Practical immunology

LECTURER
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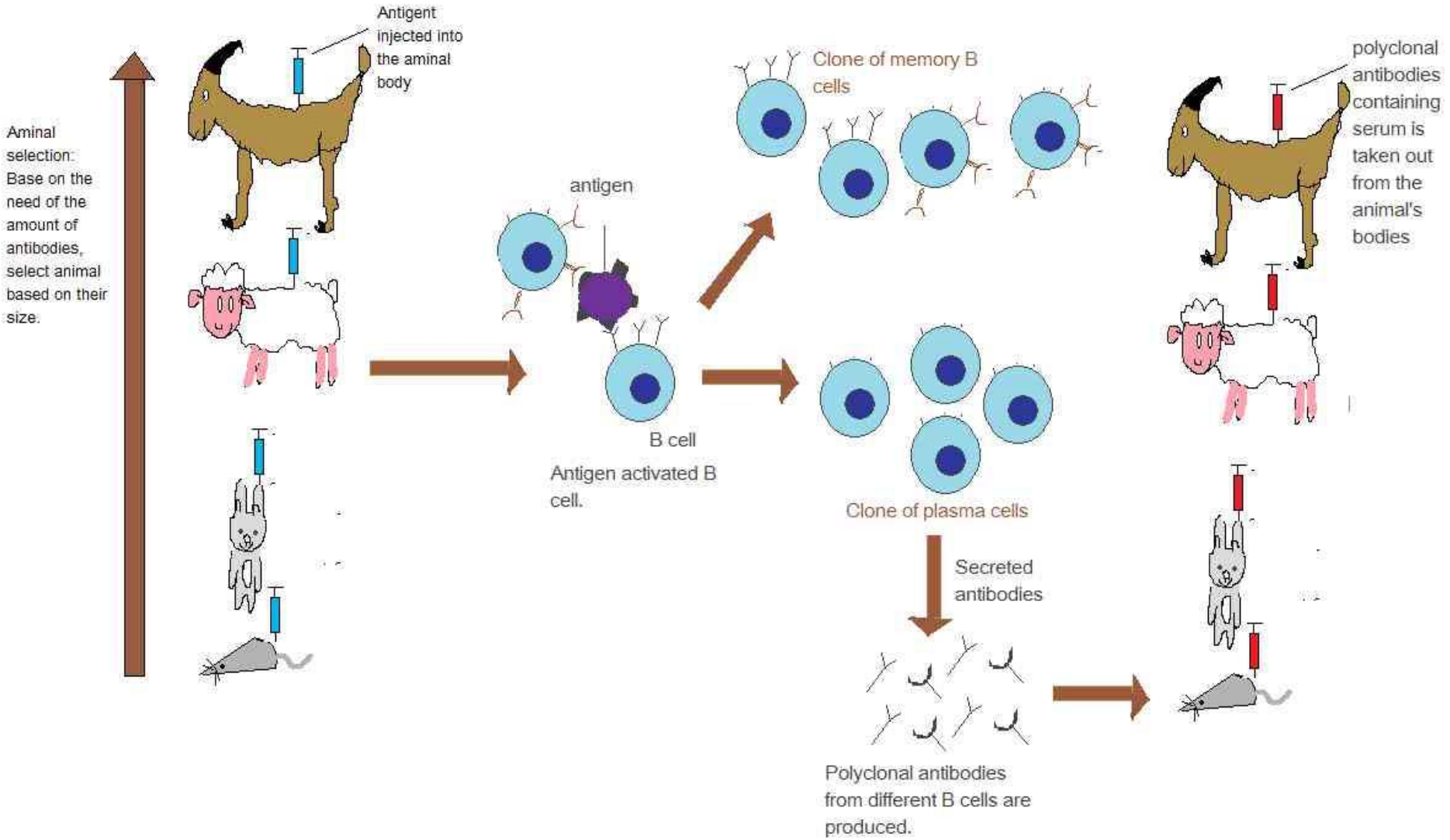
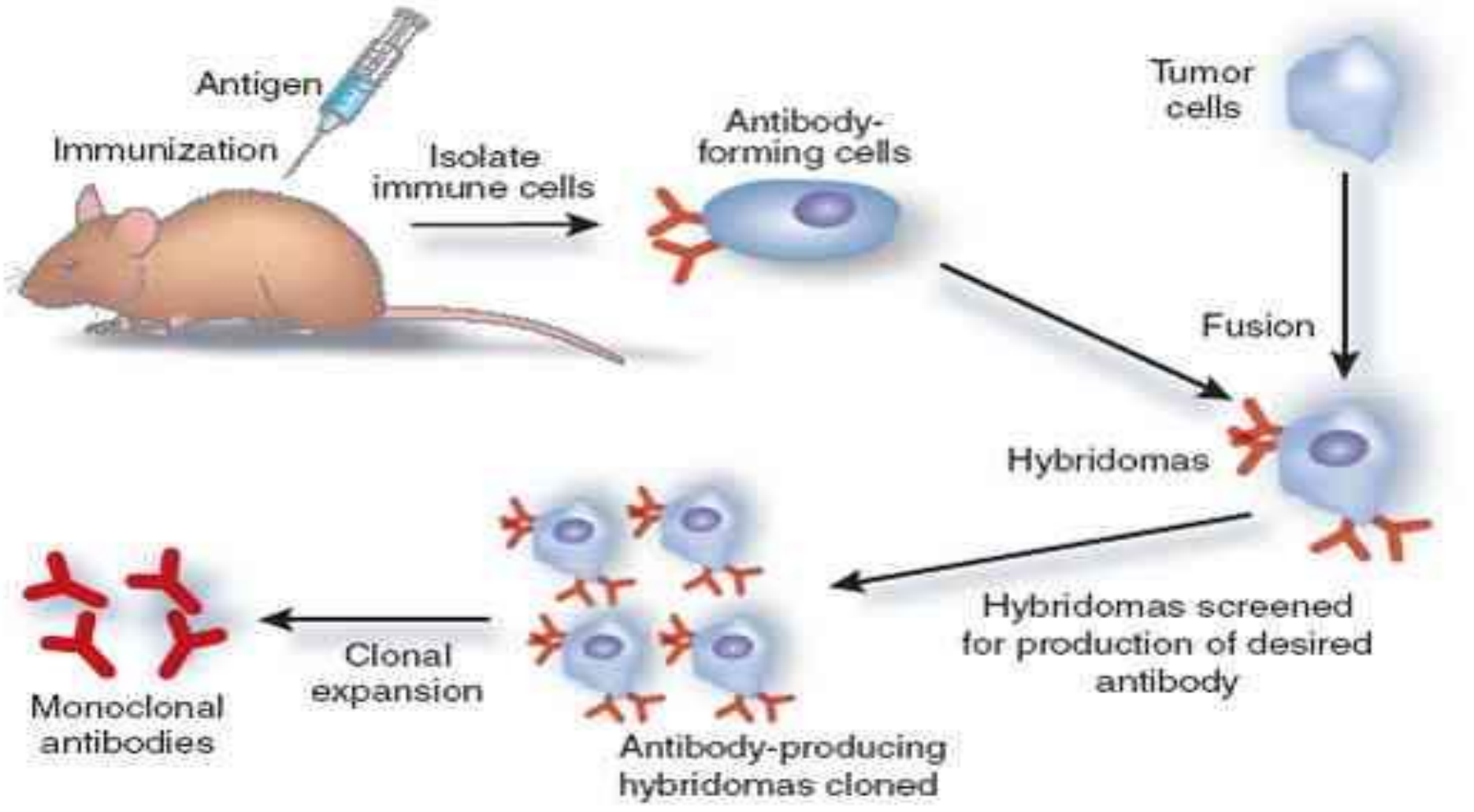
Polyclonal antiserum is produced in a normal animal hosts in response to immunogen administration. Produced by different B- cells



Monoclonal antibodies **are monospecific antibodies that are identical because they are produced by one type of immune cells that are all clones of a single parent cell.**



Immunological Methods

- Unlabelled Immunoassay: Immunoassay that doesn't use immunological markers;ex Precipitin Ab, Immunodiffusion (Double and Single), Agglutinin Ab, Latex test, CFT.

- Labeled Ab : Immunoassay that use immunologic markers ; ex: Immunoflourescent assay (IFA), Radio-immunoassay (RIA), Enzyme linked immunosorbent assay (ELISA).

Labeled immunoassay

- The technique of labeled immunoassay using labeled reagents for detecting antigens and antibodies

- Labeled immunoassay employing ligand labeled with:

1. Enzymes (Enzyme Immunoassay

Enzyme-linked immunosorbent assay; ELISA)

1. Fluorscent dyes (Fluorscent immuno assay).
2. Radioisotopes (Radioimmuno-assay)

Fluorscent immunoassay

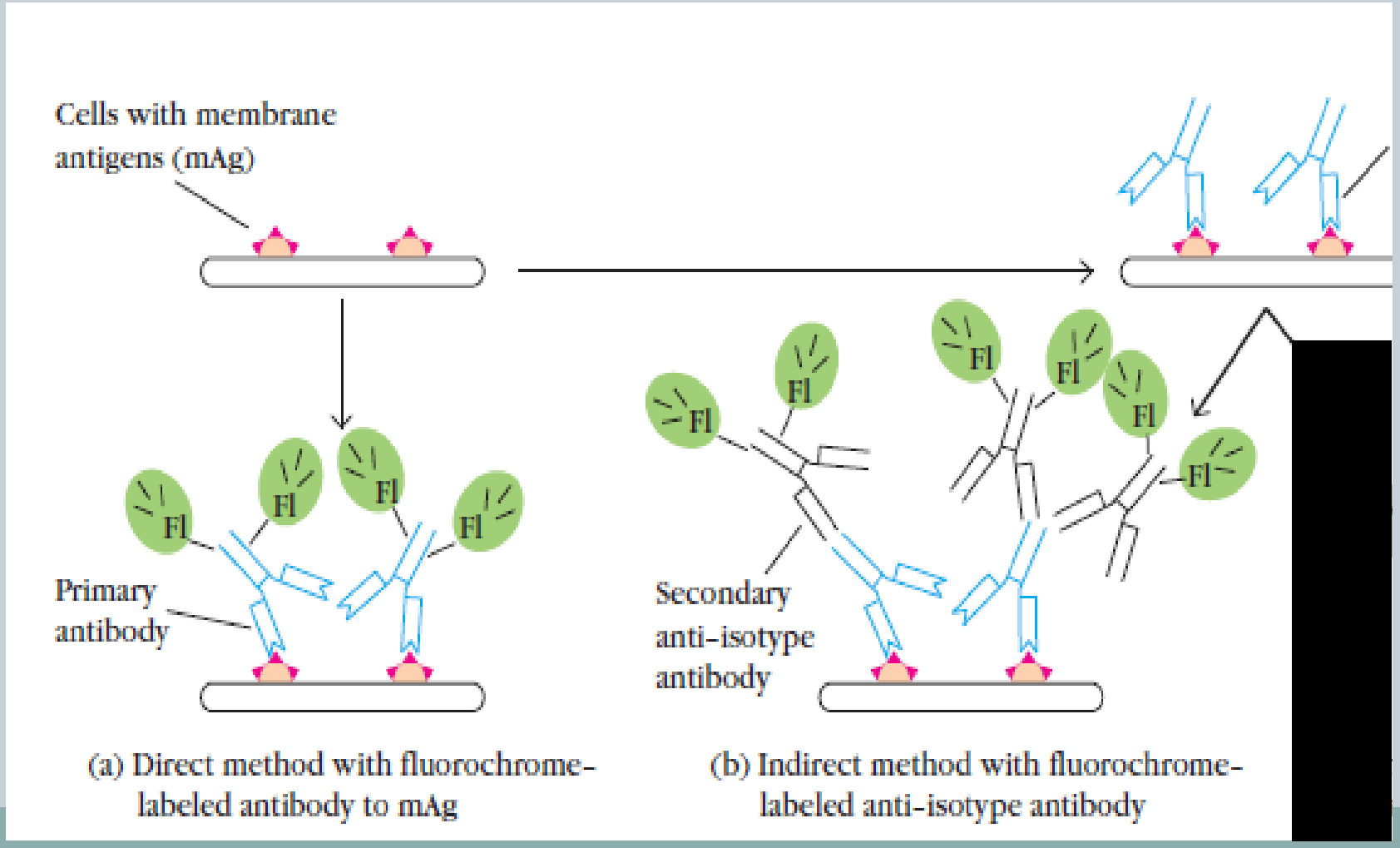
- **Fluorescent molecules:** absorb light of one wavelength (excitation) and emit light of another wavelength (emission).

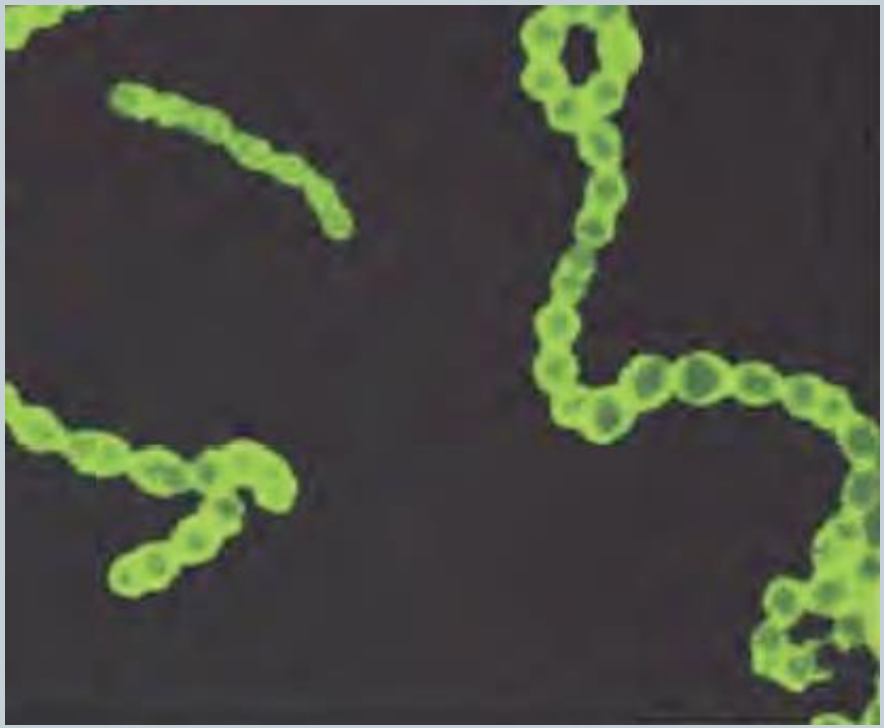
If antibody molecules are tagged with a fluorescent dye, immune complexes containing these fluorescently labeled antibodies (FA) can be detected by colored light emission when excited by light of the appropriate wavelength.

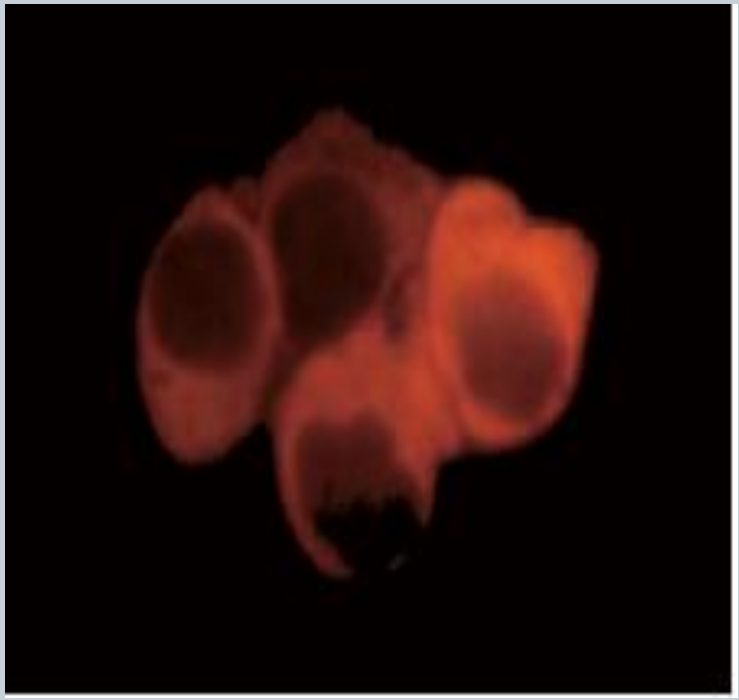
- Antibody molecules bound to antigens in cells or tissue sections can similarly be visualized.

The emitted light can be viewed with a fluorescence microscope, which is equipped with a UV light source,

- Fluorescent-antibody staining of cell membrane molecules or tissue sections can be direct or indirect.
- In **direct staining**, **the specific antibody (the primary antibody)** is directly conjugated with fluorescein;
- in **indirect staining**, **the primary antibody is unlabeled and is detected** with an additional fluorochrome-labeled reagent (secondary antibody).


Cells with membrane antigens (mAg)
Fl
Fl
Fl
Fl
Primary antibody
Fl
Fl
Fl
Fl
Fl
Secondary anti-isotype antibody
(a) Direct method with fluorochrome-labeled antibody to mAg
(b) Indirect method with fluorochrome-labeled anti-isotype antibody





1-Fluorescein, an organic dye that is the most widely used label for immunofluorescence procedures, absorbs blue light (490 nm) and emits an intense **yellow-green fluorescence** (517 nm).

2- Rhodamine, another organic dye, absorbs in the yellow-green range (515 nm) and emits a deep red fluorescence (546 nm).

3-Phycoerythrin is an efficient absorber of light (~30-fold greater than fluorescein) and a brilliant emitter of red fluorescence, stimulating its wide use as a label for immunofluorescence.

Immunofluorescence has been applied to

1- identify a number of subpopulations of lymphocytes, notably the CD4 and CD8 T-cell subpopulations.

2-The technique is also suitable for identifying bacterial species,

3-detecting Ag-Ab complexes in autoimmune disease,

4- detecting complement components in tissues, and localizing hormones and other cellular products stained in situ.

5-a major application of the fluorescent-antibody technique is the localization of antigens in tissue sections or in sub-cellular compartments.

Because it can be used to map the actual location of target antigens, fluorescence microscopy is a powerful tool for relating the molecular architecture of tissues and organs to their overall gross anatomy.

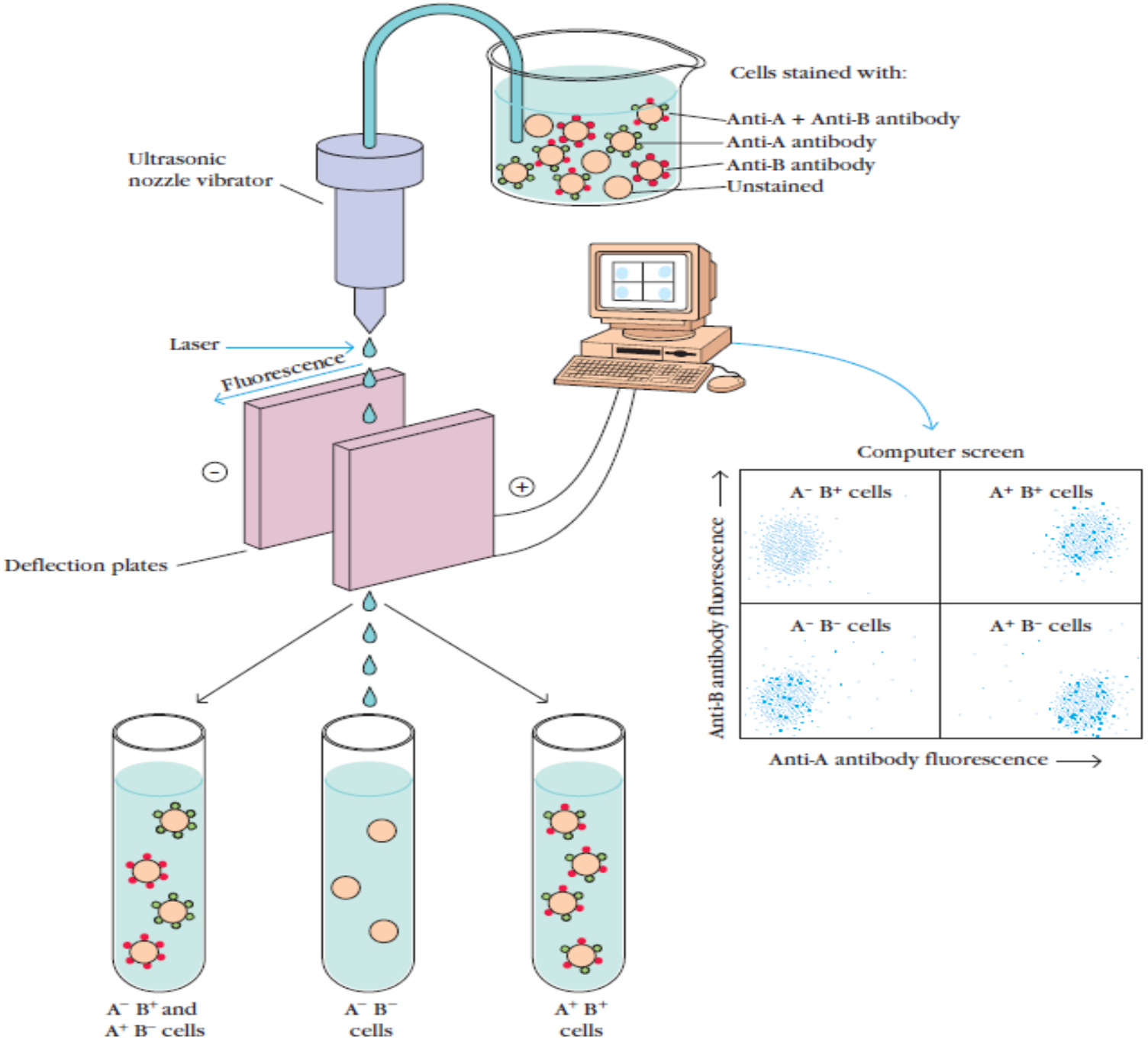
Flowcytometry

Fowcytometer, was designed to automate the analysis and separation of cells stained with fluorescent antibody.

The flow cytometery uses a laser beam and light detector to count single intact cells in suspension.

Every time a cell passes the laser beam, light is deflect from the detector, and this interruption of the laser signal is recorded.

- capable of sorting populations of cells into different containers according to their fluorescence profile.


Cells stained with:
Anti-A + Anti-B antibody
Anti-A antibody
Anti-B antibody
Unstained
Ultrasonic nozzle vibrator
Laser
Fluorescence
Deflection plates
Computer screen
A⁻ B⁺ cells
A⁺ B⁺ cells
A⁻ B⁻ cells
A⁺ B⁻ cells
Anti-B antibody fluorescence
Anti-A antibody fluorescence
A⁻ B⁺ and A⁺ B⁻ cells
A⁻ B⁻ cells
A⁺ B⁺ cells

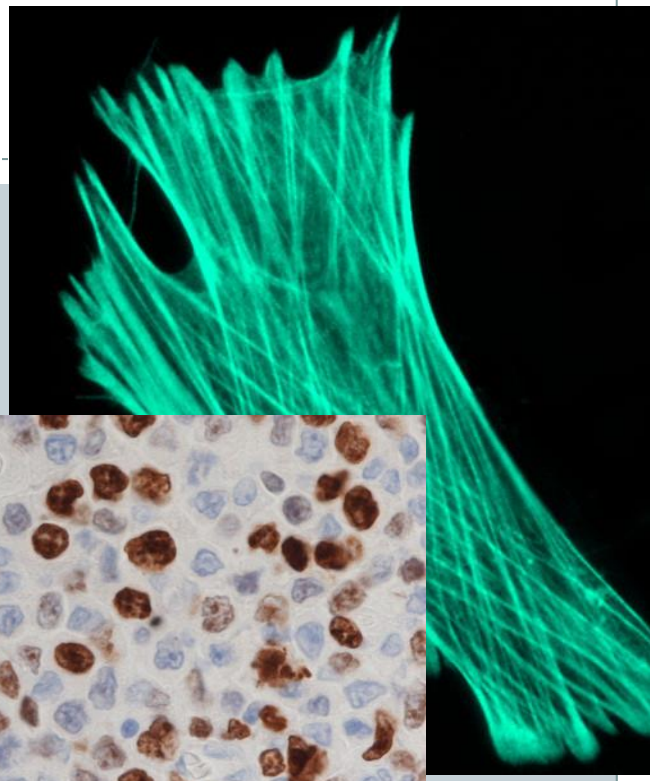
Immunohistochemistry (IHC)

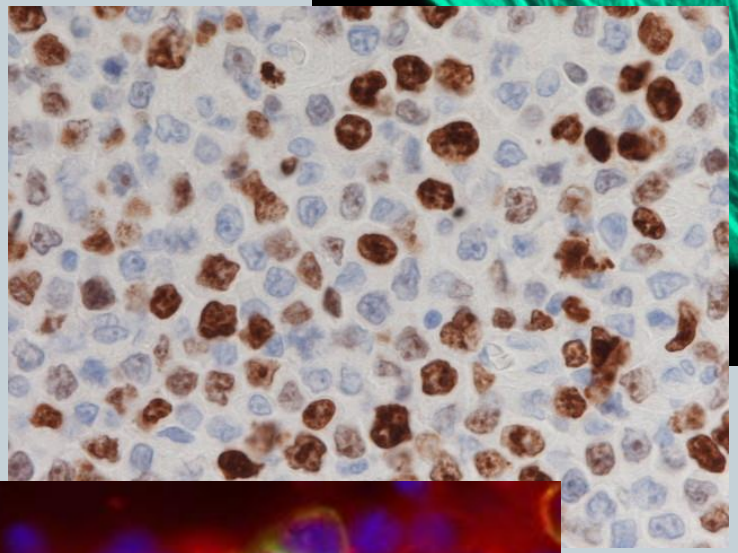
- Immunohistochemistry (IHC) combines histological, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label.
- IHC makes it possible to visualize the distribution and localization of specific cellular components within a cell or tissue.

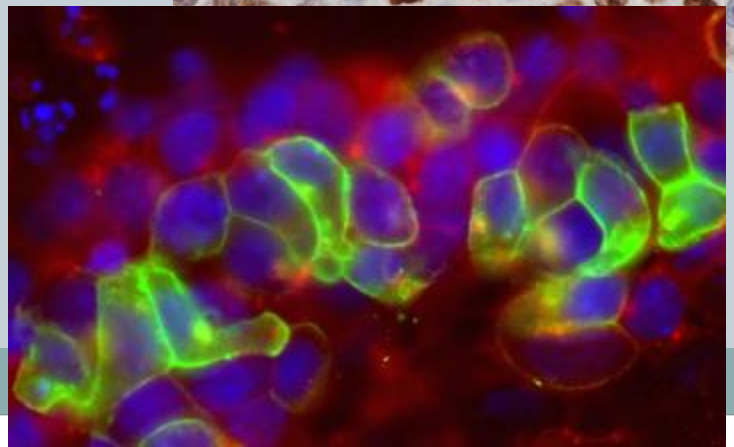
- **Applications**
- IHC is used for disease diagnosis
- drug development to test drug efficacy by detecting either the activity or the up- or down-regulation of disease targets.
- biological research.
- diagnose a cancer as benign or malignant,
- determine the stage and grade of a tumor, and identify the cell type and origin of a metastasis to find the site of the primary tumor.

What cellular antigens can be target?

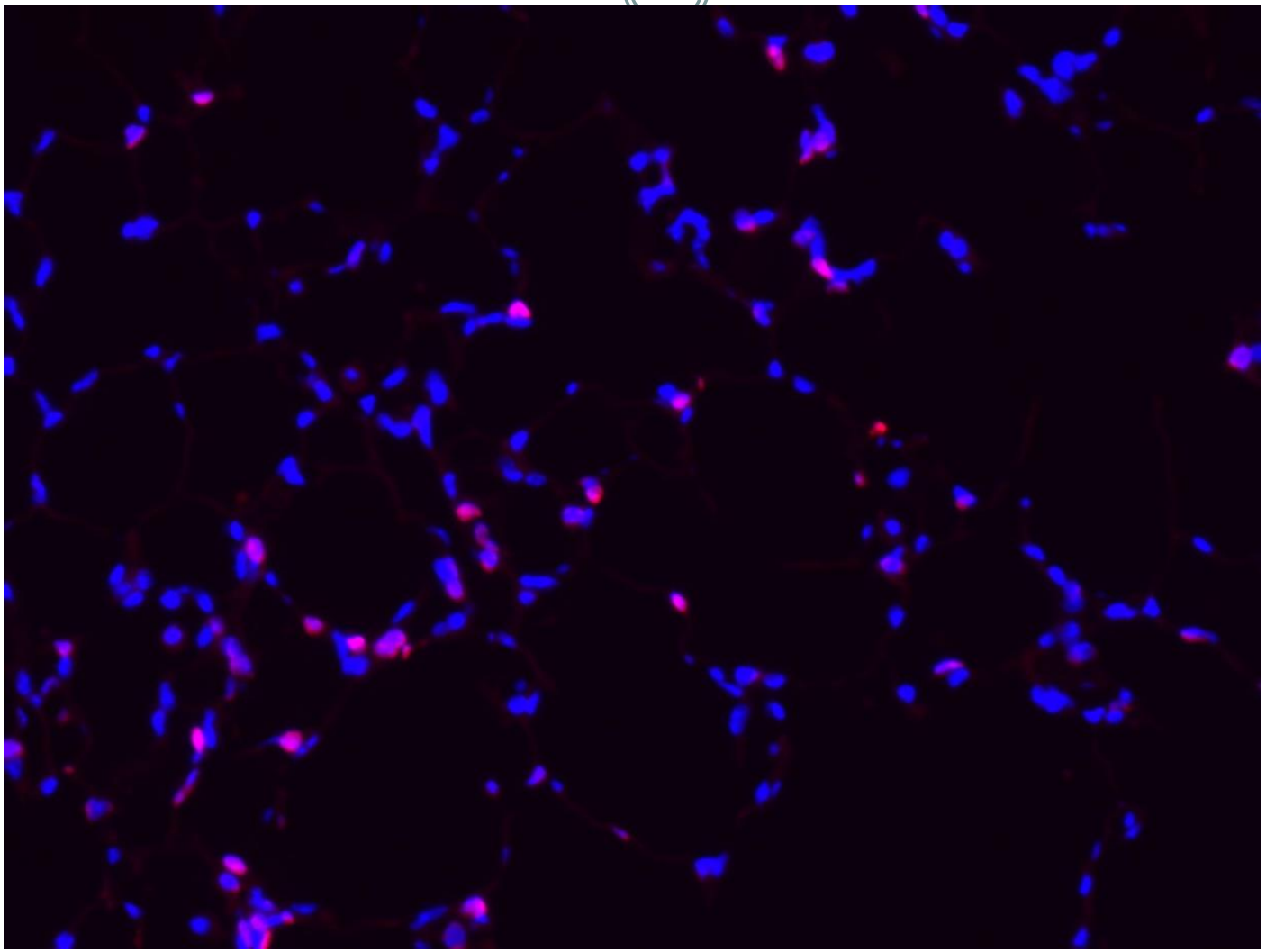
Cytoplasmic
Nuclear
Cell membrane
Lipids
Proteins



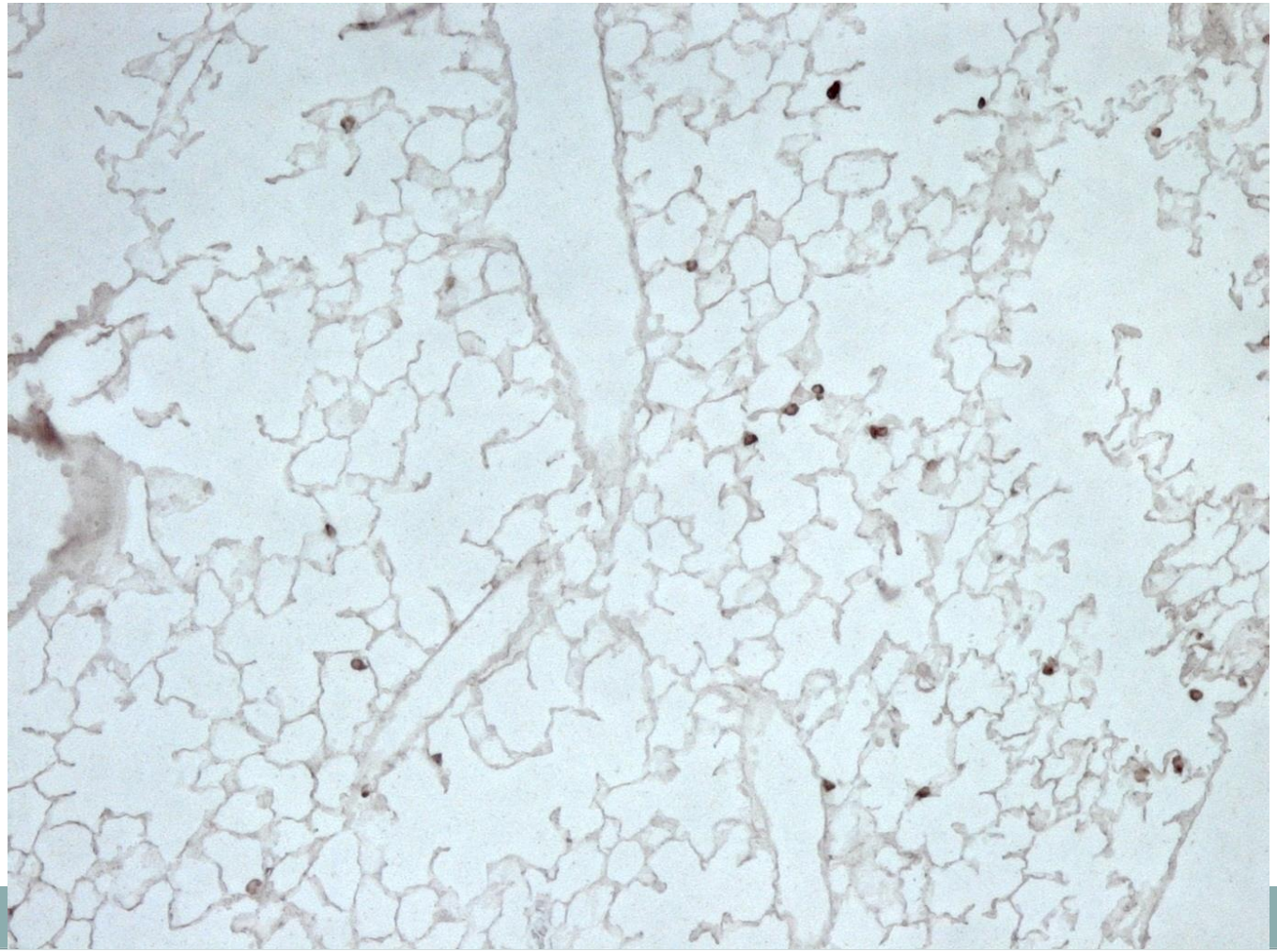




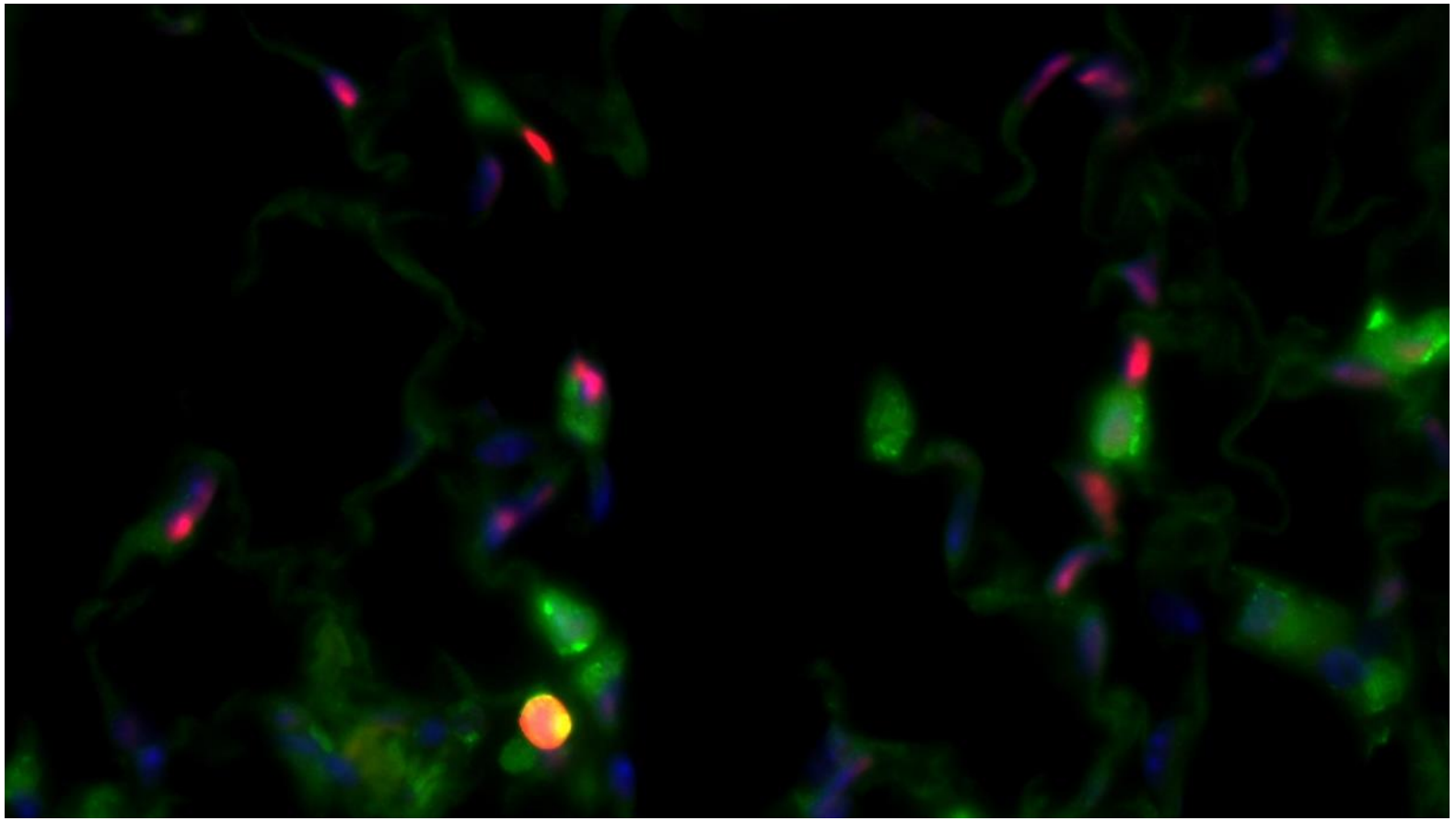
Identify replicating cells



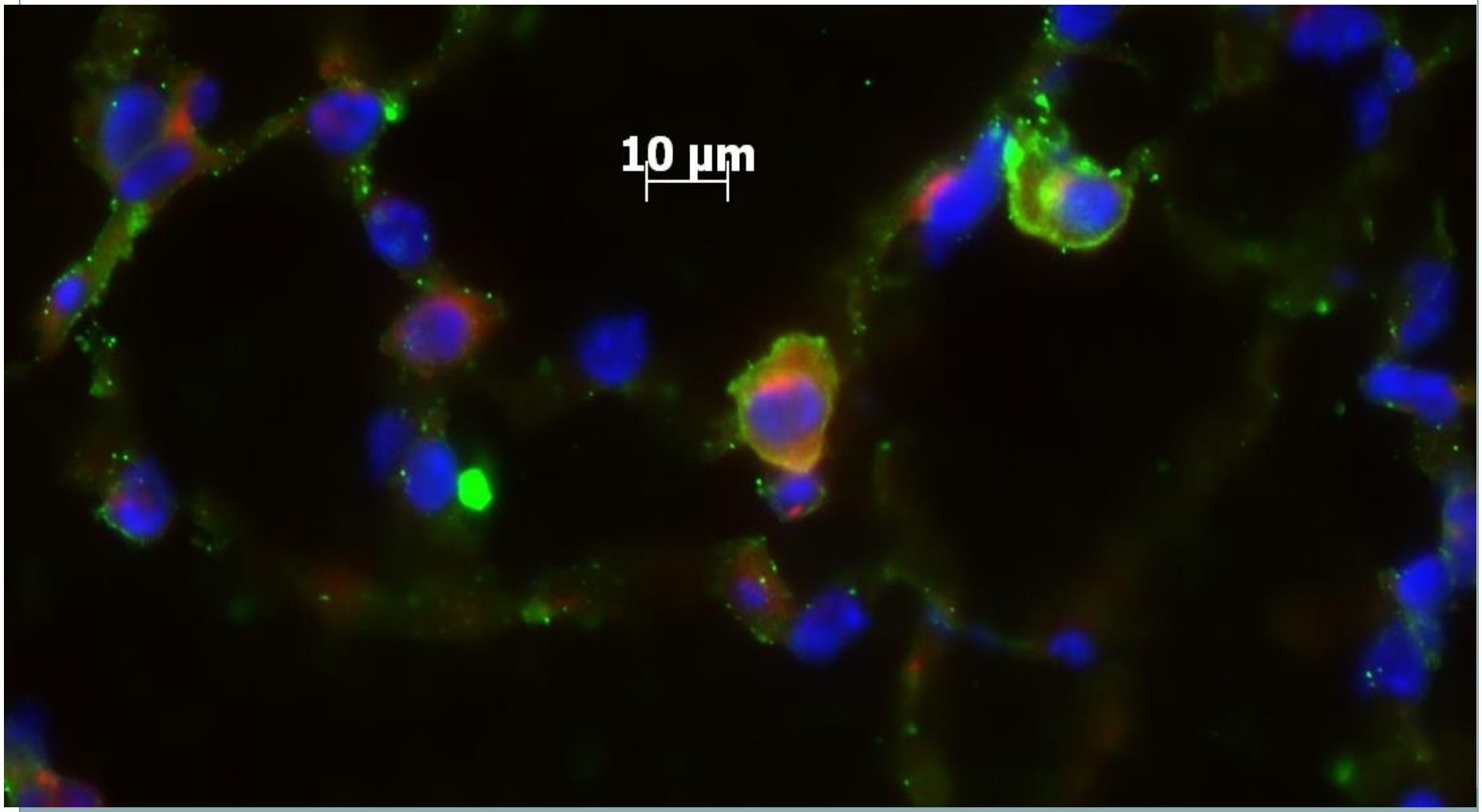
Locate cells that are signaling



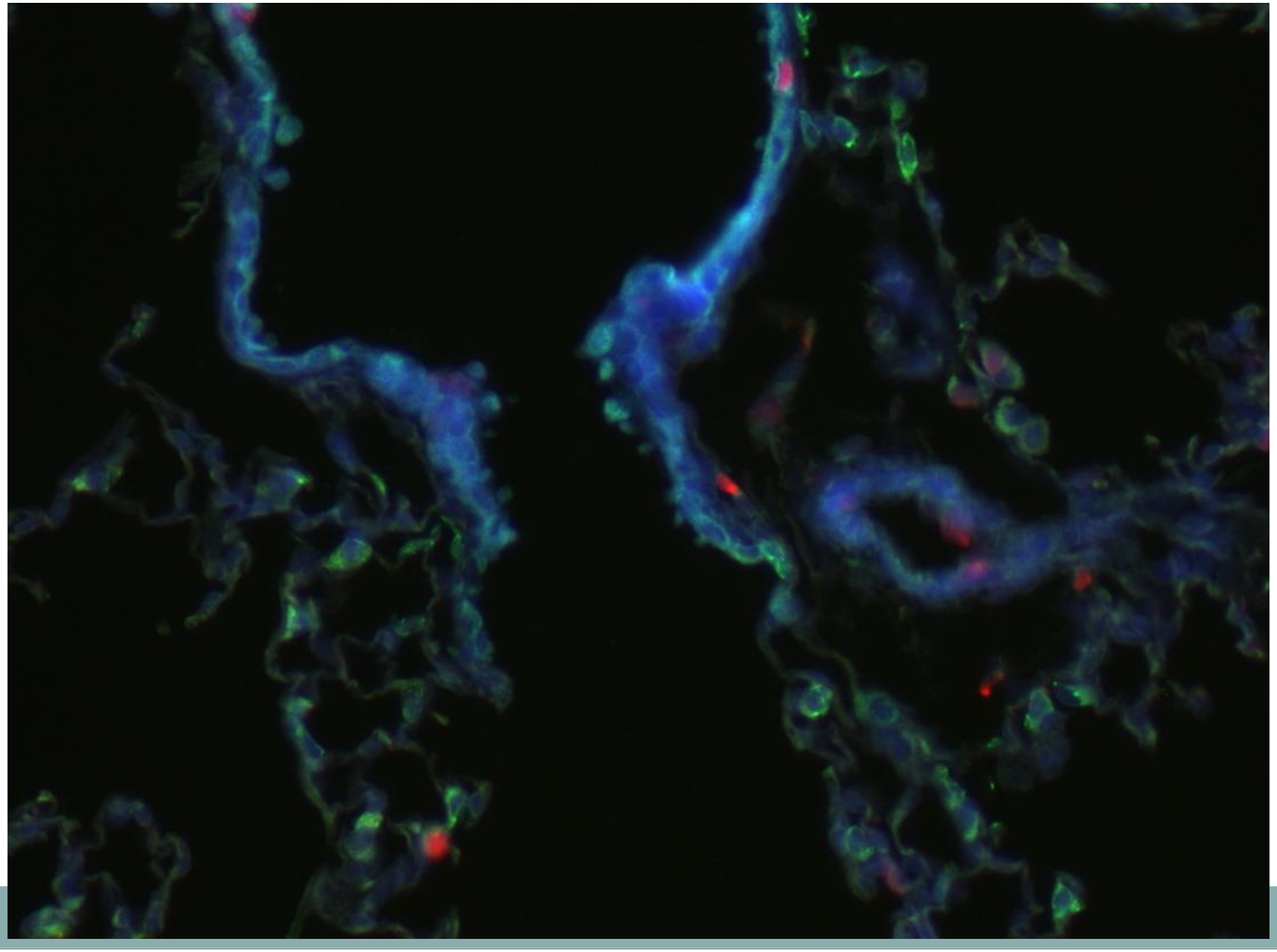
Locate apoptotic cells



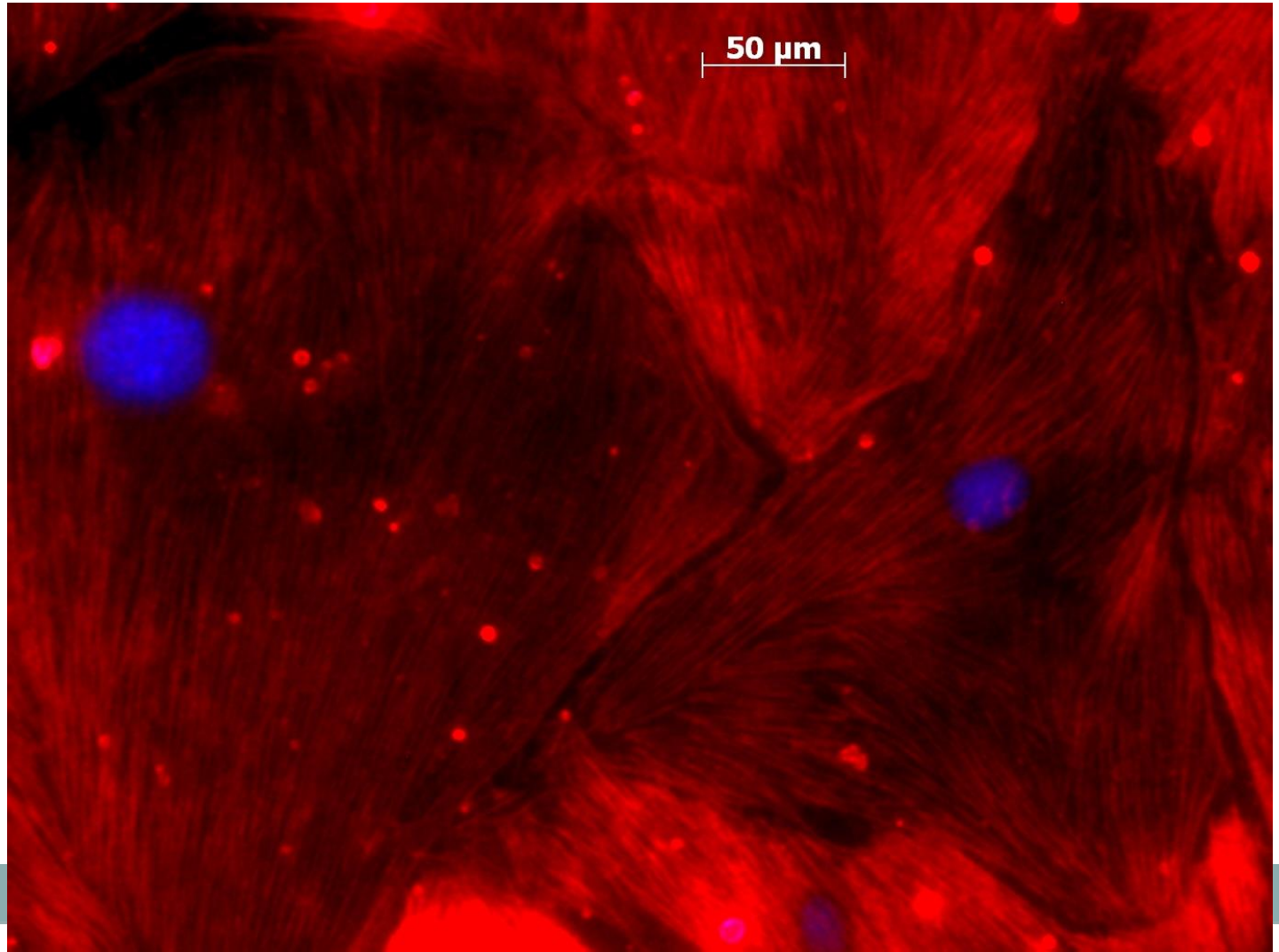
Identify activation states



Identify different types of cells in a tissue



inspect cytoskeletal structure



Protocol of IHC

1. **Tissue Collection and Perfusion**

Patient or animal biopsies, or whole animal organs, are collected for preservation and IHC analysis, depending on the requirements of the assay. Tissue must be rapidly preserved to prevent the breakdown of cellular protein and tissue architecture

- **2- Tissue Fixation**

Fixation chemically crosslink proteins or reduces protein solubility, which can mask target antigens during prolonged or improper fixation. The most common fixative is formaldehyde

3-Tissue Embedding

Fixed tissue samples are embedded in paraffin to maintain the natural shape and architecture of the sample during long-term storage and sectioning for IHC.

4-Sectioning and Mounting

The decision to section tissue is dependent upon the application used

5- **Epitope (Antigen) Recovery**

The paraffin from formalin-fixed, paraffin-embedded sections must be completely removed for the antibodies to reach the target antigens

6-Quenching/Blocking Endogenous Target Activity

quenching or masking endogenous forms of these proteins prevents false positive and high background detection.

7-Blocking Nonspecific Sites

Although antibodies show preferential avidity for specific epitopes, antibodies may partially or weakly bind to sites on nonspecific proteins. To reduce background the samples are incubated with a buffer that blocks the reactive sites to which the primary or secondary antibodies may otherwise bind. normal serum, non-fat dry milk, BSA or gelatin.

8-Sample Labeling (Immunodetection)

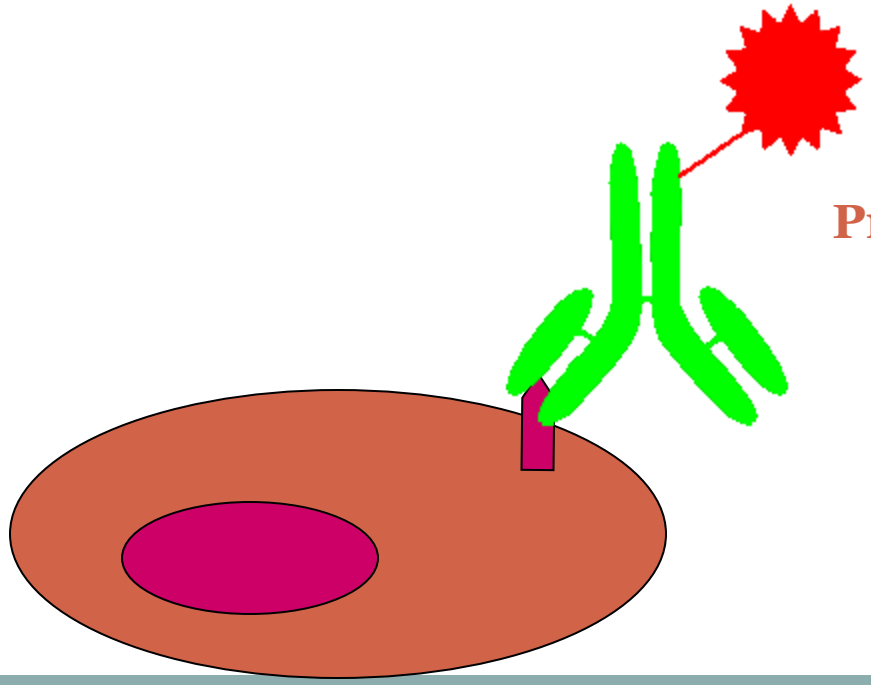
Detecting the target antigen with antibodies is a multi-step process that requires optimization at every level to maximize the signal detection

9-β-Galactosidase Staining

10-Counterstaining

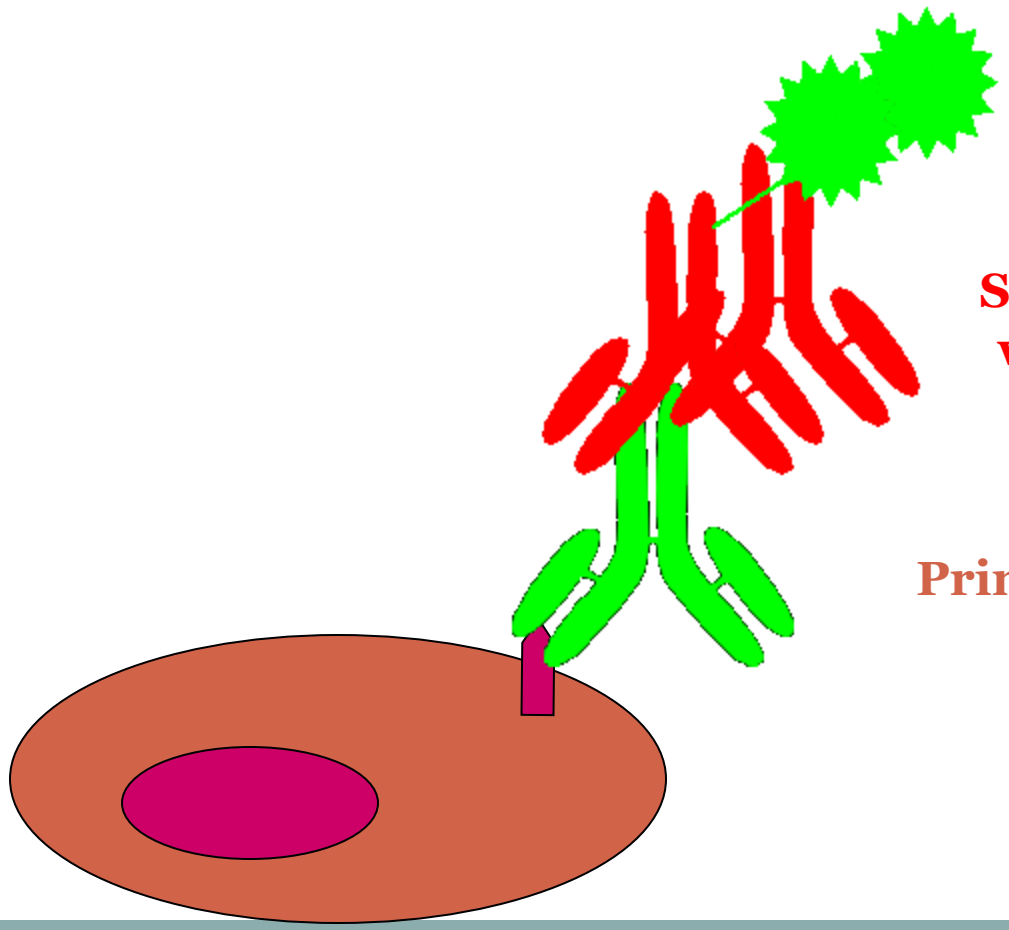
- **11-Sealing the Stained Sample**
- Sealing the sample by mounting a coverslip with an appropriate mounting stabilizes the tissue sample and stain. The coverslip can then be sealed with clear nail polish or a commercial sealant after the mountant has cured to prevent sample damage
- **12-Sample Visualization**
- Once the sections are prepared, the samples are viewed by light or fluorescent microscopy. Depending on the antibody detection method, one can perform confocal microscopy for greater detail and enhanced imaging capabilities

Direct method- primary antibody only



Primary Ab labeled with fluorescent dye

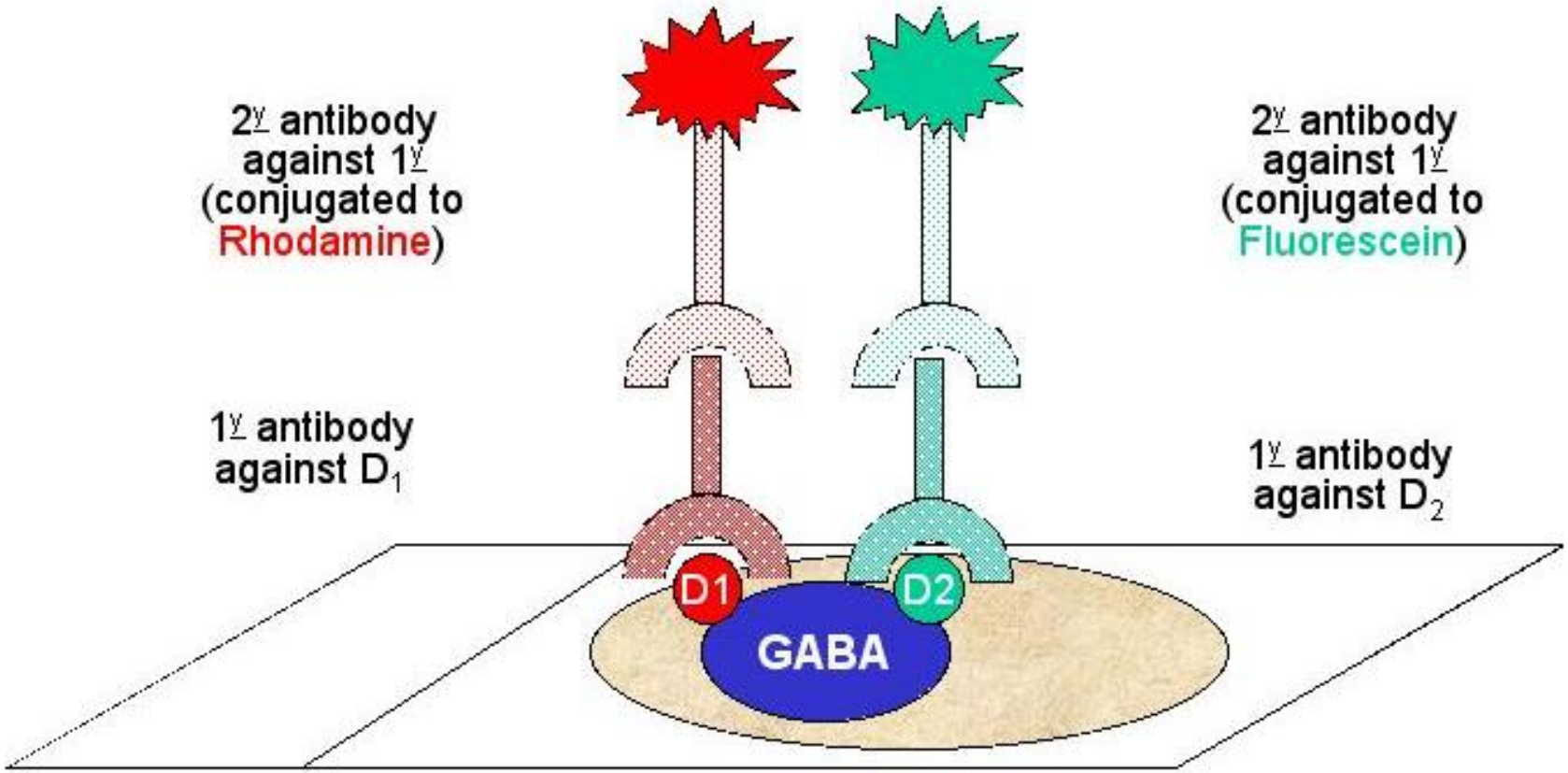
Indirect method – primary and secondary antibodies



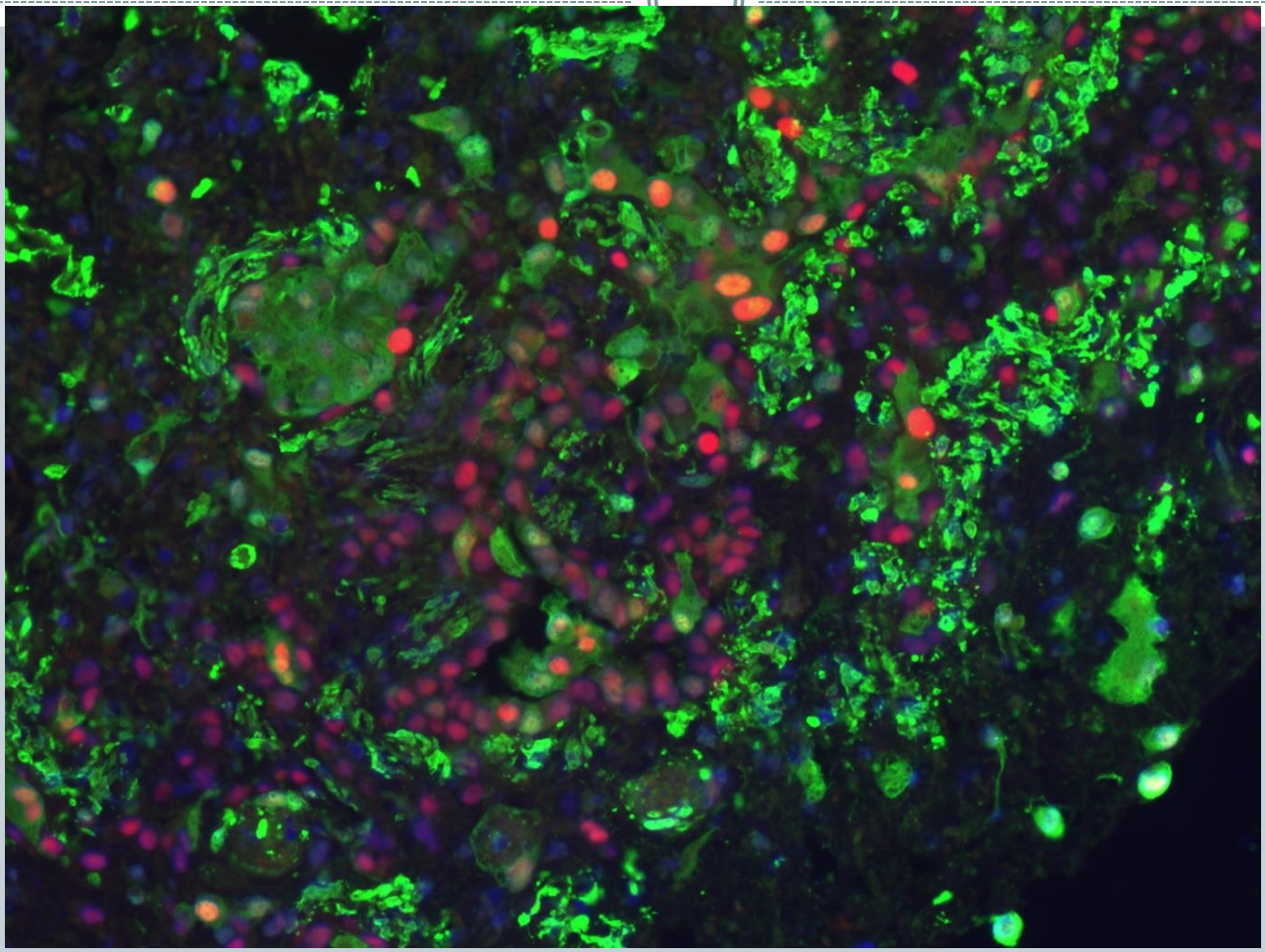
Secondary Ab labeled with fluorescent dye

Primary Ab

Multiple Immunofluorescence



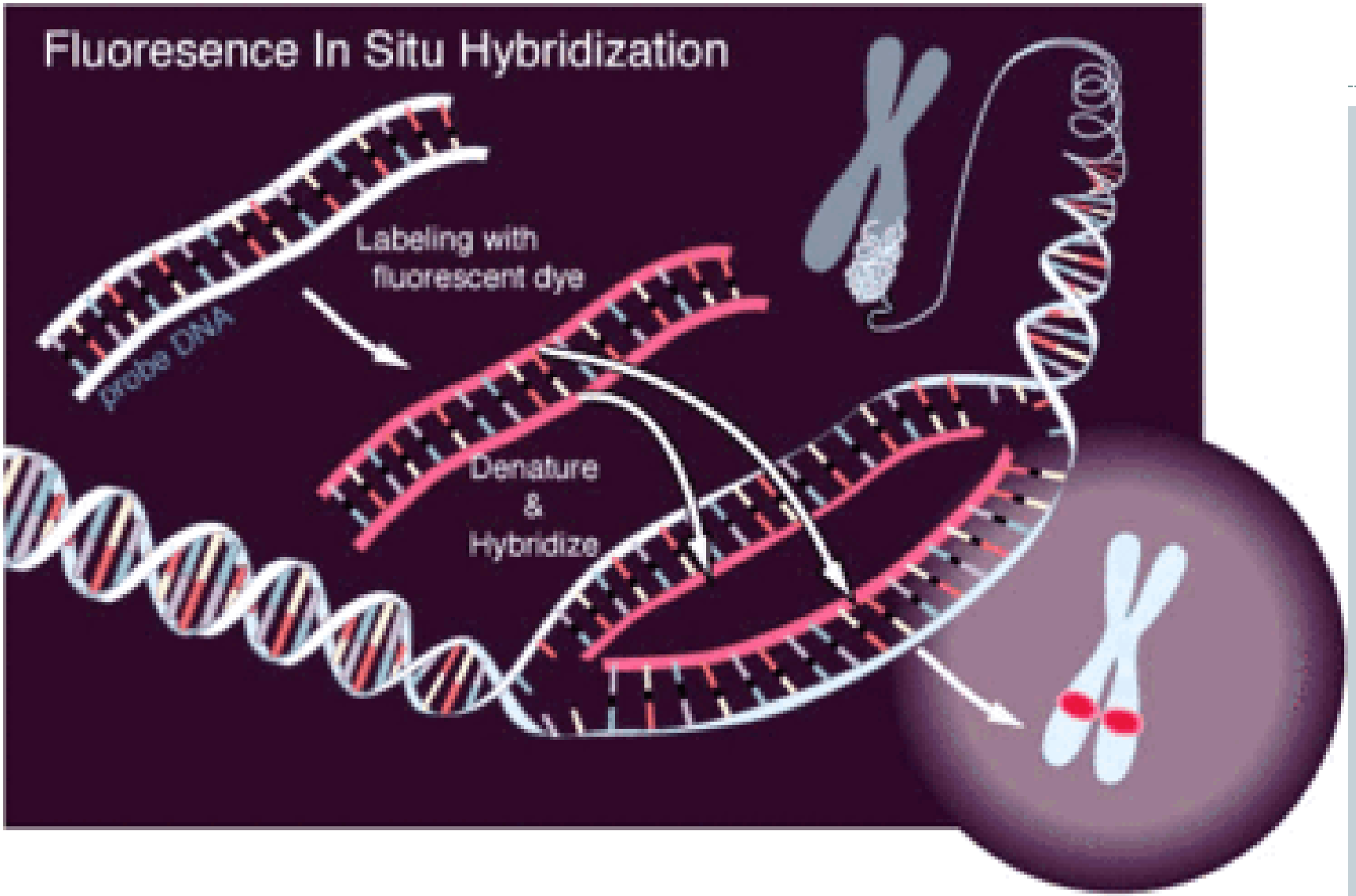
Multiple Labelling of a Tissue Section



(Fluorescence in situ hybridization (FISH

- is a cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity.
- Probe: is a segment of artificial single strand nucleic acid
- It was developed by biomedical researchers in the early 1980s and is used to detect and localize the presence or absence of specific DNA sequences on chromosomes

- Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes.
- FISH is often used for finding specific features in DNA for use in genetic analysis, medicine, and species identification.
- FISH can also be used to detect and localize specific RNA targets (mRNA, tRNA and miRNA) in cells, circulating tumor cells, and tissue samples.
- In this context, it can help define the spatial -temporal patterns of gene expression within cells and tissues.


Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize

(FISH) is a powerful technique used in

- the detection of chromosomal abnormalities
- research
- diagnosis of haematological malignancies and solid tumours
- used for understanding genetic mutations.