INTRODUCTION TO PATHOLOGICAL TECHNIQUES

1. Types of routine biopsy procedures
2. Special exams (IHC, FISH)

Biopsy-Indications

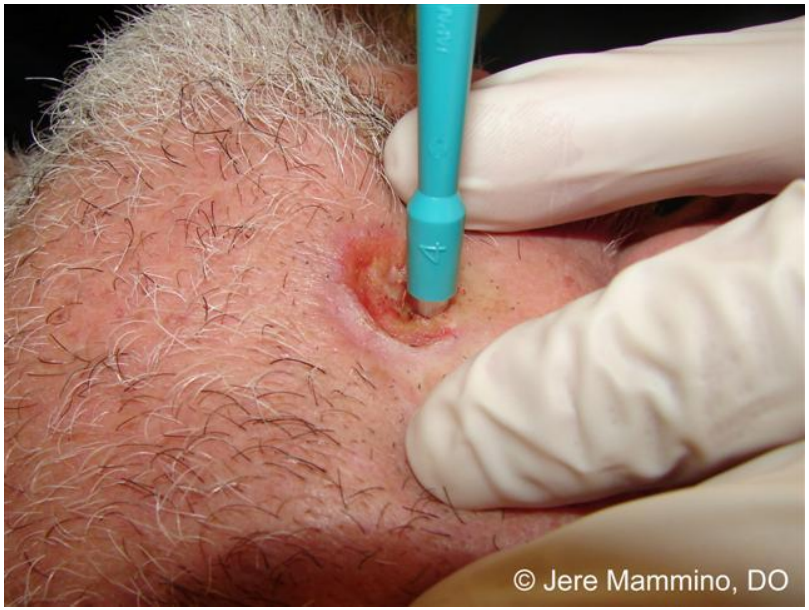
- Diffuse/multifocal lesions (neoplastic, inflammatory, etc)
  - Etiology of the disease
  - Evaluation of tumor characteristics for systemic treatment planning
- Solitary lesions (generally neoplastic)
  - Etiology, dignity assessment
  - Evaluation before surgery

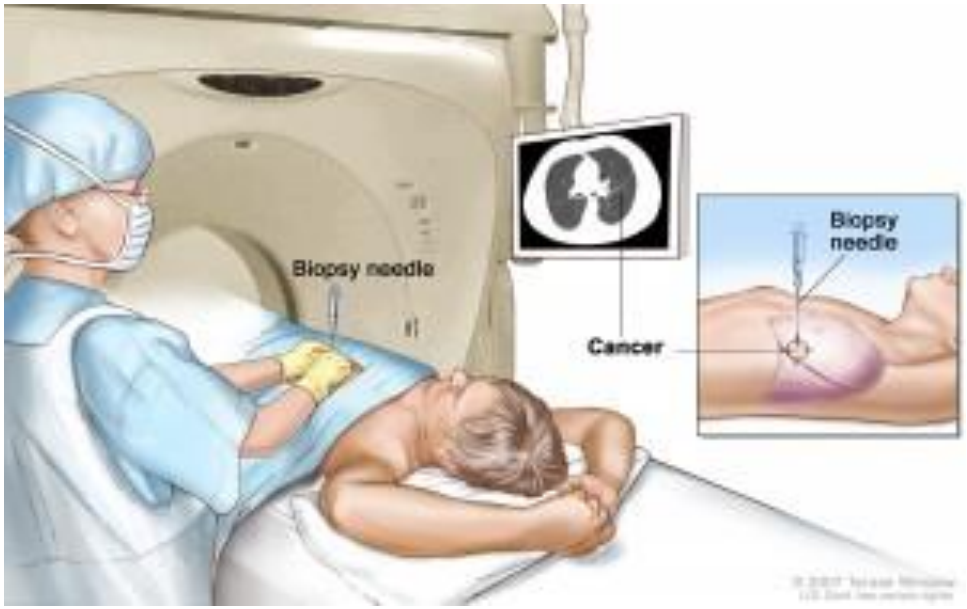
Biopsy Types

- Cytology sampling
  1. Exfoliative (brush)
  2. Liquid
  3. Fine needle aspiration
- Tissue sampling
  1. By excision (direct, open surgical, video-assisted)
  2. Core needle biopsy
  3. By endoscopy

Biopsy-Guidance

- Visual
  - Superficial localization, body cavities, hollow organs
- By imaging (US, CT, MRI)
  - Deep localization





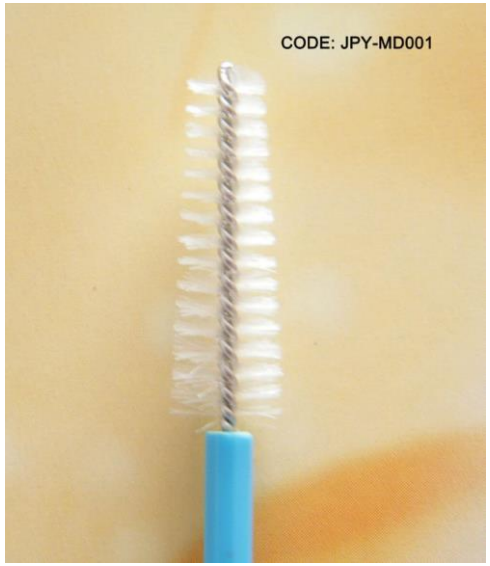
Cytology sampling

- Result: SMEAR= cell samples spread on a glass slide
  - Cellular elements: from the lesion and surrounding tissue
  - „Background”: blood, inflammatory cells, extracellular substance (mucus, colloid etc)
- Fast results (bedside diagnosis)
- Sample processing:
  - Wet fixation(alcohol)+staining (HE, Papanicolaou): preserved cellular morphology
  - Air drying+staining (Giemsa, Diff-Quik): fast and simple but alters cellular morphology

Cytology sampling- types

Exfoliative cytology (brush)

- Superficial lesions of hollow organs =intraepitelial or invasive tumors (cervix, small bronchus, biliary duct system)
- Cellular components: numerous normal/reactive epithelial cells>generally relatively few abnormal cells
- Limitations
  - Reactive or malignant?
  - Dysplasia or invasive tumor?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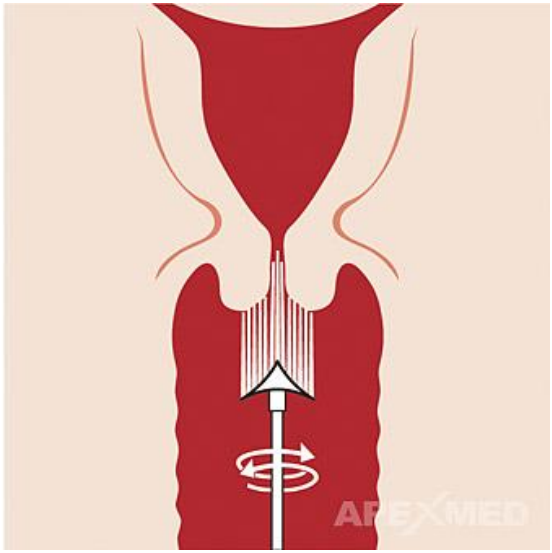
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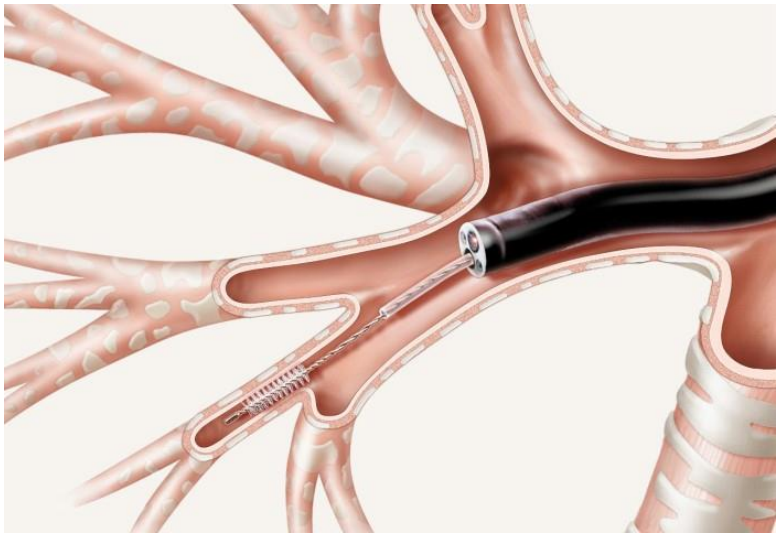
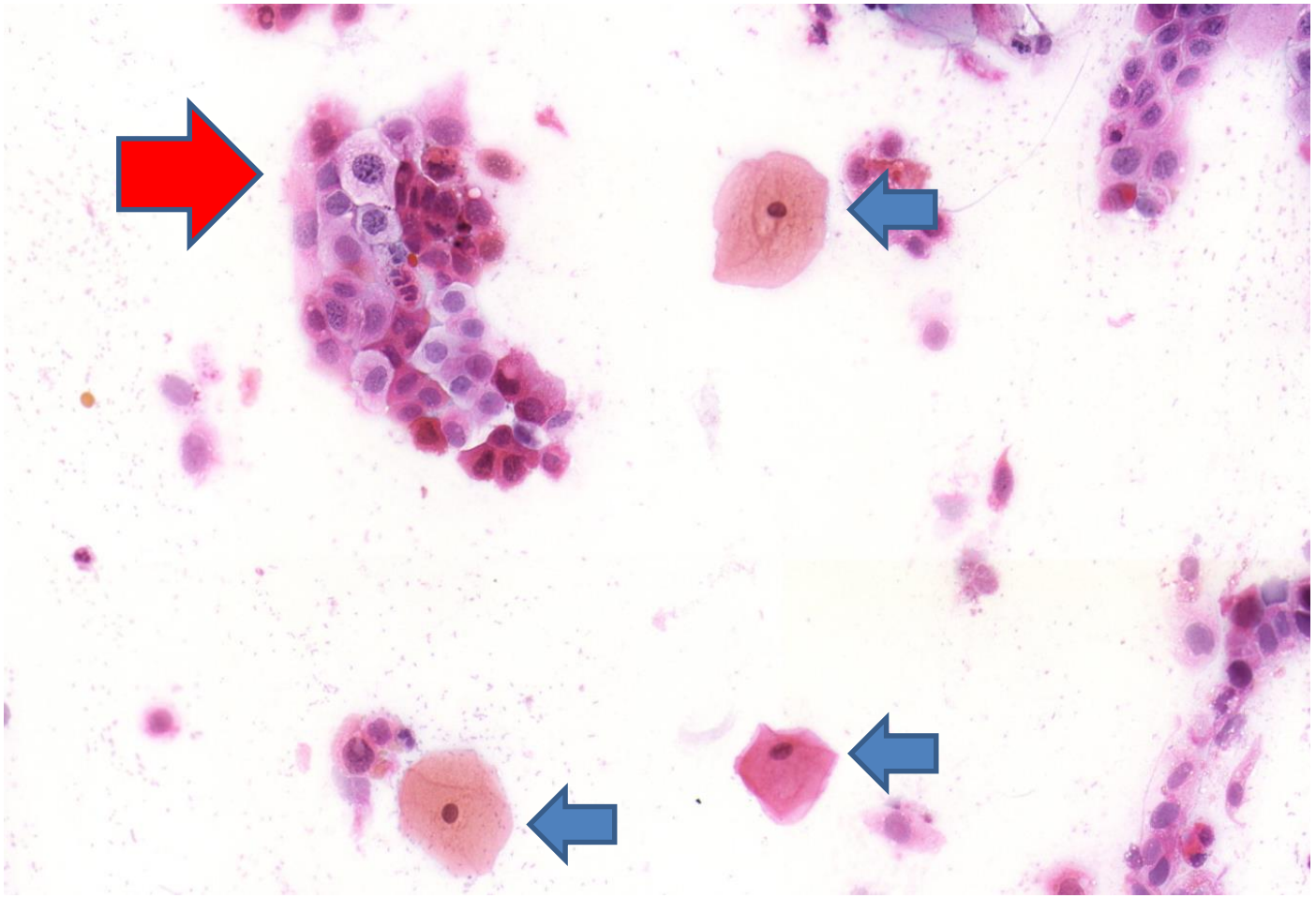


Pap smear

Normal superficial cells: blue arrow

Atypical cells (HSIL): red arrow



Cytology Sampling - Types

Cytology of Liquids

- Body cavity effusions of neoplastic or of inflammatory origin, cyst content, other fluids than blood (e.g. peritoneal, pleural, pericardial, urine)
- Cellular components
  - Numerous normal/reactive mesothelial or epithelial cells altered by liquid environment
  - Numerous inflammatory cells (neutrophils, histiocytes)
- Limitations
  - Reactive or malignant?

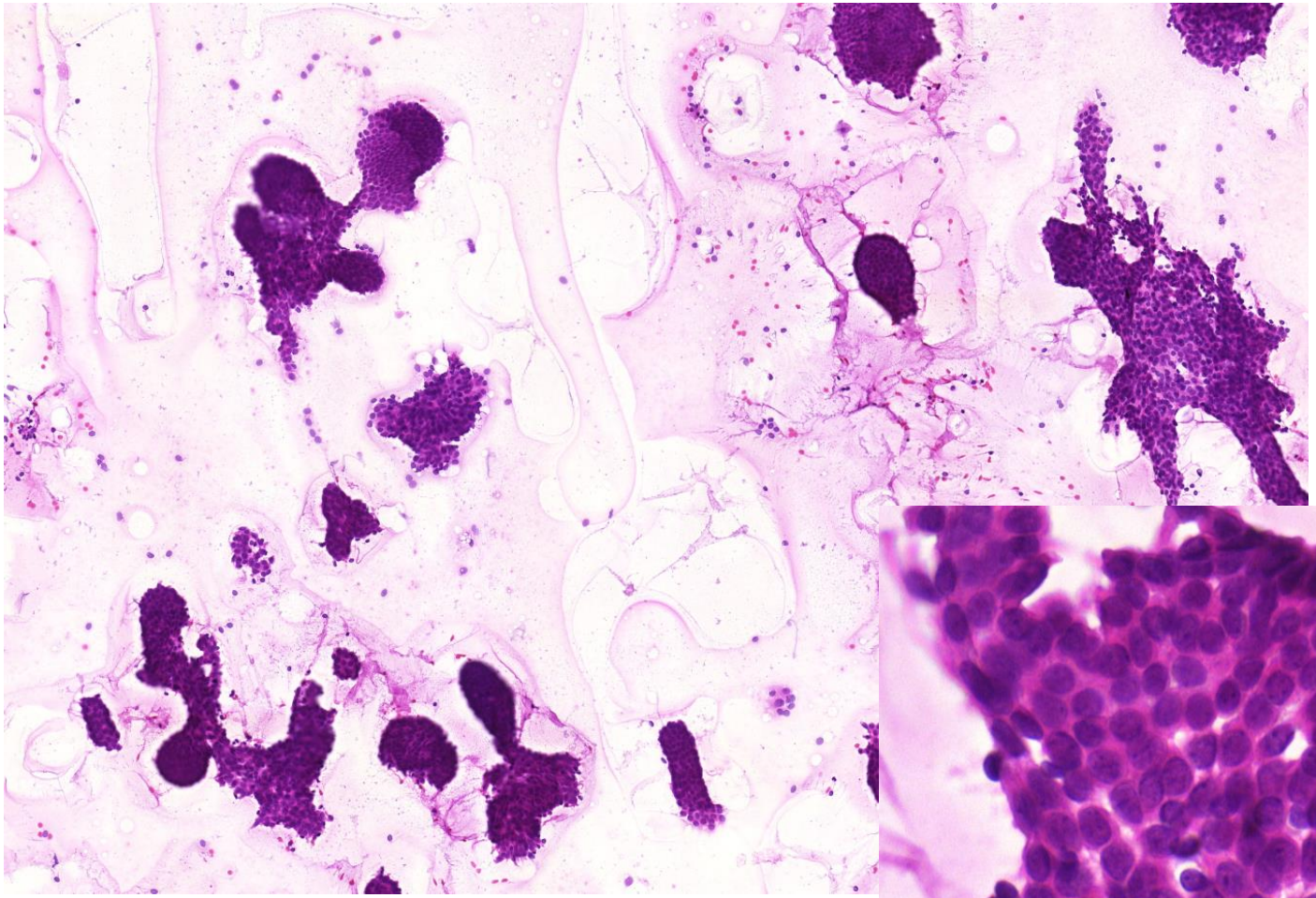
Cytology Sampling- Types

Fine needle aspiration (FNA)

- Solitary/multifocal solid lesions
- Cellular components
  - Tumor cells mainly (in case of a neoplastic process)
  - Surrounding tissue cellular elements in varying proportion
  - Contamination from needle track
- Limitations
  - Sample not representative (missed targeting, necrosis, etc.)

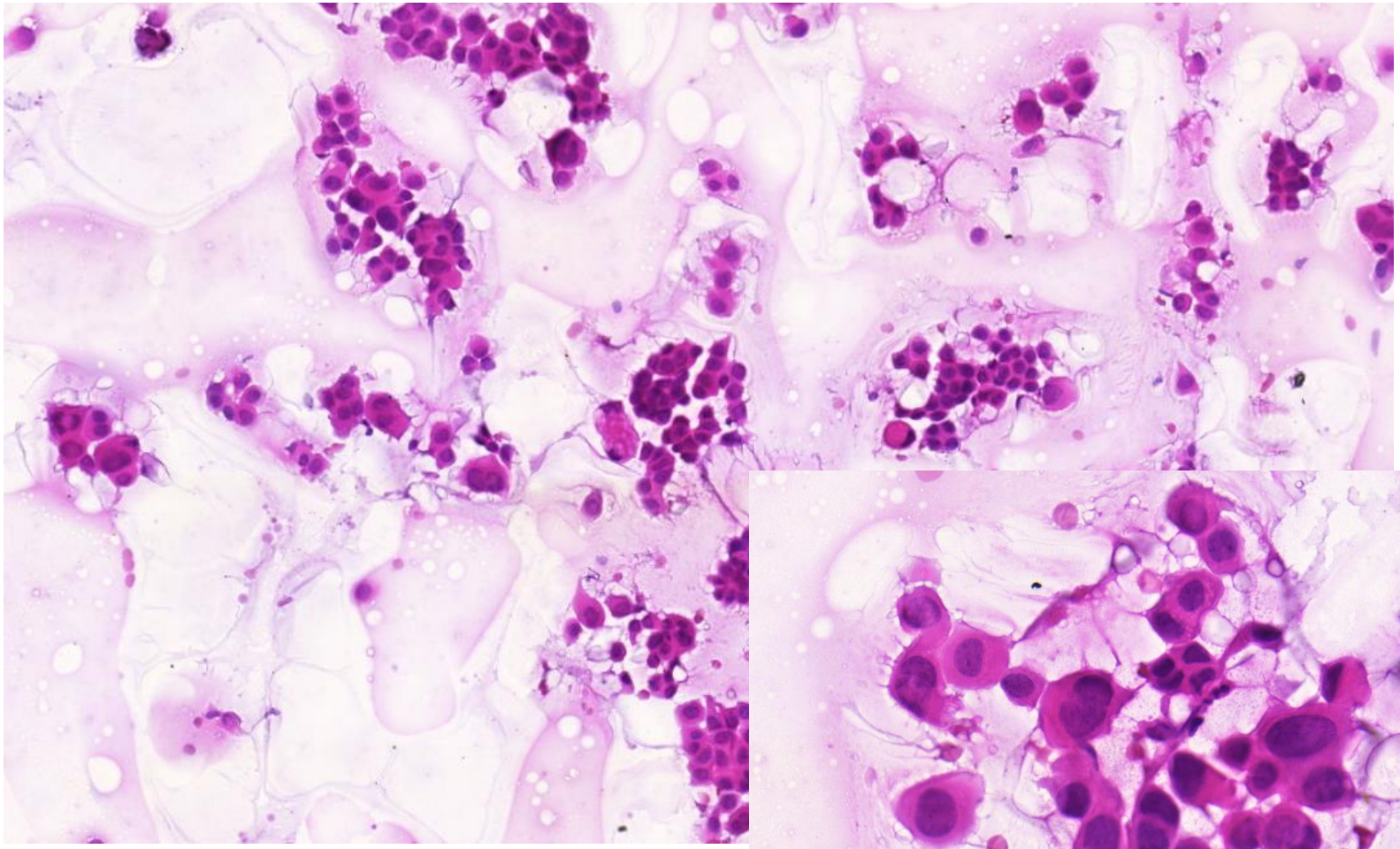
FNA: benign tumor (breast fibroadenoma)

- well formed glandular and cohesive cell nests
- no atypia
- only tumor - normal breast tissue is not represented



FNA: malignant tumor (invasive breast carcinoma)

- Discohesive cells nests – single cells
- significant atypia
- only tumor - normal breast tissue is not represented



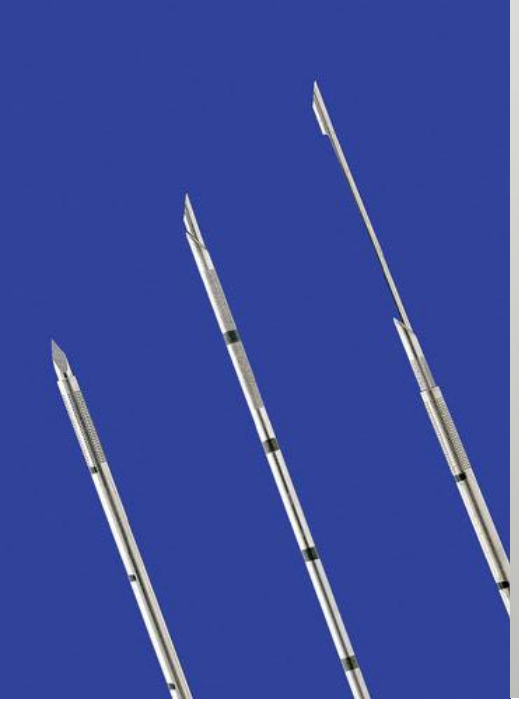
Tissue Sampling

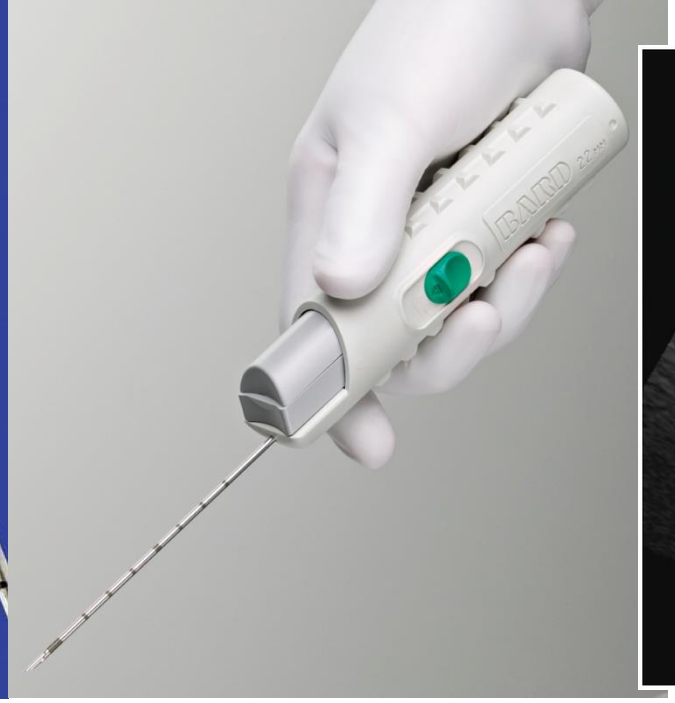
- Result: SLIDE
- Time consuming (min. 24 hours-2 days)
- Formalin fixation
  - EXCEPT:
    - fresh sample from skin or kidney sent to pathology without delay! (immunofluorescent microscopy)
    - lymphomas (ideally fresh frozen sample for molecular techniques)

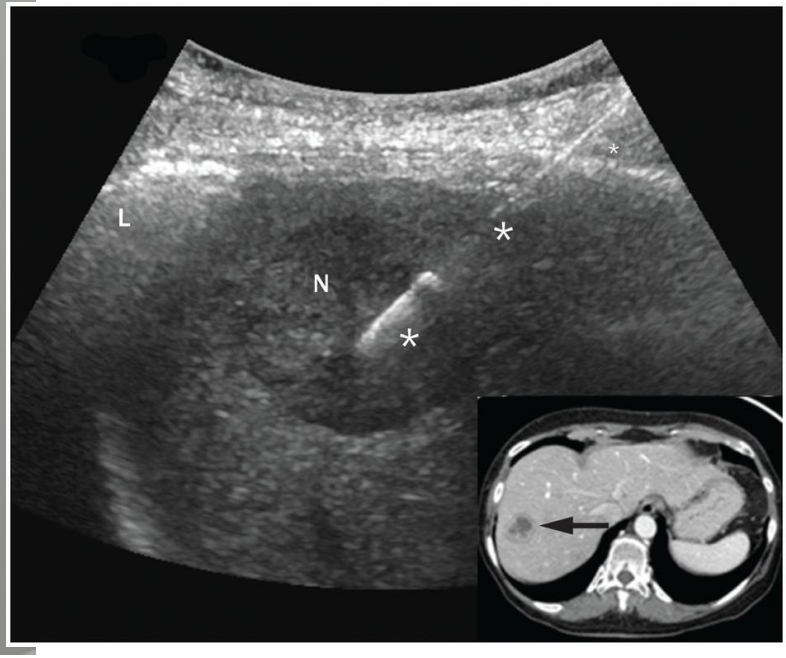
Tissue Sampling Types

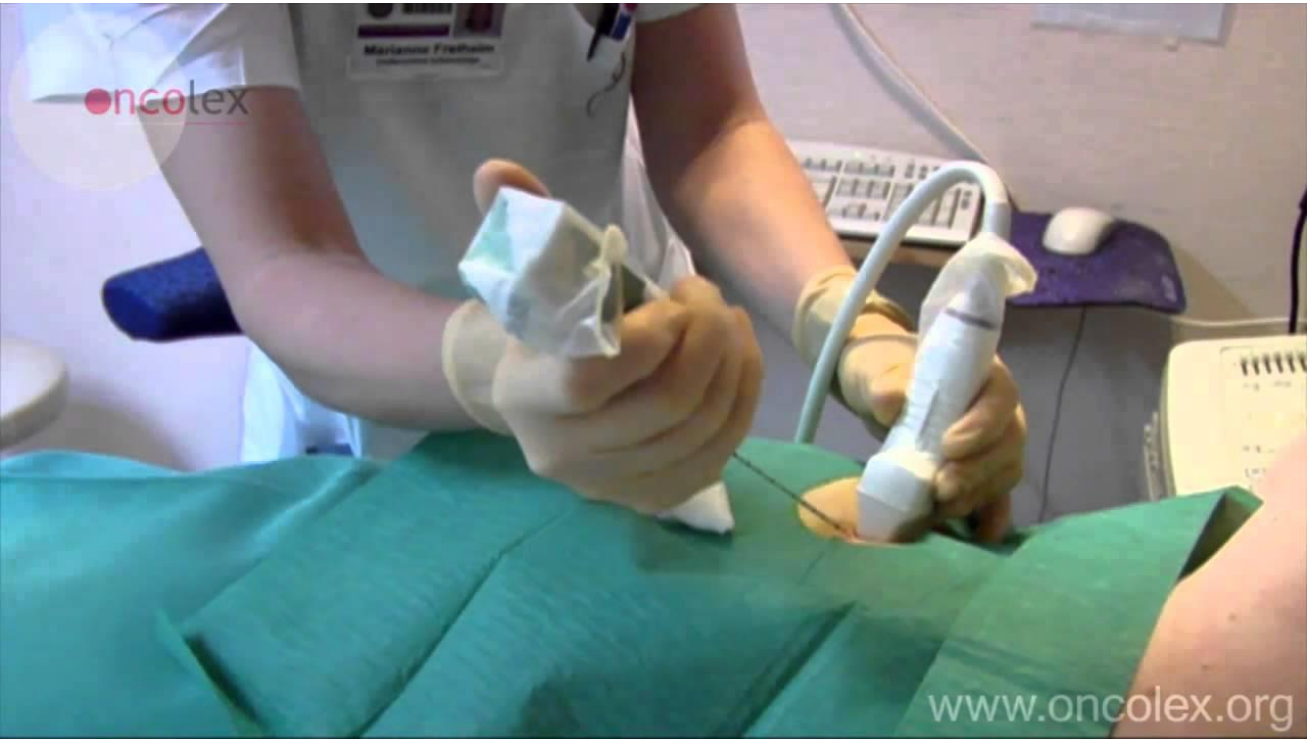
Core needle biopsy

- Focal lesion (solitary or multifocal), solid organs – may be alternative/ancillary to cytology
- Diffuse lesions in solid parenchymal organs leading to structural alterations (e.g. glomerular diseases, diffuse hepatic lesions)
- Targeting: US, CT, MRI, stereotactic







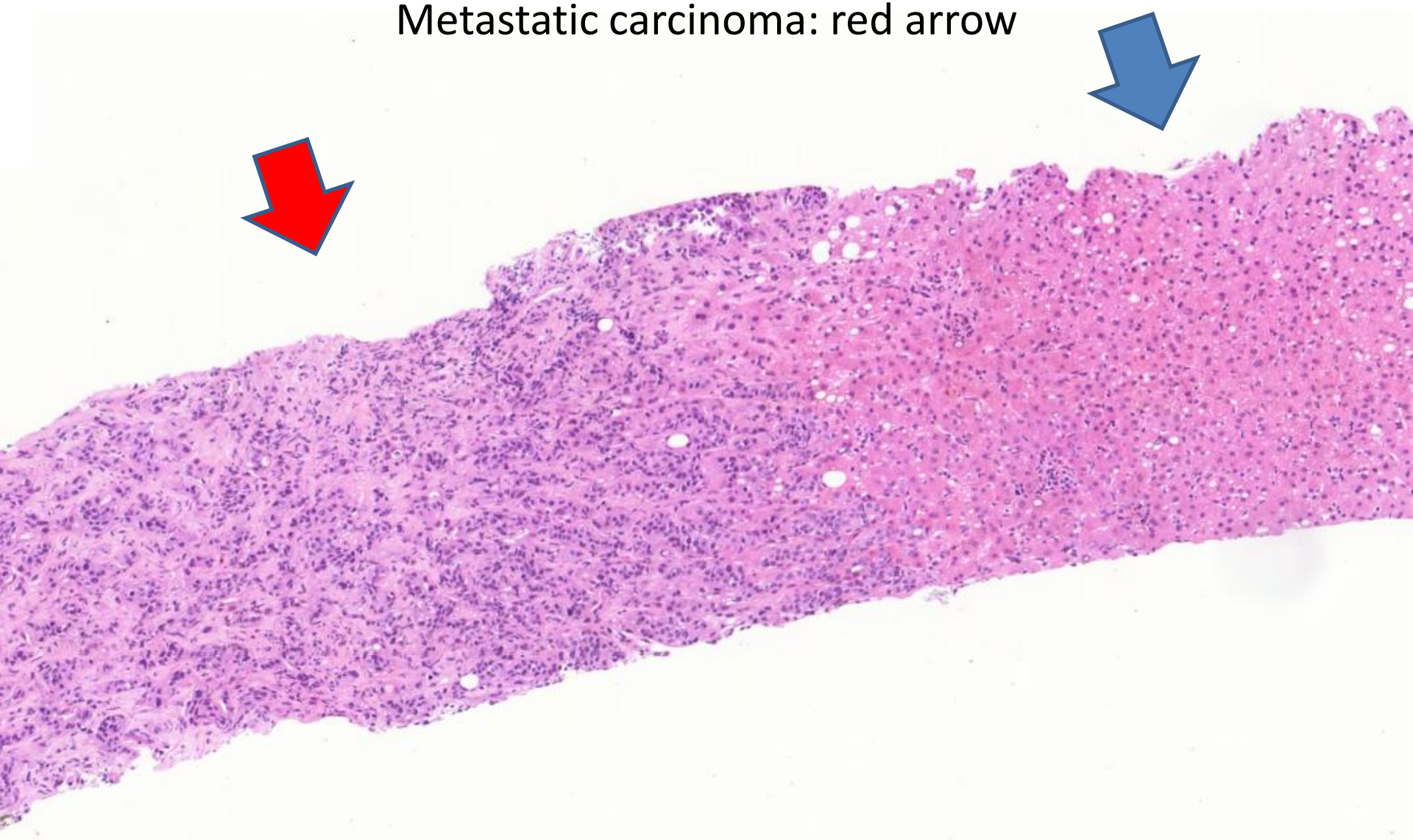


A core needle biopsy allows more tissue to be removed from the breast. This allows the pathologist to give a histological diagnosis as against a cytological diagnosis obtained by FNAC

CNB: liver metastasis

Normal liver: blue arrow

Metastatic carcinoma: red arrow



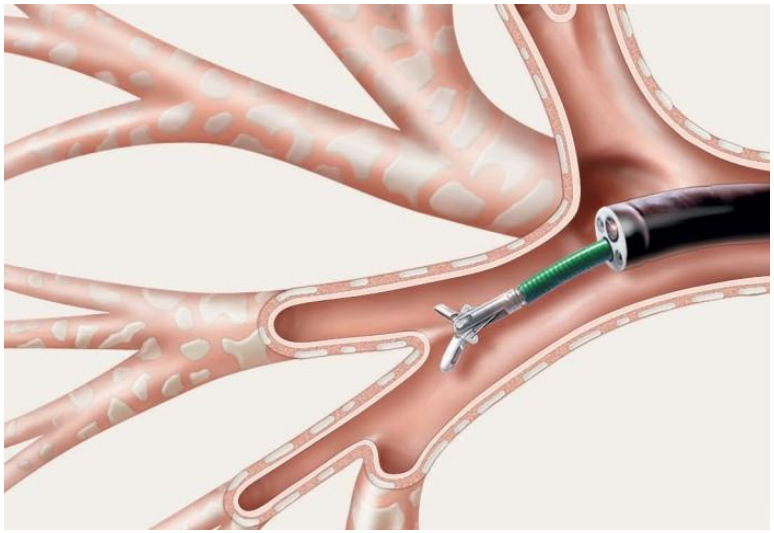
Tissue Sampling Types

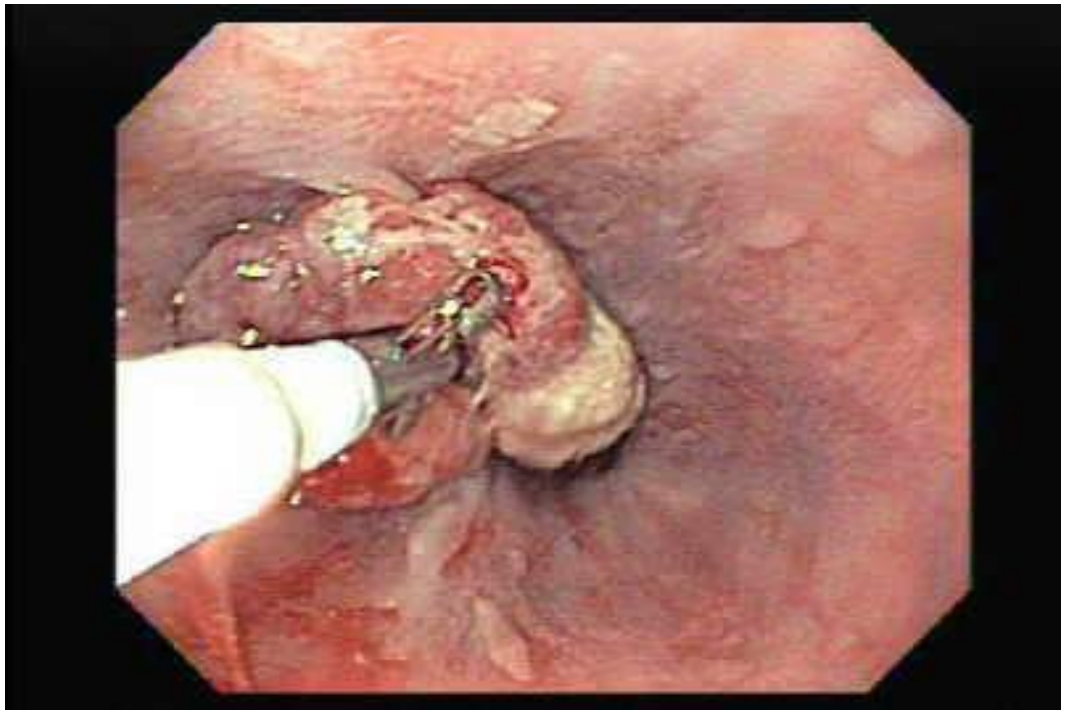
Biopsy by endoscopy

- Gastroscopy (esophagus-duodenum)
- Colonoscopy (terminal ileum-anus)
- Laryngoscopy (pharynx-larynx)
- Bronchoscopy (trachea-large bronchi)
- Cystoscopy

- Focal lesions (tumor): 2-3 representative samples, from the periphery or surface of the lesion, not from necrosis!
- Diffuse lesions (gastritis, IBD): map biopsy
  - Ideal biopsy: representative= also includes muscularis mucosae, fixation on a flat surface=better orientation of the specimen while processing…


mako
forceps

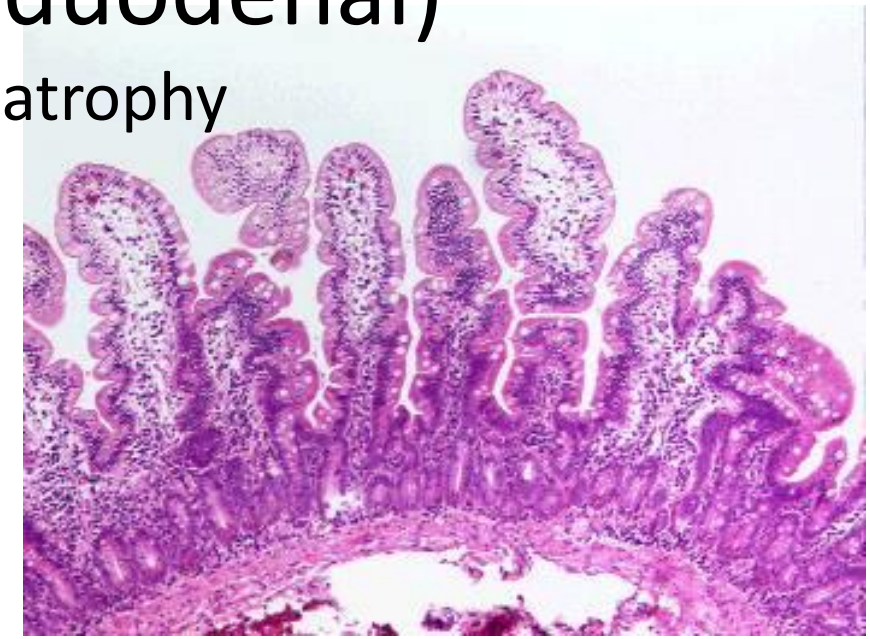




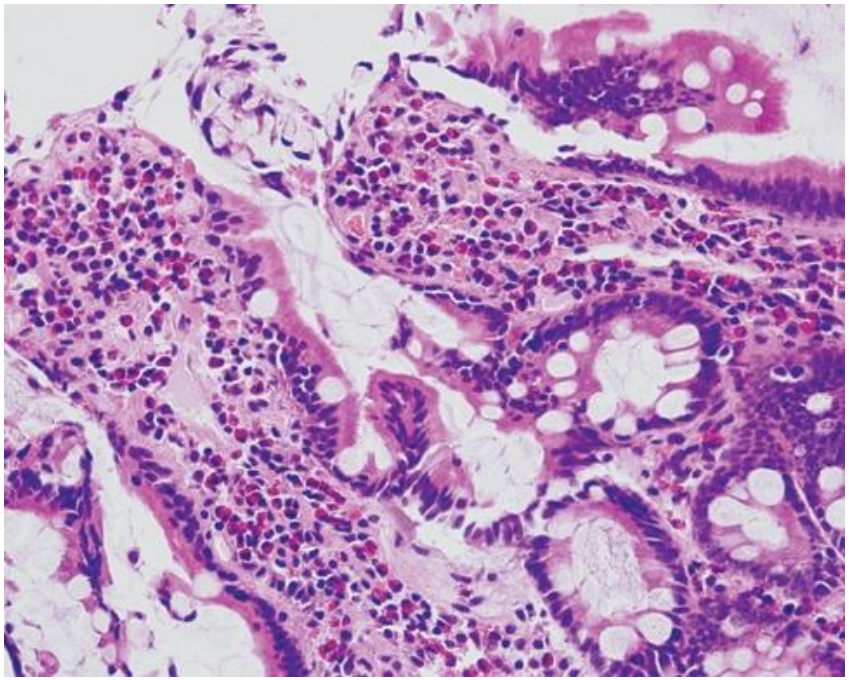
Endoscopic biopsy (duodenal)

assessment of villous atrophy

Ideal sample



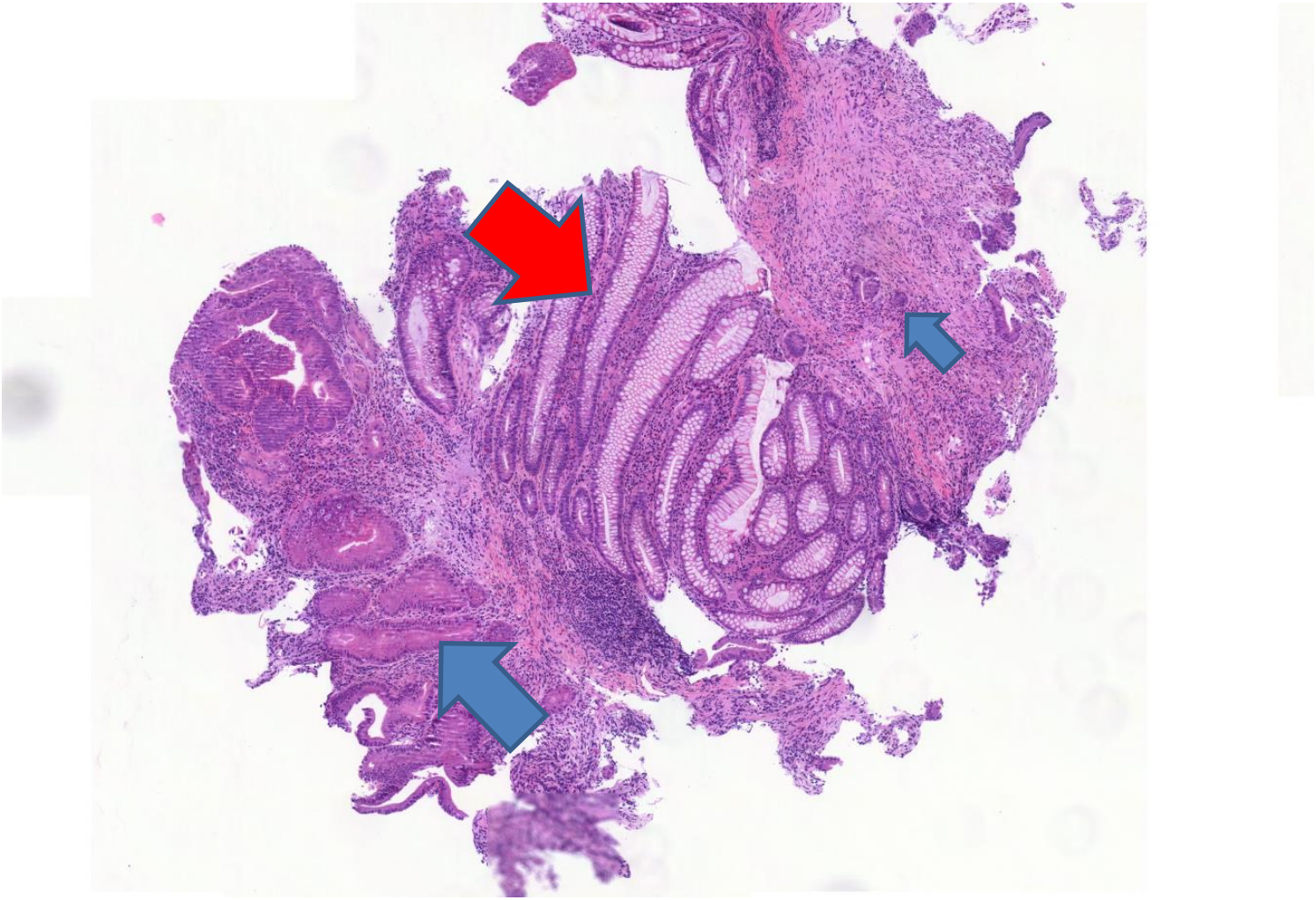
Not diagnostic sample



Endoscopic biopsy (colonic adenocarcinoma)

Normal mucosa: blue arrow

Tumor: red arrow



Cytology vs Tissue Sampling Summary

	Cytology	Histology
Advantages	•Fast (<1 hour if needed) •Simple tools •Minimally invasive, complications rare	• Several slides from the same sample • Ideal if special exams is needed •Assessment of histological structures (eg: glomeruli)
Disadvantages/ limitations	•Limited sample number(smear) •Ancillary exams (e.g. immunohistochemistry) limited	•Time consuming processing •More expensive, lab requirements •Invasive, complications may occur
Diagnostic evaluation(tumors)	•Dignity •Type – main tumor type •Low grade/high grade •Invasion – limited assessment	•Dignity •Type –more accurate tumor typing •Grade/exact assessment of proliferation •Exact assessment of invasion
Setting	•Before surgery •in case of a metastatic disease clarify etiology •Special exams are limited (depend on the number of smears)	•Before surgery •Systemic therapy planning (molecular assays) •Some special tumors (e.g.lymphomas= lot of special exams needed)
Both techniques require experience!!!! Unsatisfactory samples are not diagnostic-unnecessary invasive intervention!		

Intraoperative Examination

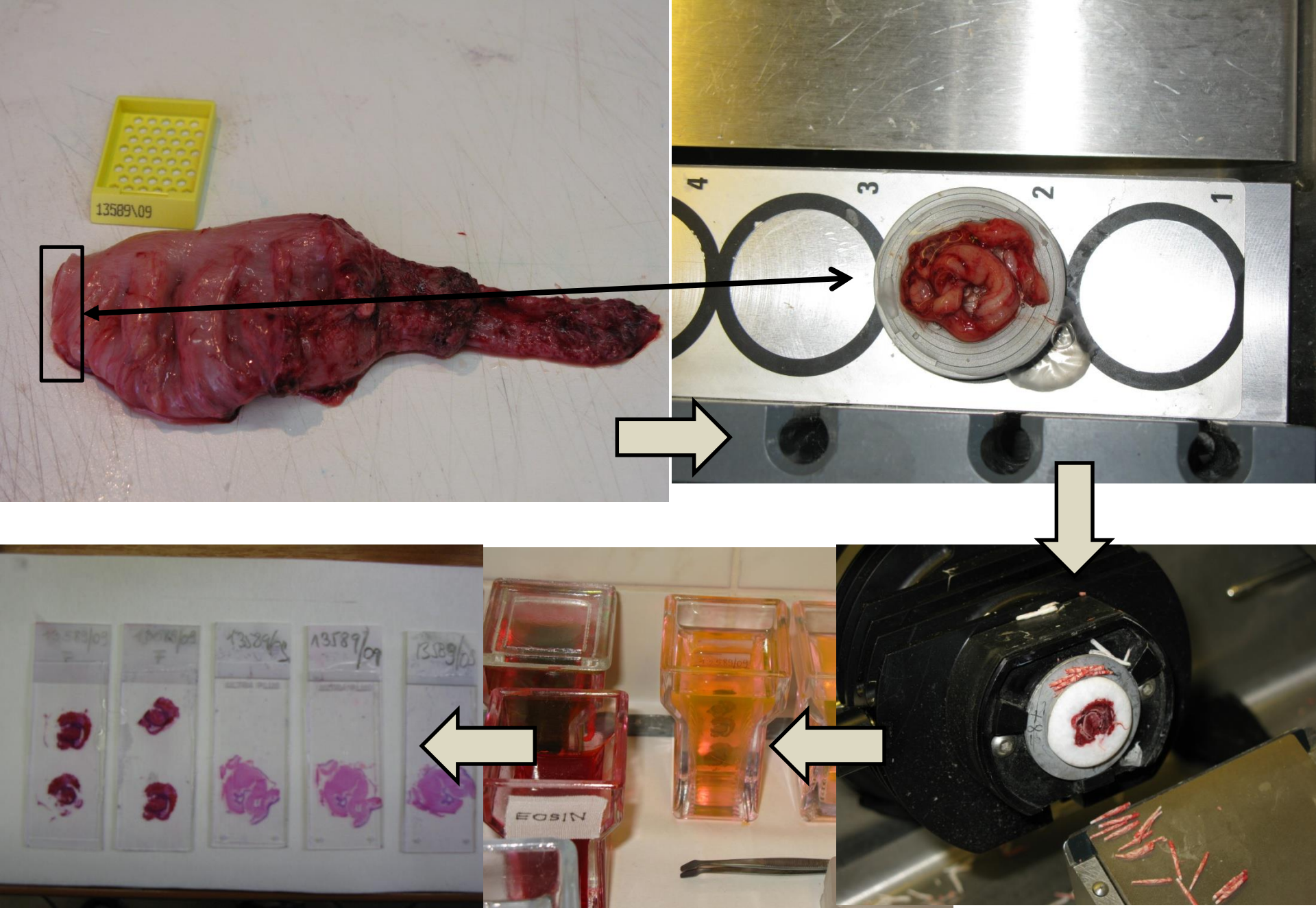
Indications

- No preoperative biopsy (e.g. pancreas, ovarium): to evaluate dignity (benign or malignant)
- In case of a known malignancy:
  - Resection margin assessment (positive or negative)
  - Sentinel lymph node biopsy (positive or negative)
  - Unrecognized lesion by preoperative imaging (e.g. liver metastasis or carcinosis)

Intraoperative Examination

Techniques

- Intraoperative cytology (FNA): by the surgeon (on palpation, US-guided)
- Intraoperative tissue sampling: quick-frozen section(cryostat), H&E staining (10-20 minutes)
- Touch prep: ancillary to frozen section: cellular morphology preserved (e.g. evaluating tumor cell nuclei)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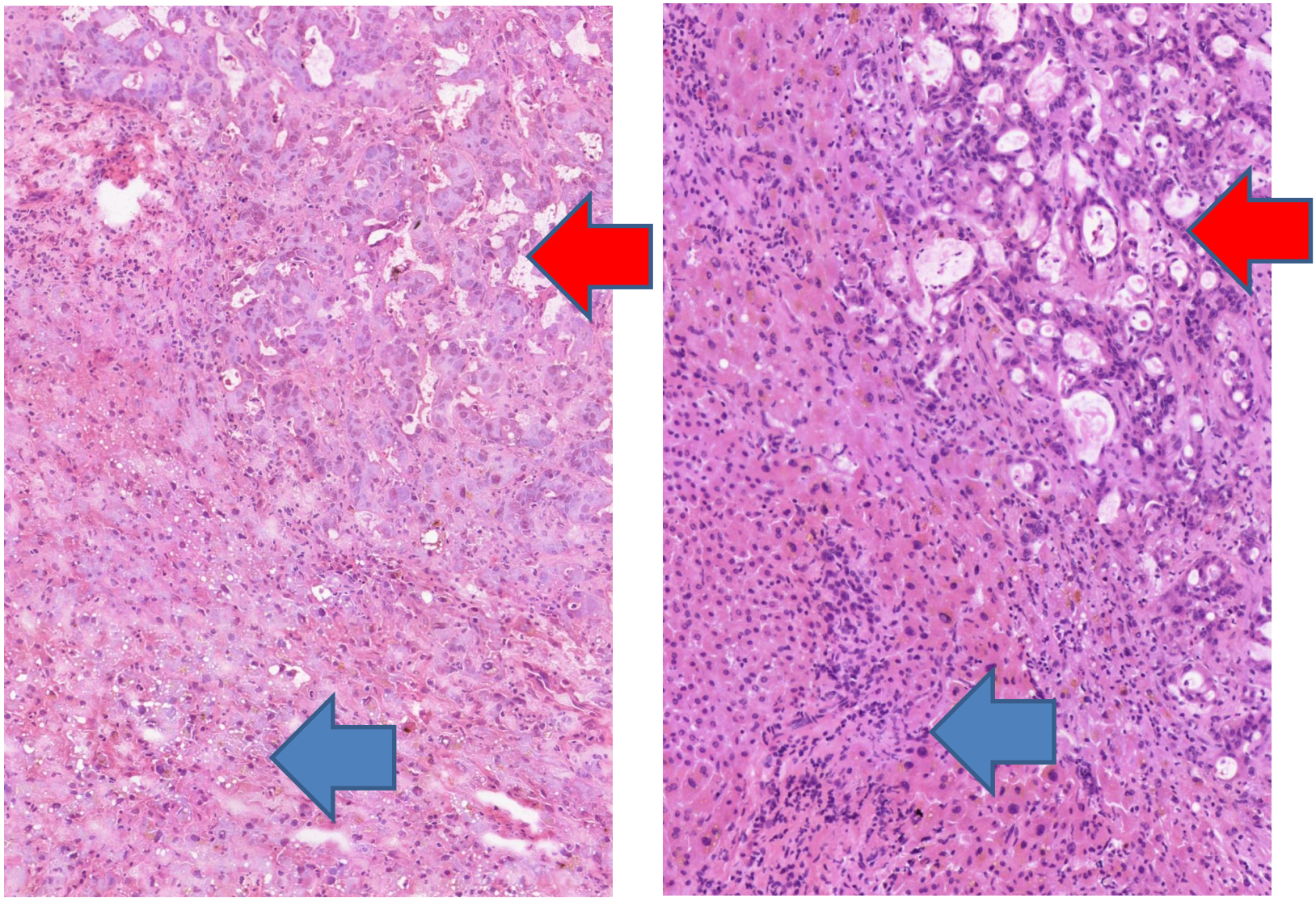
Frozen vs Processed slides

Normal liver: blue arrow

Metastatic adenocarcinoma: red arrow



Special Techniques

- Protein-based techniques: immunohistochemistry, immunocytochemistry
- Molecular pathology: DNA/RNA-based exams
  - FISH (morphology-based..)
  - Sequence analysis etc. (see lectures)

Immunohistochemical reaction (IHC)

Definition

Detection of proteins or protein fragments by immunological reaction (antigen-antibody complex). Generally used in tumor pathology

- Normal proteins which show the cellular origin of a tumor
- Abnormal accumulation of proteins during a pathological process (malignant transformation)

Diagnostic markers

Tumor type	**Marker(s)**
Epithelial tumors (carcinoma)	Cytokeratin subtypes, tissue-specific markers (PSA, TTF-1, etc.)
Mesenchymal tumors	Tissue specific markers (actin, s-100, factor VIII, etc.)
Hematologic tumors	CD proteins (T/B cell markers, etc.)
Undifferentiated tumors	CK, vimentin, Melan-A, CD45 = LCA

Prognostic/predictive markers

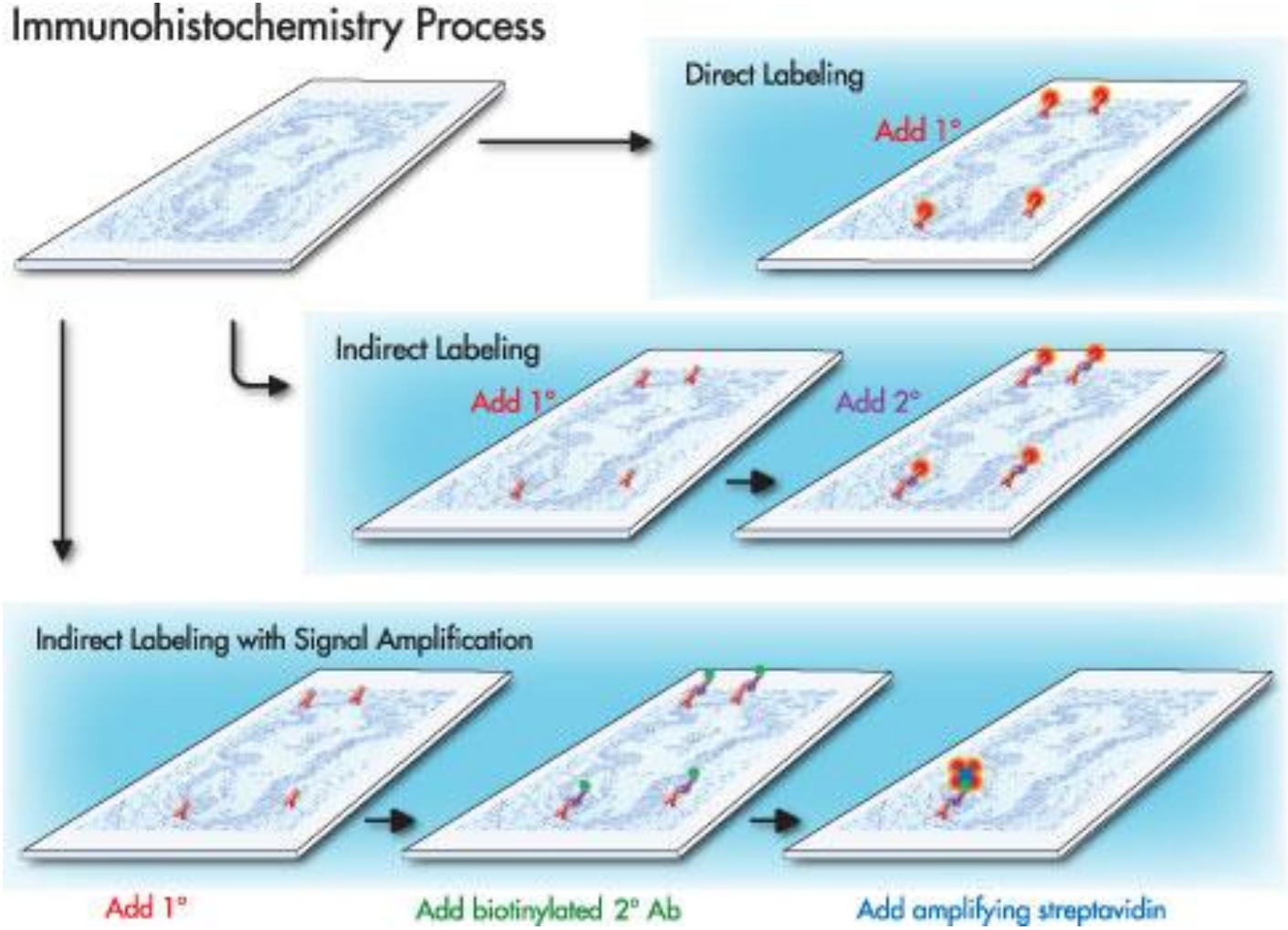
Prognosis	Proliferation: Ki-67 Oncoprotein mutation, accumulation: p-53
Predictive markers (to targeted therapies)	Hormon receptors: ER Growth factor receptors: EGFR, HER2, c-KIT

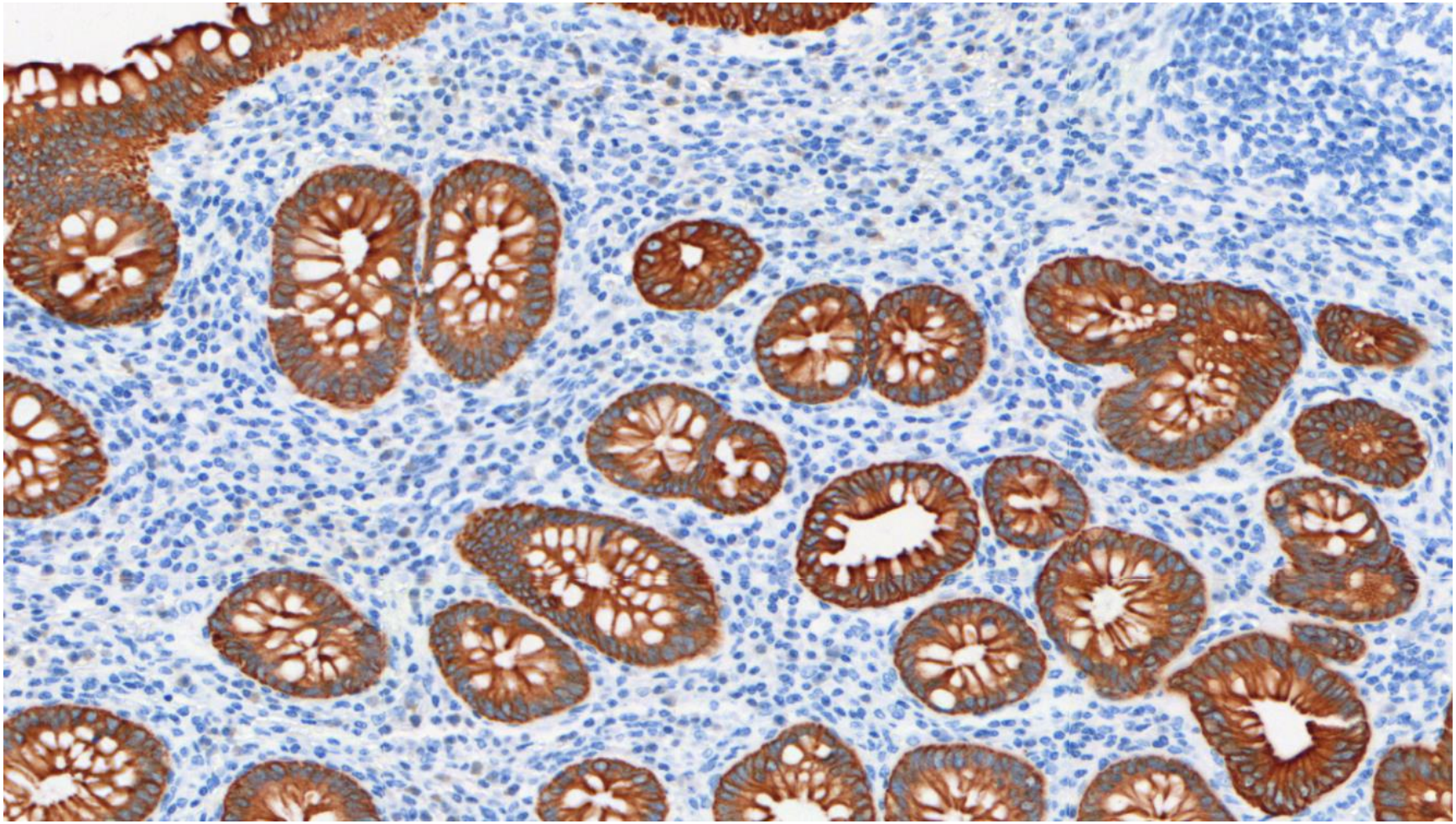
Commonly used IH reactions

- Normal proteins
  - Cytoskeleton (*cytoplasmic reaction*): cytokeratin (**epithelium**), vimentin (**mesenchymal cell**), S-100 (**neuron**), actin (**muscle**) etc..
  - Receptor (*membrane or nuclear reaction*): estrogen receptor, progesteron receptor (breast), CD proteins (hemato-lymphogen cells)
  - Cell cycle regulators (*nuclear reaction*): MIB-1/Ki-67
  - Other (cellular adhesions, cytoplasmic compartment, enzymes etc..)
- Abnormal protein accumulation
  - Oncoproteins (p-53, growth factor receptors: EGFR, HER2)
  - Infective agents (viral compartments)
  - Other (tau proteins in neurodegenerative diseases)

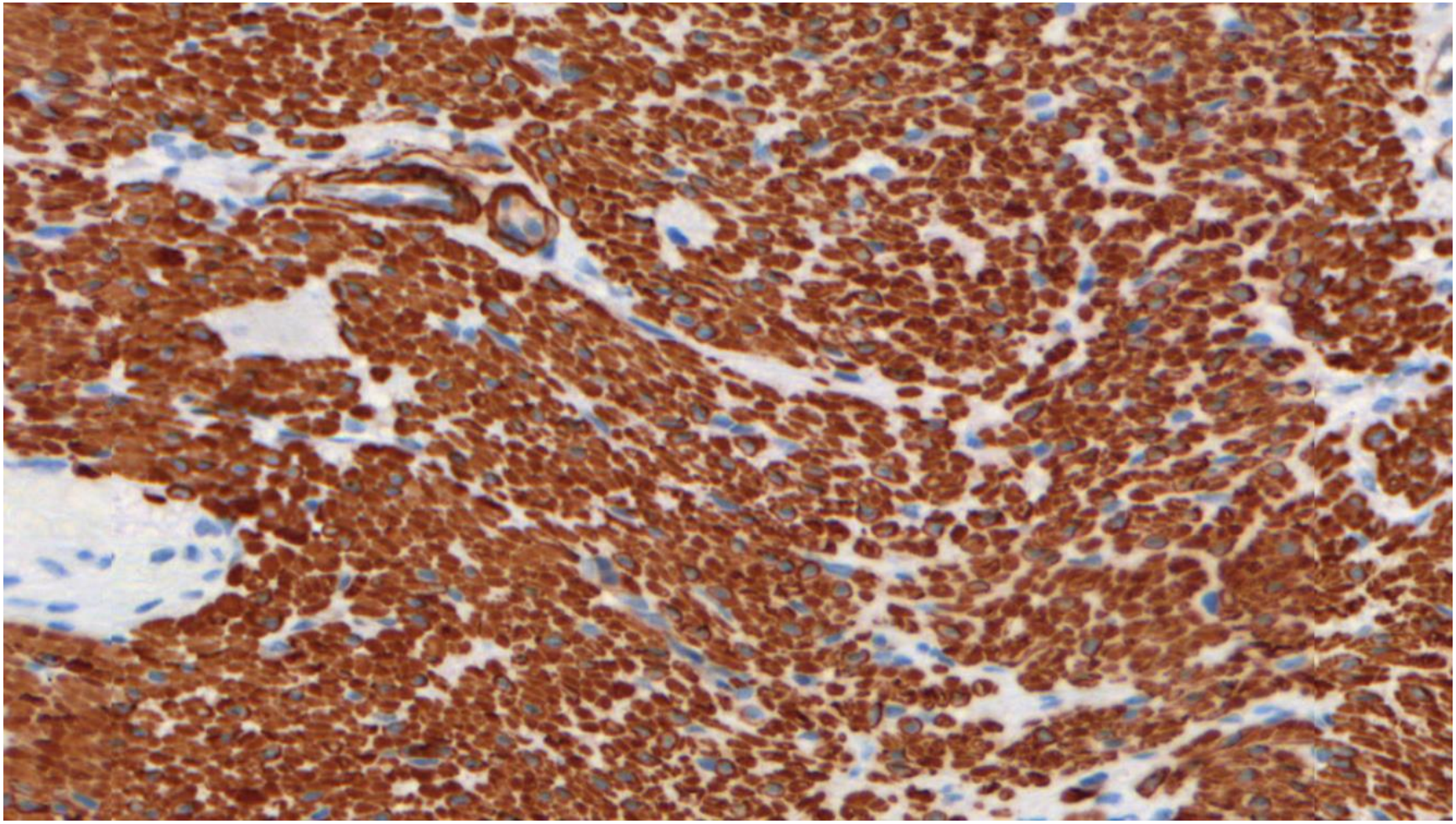
Method of immunohistochemistry

- Primary antibody (antigen specific)
- Secondary antibody+chromogen (visual detection)

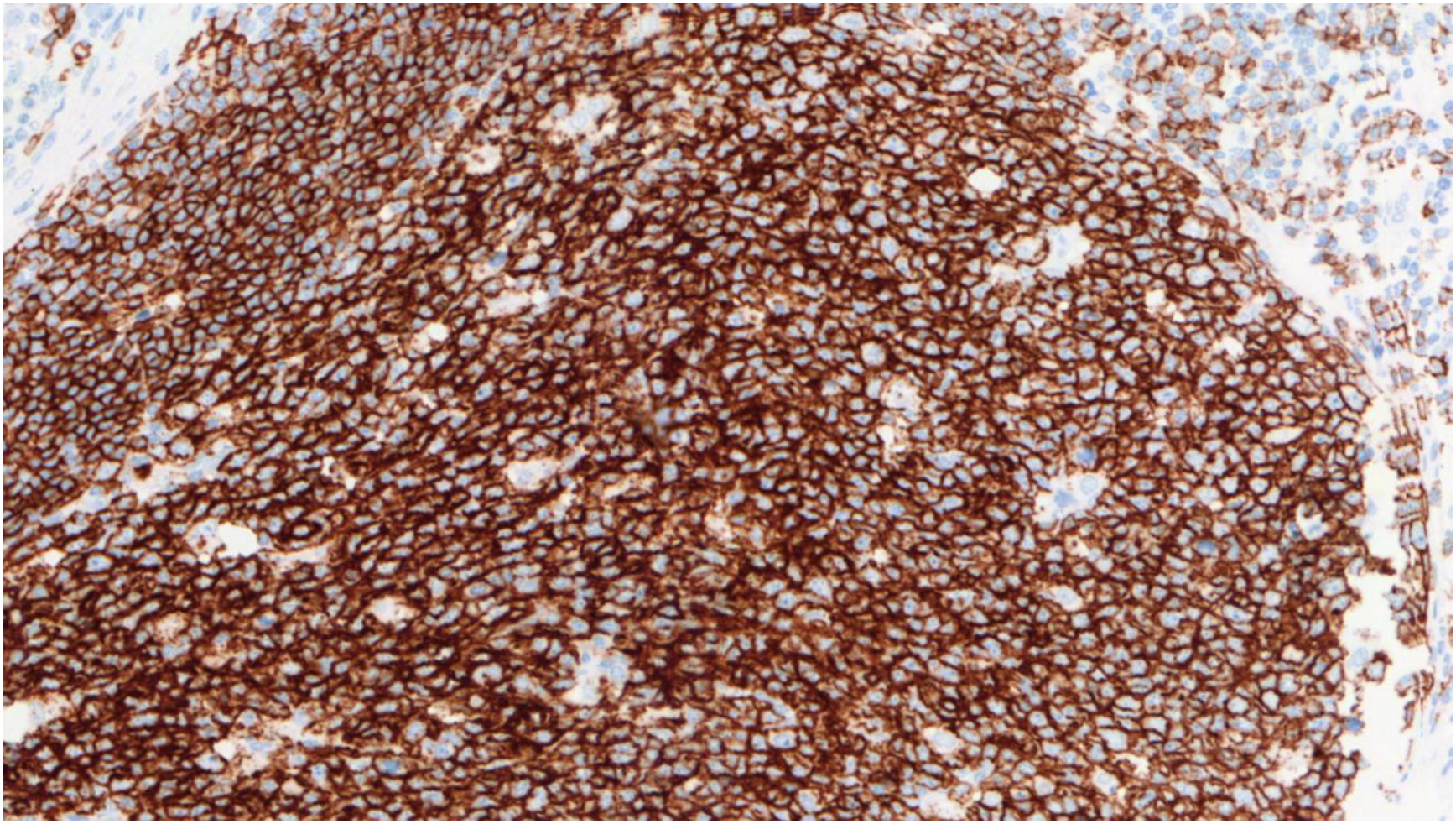




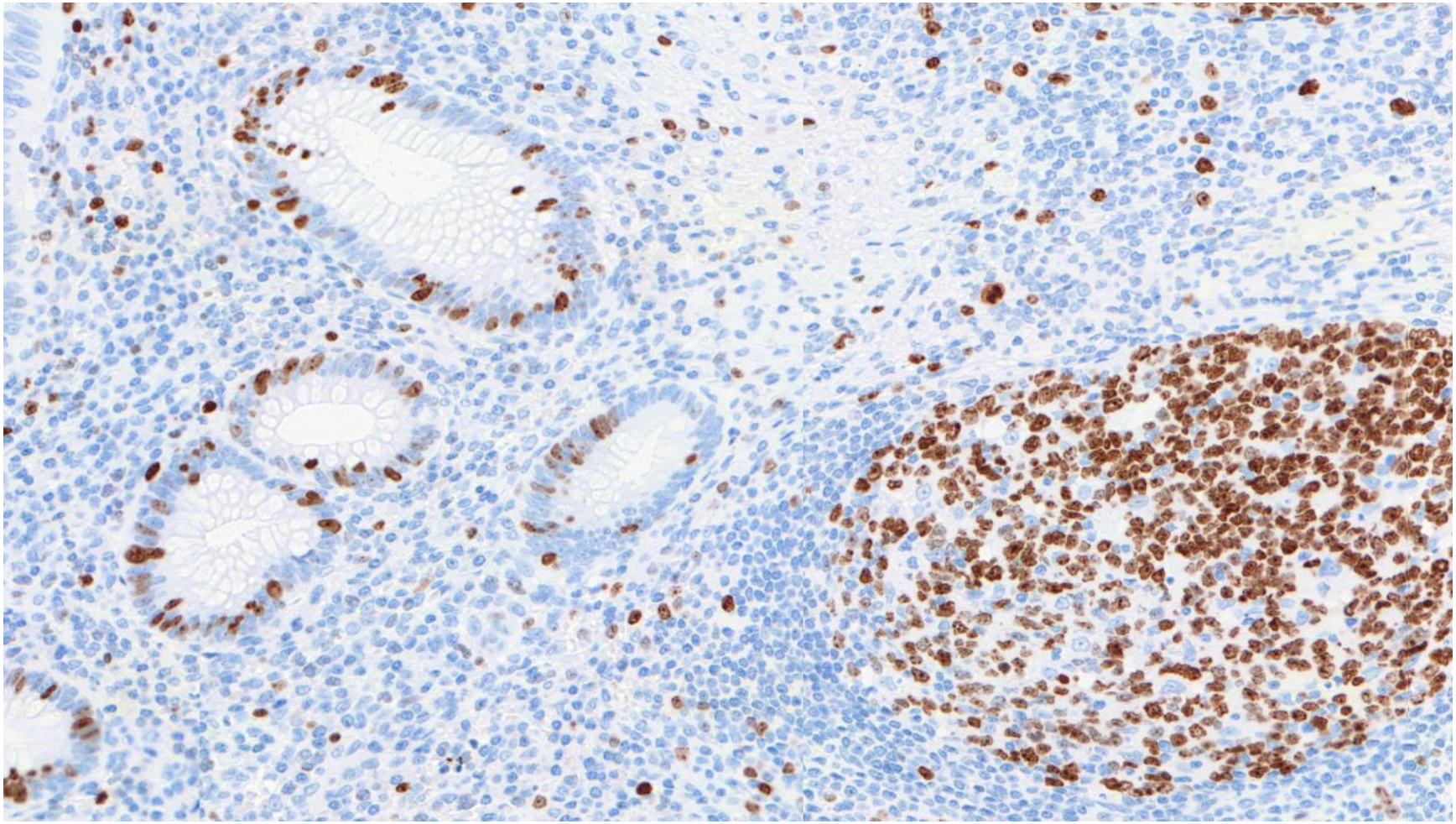
Cytokeratin



Smooth muscle actine



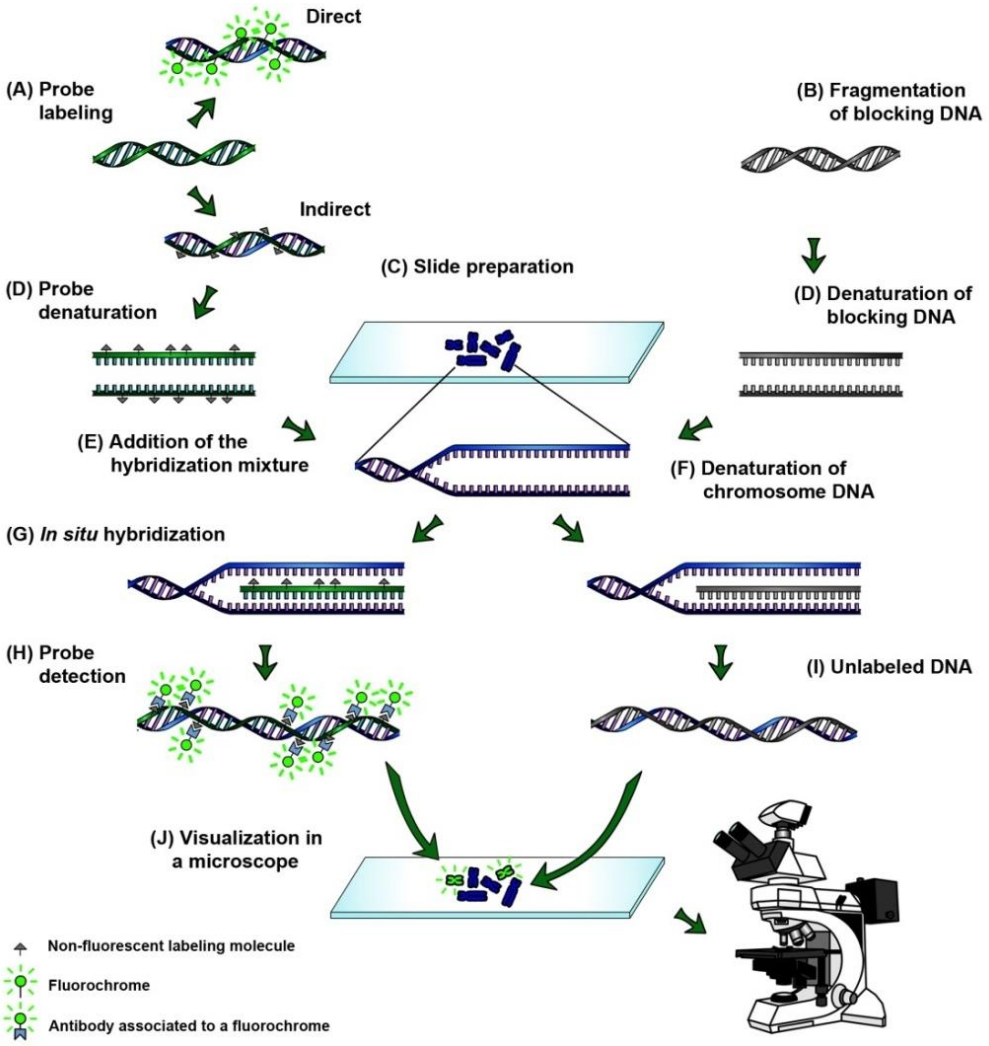
B-cell marker (CD20)

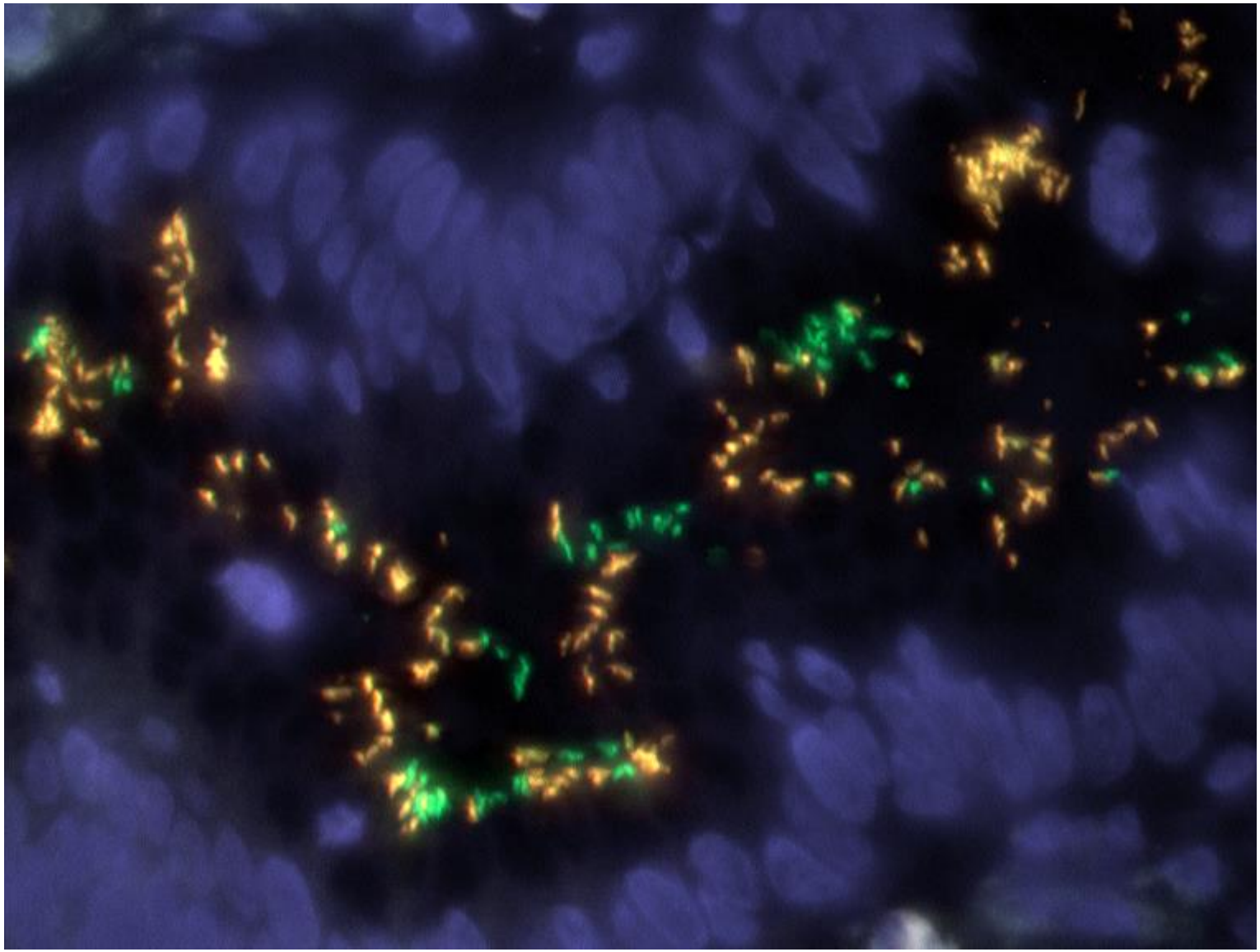


Proliferation: Ki-67

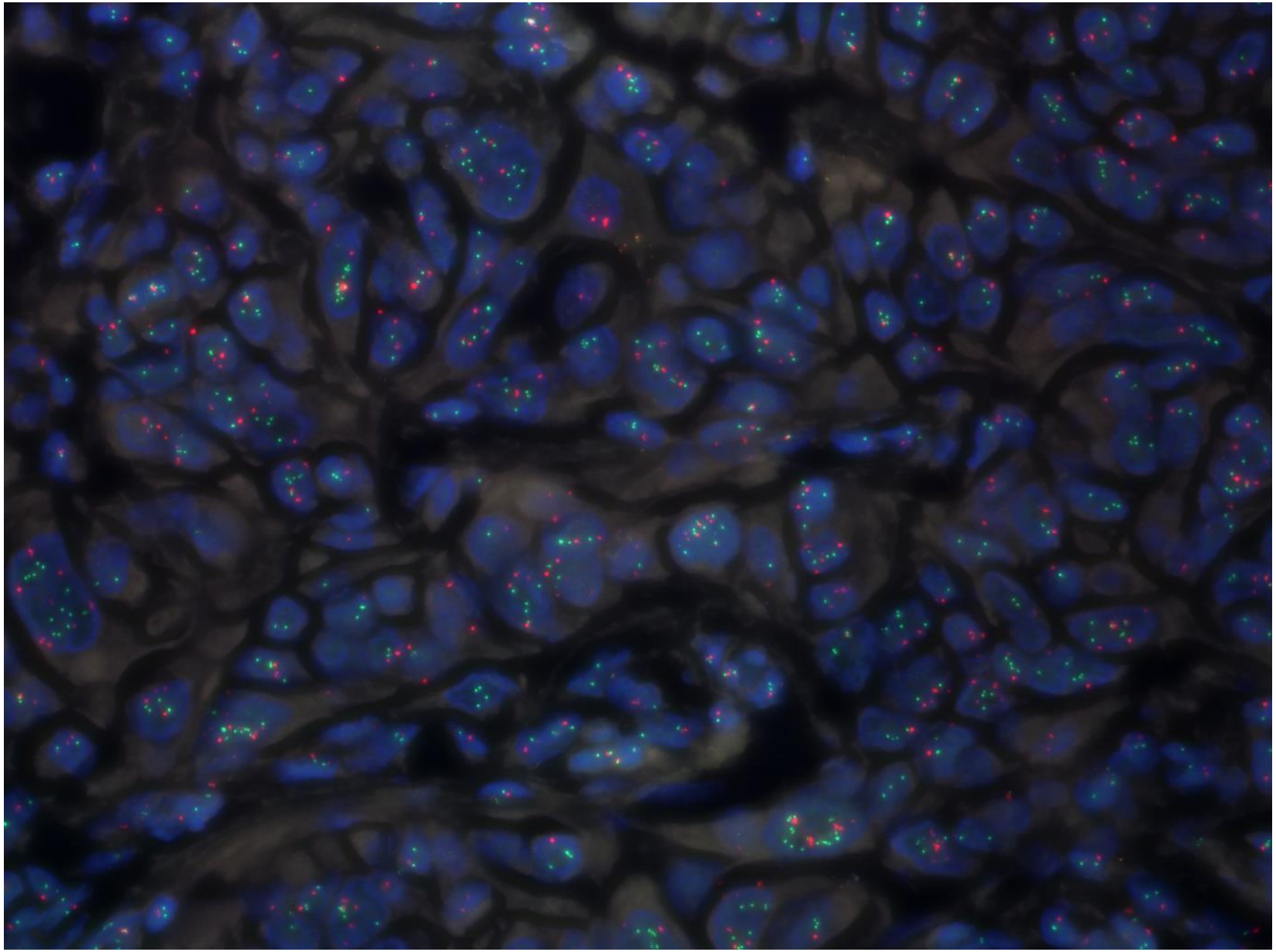
FISH (fluorescent in situ hybridisation)

- Detecting specific DNA sequences within chromosomes
- Tumor patology
  - Amplification, deletion, translocation detections
  - Predictive and diagnostic exams
- Microbiology
  - Species specific

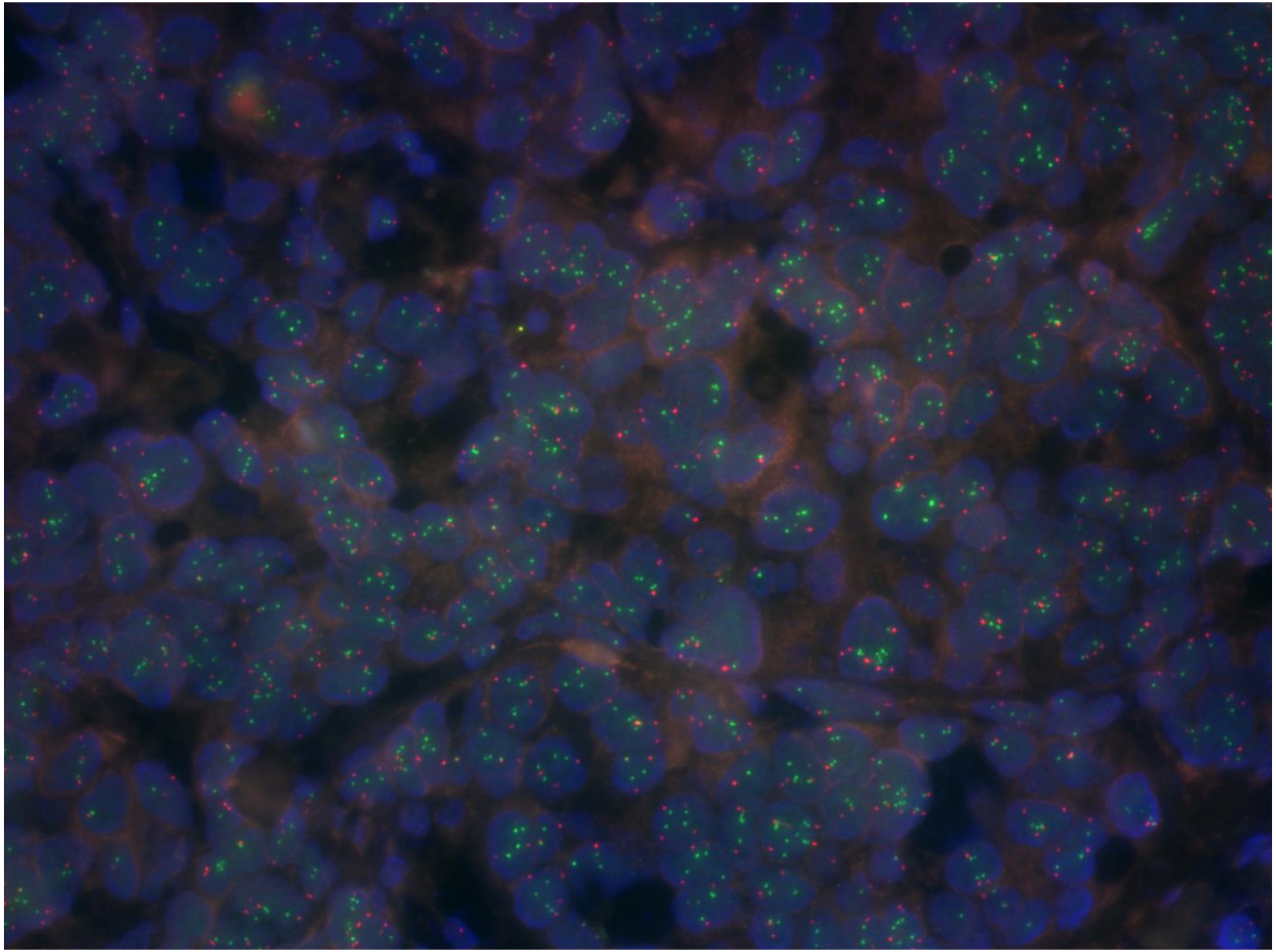




H. pylori detection



Assessment of HER2 amplification in breast carcinoma



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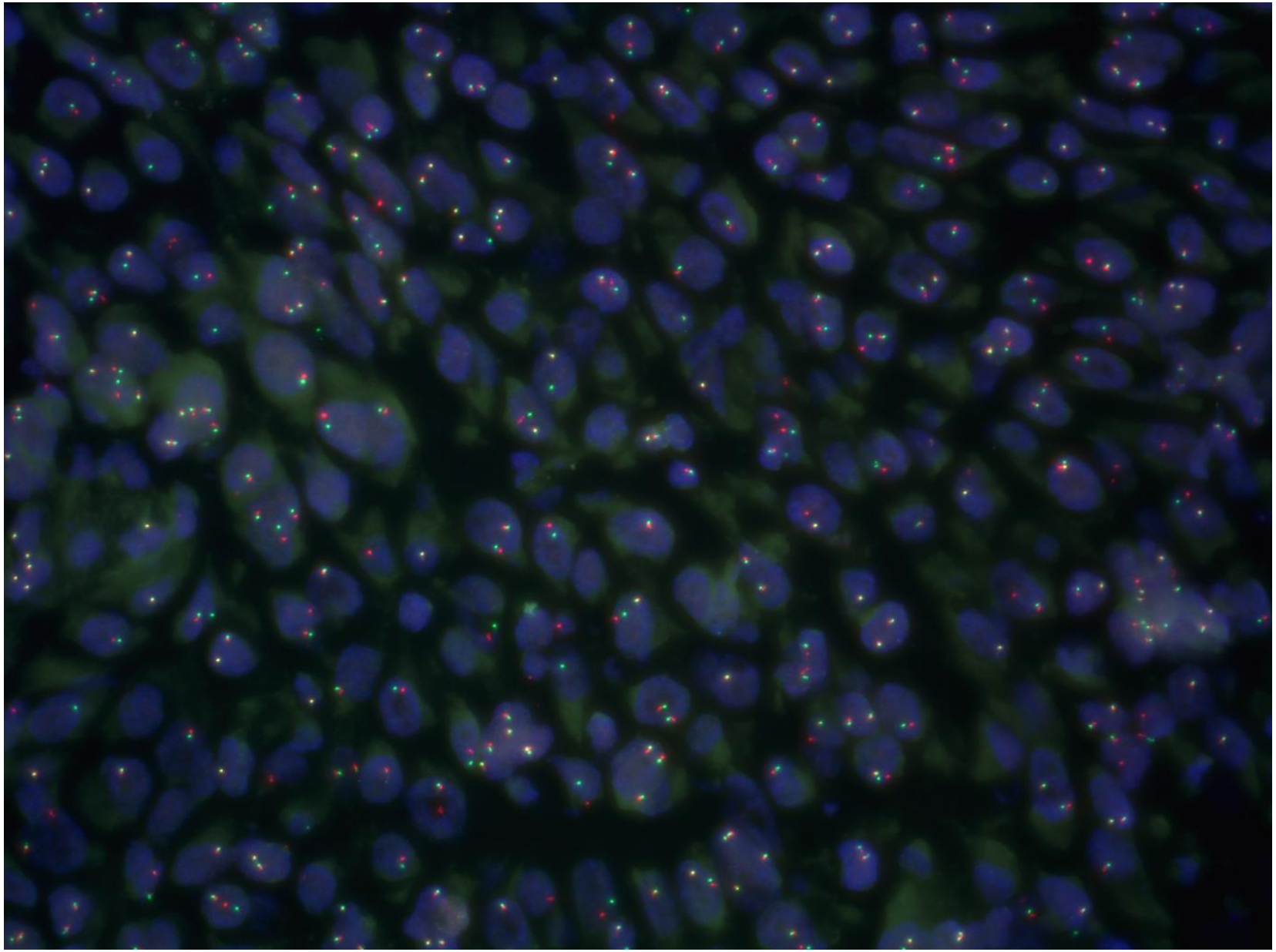
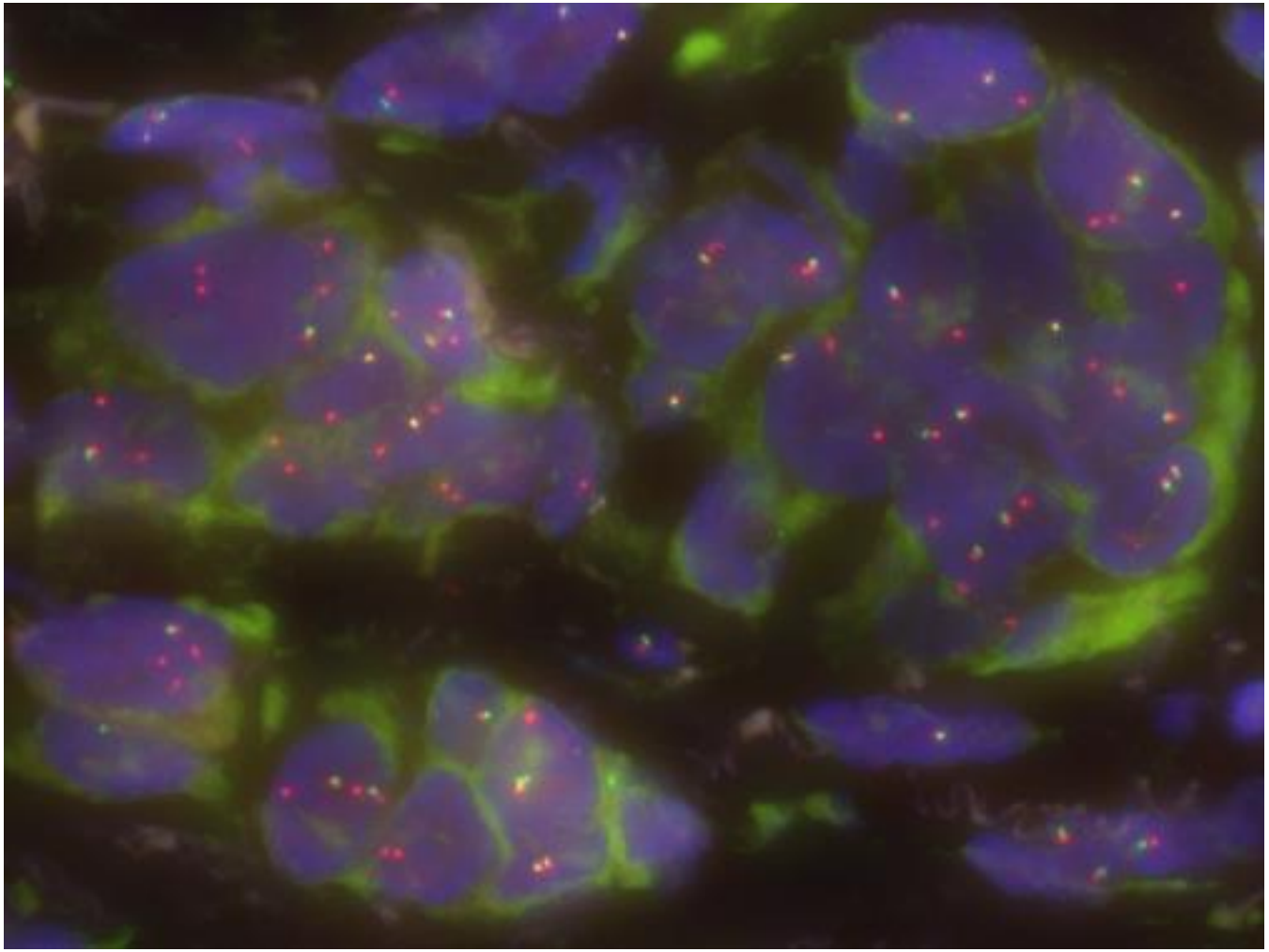


ALK break apart probe in lung adenocarcinoma



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