

Immunohistological procedures

Dr. Péter Balogh

Department of Immunology and Biotechnology

Similarities and differences between immunohistochemistry and immunofluorescence

What do we see? IF: signal/autofluorescence; IHC: signal/endogenous

EA

For how long can we see it? IF: as long as the Ab is bound; IHC: as long as the precipitate lasts

What do we assess?

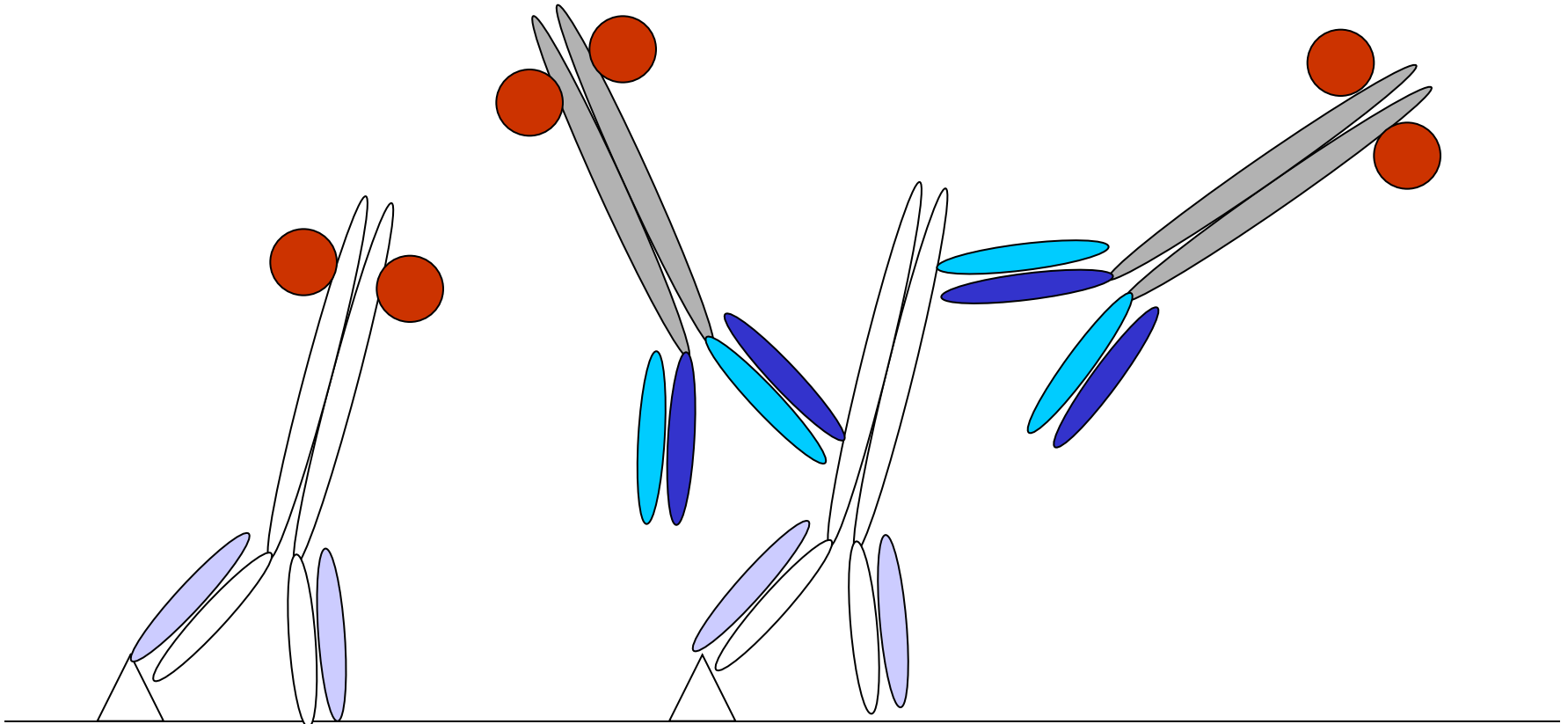
How do we improve signal strength?

Quantification?

Direct, indirect immunohistochemistry

▲ : antigen

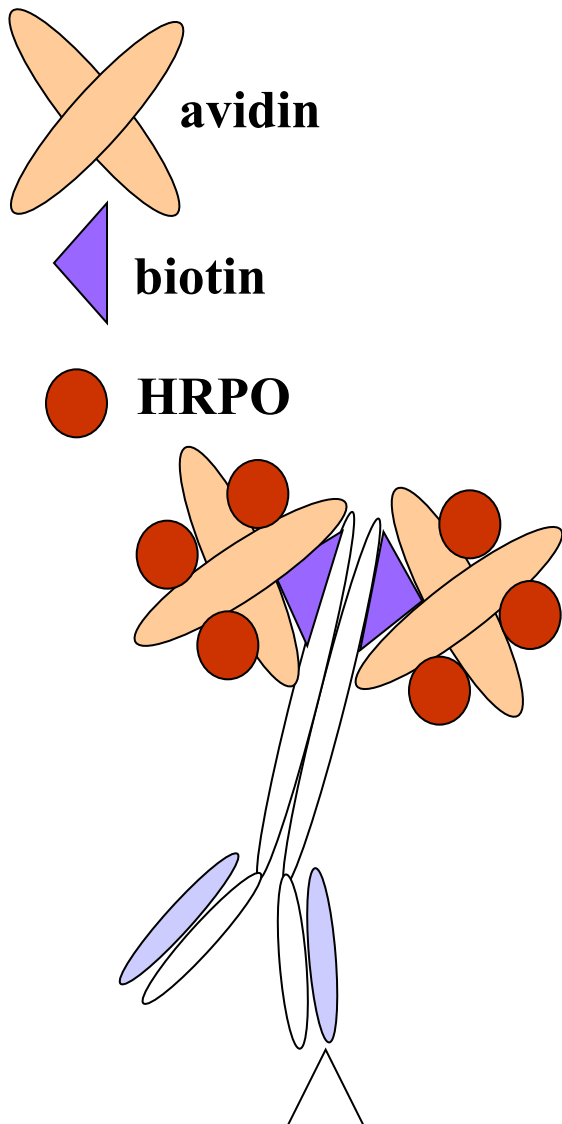
● : enzyme (HRPO, ALP), fluorochrome (FITC, TRITC, PE)



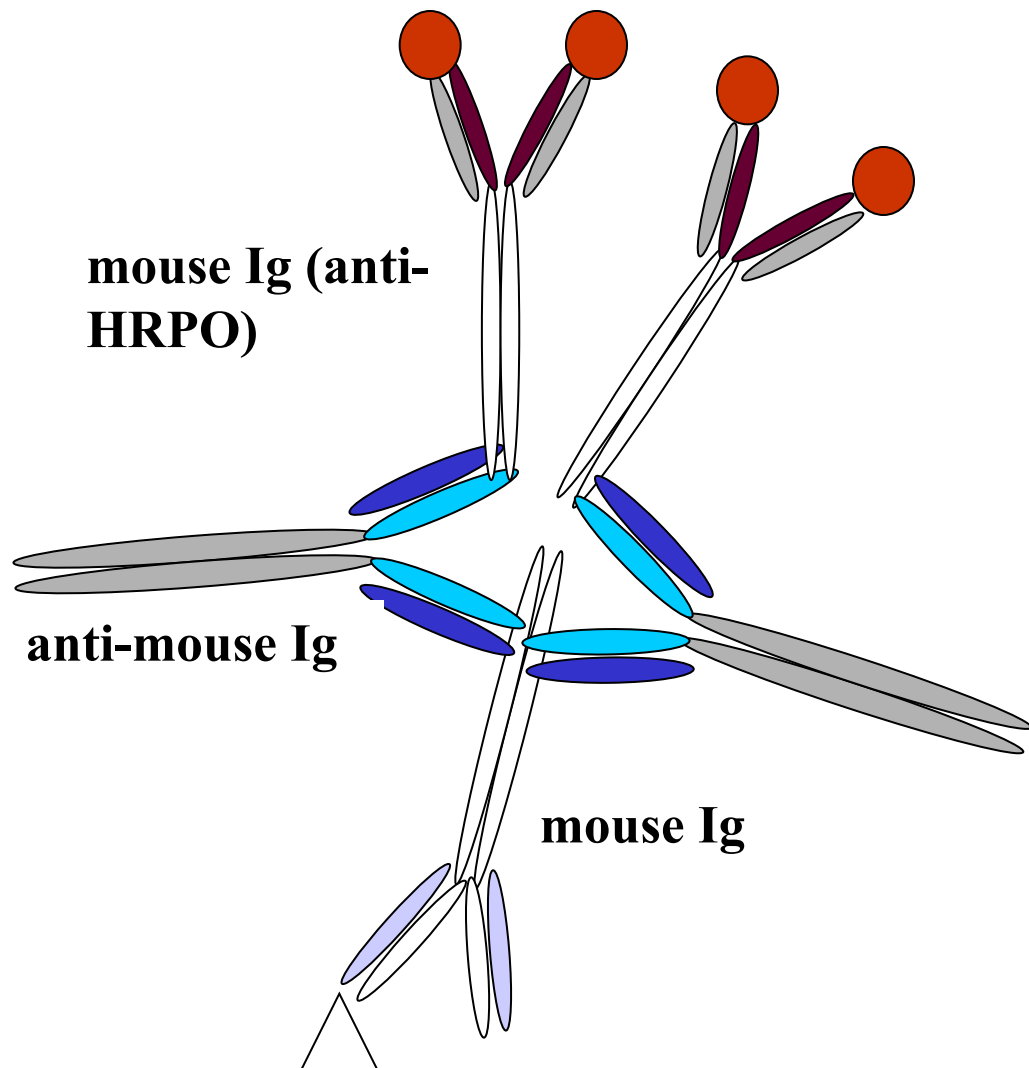
Direct method

Indirect method

Complex detection systems



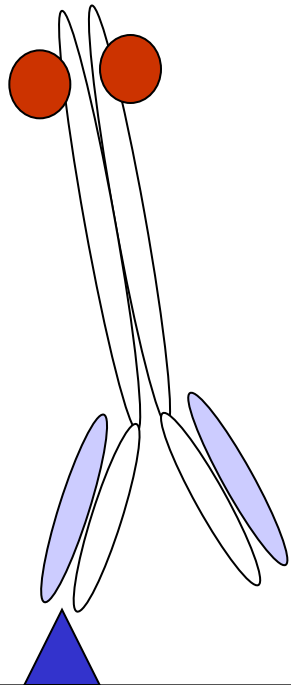
**(Strept)avidin-biotin-
HRPO complex**



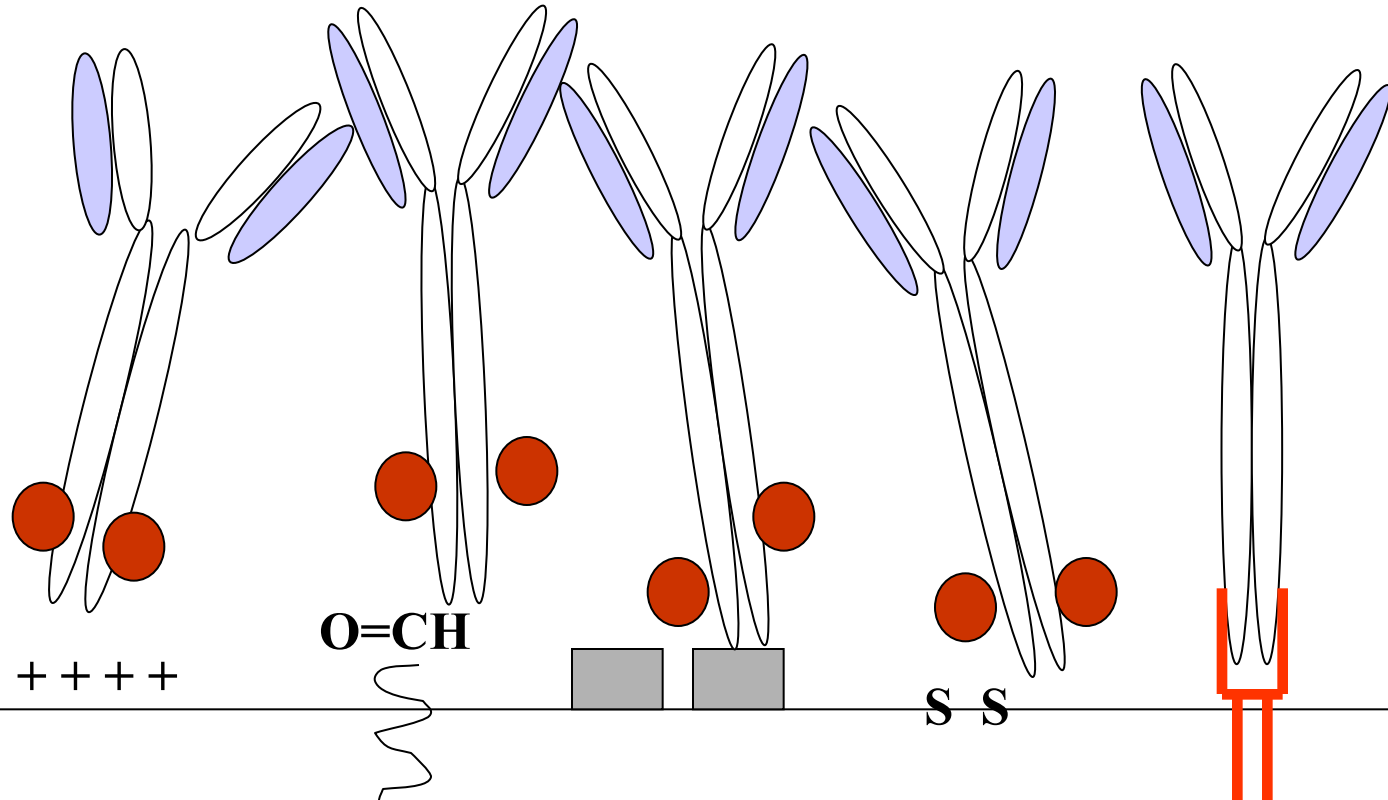
**Peroxidase-antiperoxidase
complex**

Specific and non-specific interactions

Specific Ag-Ab binding



Non-specific Ag-Ab binding



Ionic

Aldehyde

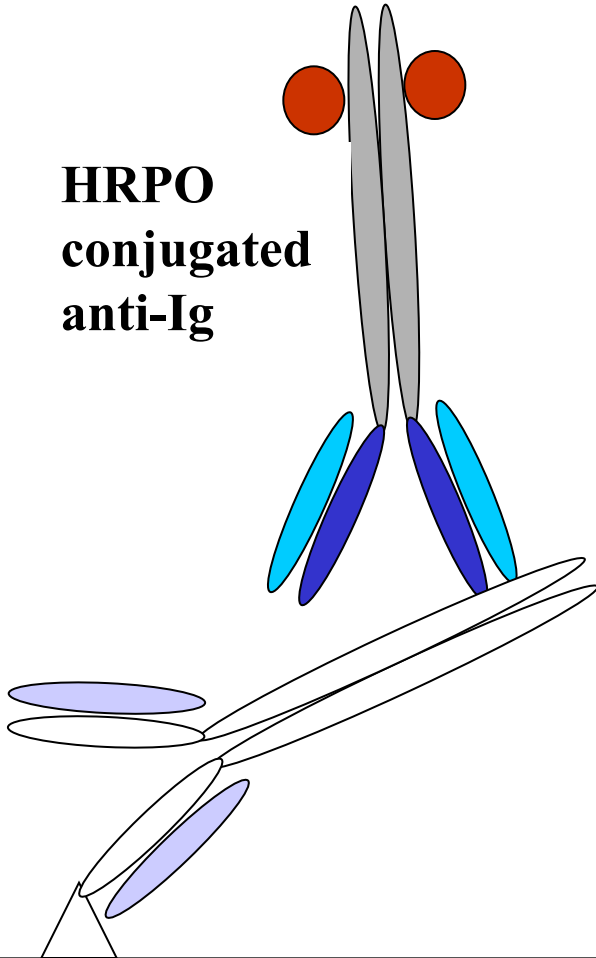
Hydrophobic

Sulfid

Fc-receptor

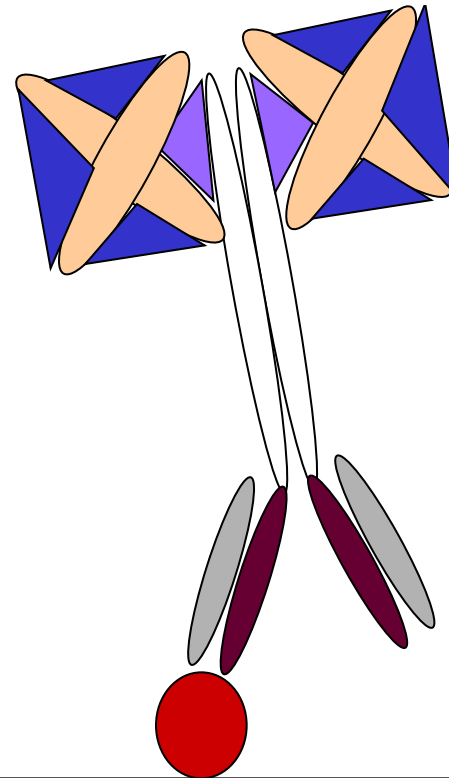
Double labeling

**HRPO
conjugated
anti-Ig**



Ag 1

**(Strept)avidin-biotin AP
complex**



Ag 2

Most frequently used enzyme-substrate systems

Peroxidase (HRPO)

DAB – diaminobenzidin (brown)

AEC – amino-ethyl-carbasol (red)

True Blue (blue)

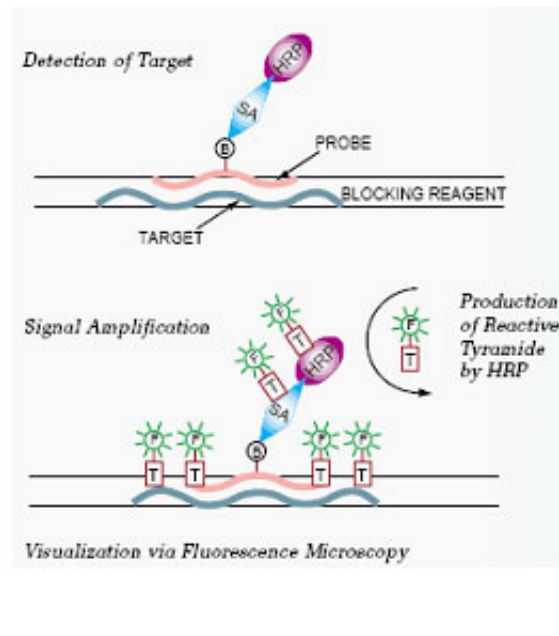
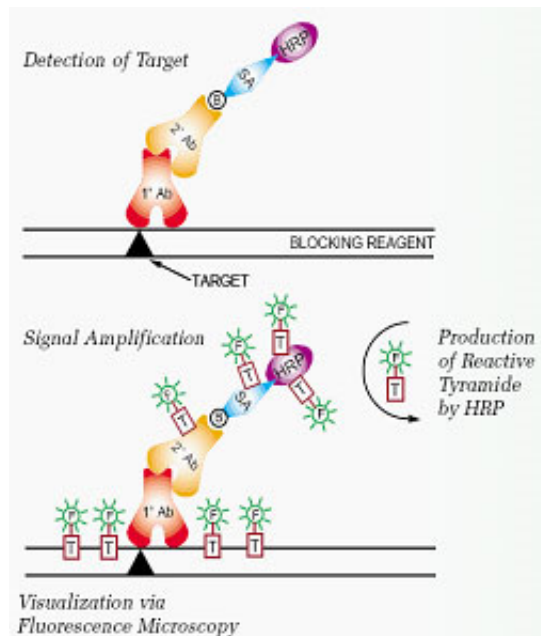
Alkaline phosphatase (AP)

NBT – nitroblue tetrasolium (blue)

BCIP – bromo-chloro-indolyl phosphate
Fast Red, Fast Blue

Amplifications:

1. Use of cocktail mAbs
2. Secondary antibodies (mono-polyclonal Abs [polymeric HRP])
3. Combination of biotinylation/secondary Abs
4. Development-intensification (DAB-metal [Ni, Co])
5. TSA:



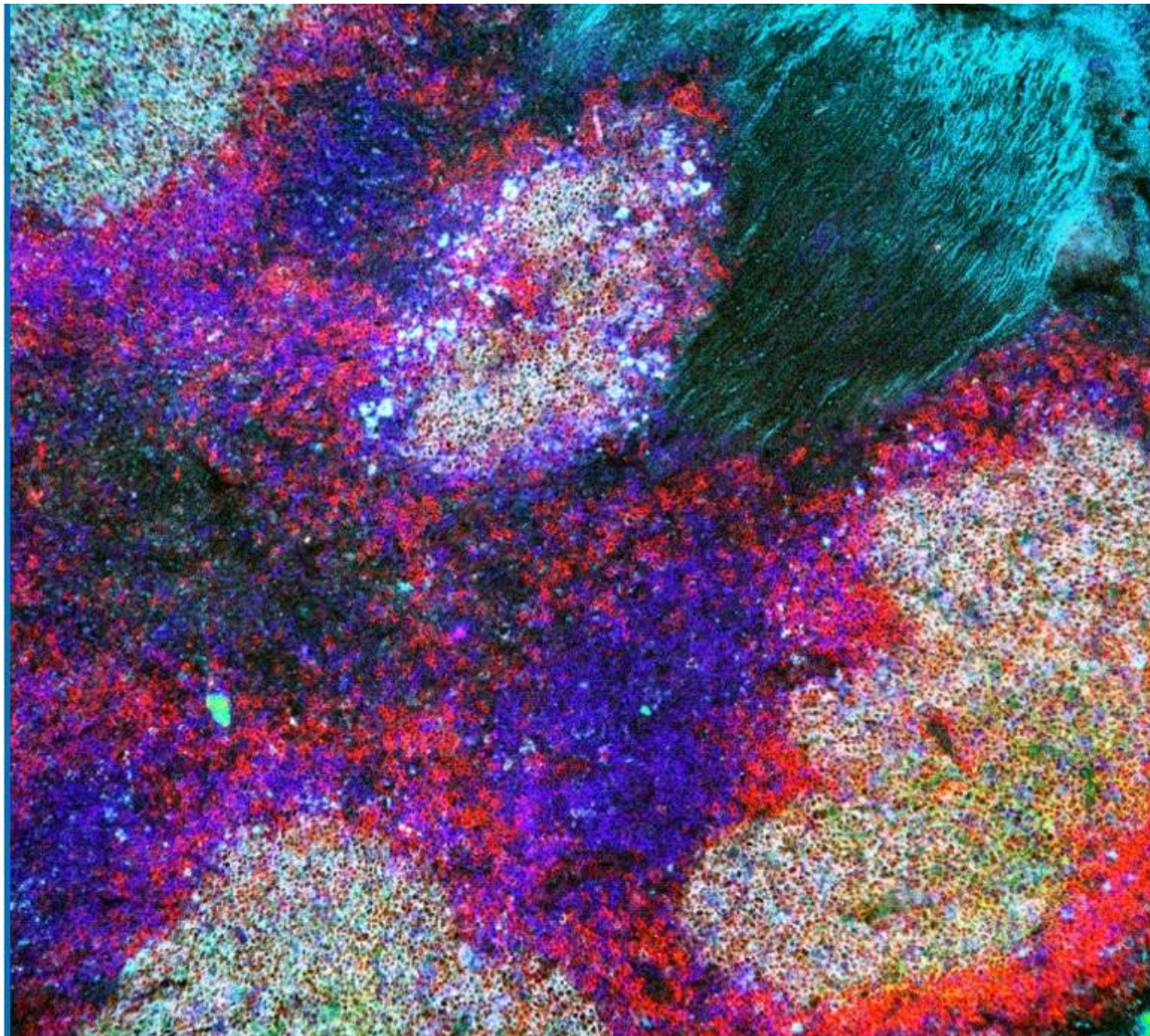
Fluorochrome Specifications

Fluorochrome	Fluorescence Emission Color	Ex-Max (nm)	Excitation Laser Line (nm)	Em-Max (nm)	BD FACScan™	BD FACSCalibur™	BD FACStar Plus™	BD FACSVantage™ SE	BD™ LSR	BD™ LSR II	BD FACSAria™	BD FACSAria™
Alexa Fluor® 405	Blue	401	360, 405, 407	421				✓		✓	✓	
Pacific Blue®	Blue	410	360, 405, 407	455				✓		✓	✓	
Alexa Fluor® 488	Green	495	488	519	✓	✓	✓	✓	✓	✓	✓	
FITC	Green	494	488	519	✓	✓	✓	✓	✓	✓	✓	
PE	Yellow	496, 546	488, 532	578	✓	✓	✓	✓	✓	✓	✓	✓
PE-Texas Red®	Orange	496, 546	488, 532	615	✓	✓	✓	✓	✓	✓	✓	
Texas Red®**	Orange	595	595	615			✓	✓				
APC*	Red	650	595, 633, 635, 647	660		✓	✓	✓	✓	✓	✓	✓
Alexa Fluor® 647	Red	650	595, 633, 635, 647	668		✓	✓	✓	✓	✓	✓	✓
PE-Cy5*	Red	496, 546	488, 532	667	✓	✓	✓	✓	✓	✓	✓	
PerCP	Red	482	488, 532	678	✓	✓			✓	✓	✓	
PerCP-Cy5.5	Far Red	482	488, 532	695	✓	✓	✓	✓	✓	✓	✓	✓
PE-Cy7	InfraRed [†]	496, 546	488, 532	785	✓	✓	✓	✓	✓	✓	✓	✓
APC-Cy7	InfraRed [†]	650	595, 633, 635, 647	785			✓	✓	✓	✓	✓	✓

[†] *InfraRed* detection requires a Hamamatsu R3896 Photomultiplier Tube (comes with detector option).

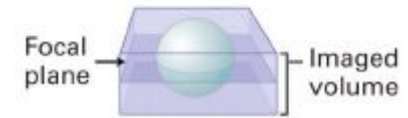
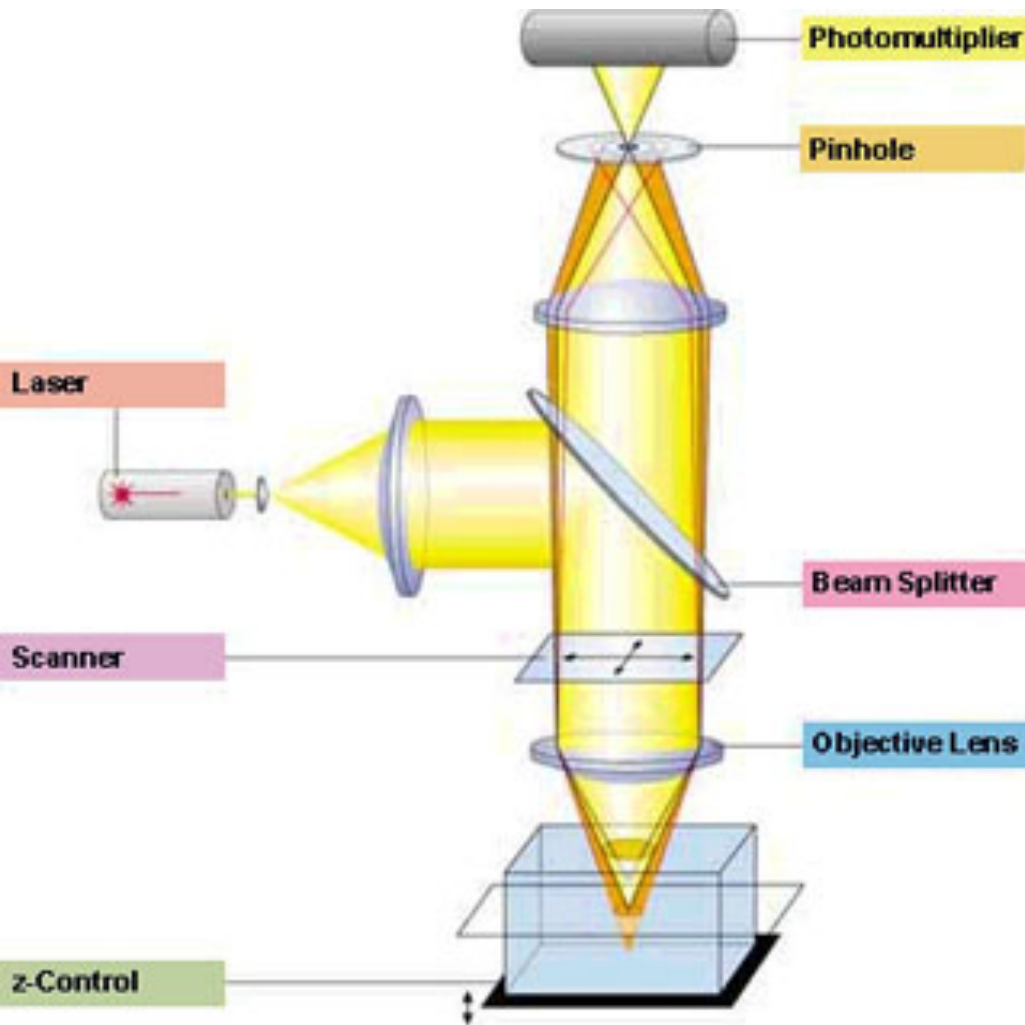
* APC and PE-Cy5 may be used together on instruments with cross-beam compensation.

** Texas Red® detection requires a dye laser.

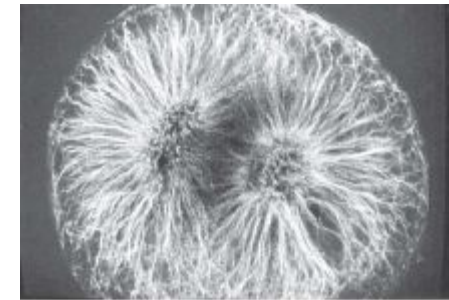


IgD/PNA/CR1/2/Thy-1

Confocal microscopy

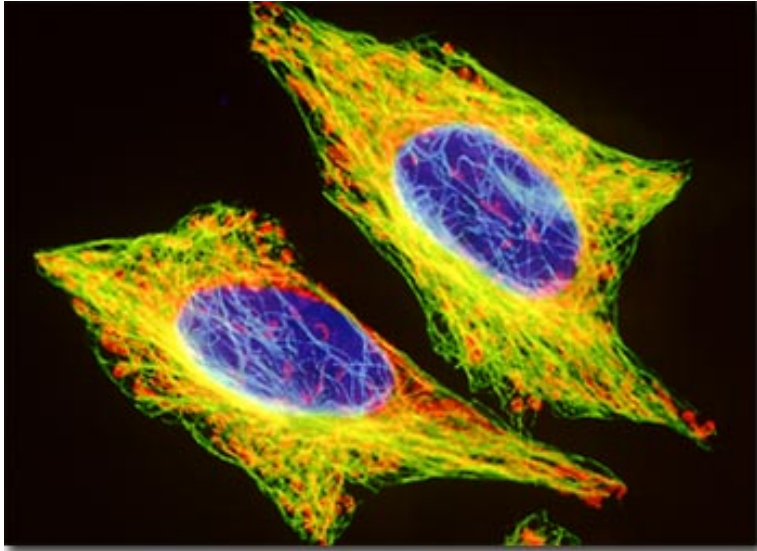


Conventional epifluorescence (anti-tubulin, mitotic cells)

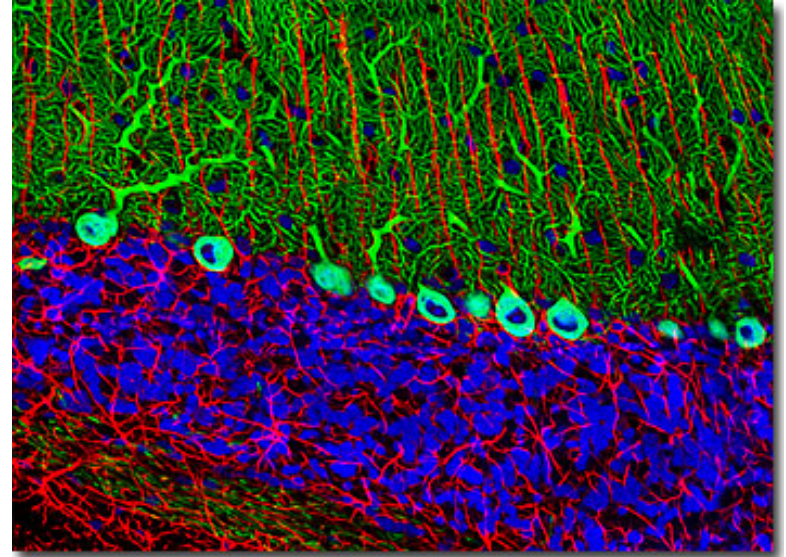


Confocal microscopy

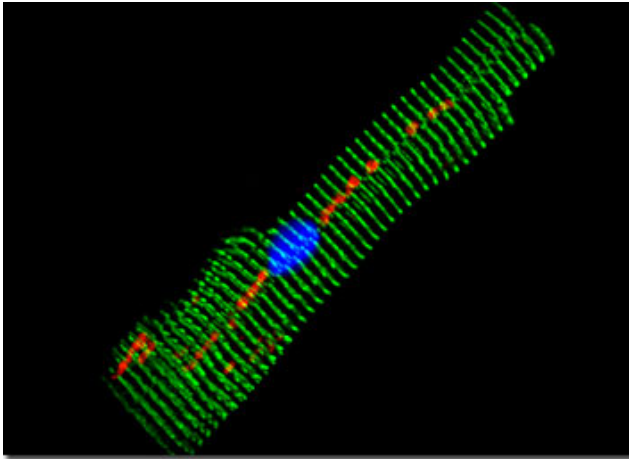
Confocal microscopy



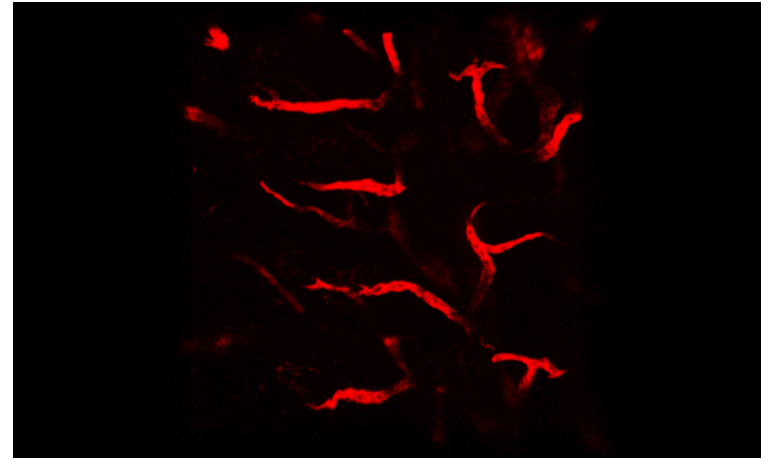
HeLa cells



Rat cerebellum

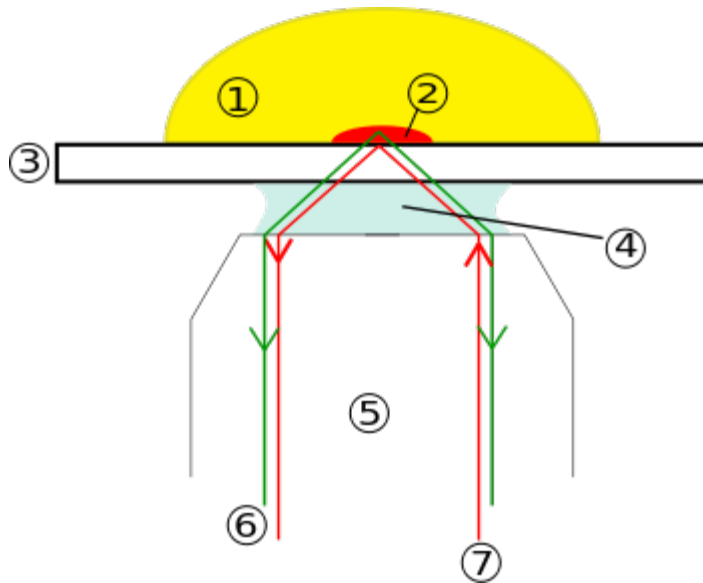


Myocardium

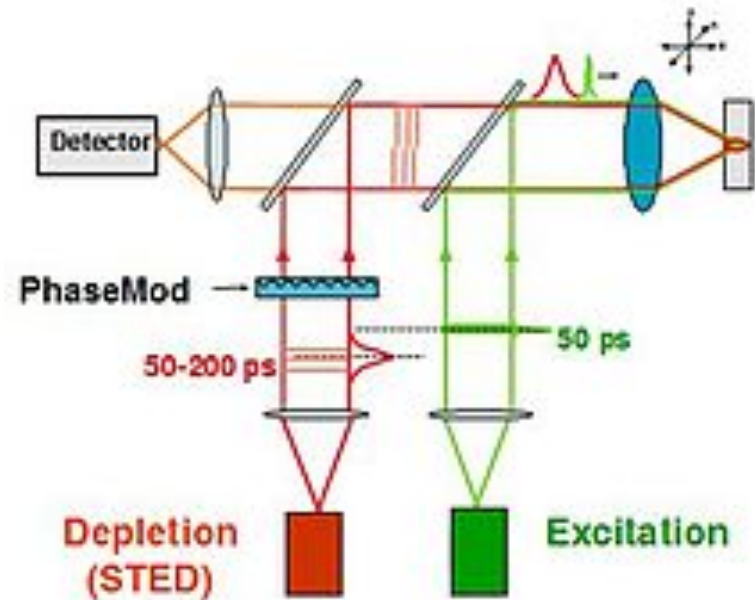


Mouse Peyer's patch

High resolution/subcellular microscopy



**Total internal reflection
fluorescence microscope
(TIRF)**
Single molecule detection



**Stimulated emission depletion
microscopy (STED)**
Structure analysis
(resolution > 50nm)