Cihan University/ Sulaimaniya

College of Health Science

Medical Laboratory Analysis

4th Stage- 1st Semester

Pr. Clinical Immunology

Lab- 7: Immunofluorescence

2024- 2025

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Immunofluorescence

- Immunofluorescence (IF) is a common laboratory technique used for detection of antigens in various tissues and on cell surfaces.
- In 1944, Albert Coons demonstrated that antibodies can be labelled with molecules which have ability to fluoresce.
- Antibodies are chemically tagged with fluorescent dyes such as fluorescein isothiocyanate (FITC) or tetramethyl rhodamine isothiocyanate (TRITC).
- These labelled antibodies bind **directly** or **indirectly** to the antigen of interest that allow for detection and localization of the antigen through visualization of fluorescence emitted.

Immunofluorescence

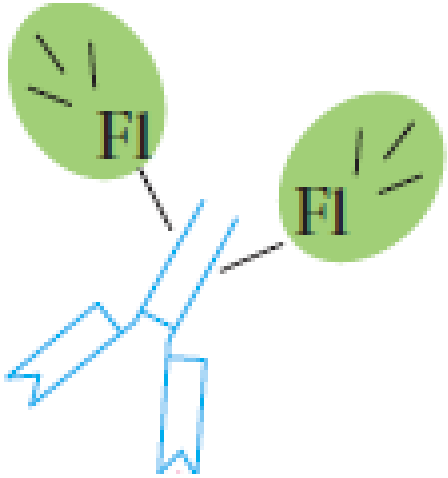
- The fluorescence can be visualized using **Fluorescence** or **Confocal Microscopy**.
- Fluorochroms are dyes that absorb ultra-violet rays and emit visible light. There are two widely used fluorochromes:

1. **Fluorescein**: an organic dye which is commonly used for labelling of antibody in immunofluorescence procedures, absorbs blue light (490 nm) and emits an intensive yellow-green fluorescence (517 nm).
2. **Rhodamine** : an organic dyes which absorbs yellow-green light (515 nm) and emits a deep red fluorescence (546 nm).

Direct IF

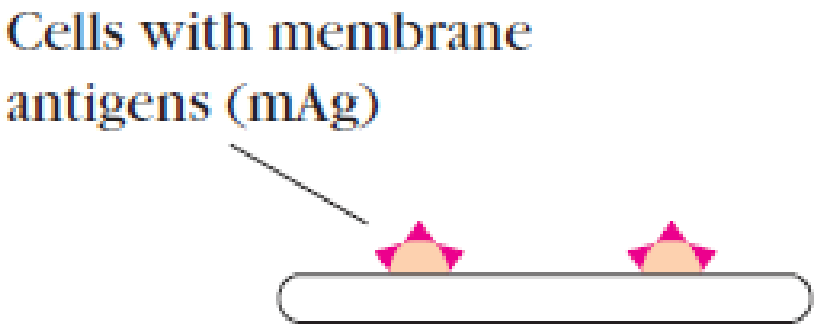
- Antibodies are conjugated with fluorescent dye

  e.g. Fluorochrome-labelled antibody.

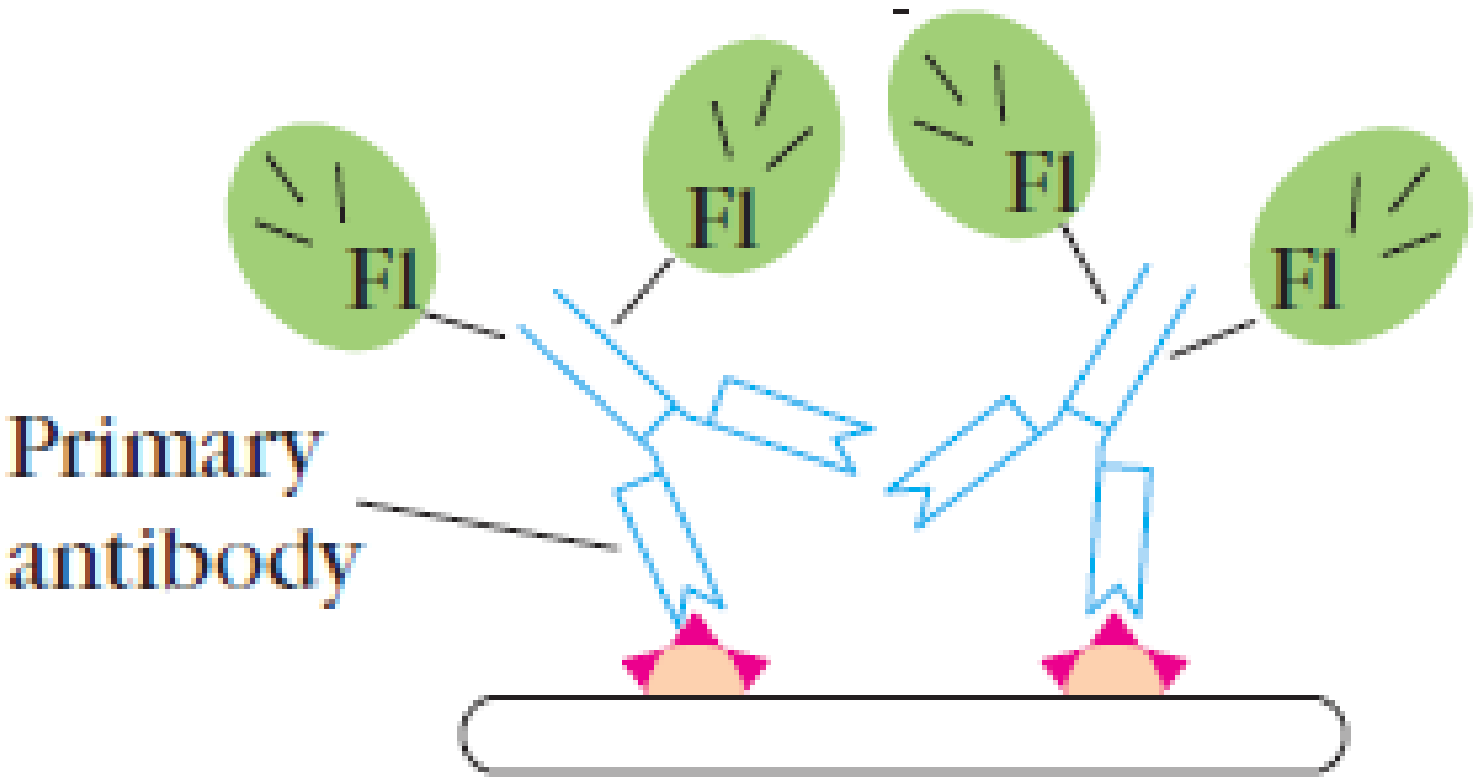


- Conjugated antibody directs against antigen of interest.

  e.g. Cell membrane antigen



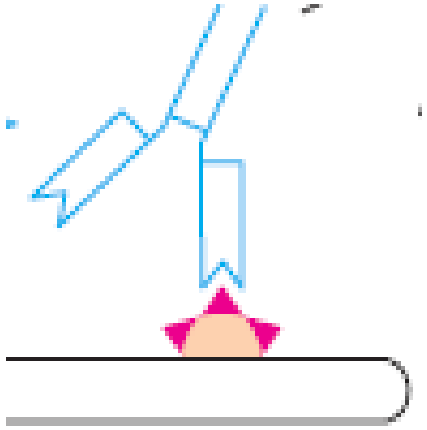
Direct IF



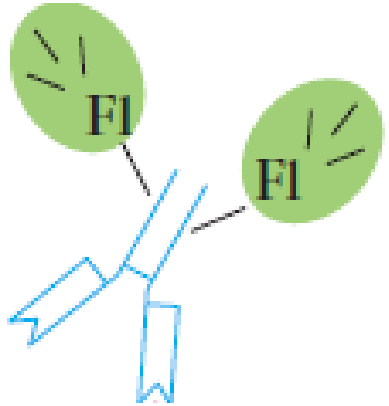
(a) Direct method with fluorochrome-labeled antibody to mAg

Indirect IF

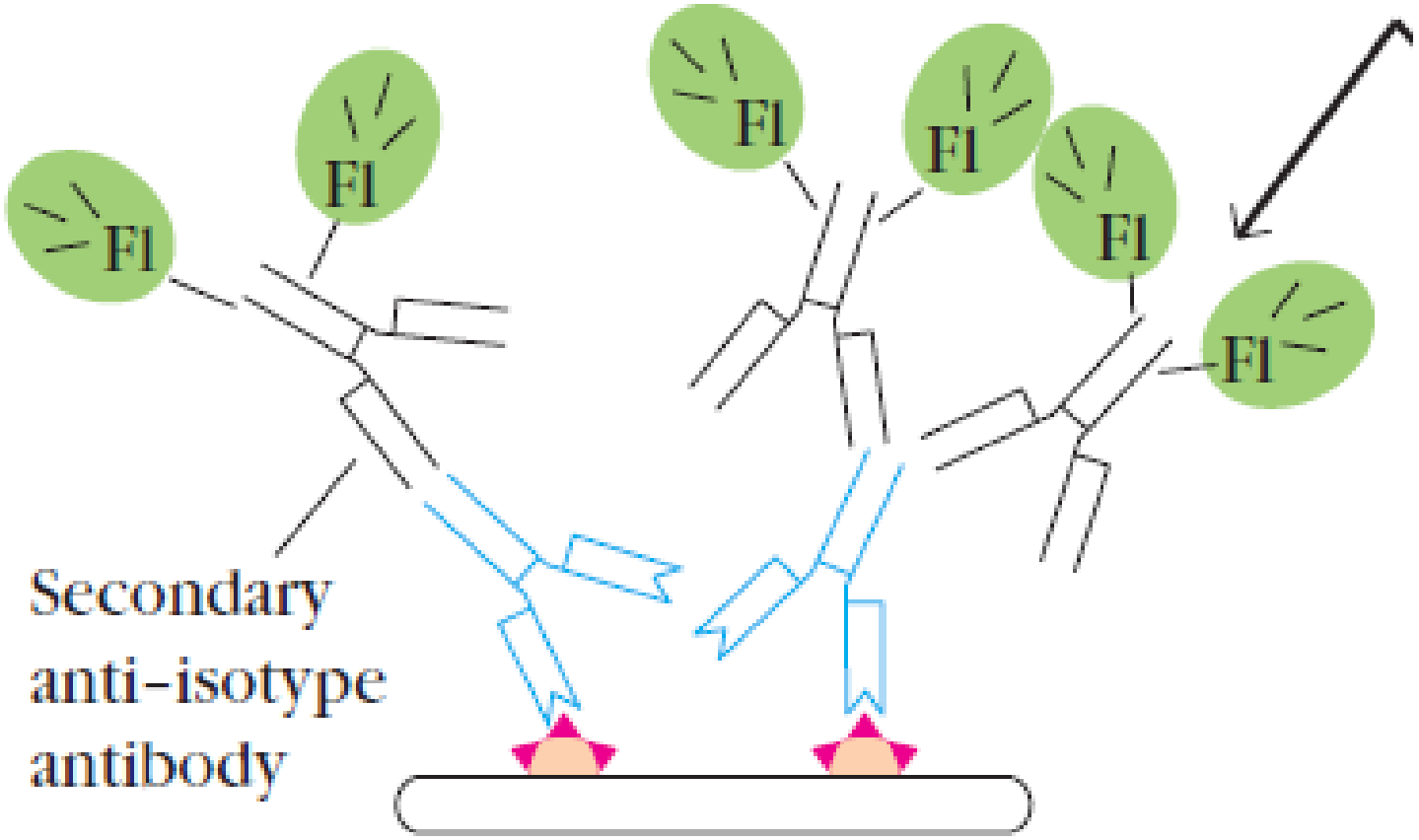
- Primary antibody is unlabelled and directed against antigen of interest.
- Primary antibody is raised from mouse.



- Secondary antibody is conjugated with fluorescent dye and directed against primary (unlabelled) antibody.
- Secondary antibody is anti-mouse antibody raised from rabbit.

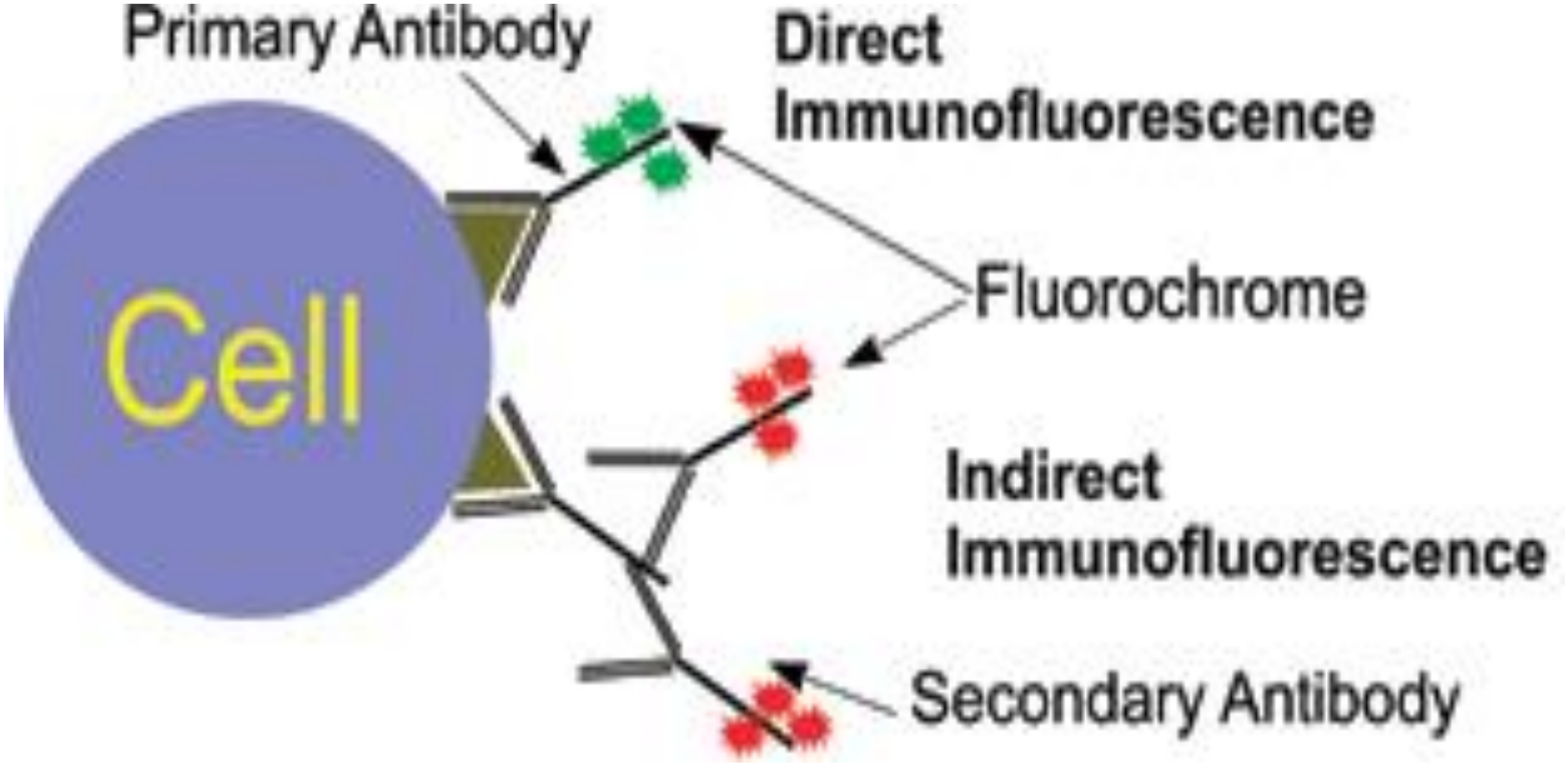


Indirect IF



(b) Indirect method with fluorochrome-labeled anti-isotype antibody

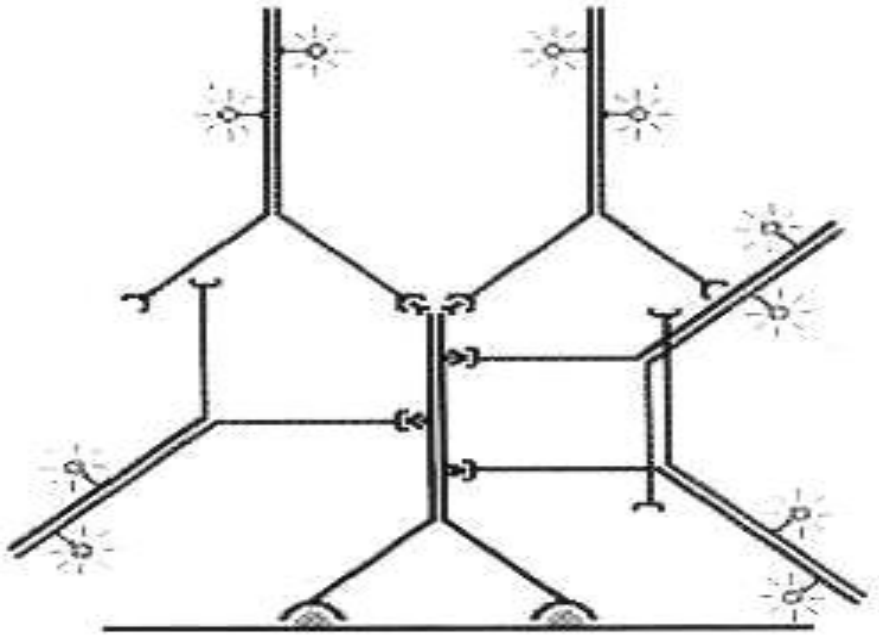
Indirect IF


Primary Antibody
Direct
Immunofluorescence
Cell
Fluorochrome
Indirect
Immunofluorescence
Secondary Antibody

Complement Indirect IF

- A normal tissue substrate is overlaid with heated plasma, serum or any tissue fluids at 56 °C for 30 minutes.
- Specimens are then washed.
- Fresh complement (such as fresh human serum) will be added to the tissue substrate and incubate to allow binding between complement (C3) with antigen-antibody complex binding site in the tissue.
- Wash again to remove unbound complement
- Sections are then incubated with fluorescein-labelled anti-human C3 antibodies which bind to C3 bound antibody-antigen complex.

Complement Indirect IF



Legend:

- antigen
- primary antibody
- secondary antibody
- complement
- fluorochrome

- After washing, the sections would be examined under a fluorescent microscope

Advantage and disadvantage of Direct IF

- **Advantage:**
  - Shorter staining sample time and simpler dual and triple labelling procedure
- **Disadvantage:**
  - Lower signal and higher cost.

Advantage and disadvantage of Indirect IF

Advantage:

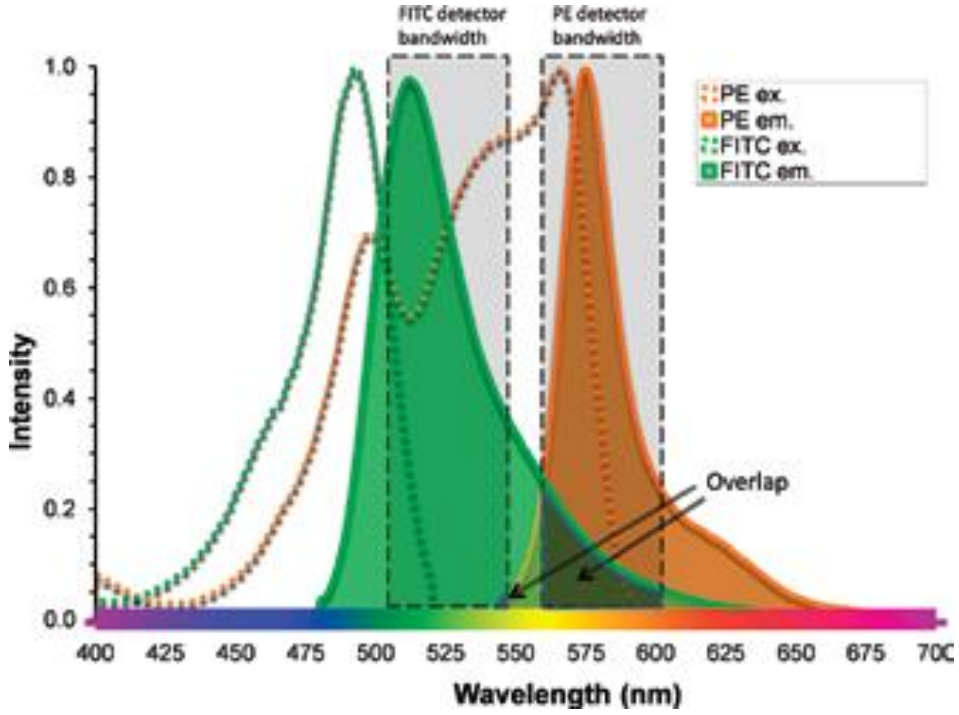
- More sensitive than direct IF
- There is amplification of signals in indirect IF.
- Commercially produced secondary antibodies are inexpensive, available in different colours.

Disadvantage:

- Cross reactivity is a main problem.

Limitation of IF technique

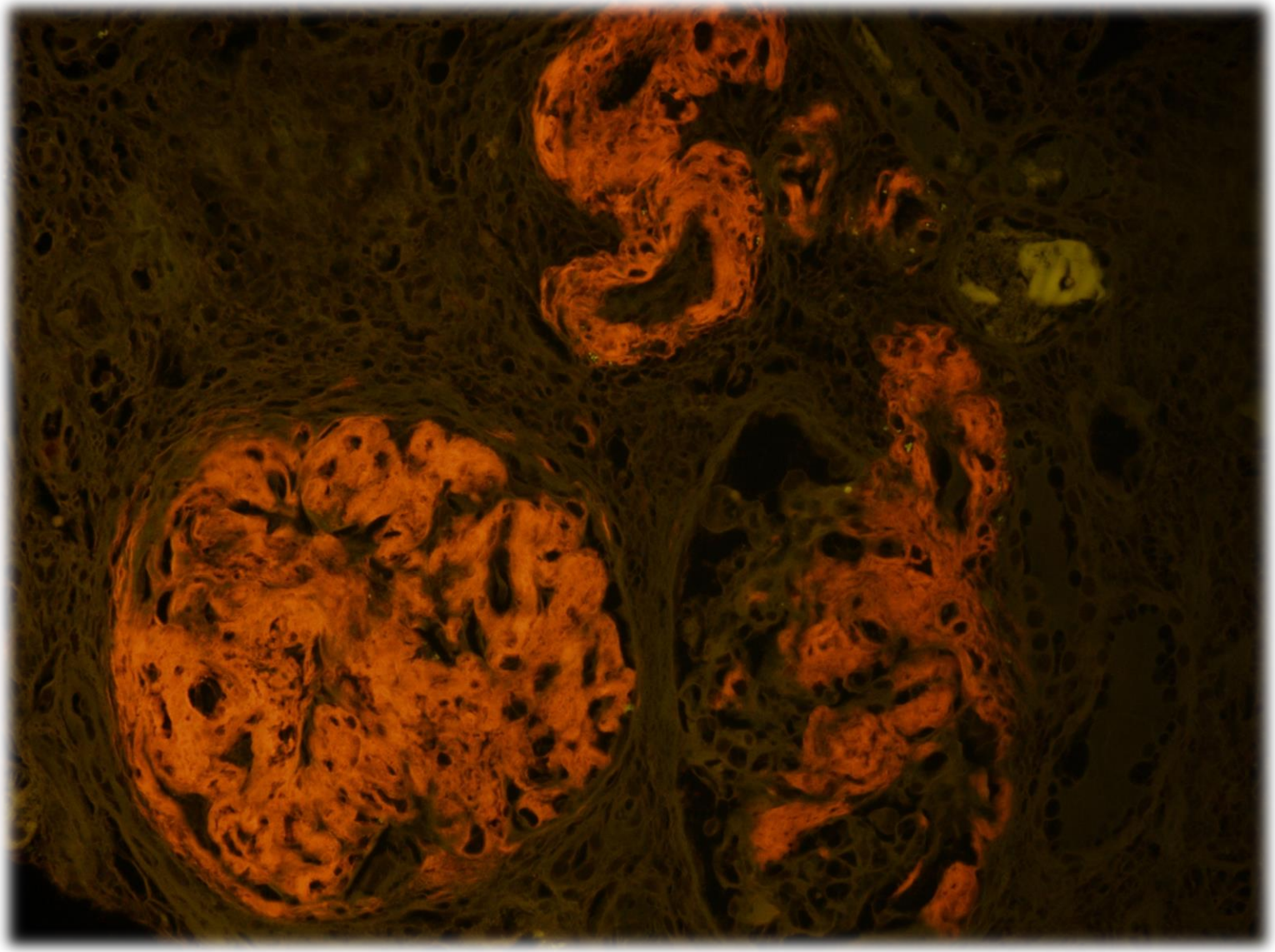
- Photobleaching (reactive oxygen species)
- Autofluorescence (flavin coenzymes (FMN & FAD) and reduced pyridine nucleotide (NADH))
- Fluorescence overlap

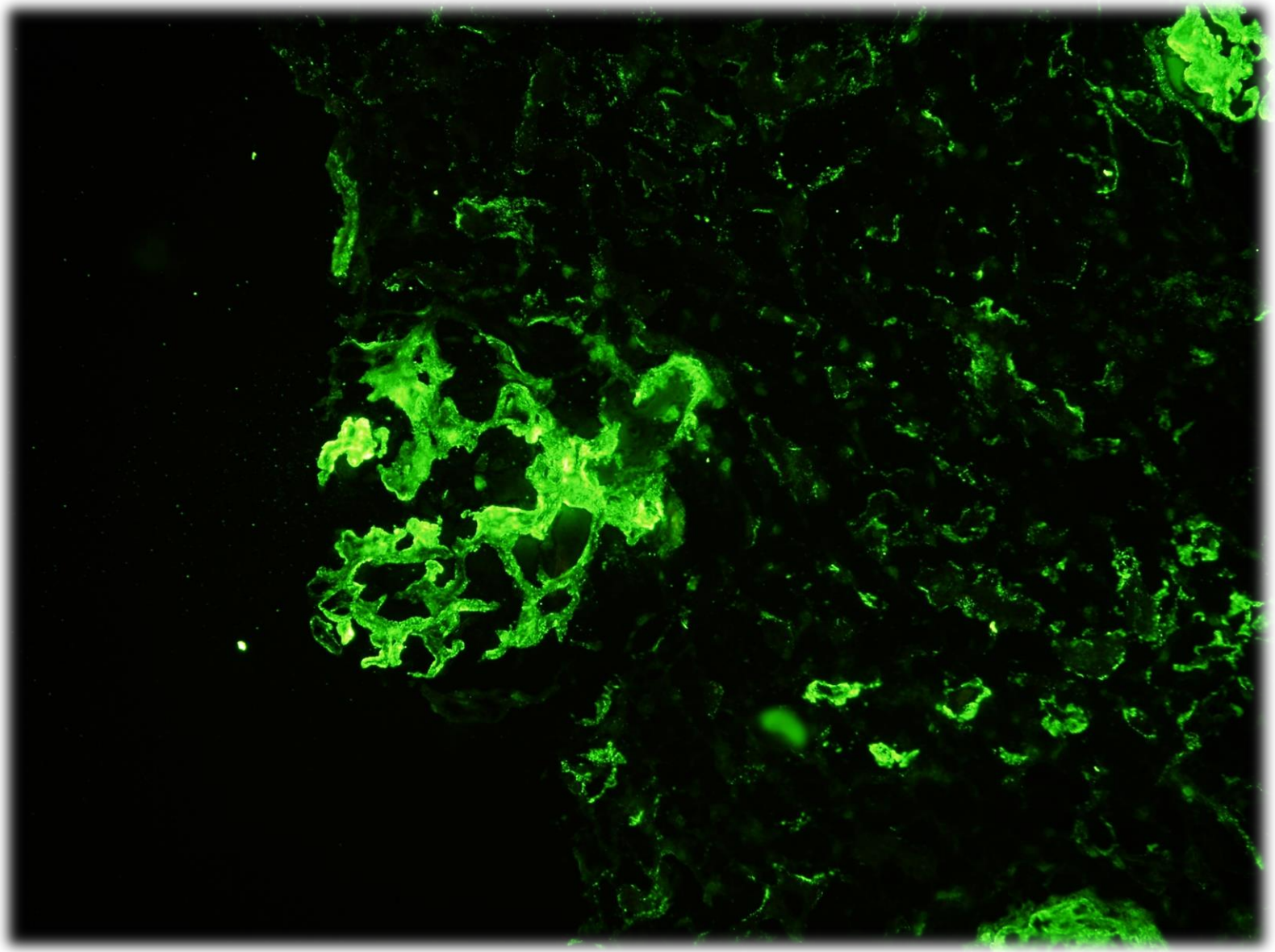


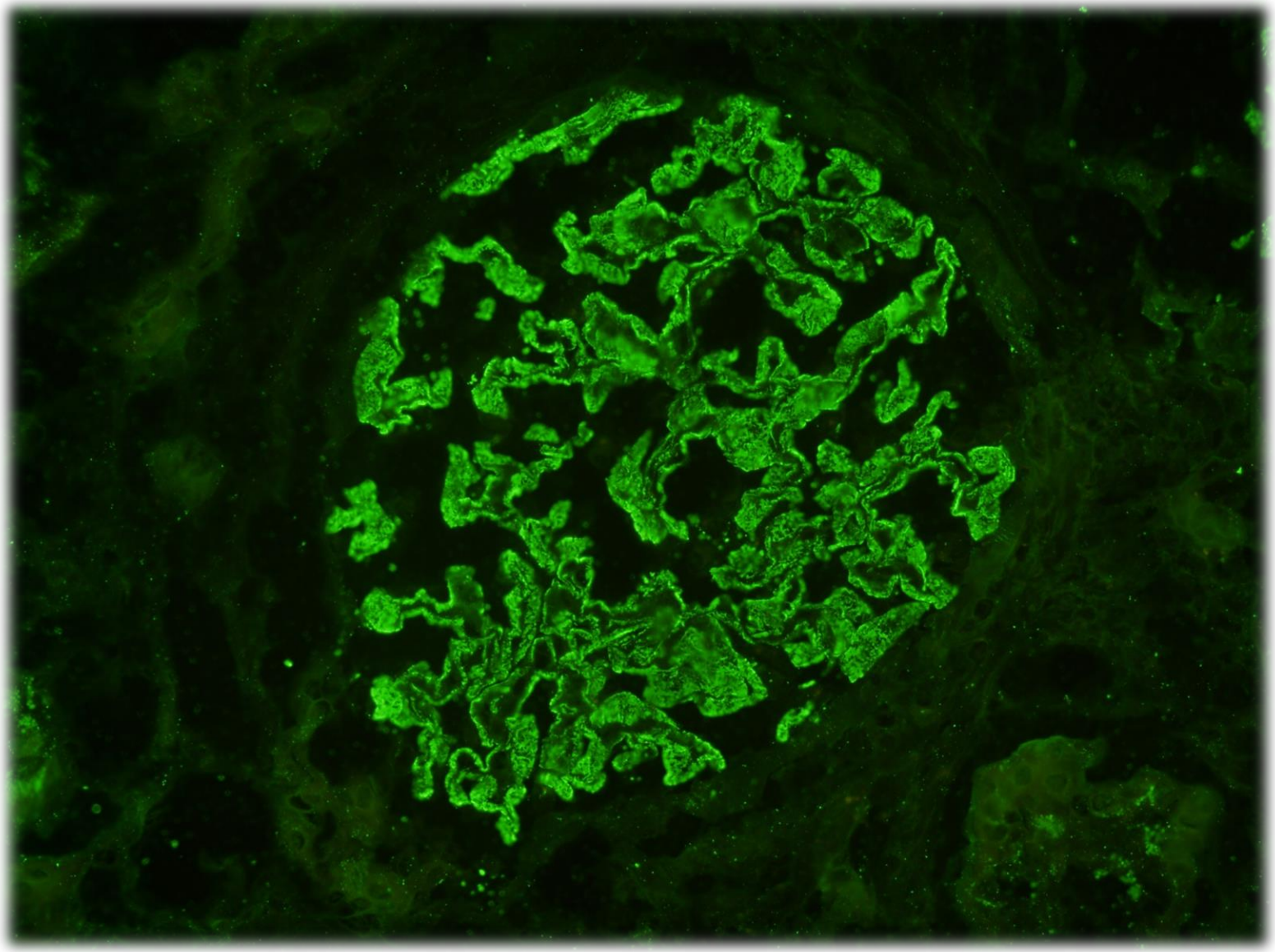
Application of IF

- Detection of antigen in fresh, frozen or fixed tissues.
- Localization of cellular organelles by detection of antigens covered them.
- IF is also suitable for detection of antibody-antigen complex in autoimmune disease.
- Detection and localization of specific DNA sequences on chromosome using Hoechst 33342 fluorochrome.
- Defining the pattern of gene expression within cells or tissues.


DELL







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