* Enzyme Multiplied Immunoassay Technique :-

Ag - enzyme + Ab $\xrightarrow{Ag}$ Ag : Ab + Ag-enzyme

↓ No Ag

Ag - Enzyme : Ab

No enzyme activity

↓ active enzyme

→ CEDIA

(Cloned enzyme Donor ImmunoAssay)

Ab + EA + ED - Ag $\xrightarrow{Ag}$ Ag : Ab + (EA : ED - Ag)

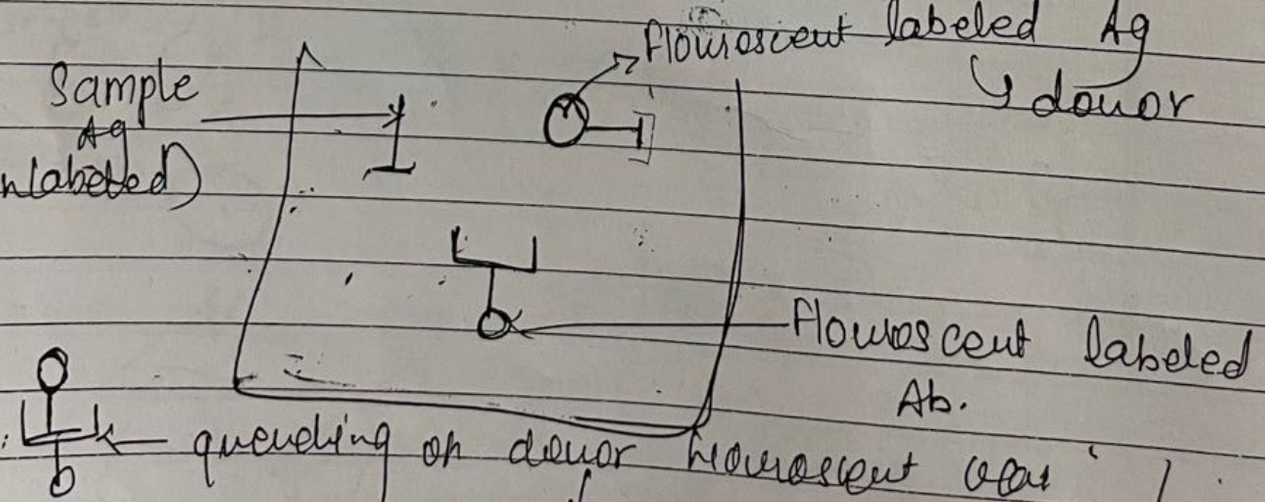
↓ No Ag.

Ab : ED - Ag

↓

No enzyme activity

↓ enzyme activity not

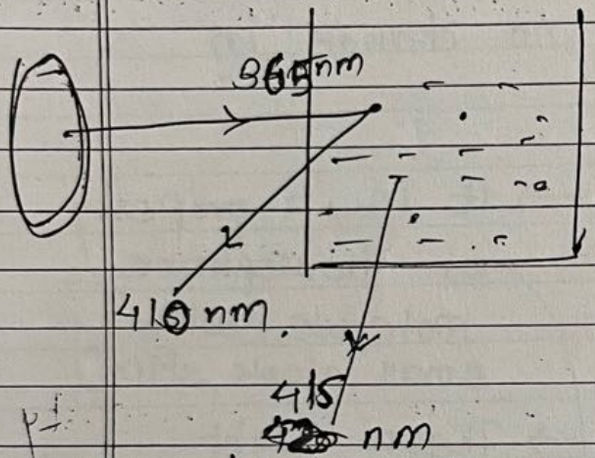


→ Raman scattering ~~emit~~ light ~~longer~~ than (Scatter) ~~excited~~ light.
emitted

→ ~~Raman scattering~~ It is a property of solvent.

→ It mainly interfere with result when flourophore concentration is very low.

→ Ex:-



→ Water shift wavelength about 50 nm at 365 nm

So If emitted light of flourophore is 415 nm when excitation light is 340 nm & light scattered by solvent (water) is about 425 nm

- So there is overlapping of spectra between solvent emitted wavelength & flourophore emitted wavelength.

↓

So these will be interference when narrow spectrum of light is emitted by solvent (substance) other than flourophore

↓

So positive interference occur.

* Controlled by narrowing the slit width on the excitation monochromator.

↓

But it can decrease sensitivity also.

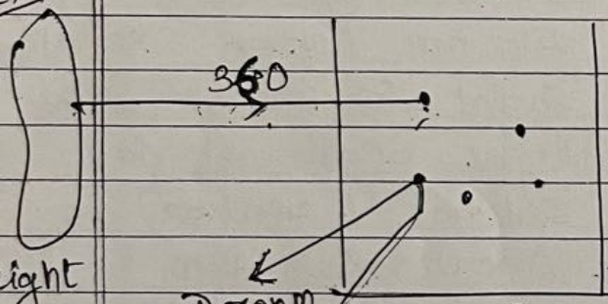
* Light Scattering :-

→ two type of light scattering : Raman & Rayleigh scattering which limits the use of fluorescence measurement.

* Rayleigh Scattering :-

→ Scattering occurs with no change in wavelength.

ex :-



→ it is a property of fluorophore molecule (with small stoke shift)

→ These light spectra overlaps.

→ Excitation & emission spectra overlap & susceptible to loss of sensitivity.

→ This will be controlled by use of well defined emission & excitation interference filter, By appropriate monochromator setting & use of polarizers.

B'cz of background light scatter.

* Raman Scattering :-

→ Occurs with lengthening of wavelength.

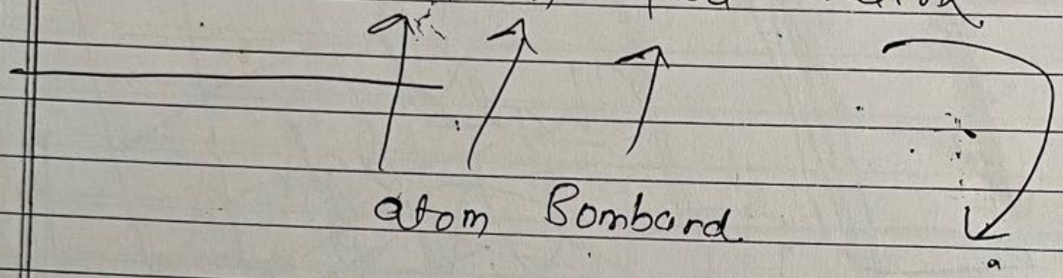
→ This scattering independant of excitation source.

Soft Ionization, Fast

→ utilizes atom bombardment

→ Not utilize electrons

Sample + dissolved in liquid matrix



molecular Ion

↓

Solvent is released from it

→ requires in Heat labile substance.
∴ fragile sample.