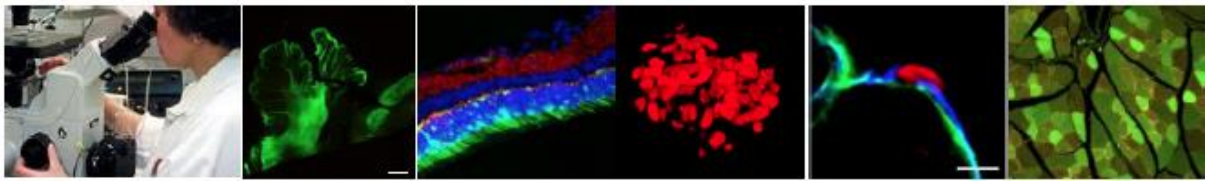
Fluorescence Bio-imaging practical courses

Immunohistochemistry : Principle

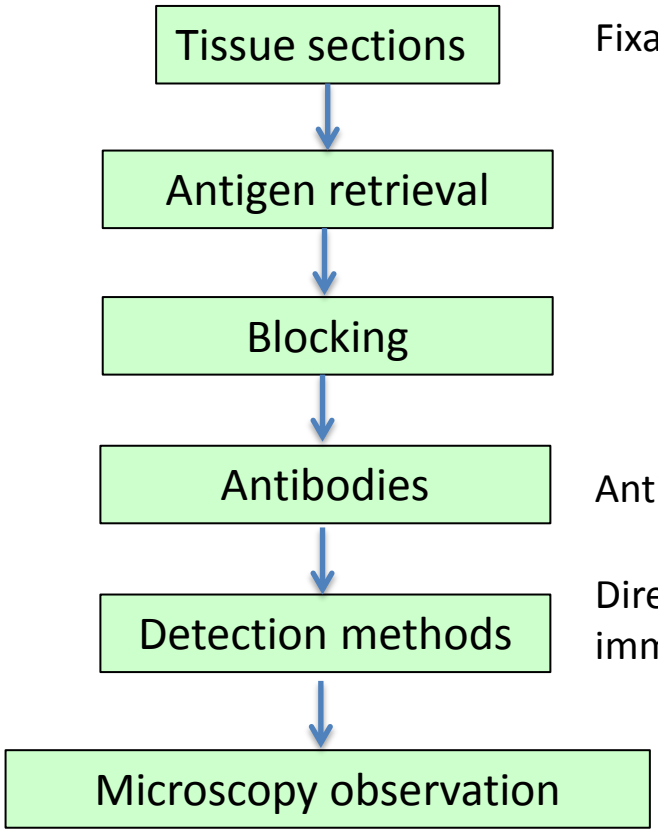
Laurence Dubreil/Steven Nedelec/Johan Deniaud/Candice Babarit/Helicia Goubin



Immunohistochemistry- what's good about it?

- Uses antibodies to detect and visualize antigens in cells from tissue section
- Antibodies bind to antigen in specific manner
- Gives you a *spatial location*
- Can be used to locate particular cells and proteins
- Can be used to identify cellular events – e.g.apoptosis

IHC steps and important considerations



Fixation , sectioning

Antibodies selection

Direct or indirect methods, immunoenzyme, fluorescence, controls

You actually need to care about all this now because it may affect how you harvest your samples !

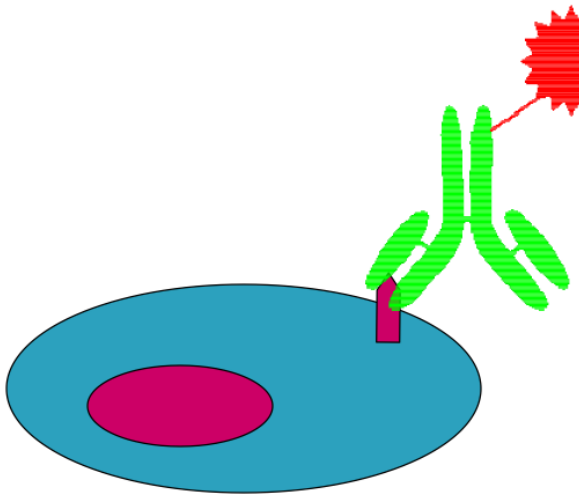
Sectioning

Paraffin	Frozen
(-) Must heat and process through xylenes and alcohols – ruins some antigens (+) Good morphology	(-) Poor morphology (+) Better survival of many antigens

Detection methods

- **Direct Immunofluorescence method**

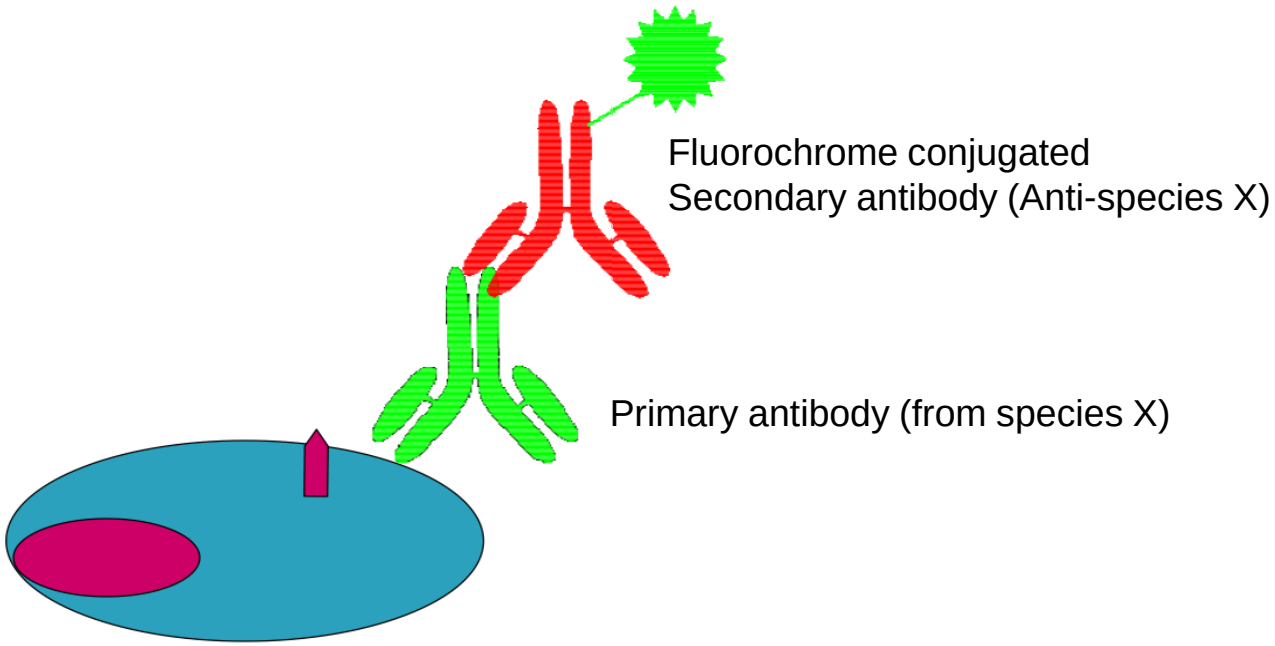
(-) Easy application, only few steps
(+) Not very versatile



Primary antibody conjugated with a fluorochrome

- **Indirect Immunofluorescence method**

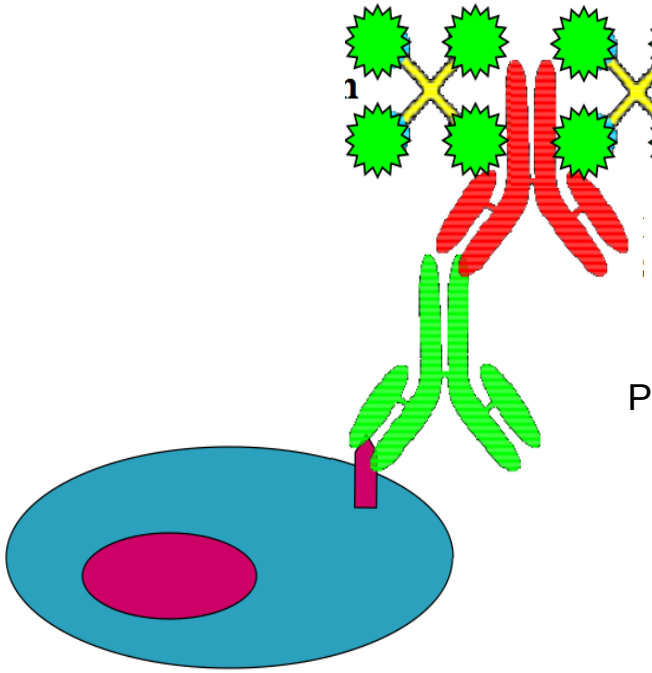
(-) More steps
(+) More versatile (= different combination of fluorophores possible)



Detection methods

- **Indirect immunofluorescence using biotinylated secondary antibody**

(+) Biotin binds to avidin with high affinity (good linker system)
(+) very high sensitivity



Fluorochrome conjugated Streptavidin

Biotinylated Secondary antibody (Anti-species X)

Primary antibody (from species X)

Detection methods

- **Multiple Immunofluorescence Labelling**