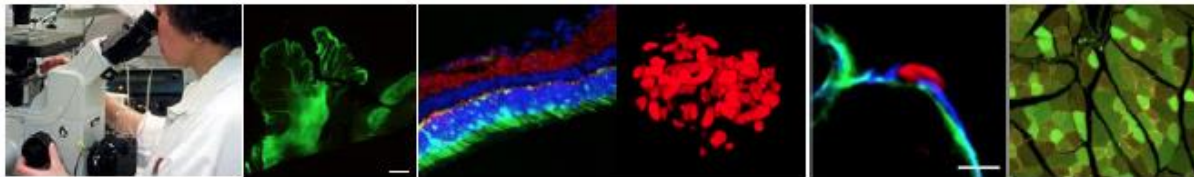




Fluorescence Bio-imaging practical courses

Immunohistochemistry : Principle

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

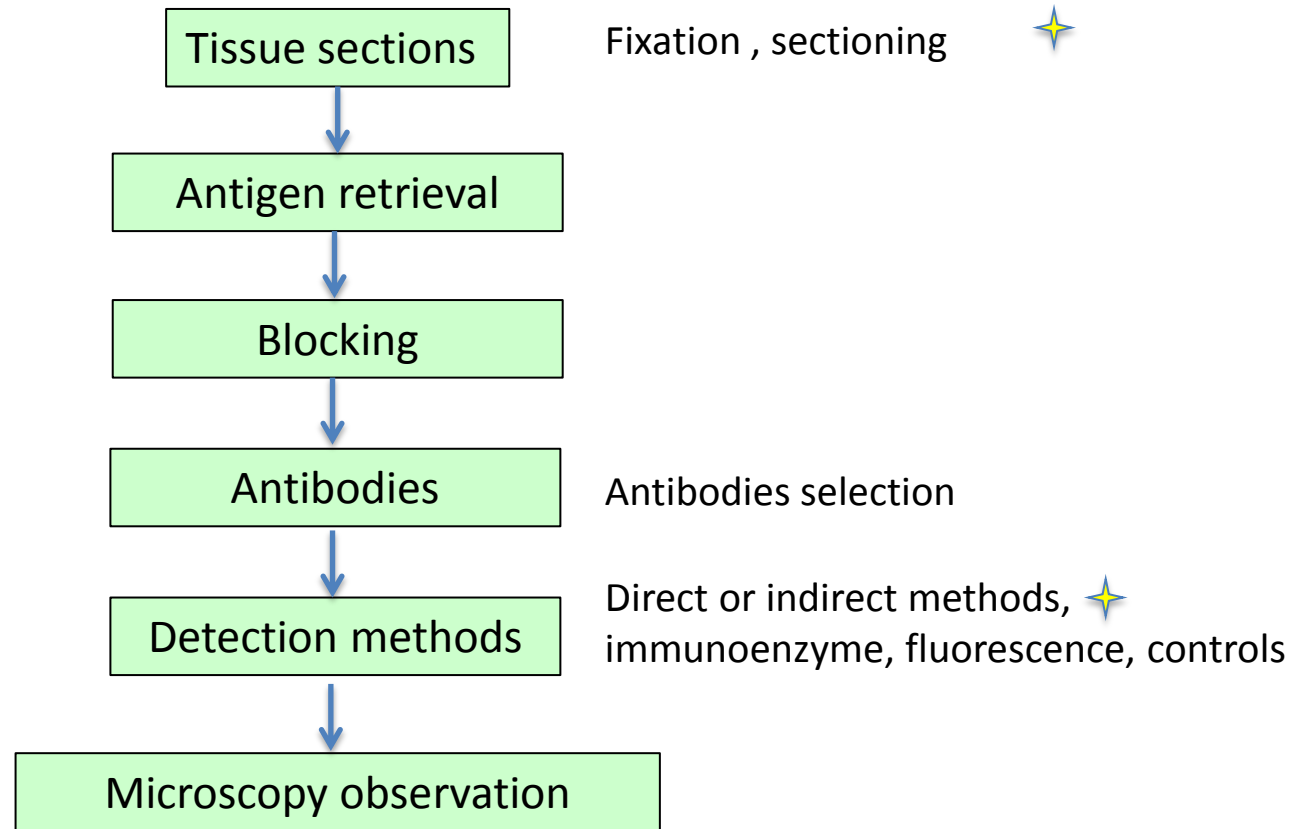




Immunohistochemistry- what's good about it?

- 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



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

Sectioning

Paraffin

(-) Must heat and process through xylenes and alcohols – ruins some antigens

(+) Good morphology



Frozen

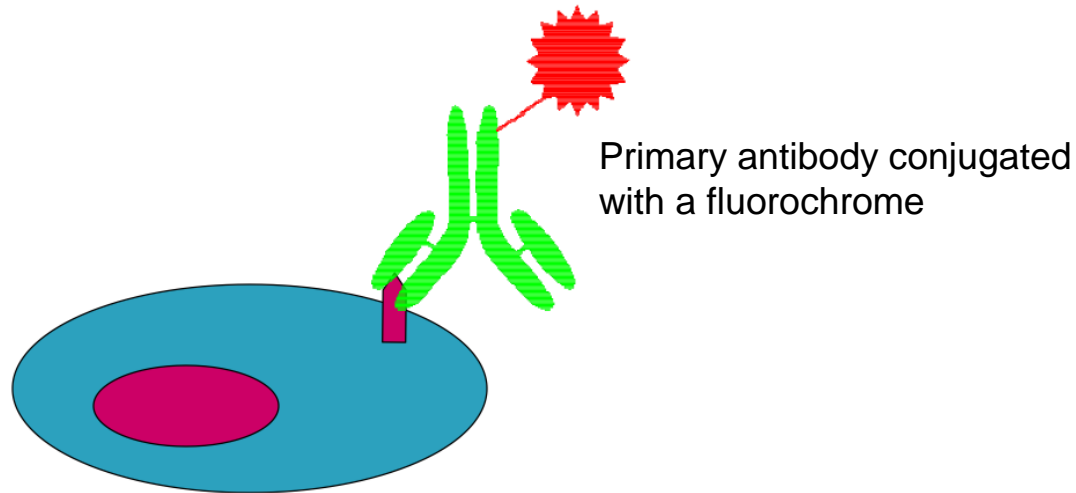
(-) Poor morphology

(+) Better survival of many antigens



- **Direct Immunofluorescence method**

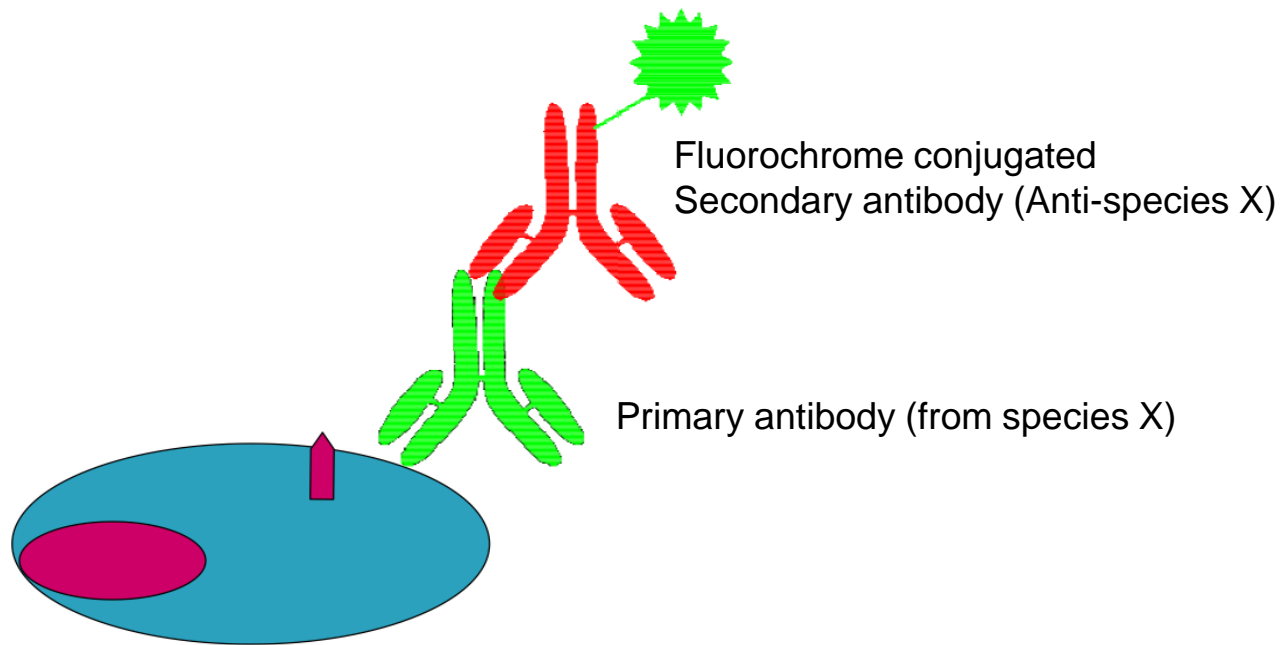
- (-) Easy application, only few steps
- (+) Not very versatile



- **Indirect Immunofluorescence method**

(-) More steps

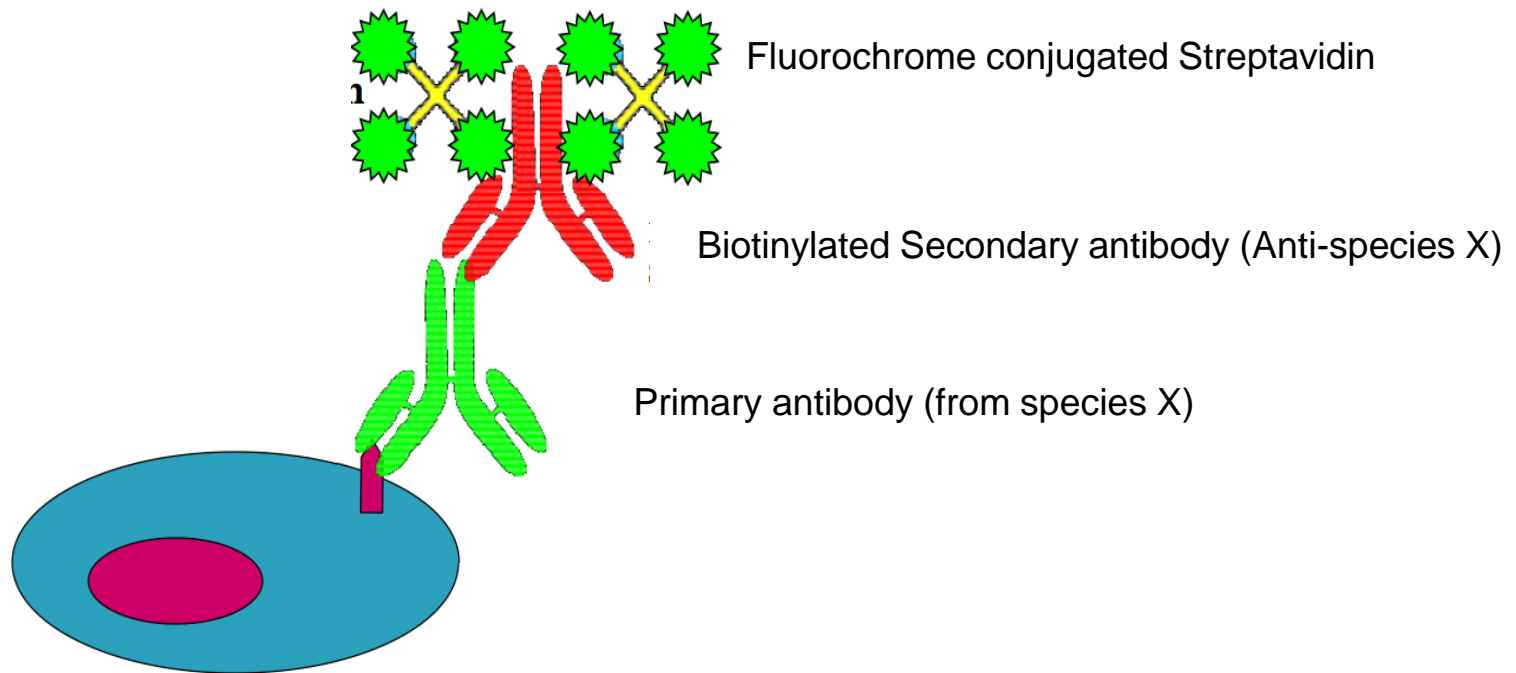
(+) More versatile (= different combination of fluorophores possible)



- **Indirect immunofluorescence using biotinylated secondary antibody**

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

(+) very high sensitivity



- Multiple Immunofluorescence Labelling

