Photo / Luminescence: Fluorescence, Phosphorescence Chemiluminescence

Fluorescence / Phosphorescence

- Fluorescence:

an effect of **emission** of longer wavelength radiation (λ_{em}=700nm)

by a substance

as a consequence of **absorption** of energy from a shorter wavelength radiation (λ_{exc}=400nm)

continuing only as long as the stimulus is present

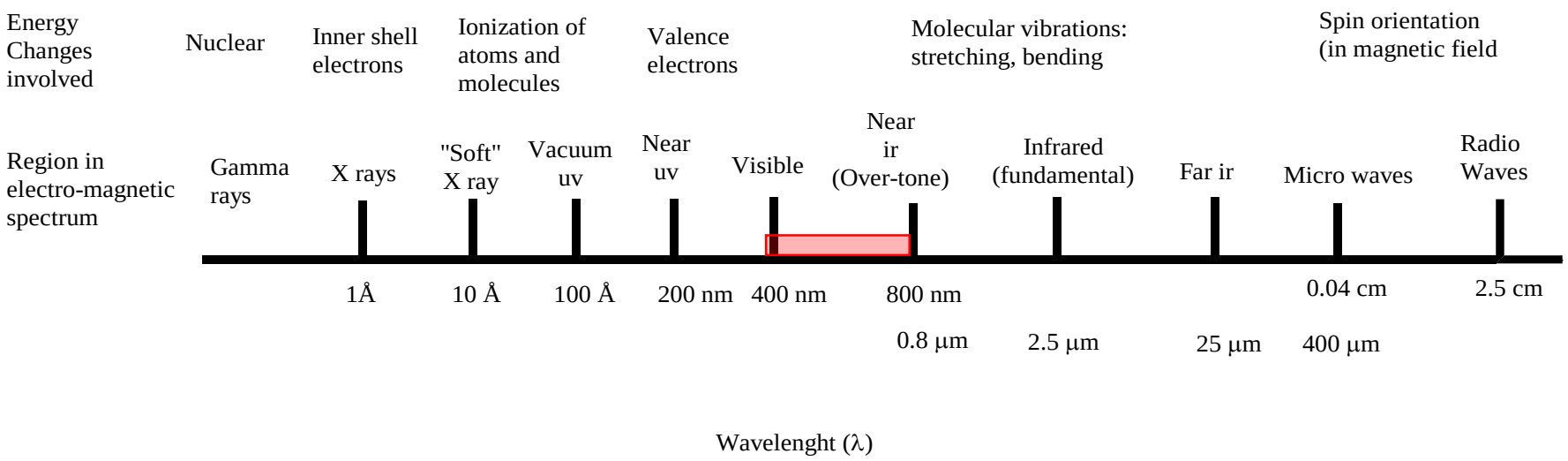
- Phosphorescence:

emission persists for a perceptible period of time

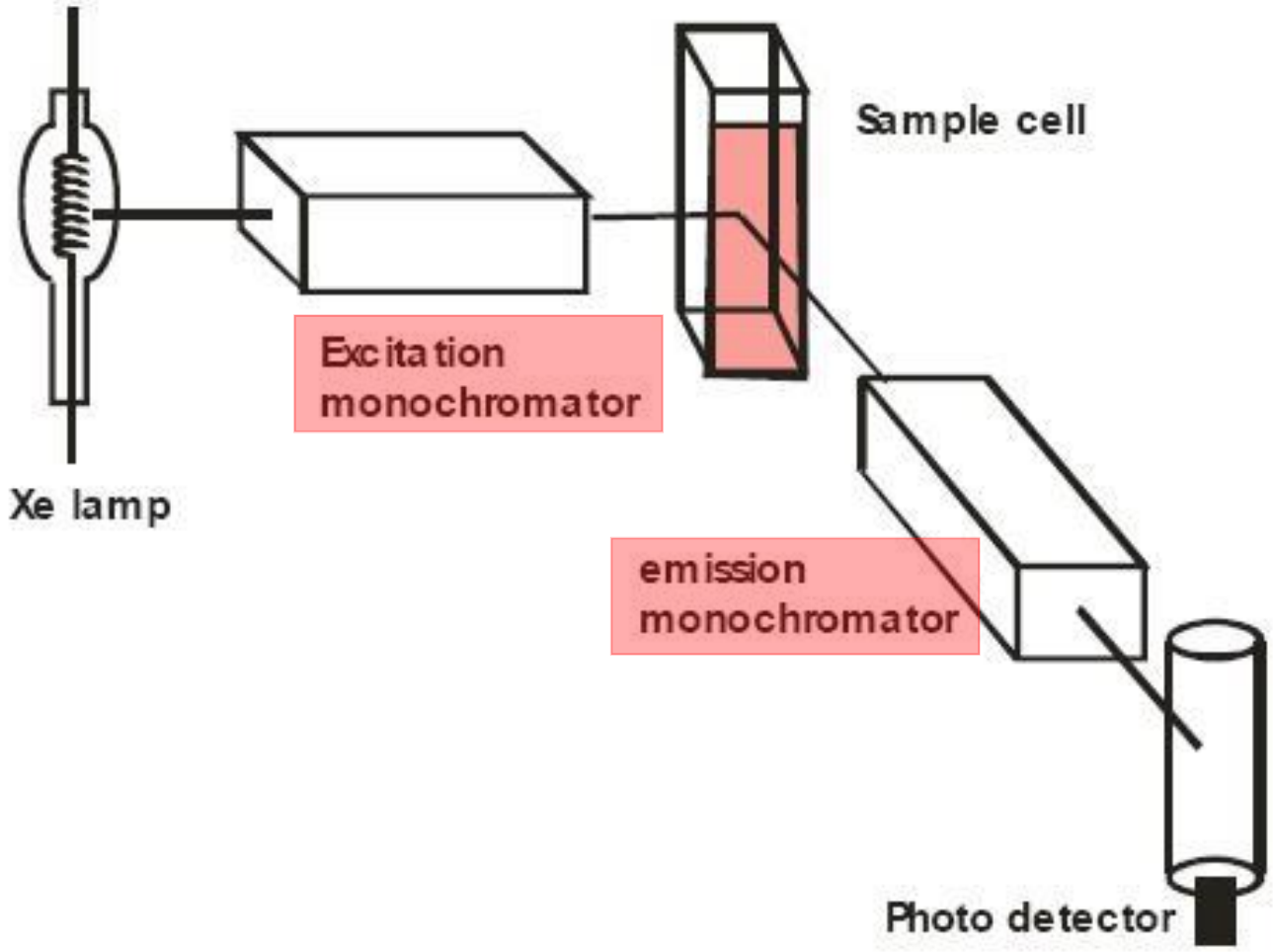
after the stimulus has been removed

Electromagnetic Diagram

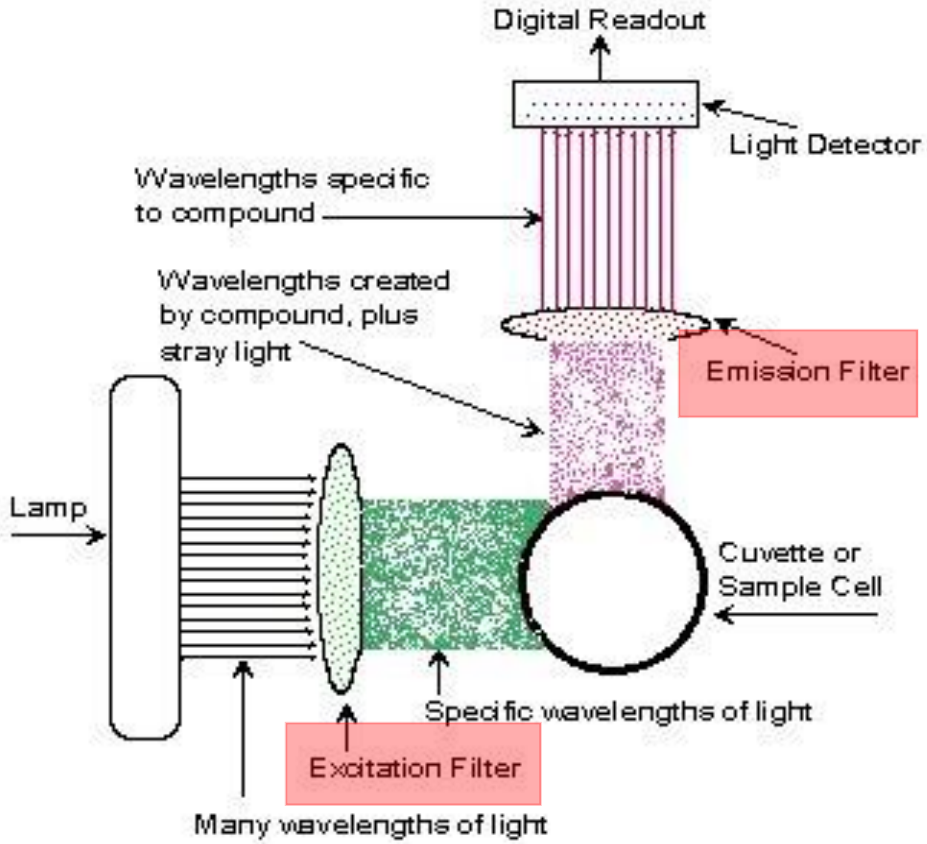
- λ_{exc} = 400-700nm
- $\lambda_{em} > \lambda_{exc}$ nm

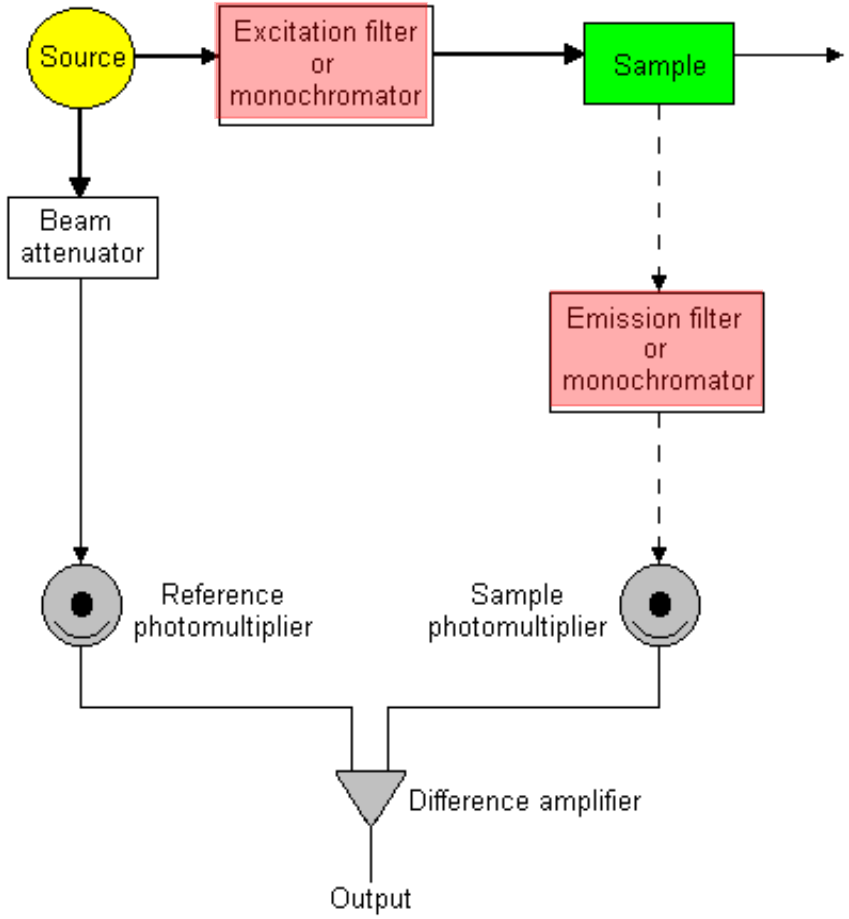


Fluorimeter in Schematic Image



Fluorimeter / Spectrofluorimeter

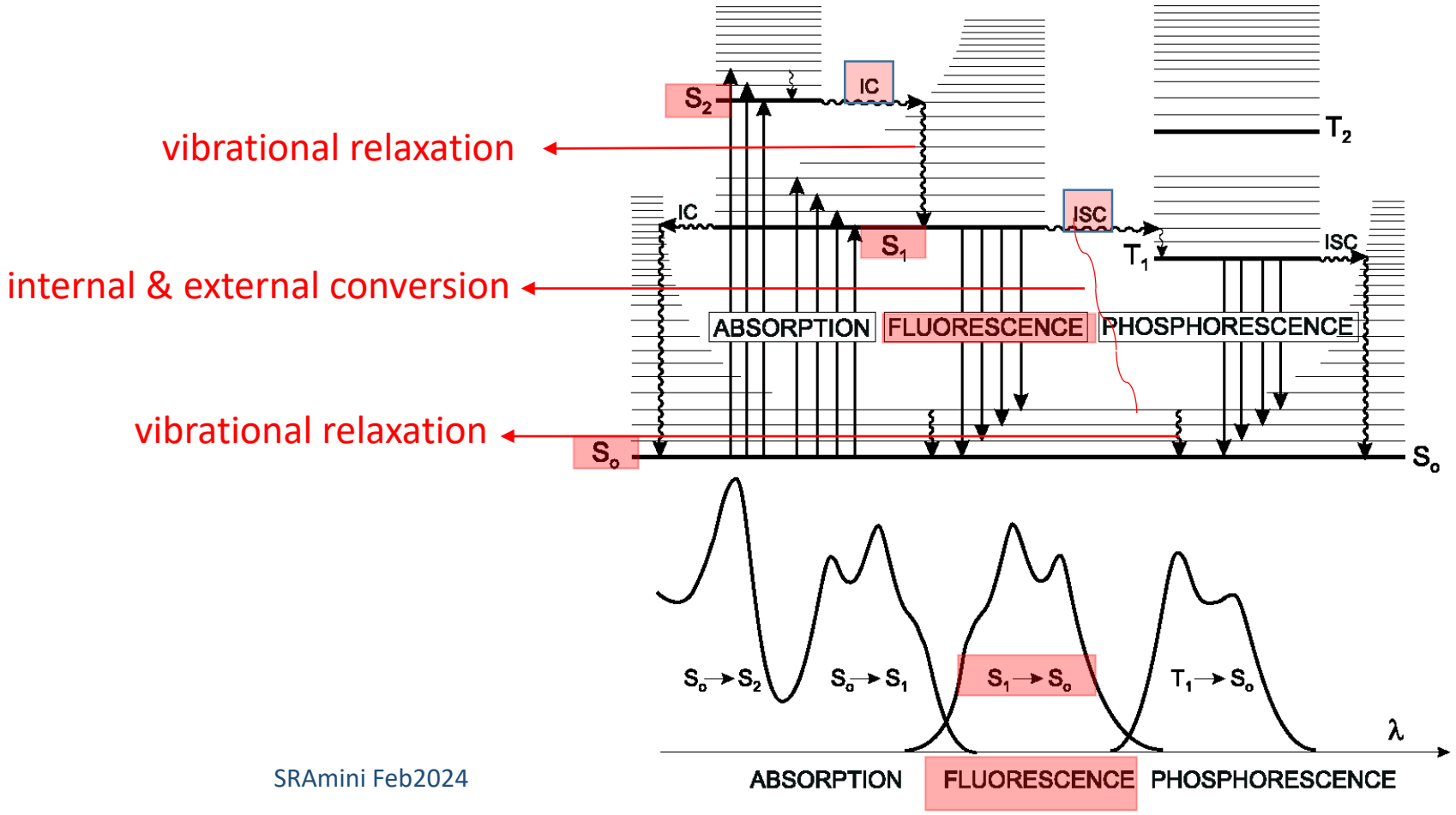




Electron Energy Diagram (Perrin-Jablonski Diagram)

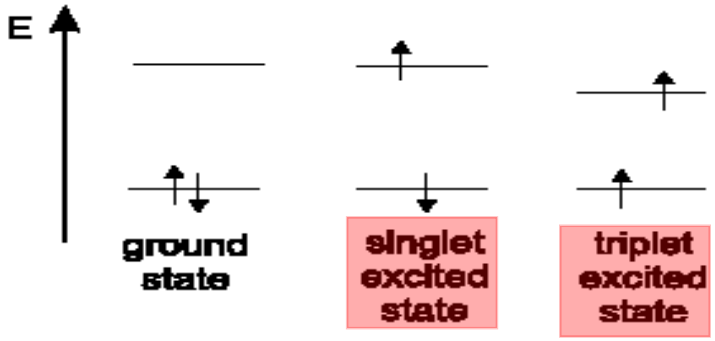
- Particular emission wavelength (λ_{em}) range for each compound
- Continuous wavelengths: due to electrons in various energy states

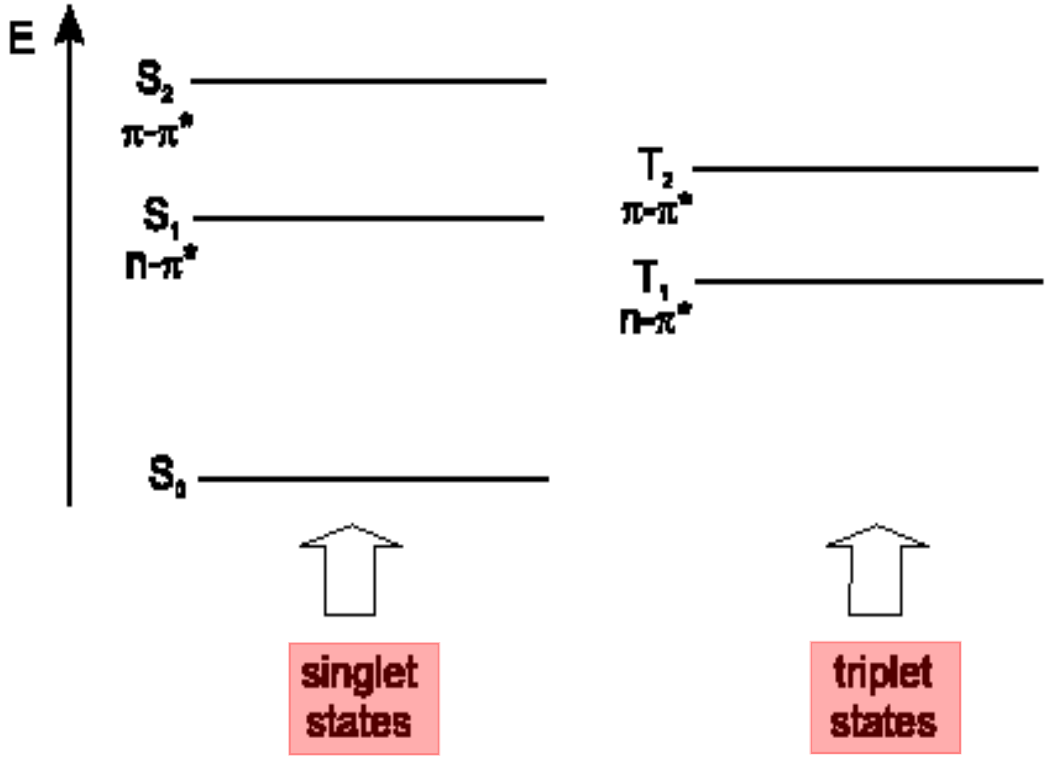
- IC: internal conversion
- ISC: inter-system crossing
- S: singlet
- T: triplet



Singlet &Triplet Energy States for Electrons in Orbitals

- Singlet state: diamagnetic
- Doublet spin
- Triplet state: paramagnetic





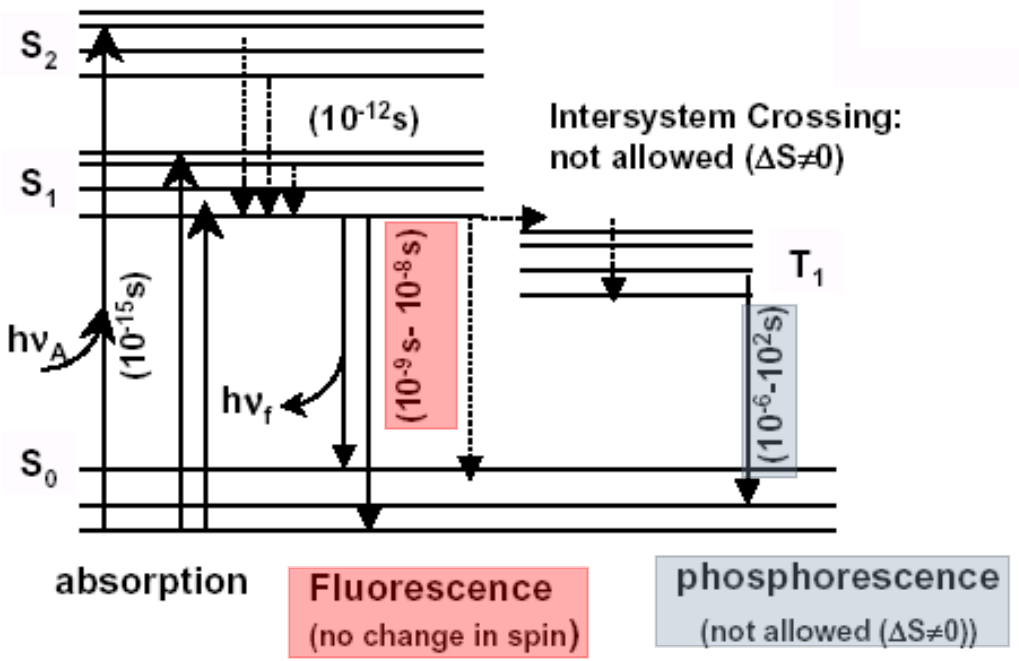
Factors Decreasing Fluorescent Intensity

- Internal & external conversion
- Vibrating relaxation
- Inner filter effect: particularly in the concentrated solutions

Comparing Absorption, Fluorescence & Phosphorescence Regarding their Individual Life Time

CHARACTERISTIC TIMES

absorption	10^{-15} s
vibrational relaxation	10^{-12}-10^{-10} s
lifetime of the excited state S_1	10^{-10}-10^{-7} s → fluorescence
intersystem crossing	10^{-10}-10^{-8} s
internal conversion	10^{-11}-10^{-9} s
lifetime of the excited state T_1	10^{-6} -1 s → phosphorescence



Some Applied Terms in Fluorescence Analysis

- Luminescent chromophore
- Fluorophore: fluorescence chromophore
- Mono/poly-chromator
- Quantum yield / efficiency:

Quantum yield is the ratio of photons emitted to photons absorbed by the system

- Lifetime
- Quench: decreases efficiency
- Quantitative / qualitative fluorescence analysis

Fluorescence Influencing Factors

- Molecular structure: aromaticity, rigidity, ionized/non-ionized forms

✓metal chelates; poly-aromatic

- Concentration of compound
- Chemical environment: solvent, pH, temperature
- Viscosity of solvent or compound
- Intensity of excitation: reflects intensity of emission

Wavelengths to Be Detected in Fluorimeter

- Particular (single point) wavelength

- Continuous range of wavelength:

✓due to energy states of various electrons in the studied structure

Fluorimeter / Spectrofluorometer

- Excitations sources:

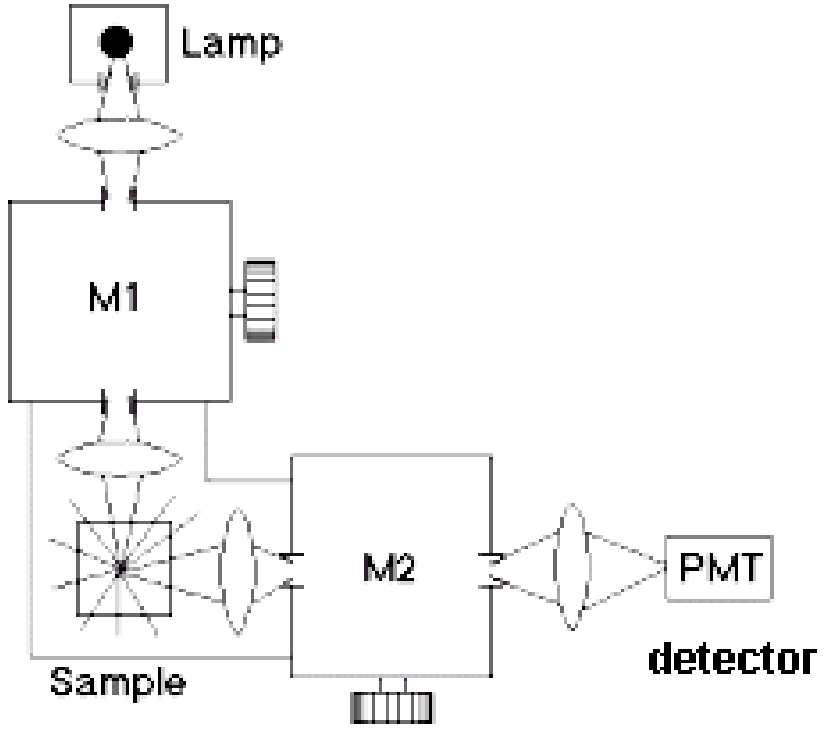
lamp, laser

- Cells, cuvette

➢Wavelength range scan

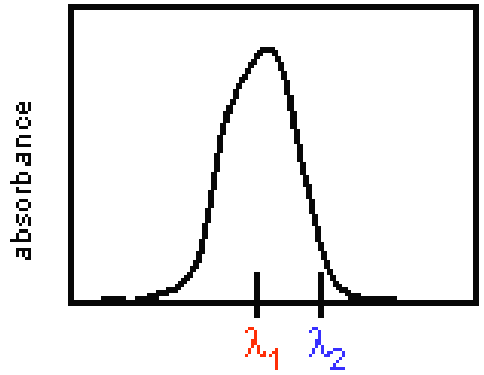
or

mono wavelength detection

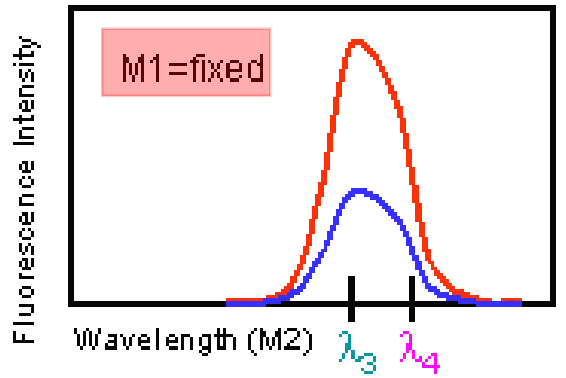


M1: excitation monochromator
M2: emission monochromator

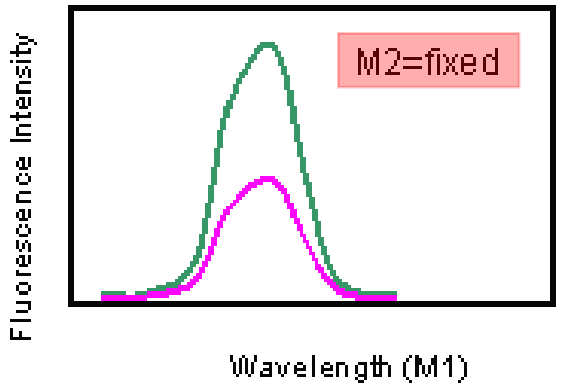
Absorption spectrum



Fl. Emission spectrum

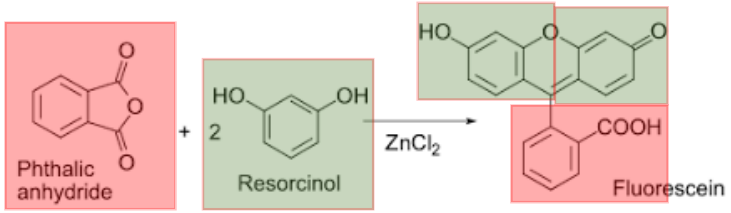


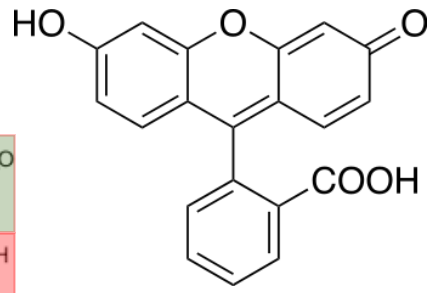
Fl. Excitation spectrum



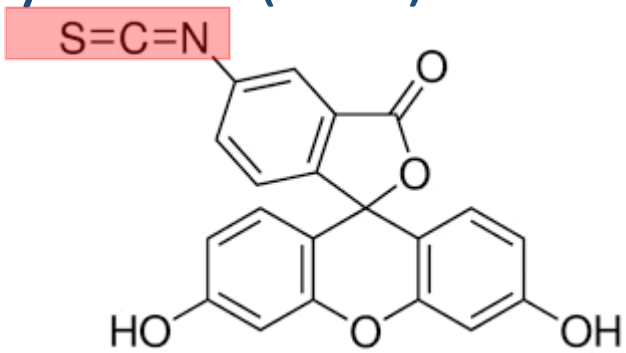
Common Fluorescent Substances or Fluorescent Staining Agents

- Fluorescein:

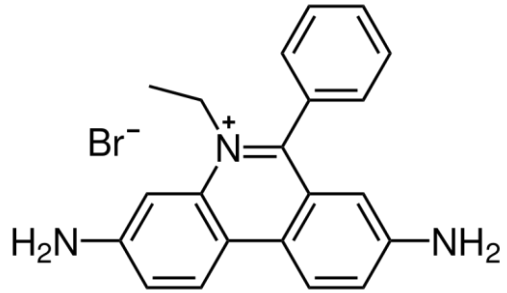




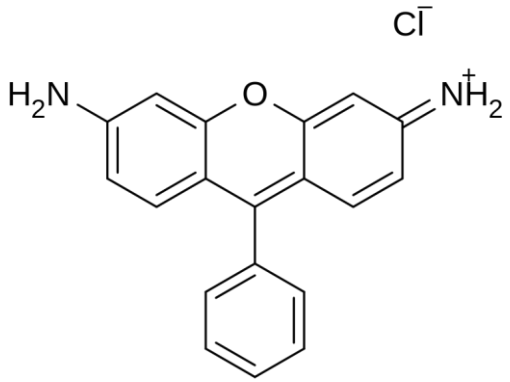
Fluorescein isothiocyanate: (FITC)



- Ethidium bromide: λ_{max}=210,285nm; λ_{em}=605nm

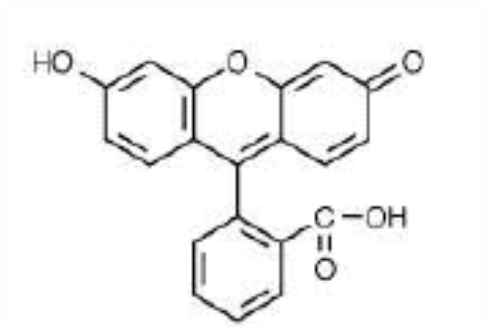


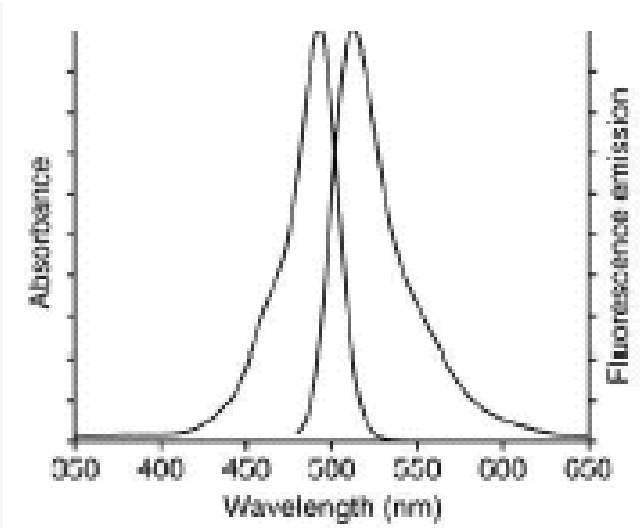
- Rhodamin B



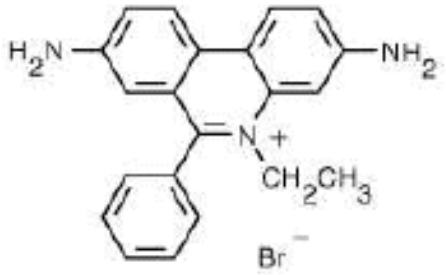
Fluorescence Curves for Fluorophores

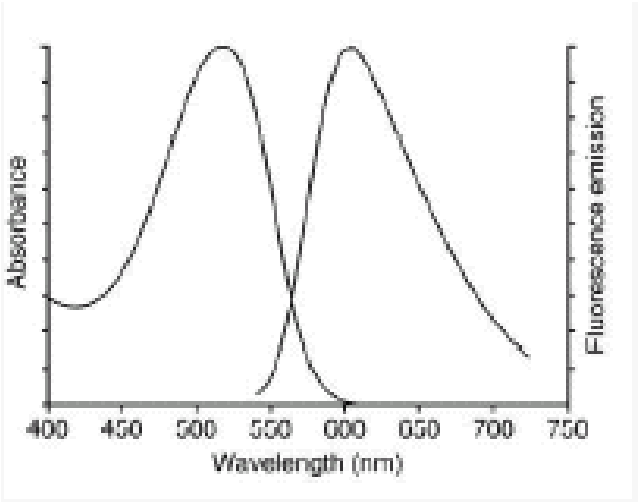
- Fluorescein





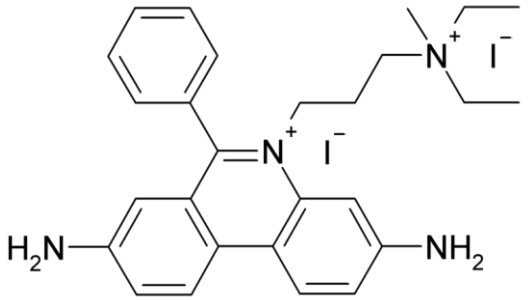
- Ethidium bromide: bound to DNA.



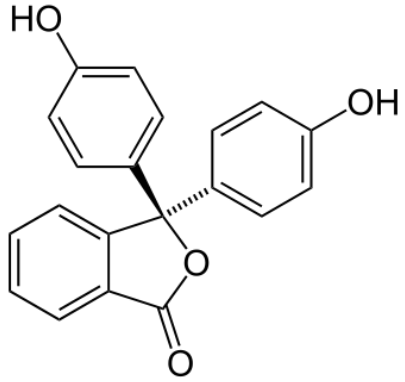


Common Fluorescent Substances or Fluorescent Staining Agents- contd.

- Propidium iodide (PI)



- Phenolphthalein



- GFP: Green Fluorescent Protein as a fluorophore:

✓a peptide isolated from jelly fish: ---Ser65,Tyr66,Gly67---

✓central role in cell biology: in vivo gene reporter

A Introductions to Two Common Fluorescence Coupled Systems

- FACS: Fluorescence Activated Cell Sorting

- FERT: Fluorescence Energy Resonance Transfer