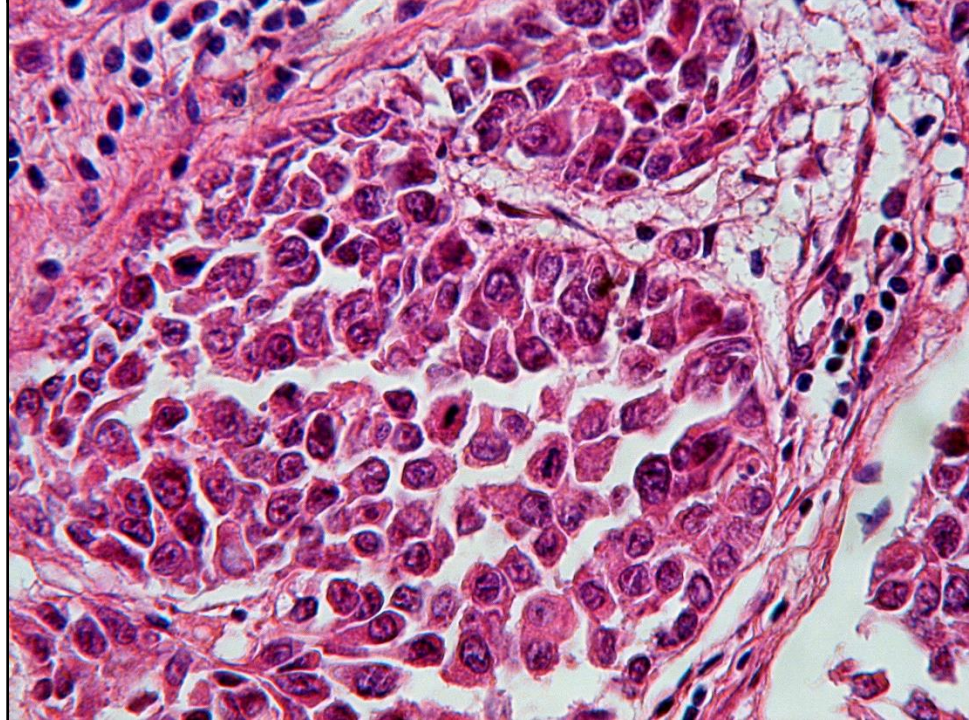
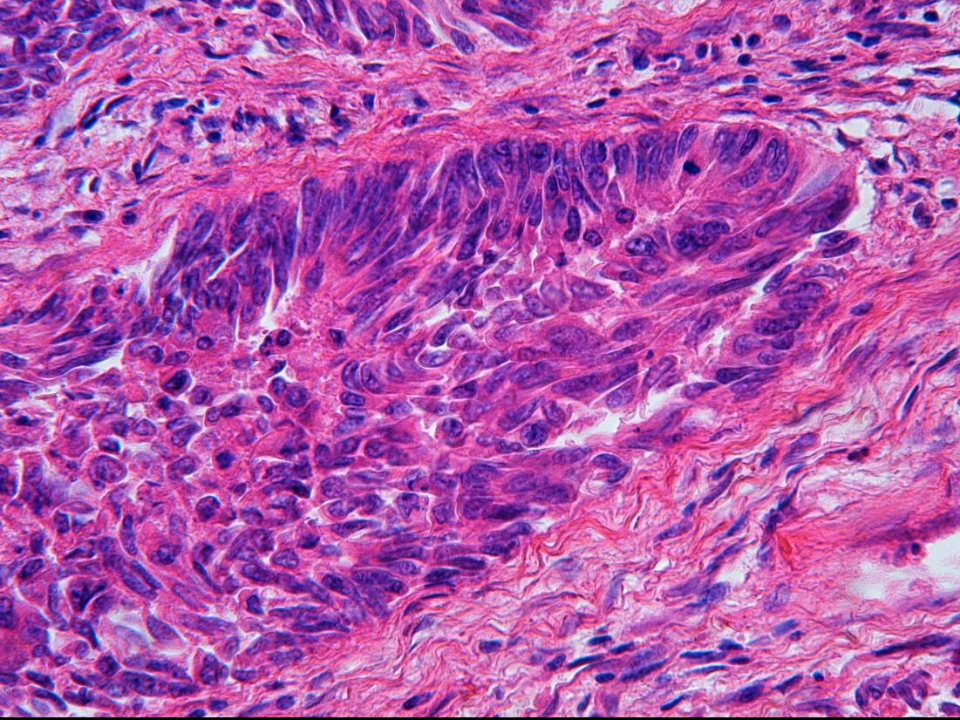
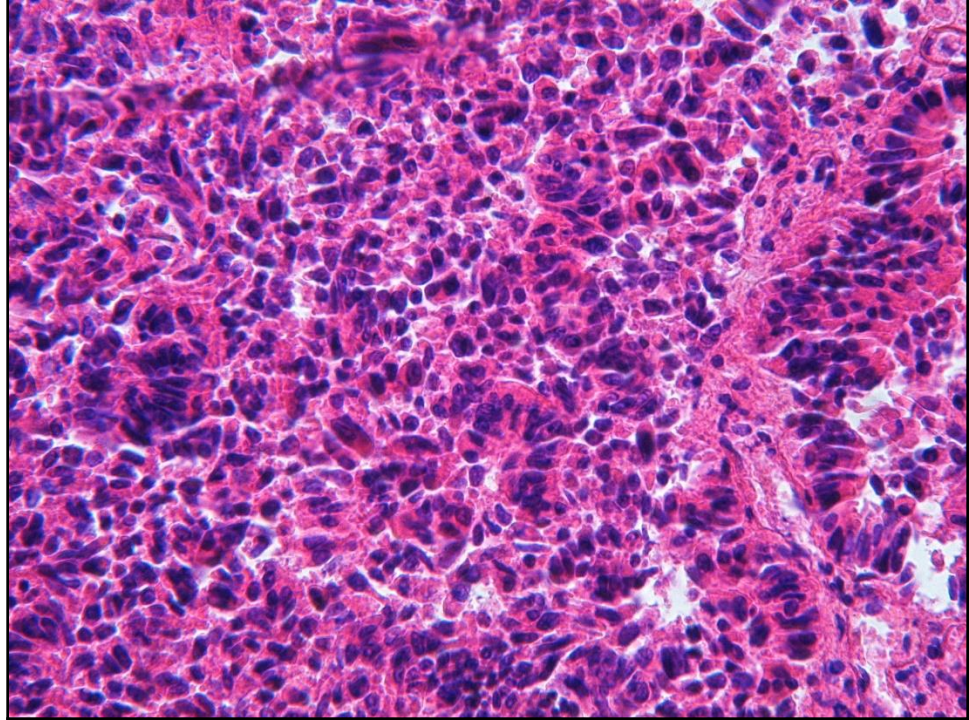
The patient hospitalized with symptoms of sepsis, pain in the abdomen. Multi-organ failure due to probable acute cholecystitis. Hypostatic bronchopneumonia of lung.

Conclusion: Multiorgan failure in sepsis

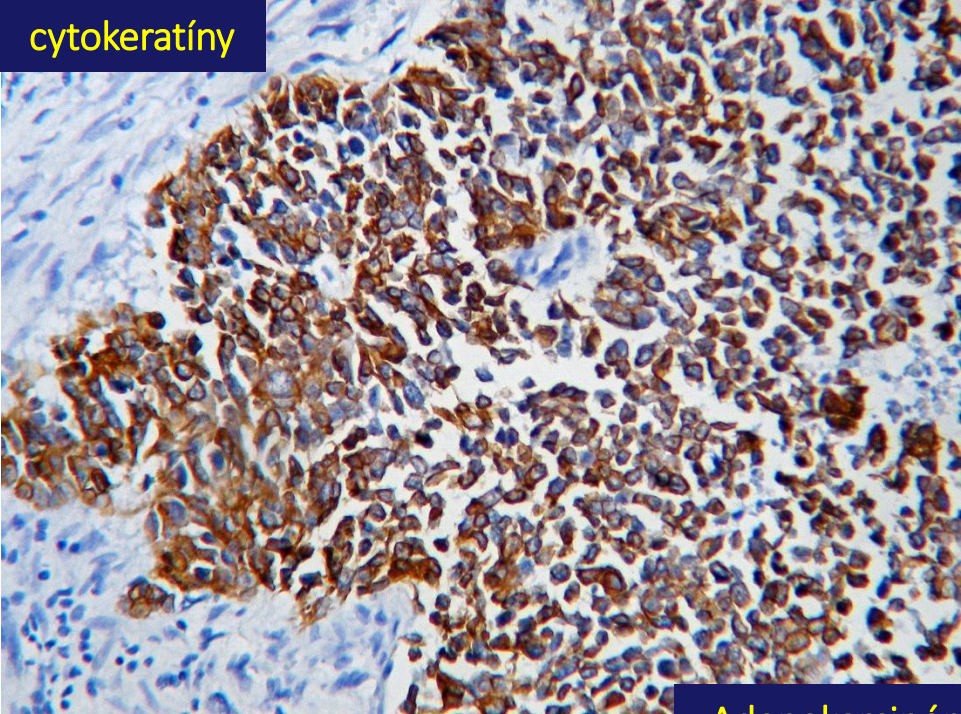




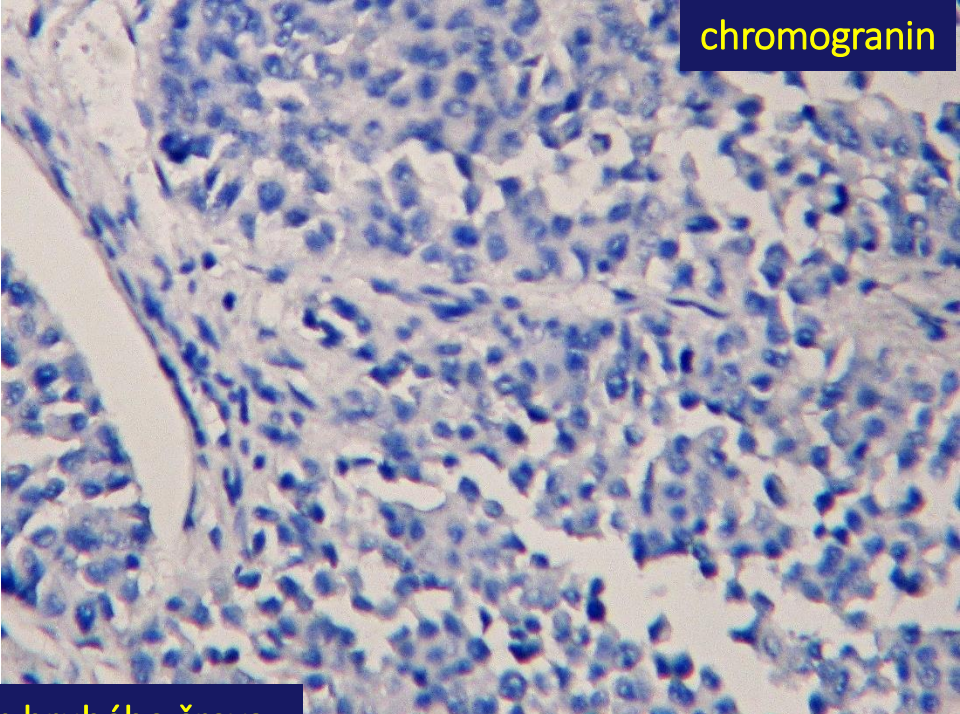




cytokeratíny

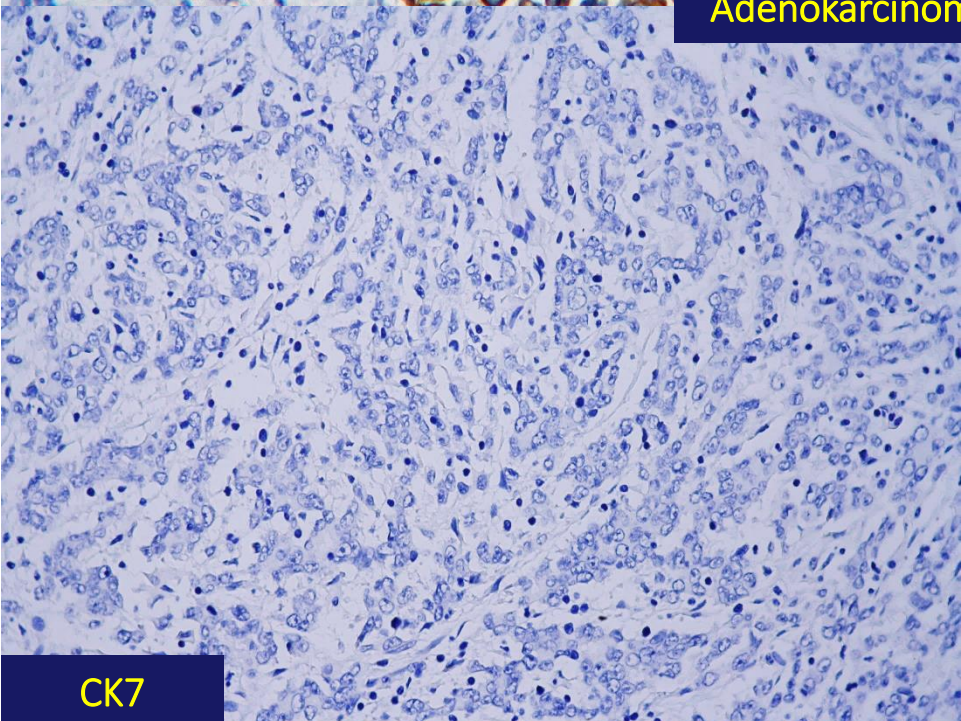


chromogranin

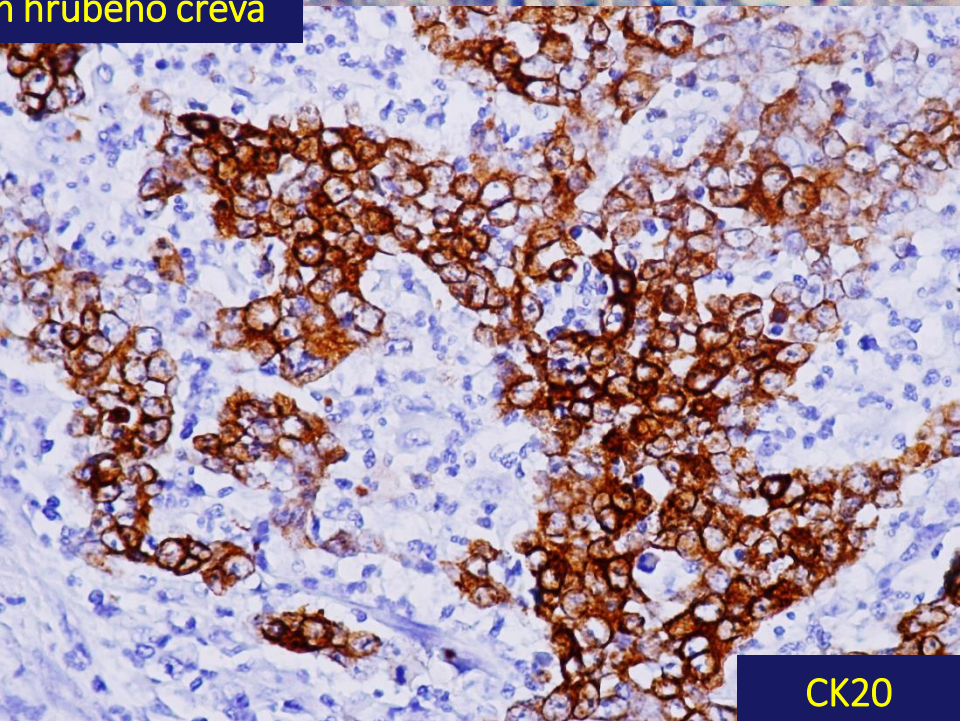


Adenokarcinóm hrubého čreva

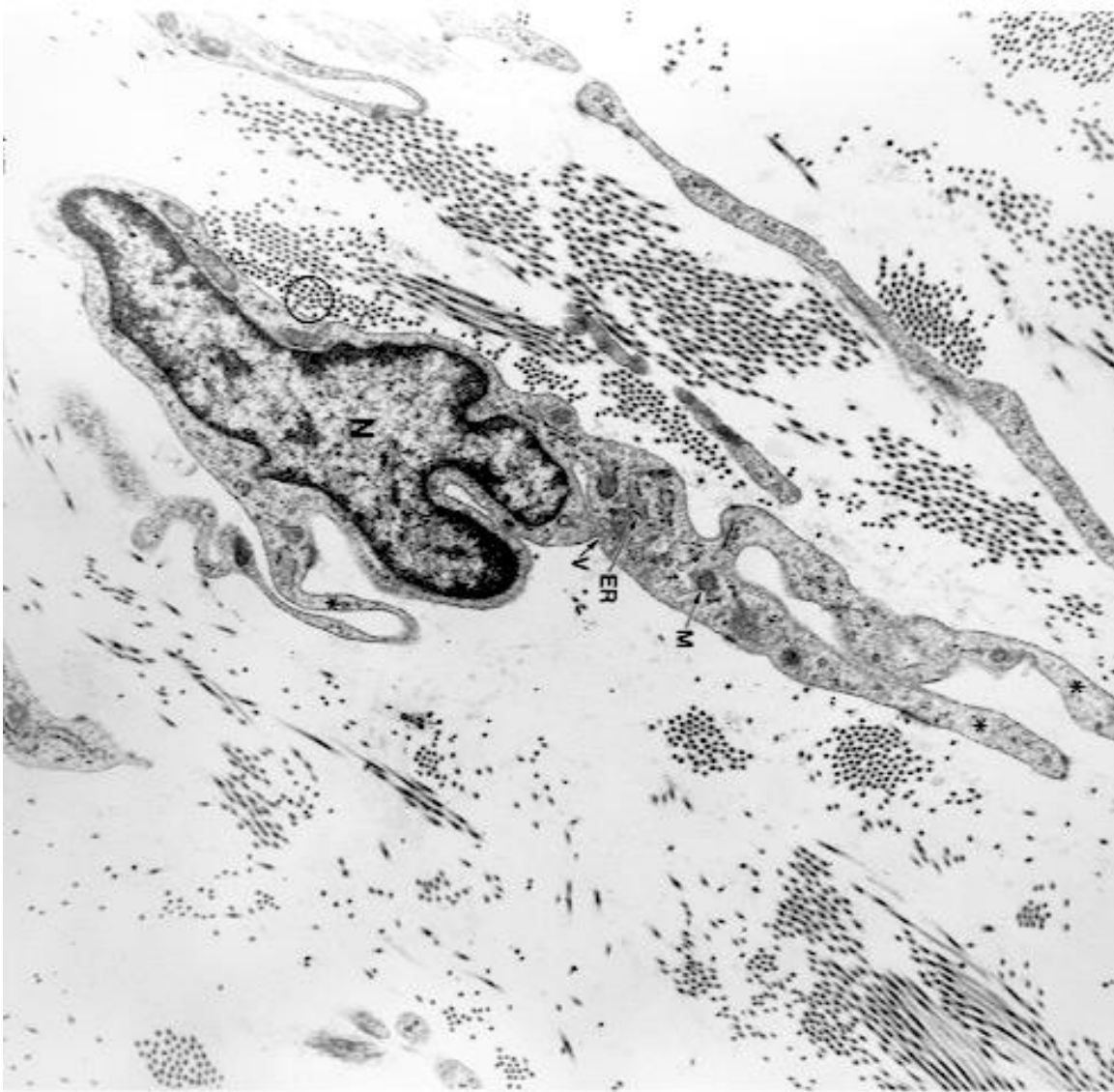
CK7



CK20



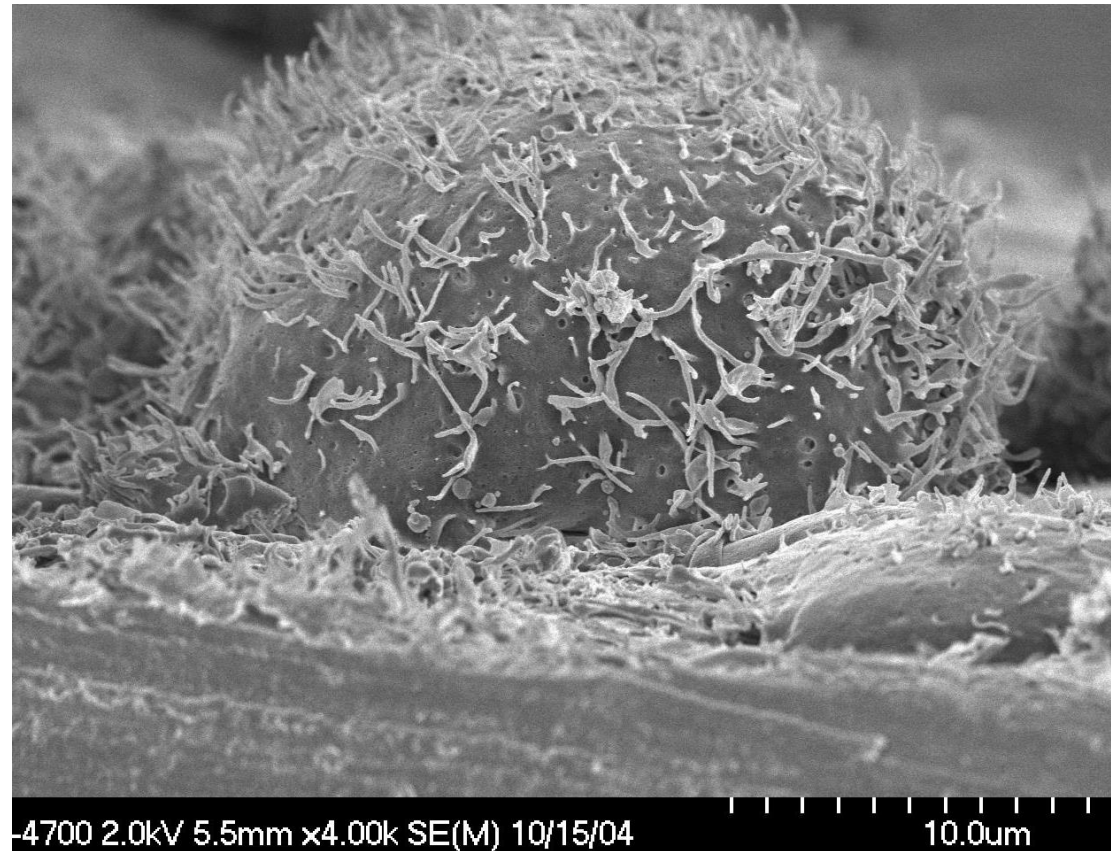
- Diagnostic markers
- Therapeutic markers
- Prognostic markers



Elektron microscopic methods

Transmission Electron Microscopy (TEM)

- **Scanning electron microscope (SEM)**



**I DON'T KNOW WHAT
A BASEMENT MEMBRANE IS**



**AND AT THIS POINT
I'M TOO AFRAID TO ASK**