

# Contributions to Anatomic Pathology, over the years

Author	Year	Contribution
Hippocrates	III - IV BC	Etiology
Galen	131 - 203	Pathogenesis
Antonio Benivieni	1440-1502	Clinical-functional correlation
Jean Fernel	1479-1558	Organic Pathology
J.B. Morgagni	1682-1771	Clinical-pathologic correlation
Xavier Bichat	1771-1802	Tissue Pathology
Rudolf Virchow	1821-1902	Cellular Pathology
C. Milstein	1975	Hybridomas - Monoclonal Ab
KB Mullis	1986	Molecular Pathology

# Anatomic Pathology, part 1



G.B. Morgagni



Rudolf Virchow



Xavier Bichat

# Anatomic Pathology, part 1



- Anatomic pathology materials: morphological samples taken for diagnostic purposes
- Anatomic-Pathologic studies are carried out on samples from:
  1. Clinical autopsies
  2. Surgical resections
  3. Biopsies
  4. Cytological preparations

# Anatomic Pathology, part 1



## Clinical autopsy:

Aims at defining the causes of death

1. Perinatal autopsies (23-24 weeks or 500 gr >> 7 days of extrauterine life): malformations.
2. Pediatric autopsies (7 days of life >> 15 years): lymphoma, leukemia, sudden death syndromes, infections.
3. Adult autopsies: cardiovascular disease, cancer and degenerative processes.

# Anatomic Pathology, part 1



# Anatomic Pathology, part 1



## Cytopathologic studies

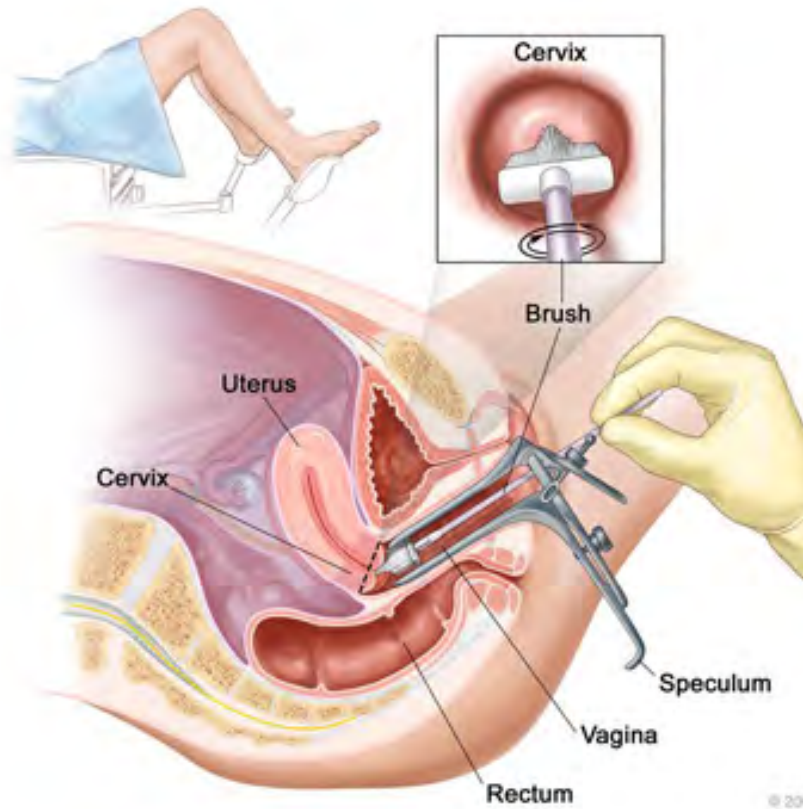
Morphologic alterations of cells obtained by:

- Spontaneous or induced exfoliation
- Fine needle aspiration

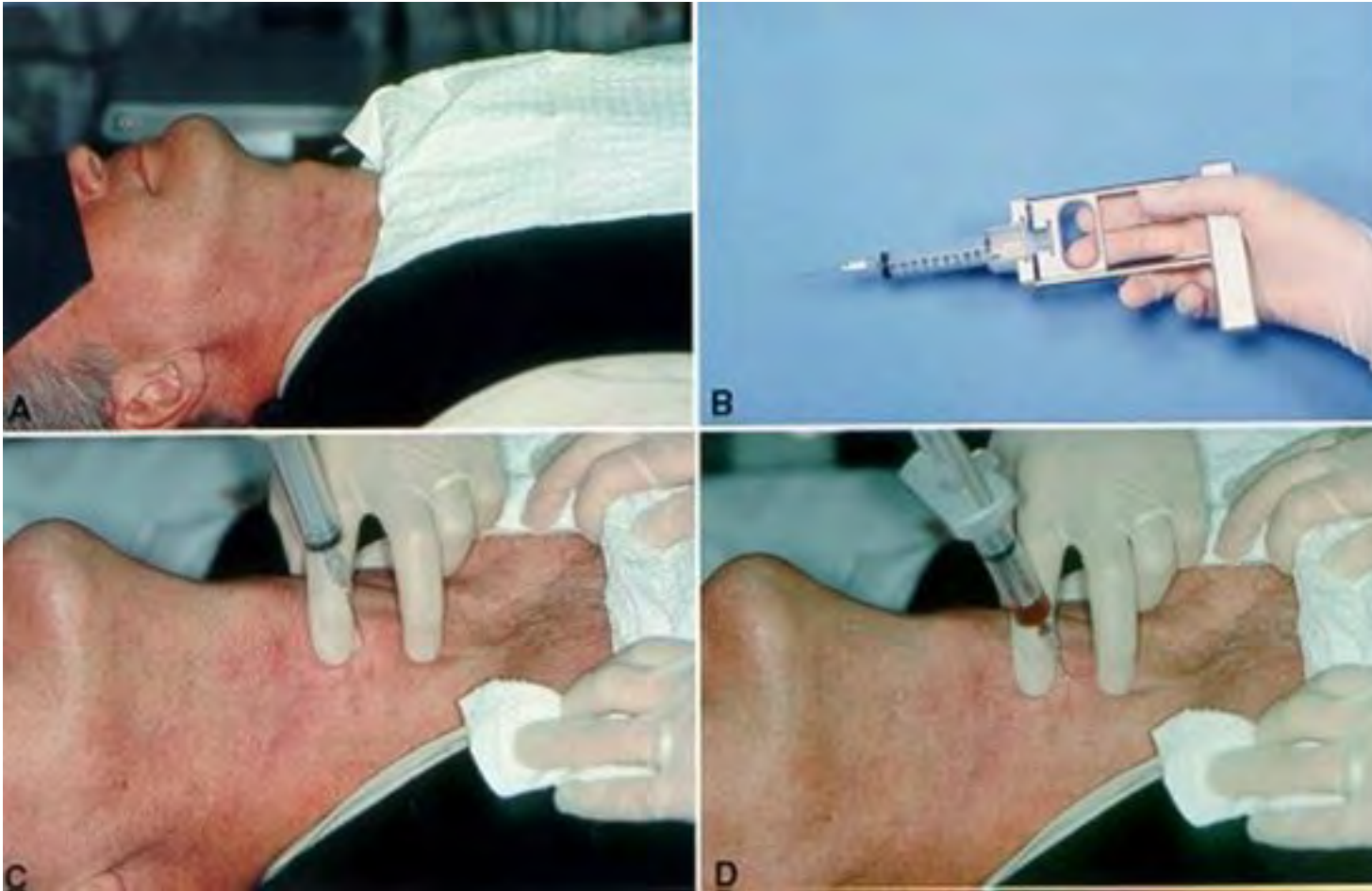
Allows the diagnosis of:

- early dysplastic processes (screening)
- neoplastic/pre-neoplastic lesions
- inflammations/infections

# Anatomic Pathology, part 1



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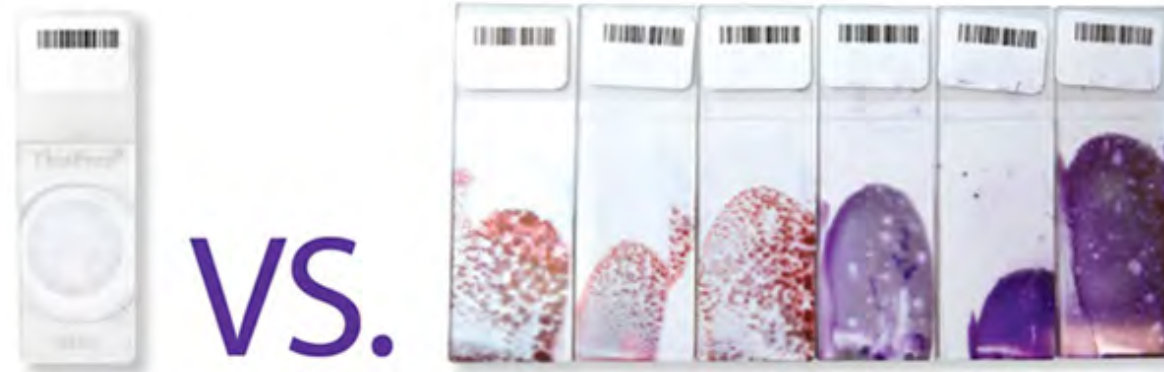




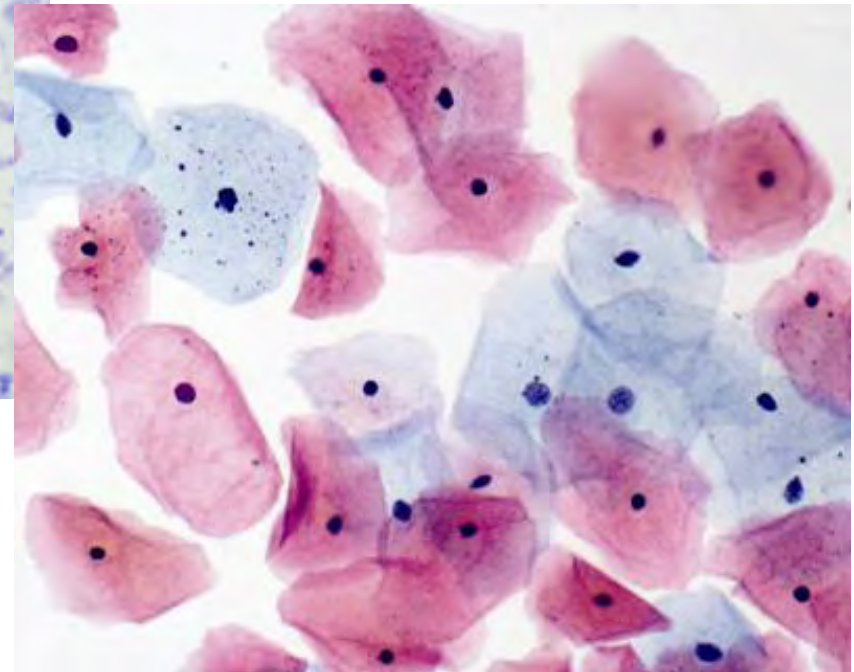
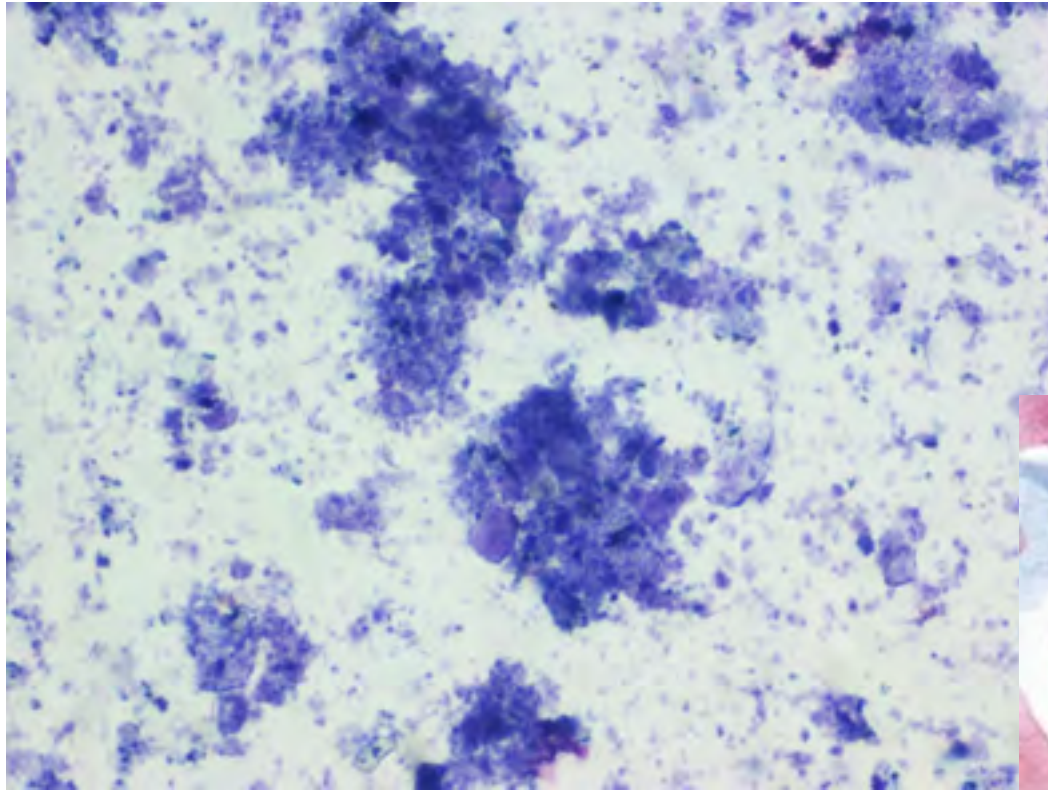
# Anatomic Pathology, part 1

Cytologic samples are processed quickly and easily:

- smearing
- centrifuging and smearing
- staining (**Papanicolaou**) and observed under the light microscope



# Anatomic Pathology, part 1



# Anatomic Pathology, part 1



- Histopathological samples:

- diagnostic biopsies

- surgical samples

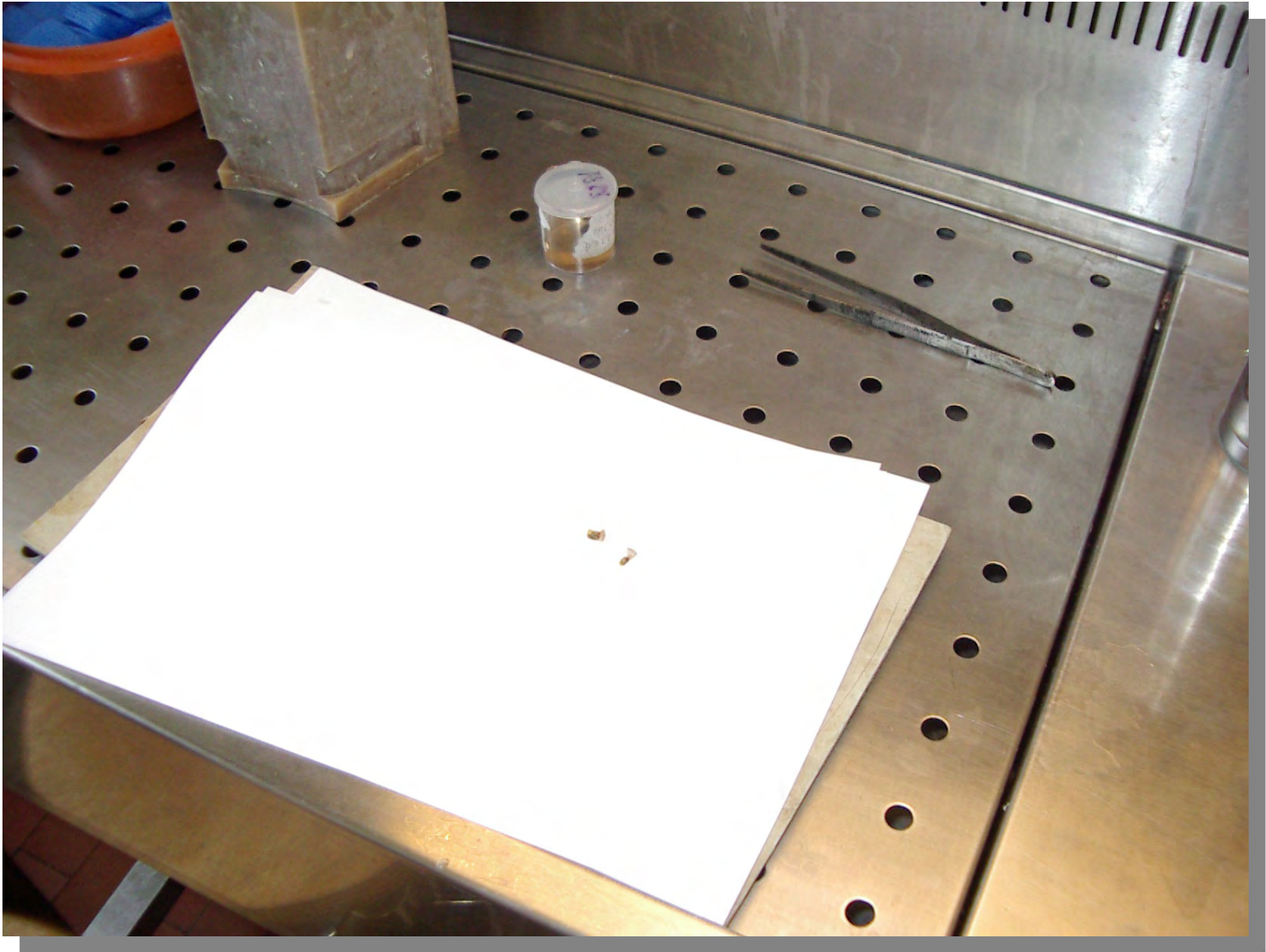
- Diagnostic biopsies allow histologic diagnoses

- small

- cylindrical (fine needle aspiration biopsy)

- only part of a lesion or an organ

Examples: renal and hepatic needle biopsies, samples from digestive endoscopy and bronchoscopy



# Anatomic Pathology, part 1



- Surgical specimens are larger >> whole organs
- The lesion is removed completely
  
- The anatomo-pathologic study is necessary for:
  - diagnosis
  - extension of the lesion and excision margins
  - prognosis
  - therapeutic strategies



WHITE FLUX

# Anatomic Pathology, part 1



- Identification and dispatch material
  - request for histologic or cytologic exam adequately filled in
  - appropriate container, correctly identified
  - appropriate fixative (type and quantity)
  - timely dispatch to the anatomic pathology labs



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 Direzione: tel. 080 5593338

RICHIESTA DI ESAME: ISTOLOGICO  CITOLOGICO  ESTEMPORANEO  CONSULENZA  ALTRO

MATERIALE DA ESAMINARE: .....

COGNOME E NOME:..... SESSO: M  F

DATA E LUOGO DI NASCITA .....

RESIDENZA:..... PROFESSIONE:..... TEL:.....

LIQUIDO FISSATORE USATO: A FRESCO  FORMALINA  ALCOOL  CYTOLYT  PRESERV-CYT

FISS. SPRAY  ALTRO

PRELEVATO DA:..... IN DATA:..... TELEFONO:.....

INVIATO DA:..... CDR..... DATA:..... TELEFONO.....

D.H.  RICOVERO  N° Cartella..... SAUB  INTRAMOENIA

**NOTIZIE CLINICHE:**

Esami istopatologici e Citologici Precedenti:.....

Terapia Praticata:..... chemioterapia ..... radioterapia .....

Compilato Modulo Allegato: Specificare.....

**REPERTO OBIETTIVO E OPERATORIO:**

Definizione Anatomica e Topografica del Materiale Inviato

**RISULTATO DEGLI ESAMI DI LABORATORIO E STRUMENTALI**

**DIAGNOSI CLINICA:**.....

Al sensi delle Leggi 675/1996 e 196/2003 autorizzo il trattamento dei dati sensibili e personali per gli scopi connessi con l'espletamento della procedura diagnostica..... Firma: .....

Accenso all'impiego del materiale biologico prelevato per usi di ricerca scientifica: SI  NO

ACCETTATO IL:..... DA:.....

**TIMBRO E FIRMA LEGGIBILE**

NB LA RICHIESTA VA COMPILATA IN STAMPATELLO IN OGNI SUA PARTE



# Check-in



- Registration:
  - ▣ Personal data (name, age, sex, occupation)
  - ▣ Privacy statement
- Payment (direct/indirect):
  - ▣ In-house (hospital)
  - ▣ Day-hospital
  - ▣ Outpatients (N.H.S. = S.S.N.)
  - ▣ Private practice

# Check-in

- Congruence
- Tacking of anomalies
- Pertinent clinical data
- Fixation time



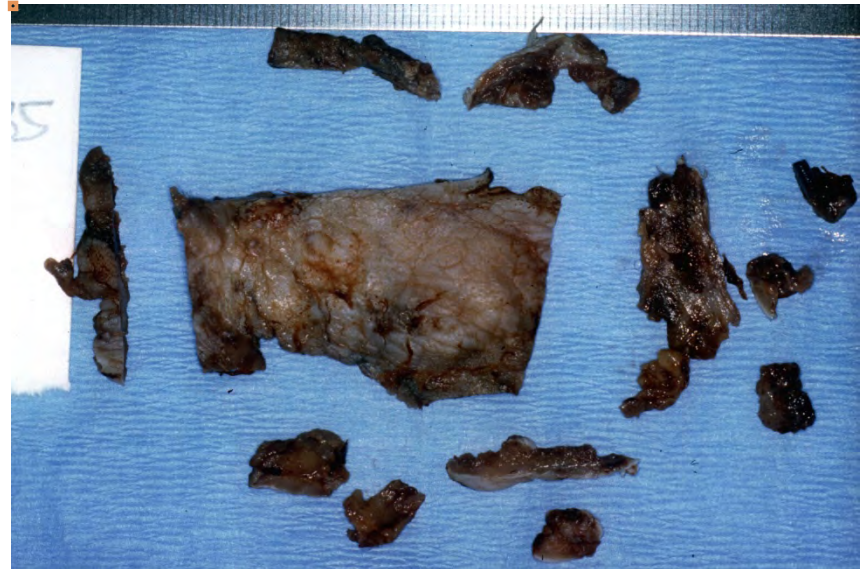
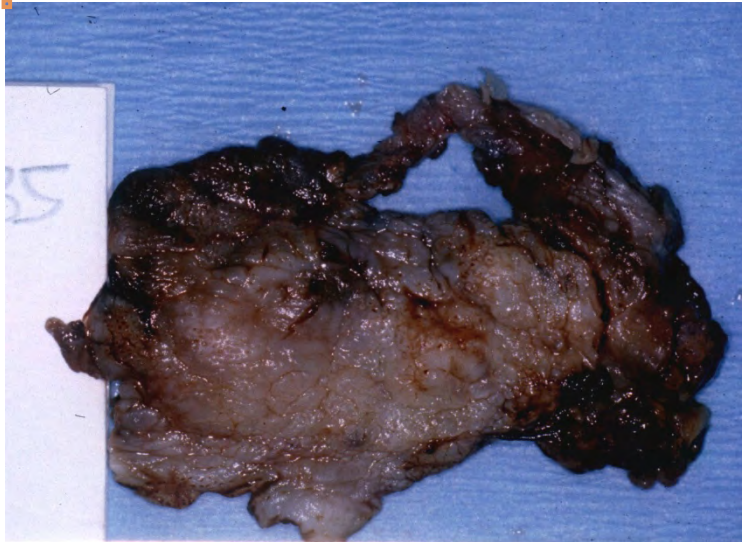


# Anatomic Pathology, part 1



- Macroscopic examination of samples aims at:
  - Exactly describing the type of material:
    - size & alterations
    - shape
    - colour
    - consistency
    - margins (china ink)
    - relations with adjacent structures
  - Selecting appropriate area for microscopic examination

# Sampling



# Sampling

- Cutting
- "Representative" tissue fragments

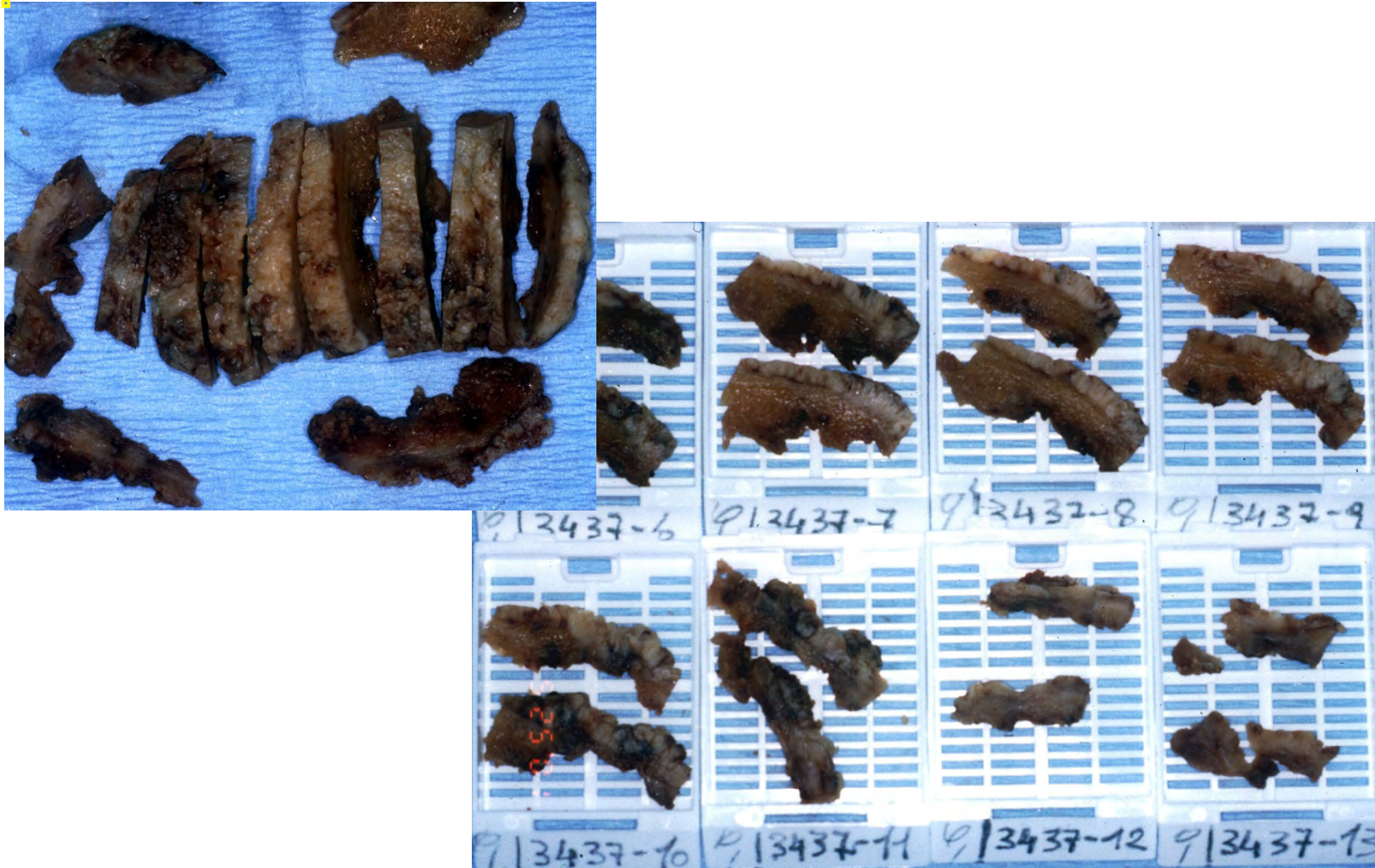


# Sampling

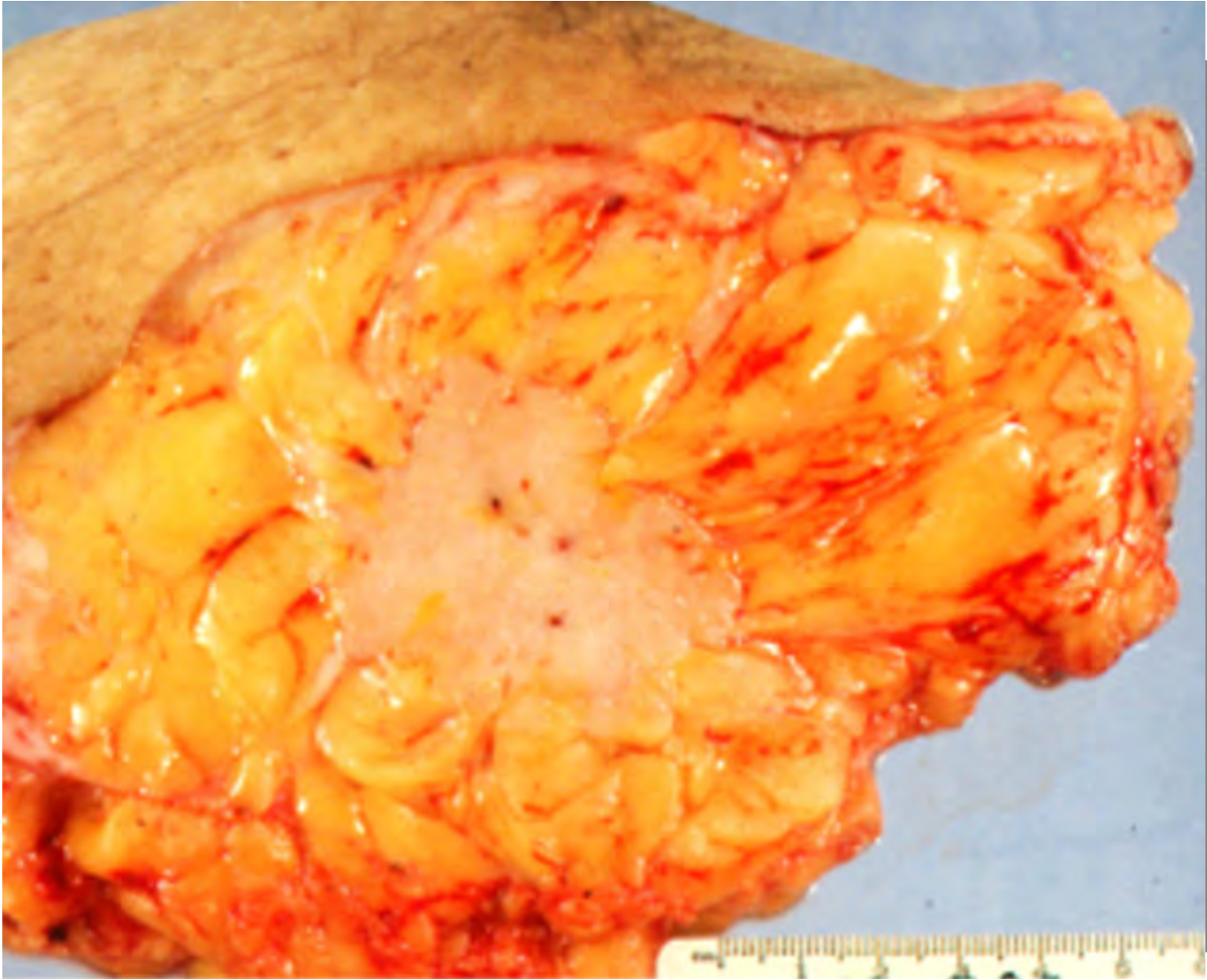
- Fragments allotted into cassettes
- Proper identification by numbers/letters



# Sampling







# Anatomic Pathology, part 1



- Processing tissues for observation with light microscope
  - tissue fixation
  - dehydration/embedding (paraffin)
  - inclusion
  - cutting
  - deparaffinization or rehydration
  - staining
  - coveslipping
  - observation under light microscope

# Anatomic Pathology, part 1



- Tissue fixation: interruption of degradation processes that start soon after cell death (autolysis and putrefaction)
  - preserving architecture
  - preserving cell composition
- Autolysis: cellular autodigestion by enzymes (rupture of lysosomal membranes)
- Putrefaction: bacterial superimposition on autolysis

# Anatomic Pathology, part 1



- Fixatives may be:
  - chemical
  - physical (freezing)
- Chemical fixatives: make tissue proteins insoluble and refractory to autolysis
  - Simple fixatives
  - Composed fixatives (fixative mixtures)
- Formalin: aqueous solution of 10% at pH 7

# Anatomic Pathology, part 1



- Sampling
- Dehydration
- Inclusion: paraffin wax
  - tissue solidity
  - preservation of architectural relationships
  - thin (3-4 $\mu$ m) regular and homogeneous sections



# Embedding

- Tissue samples are embedded in paraffin







# Anatomic Pathology, part 1



- Cut:

Microtome (sections 3-4 microns)

- Rehydration

- Staining: H&E

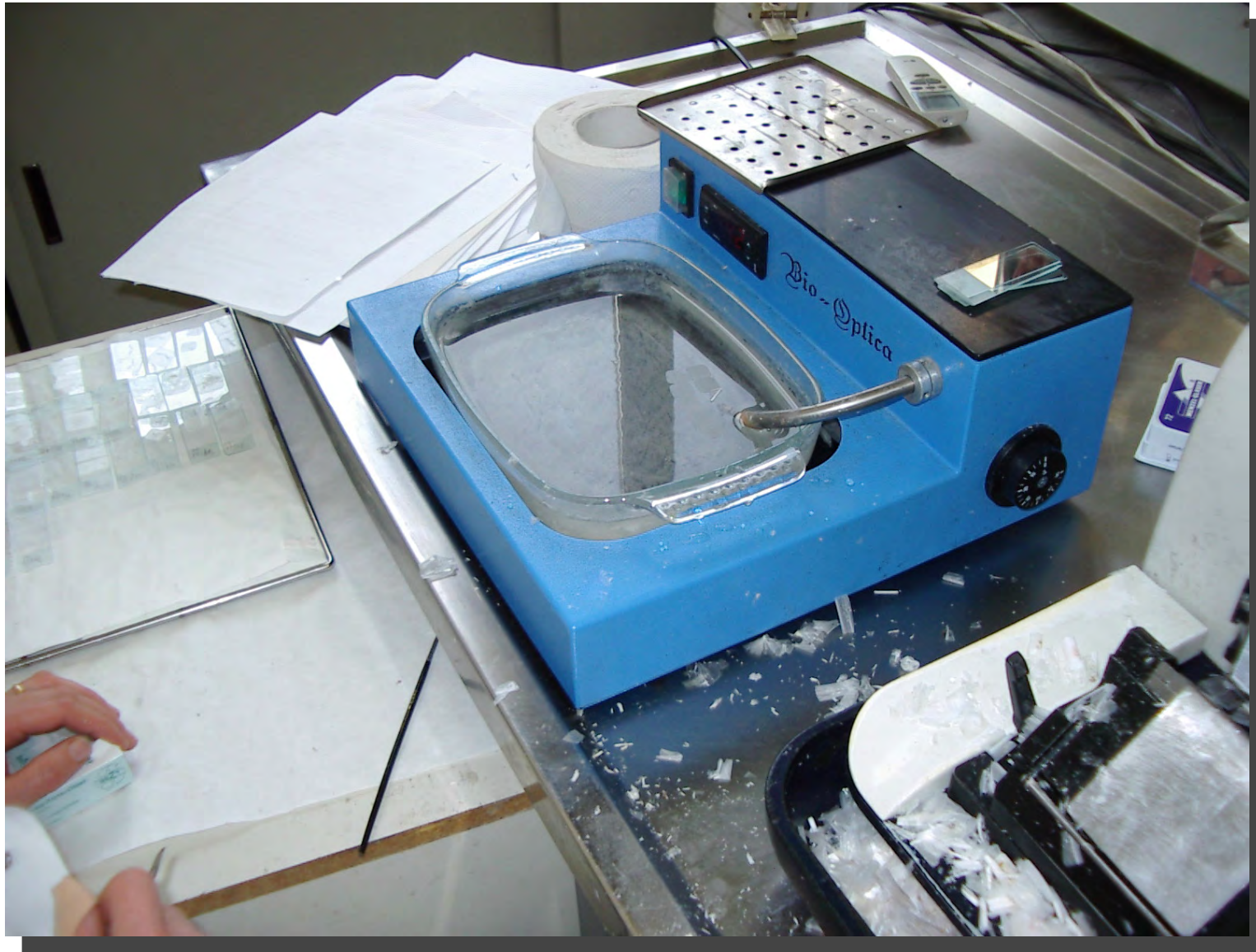
- Haematoxylin (nucleus) - Eosin (cytoplasm)

- Giemsa: blood cells; MGG; silver impregnation

- Coverslipping: synthetic resin

- Observation under a light microscope

- Histological diagnosis



# Cutting

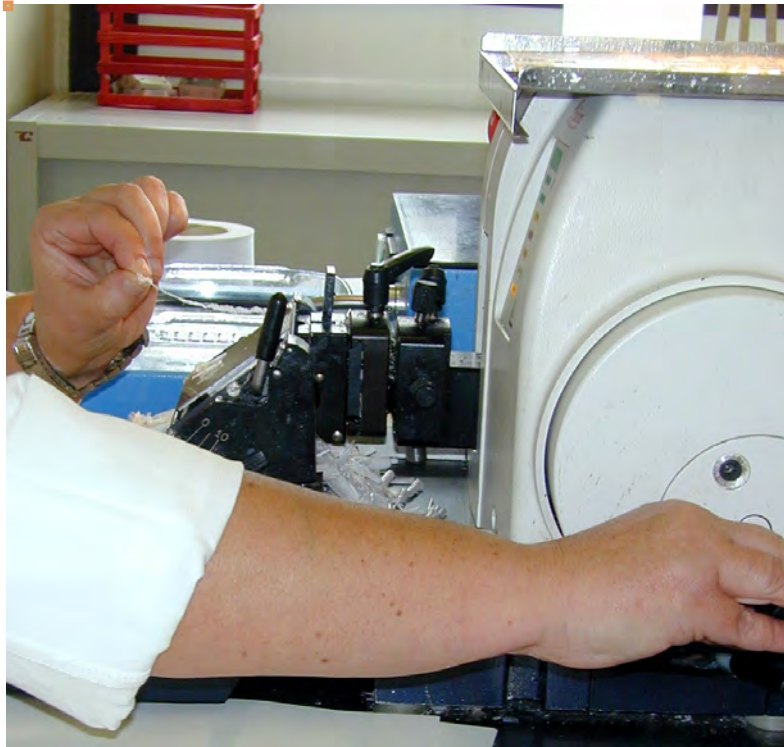
- Thin sections (3-5  $\mu\text{m}$ ) adhered to holding glass slides



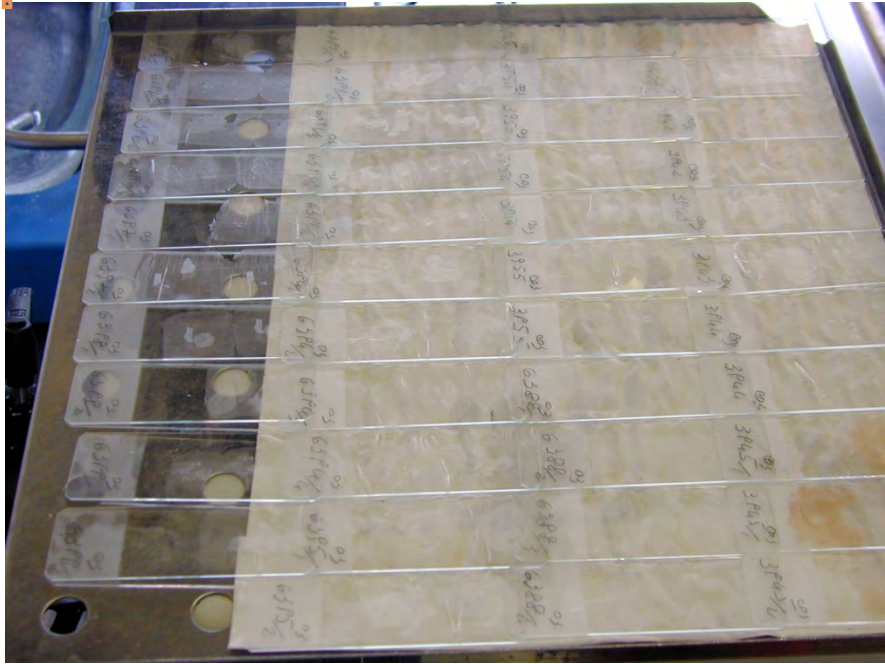
Microtome / seriotome



# Cutting



# Cutting



# Staining



# Staining



# Coverslipping



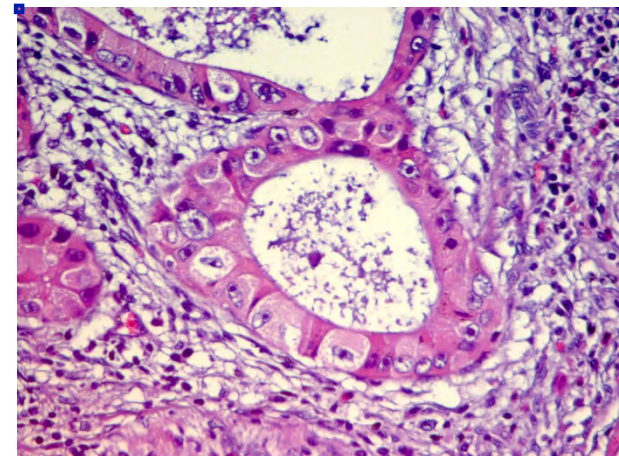
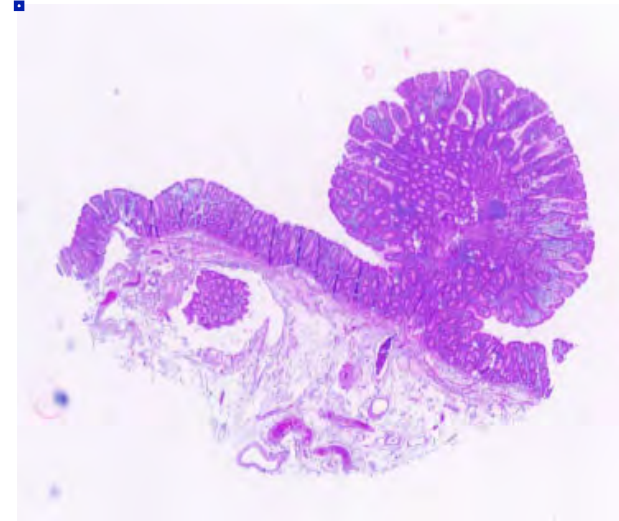


# Anatomic Pathology, part 1

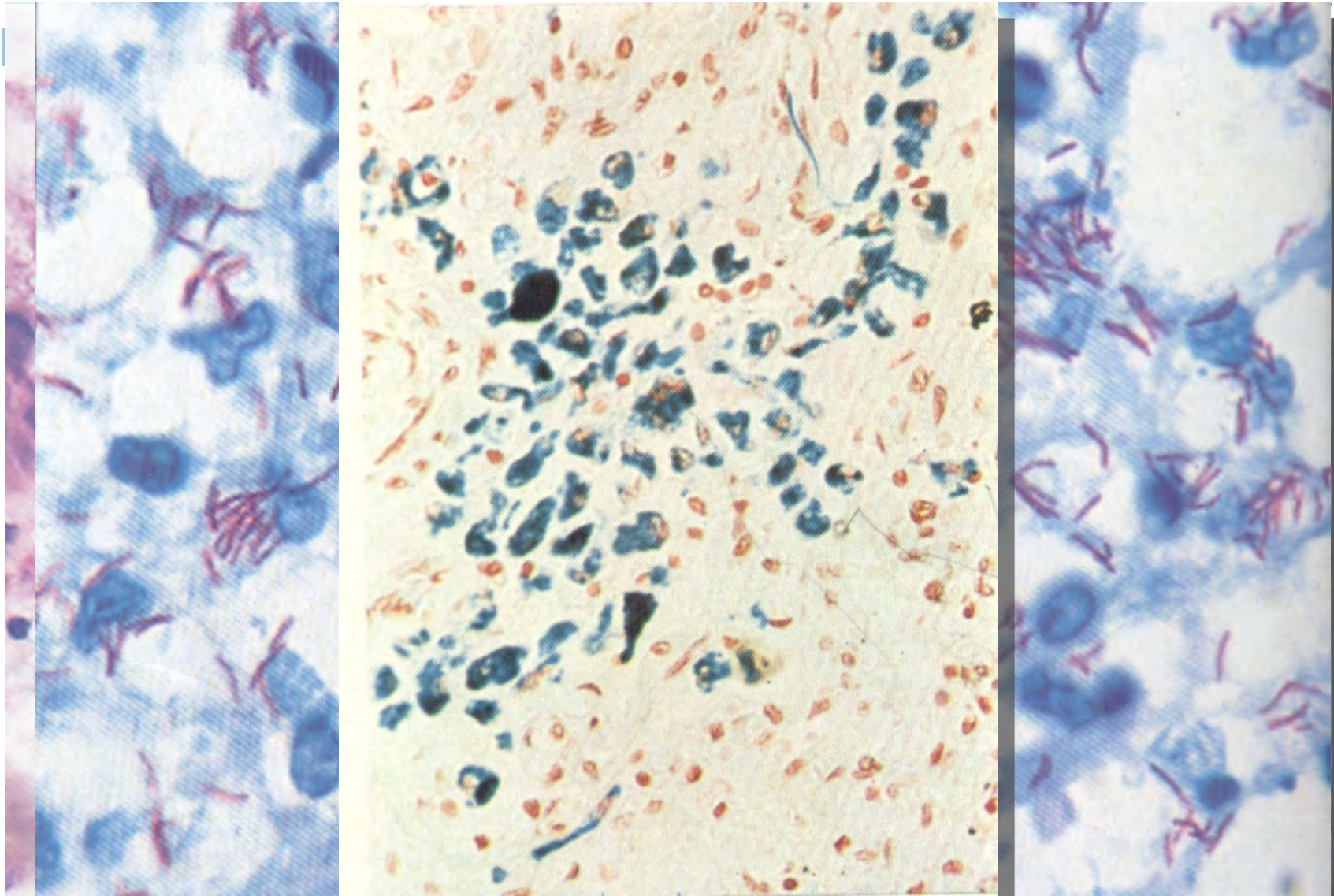
- Anatomic-pathology diagnosis uses:
  - histological examination** (morphological examination N/C)
  - histochemical methods** (chemicals linked to tissues): PAS, Sudan, Perls ....
  - immunohistochemical methods** (Ag/Ab reaction highlighted by a chromogen, studying tissue and cellular antigens)
  - electronic microscopy** (observation of subcellular structures on TEM, external morphology and molecular composition on SEM) semi-thin or ultra-thin sections
  - cytometry and flow cytometry** (based on image analyzers)
  - molecular biology** (genetic study of diseases)

# Examination and reporting

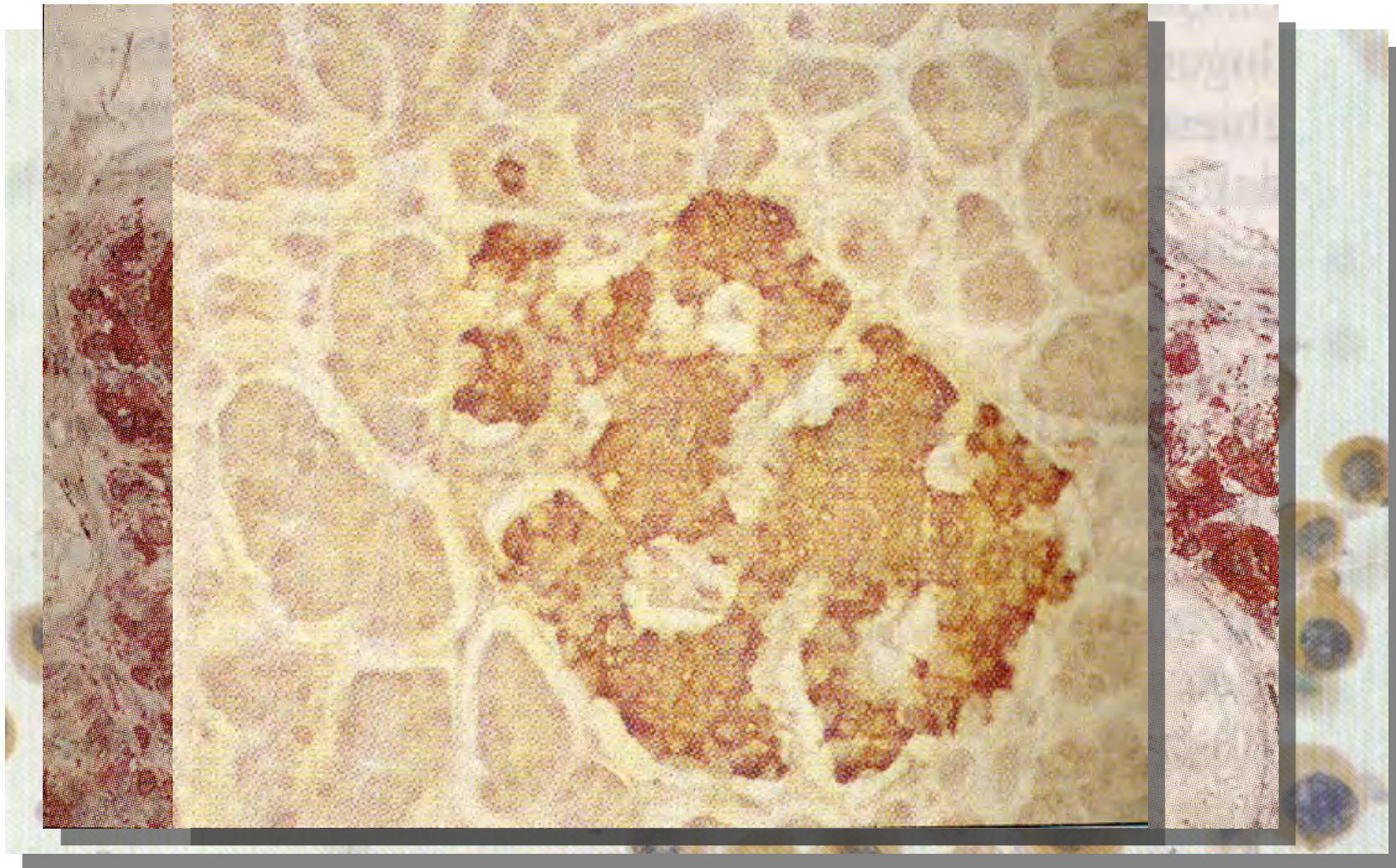
- Collection of slides
- Microscopic examination
- Additional stains
  - ▣ Histochemical
  - ▣ immunohistochemical
  - ▣ Molecular hybridization
- Report



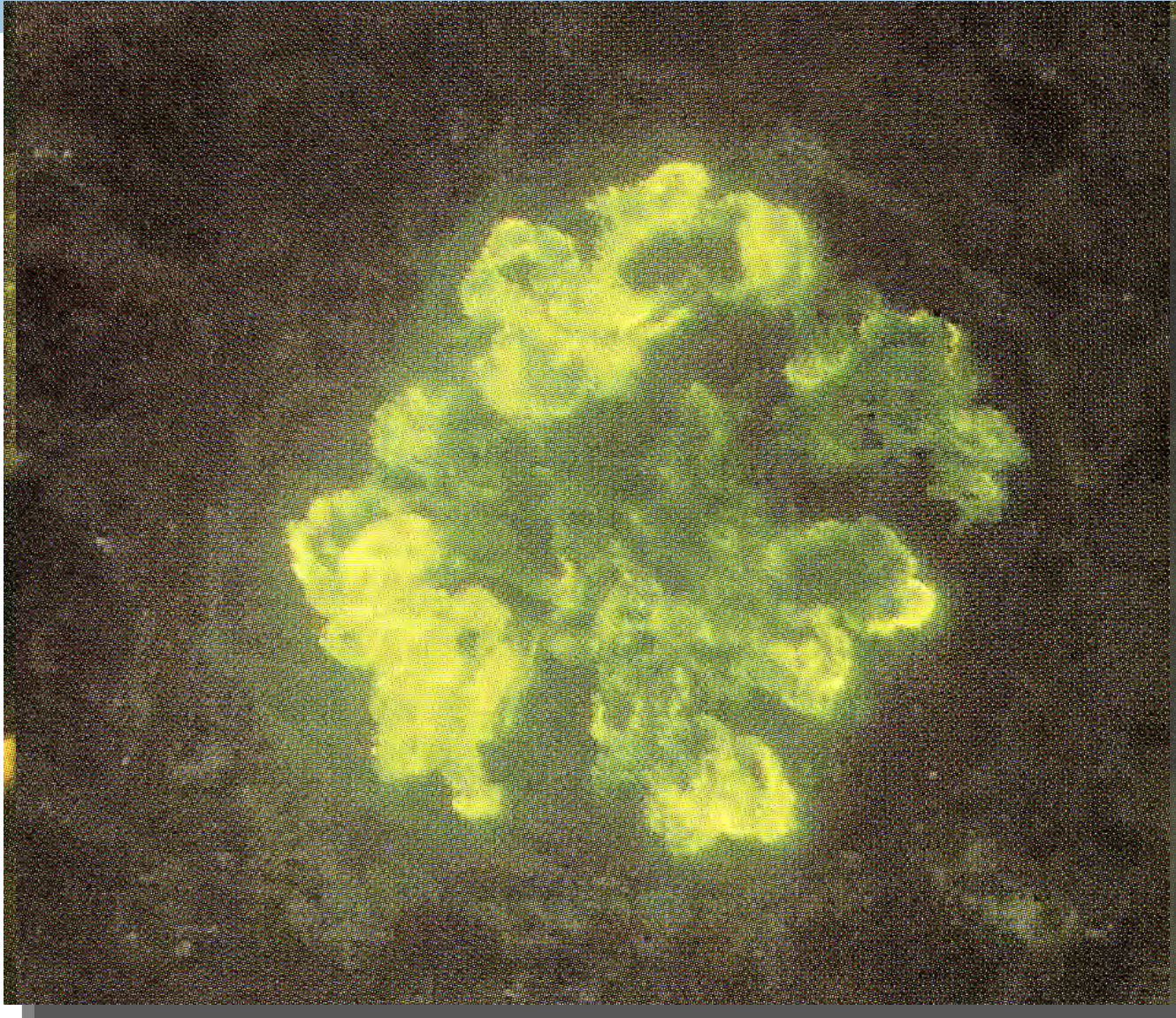
# Stains



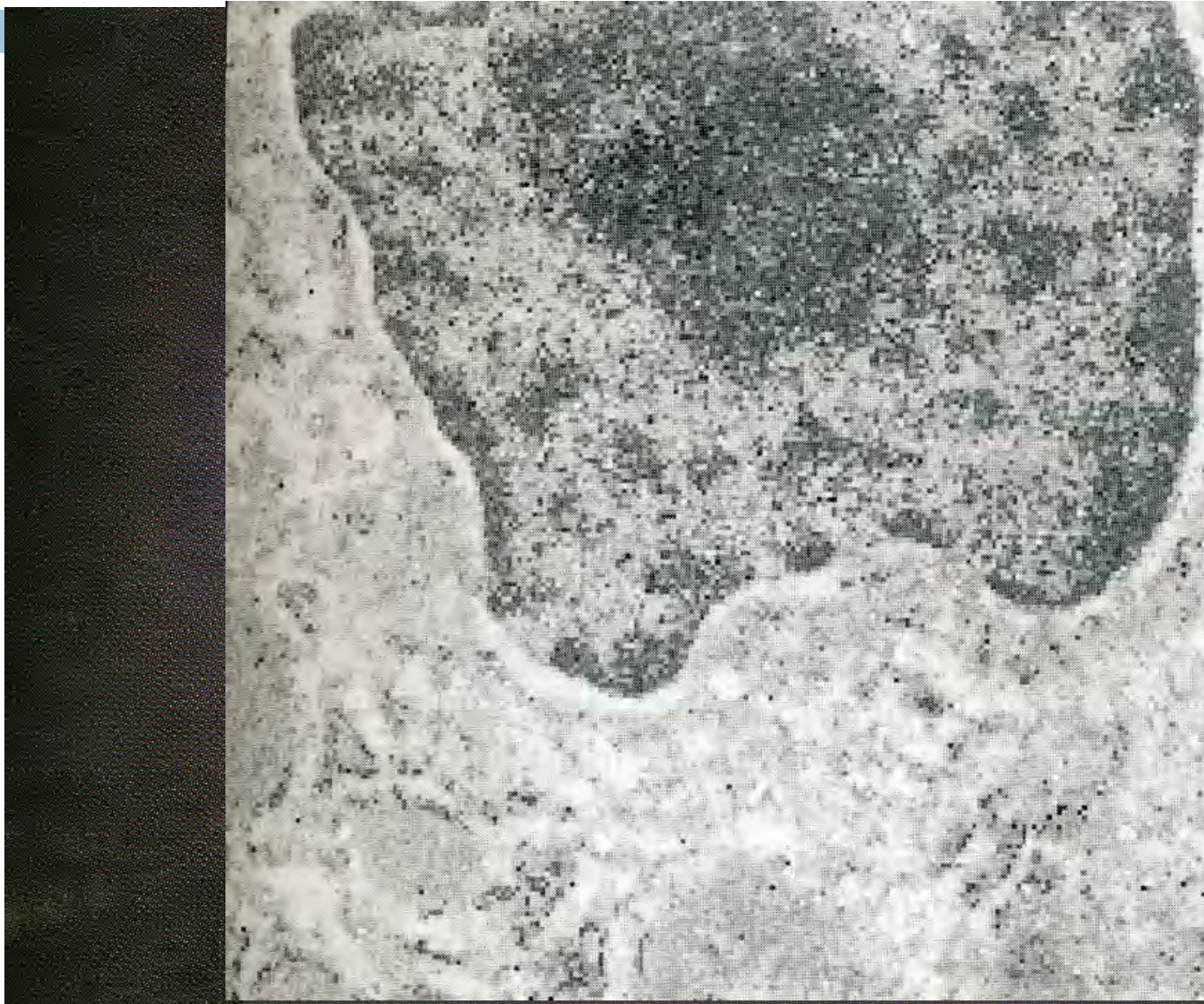
# Immunohistochemistry



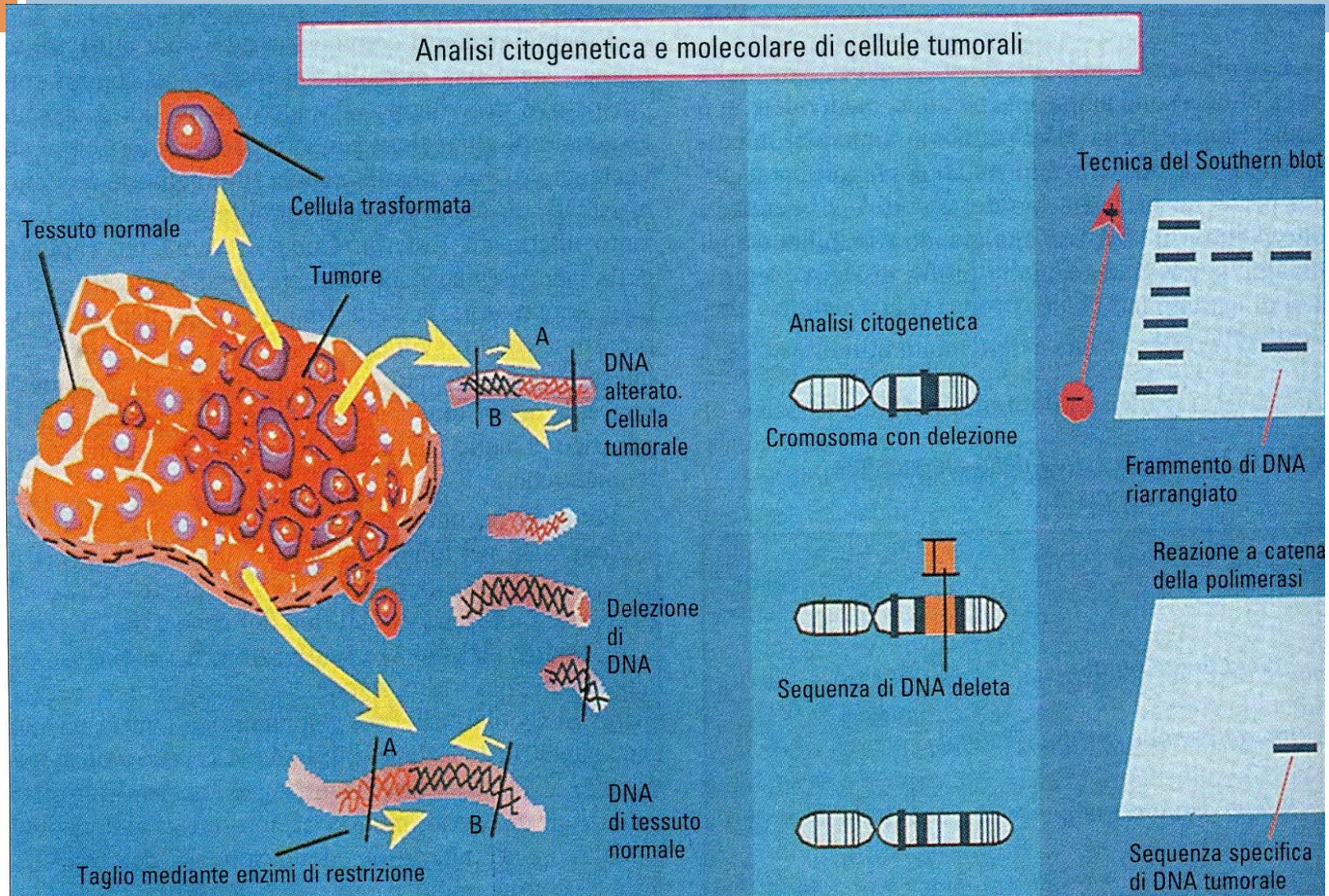
# Fluorescence



# Electronic microscopy



# Molecular biology



# Anatomic Pathology, part 1



- Intraoperative examination (frozen sections):  
histological exam required by the surgeon during a  
operation, which could modify the surgical  
approach:
  - neoplastic lymph nodes
  - margins of surgical resection
  - sample suitability (adequate cellularity)
  - confirmation of diagnostic suspicion



# Anatomic Pathology, part 1



□ Surgical samples will be:

-frozen (cryopreserved)

-sectioned by a cryostat

-stained with H&E and observed under a light microscope (OM)

Diagnosis is achieved in 70-80% of cases, may be incomplete (no grade, no stage) or only partially reflect what found on permanent sections (better morphological preservation, additional sampling, etc.)