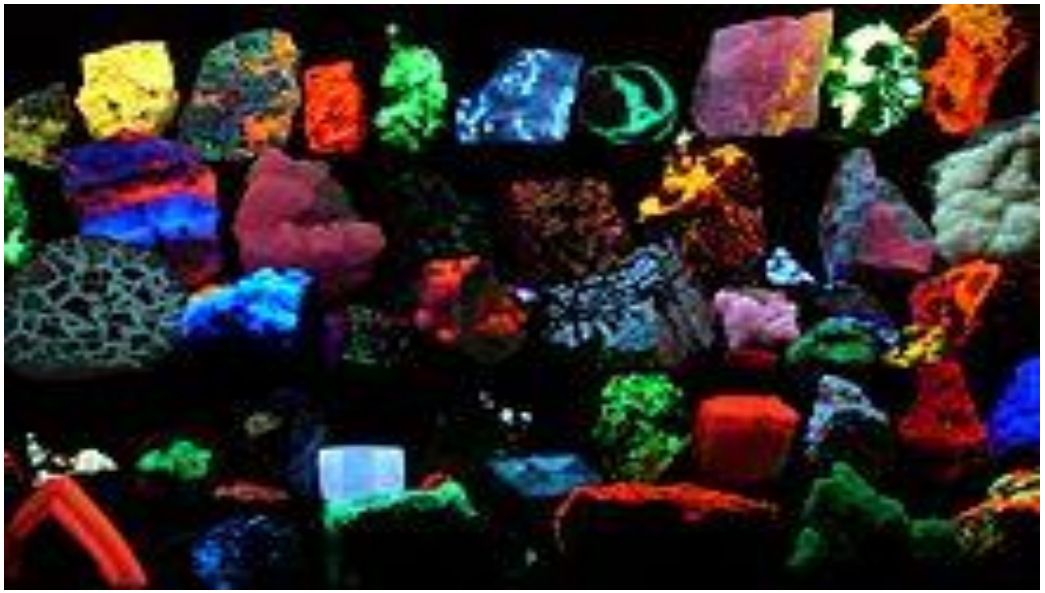
Fluorescence Spectroscopy



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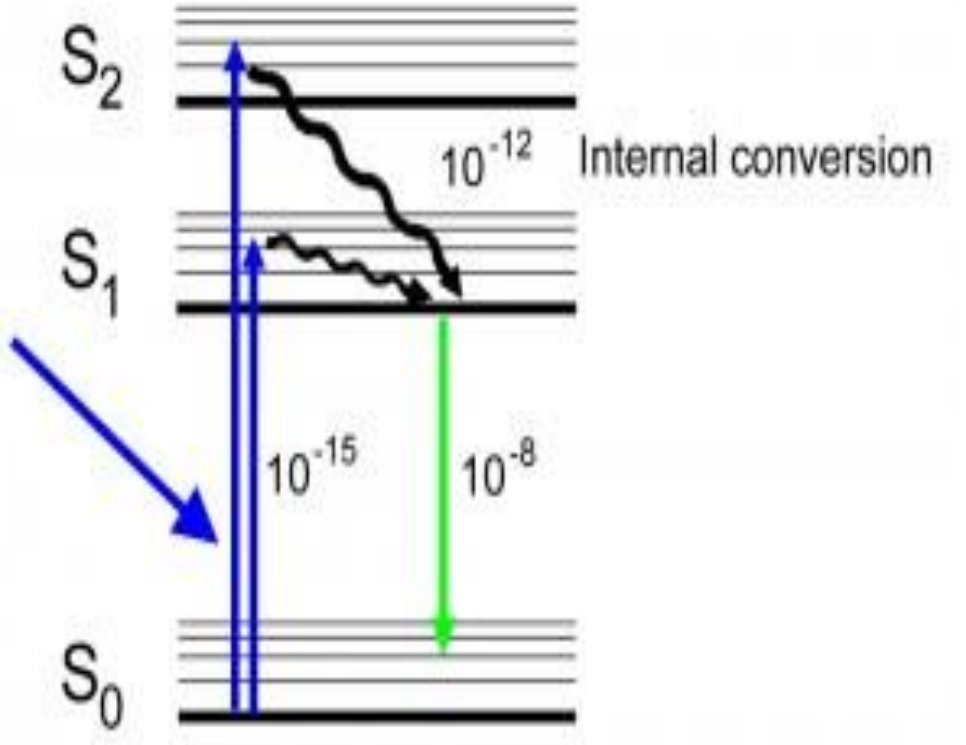
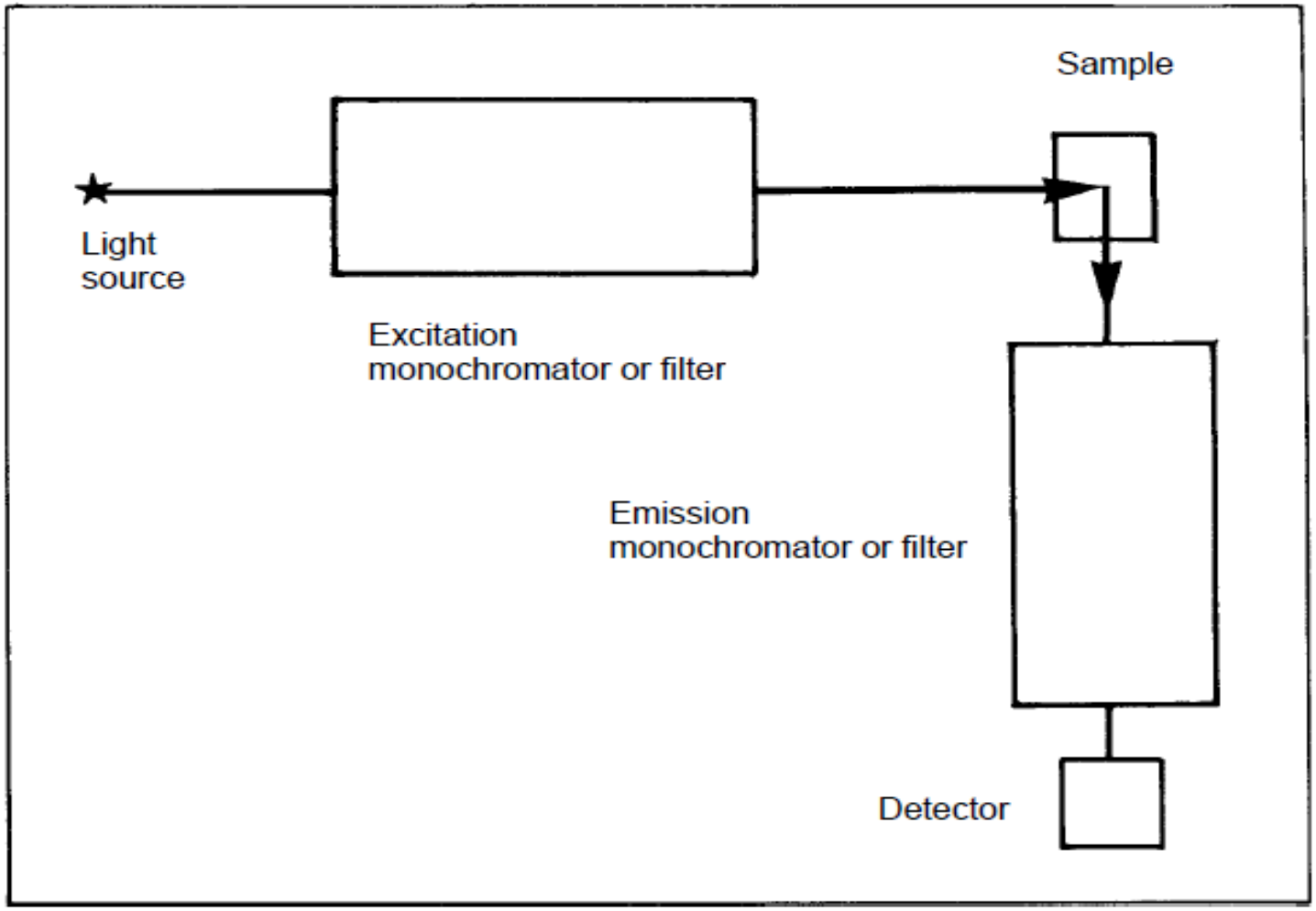
Fluorescence

- Electron in the ground state is excited to a higher energy state
- After loss of some energy in vibrational relaxation, the high energy electron returns back to the ground state by emitting fluorescent photon.



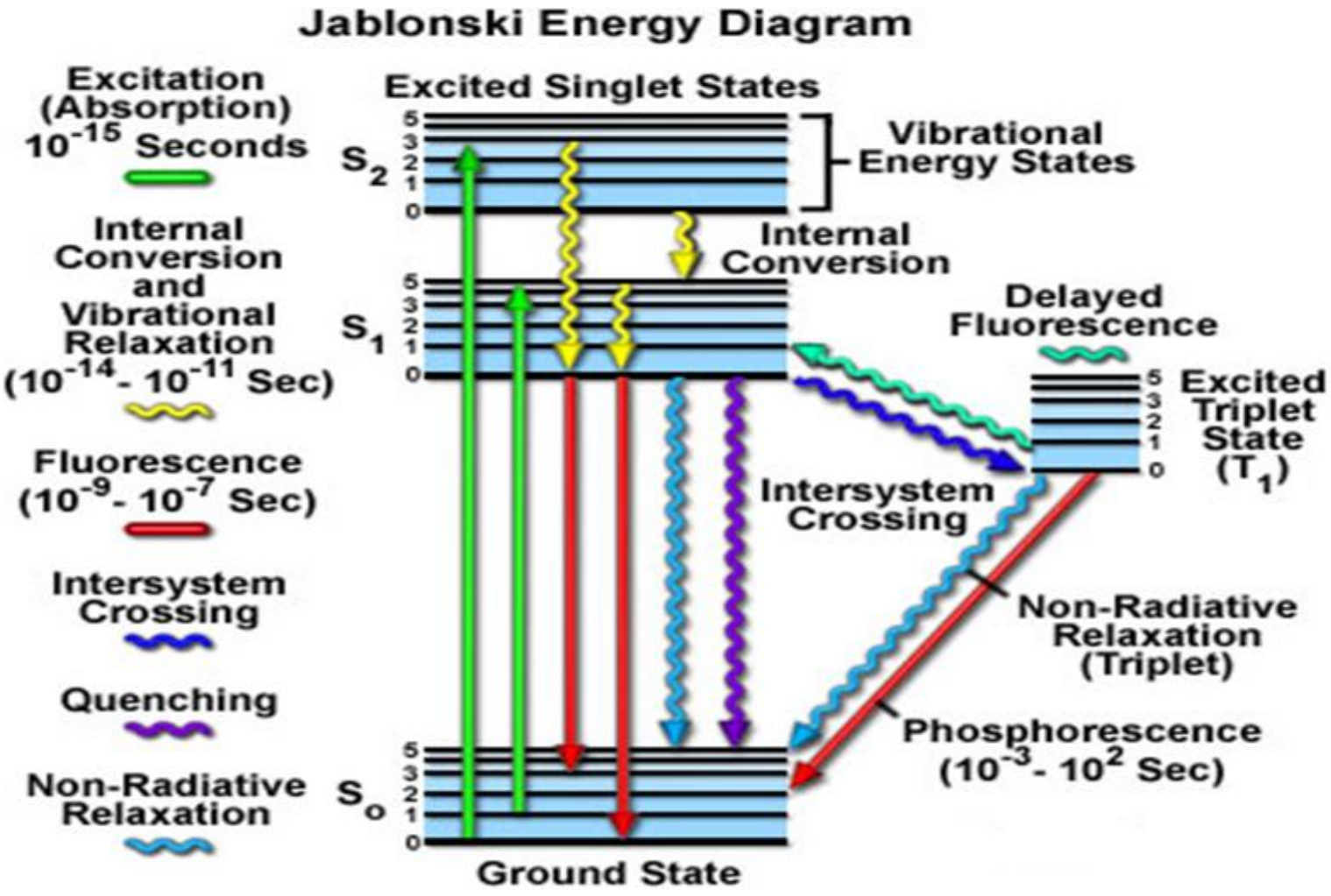
[7]

Experimental Set-up



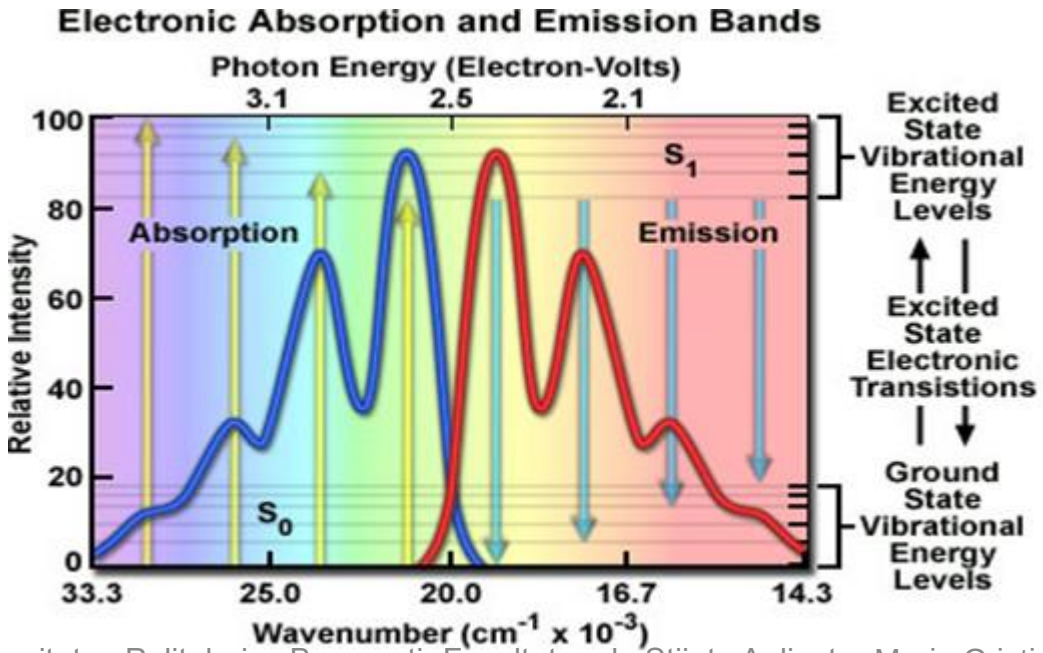
[7]

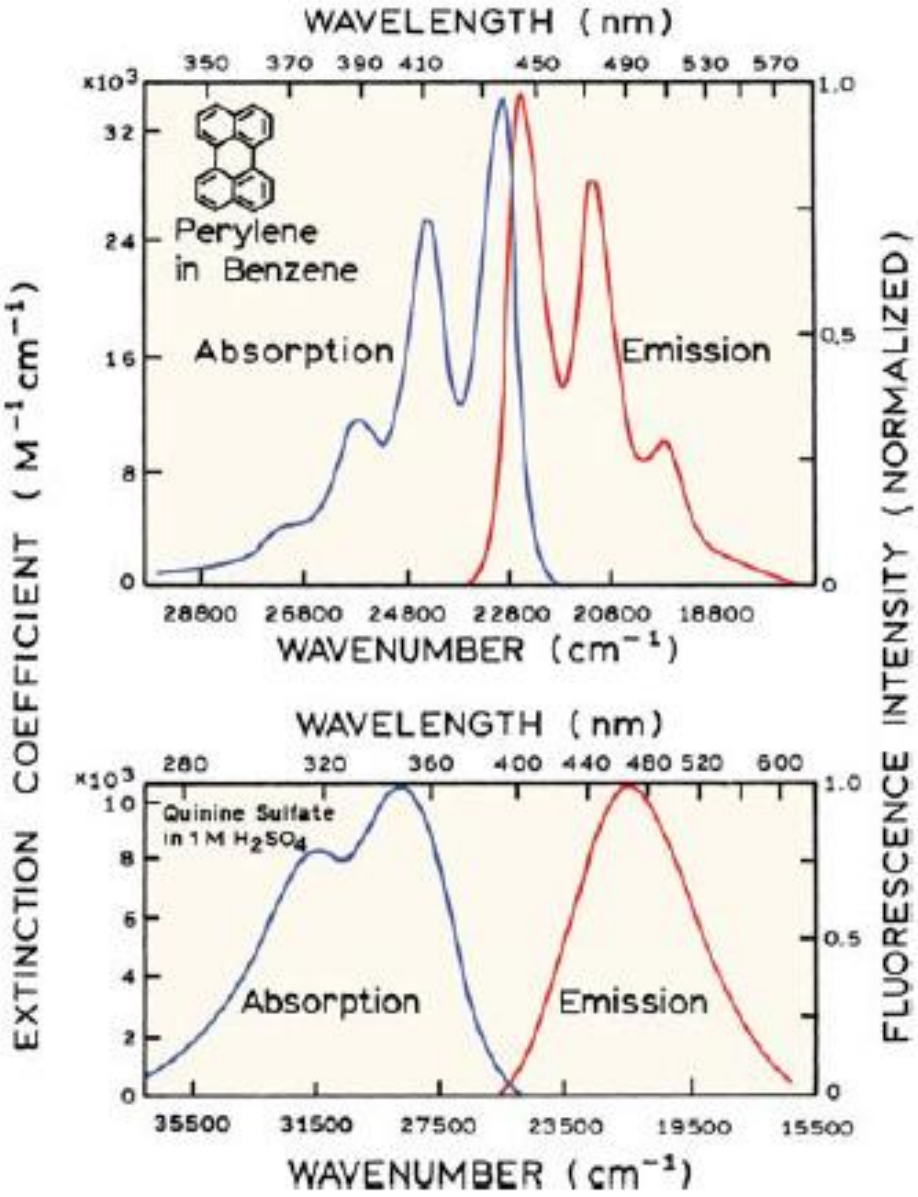
- Molecule that absorbs light = chromophor
- Molecule that emits light= fluorophor
- Fluorophor
    - Intrinsic fluorophores: aromatic amino acids
    - Extrinsic fluorophores :can be linked covalently or not to macromolecules such as peptides, proteins, membranes, or DNA.

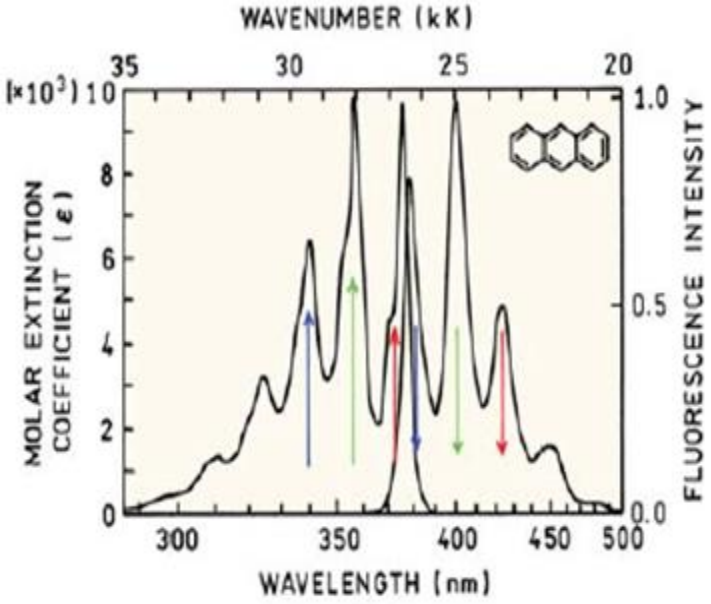

Jablonski Energy Diagram
Excitation (Absorption) 10^{-15} Seconds
Internal Conversion and Vibrational Relaxation (10^{-14} - 10^{-11} Sec)
Fluorescence (10^{-9} - 10^{-7} Sec)
Intersystem Crossing
Quenching
Non-Radiative Relaxation
Excited Singlet States
S_2
S_1
S_o
5
3
2
1
0
Vibrational Energy States
Internal Conversion
Delayed Fluorescence
Excited Triplet State (T_1)
Intersystem Crossing
Non-Radiative Relaxation (Triplet)
Phosphorescence (10^{-3} - 10^{2} Sec)
Ground State

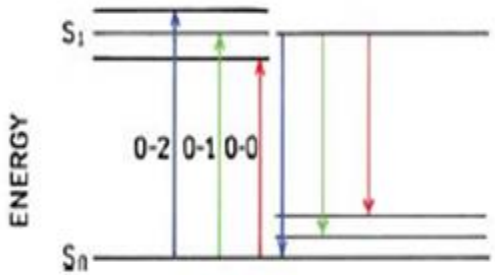
[6]

- Stokes shift
- Intensity not dependet of the excitation λ
- Mirror rule









Mirror-image rule and Franck-Condon factors. The a
sorption and emission spectra are for anthracene. The numbers 0,
and 2 refer to vibrational energy levels. From [1].

Quantum Yield. Fluorescence lifetime

- Quantum yield (*Q) is the (dimensionless) ratio of photons emitted to the number of photons absorbed.*

$$Q = \frac{\Gamma}{\Gamma + k_{nr}}$$

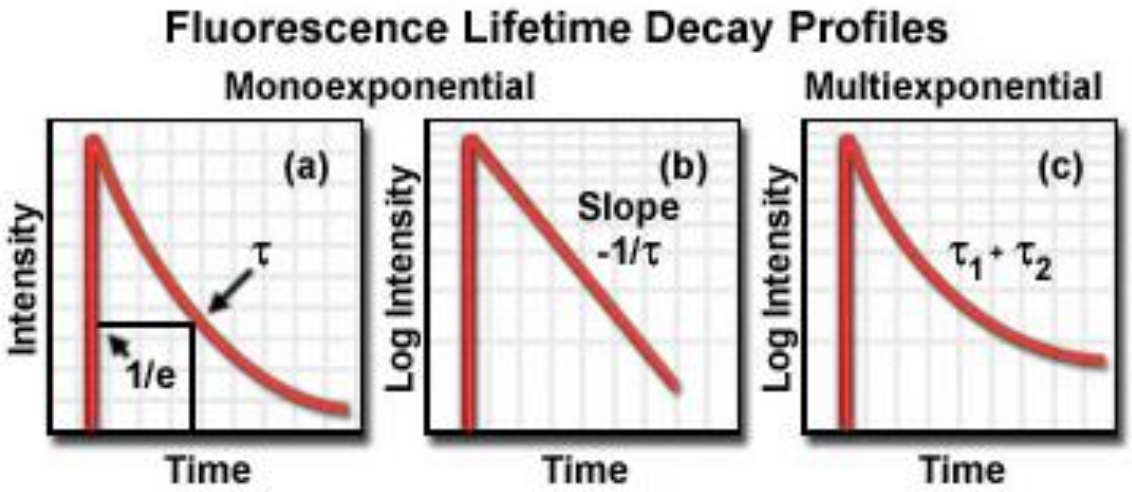
- $\Gamma$is fluorescence decay rate and k_{nr} is combined nonradiative decay rate

- Lifetime (τ) is an average value of time spent in the excited state
- The lifetime of the fluorophore in the absence of nonradiative processes is called the intrinsic or natural lifetime, τ_n

$$\tau = \frac{1}{\Gamma + k_{nr}} \qquad \tau_n = \frac{1}{\Gamma} \qquad \tau_n = \tau / Q$$

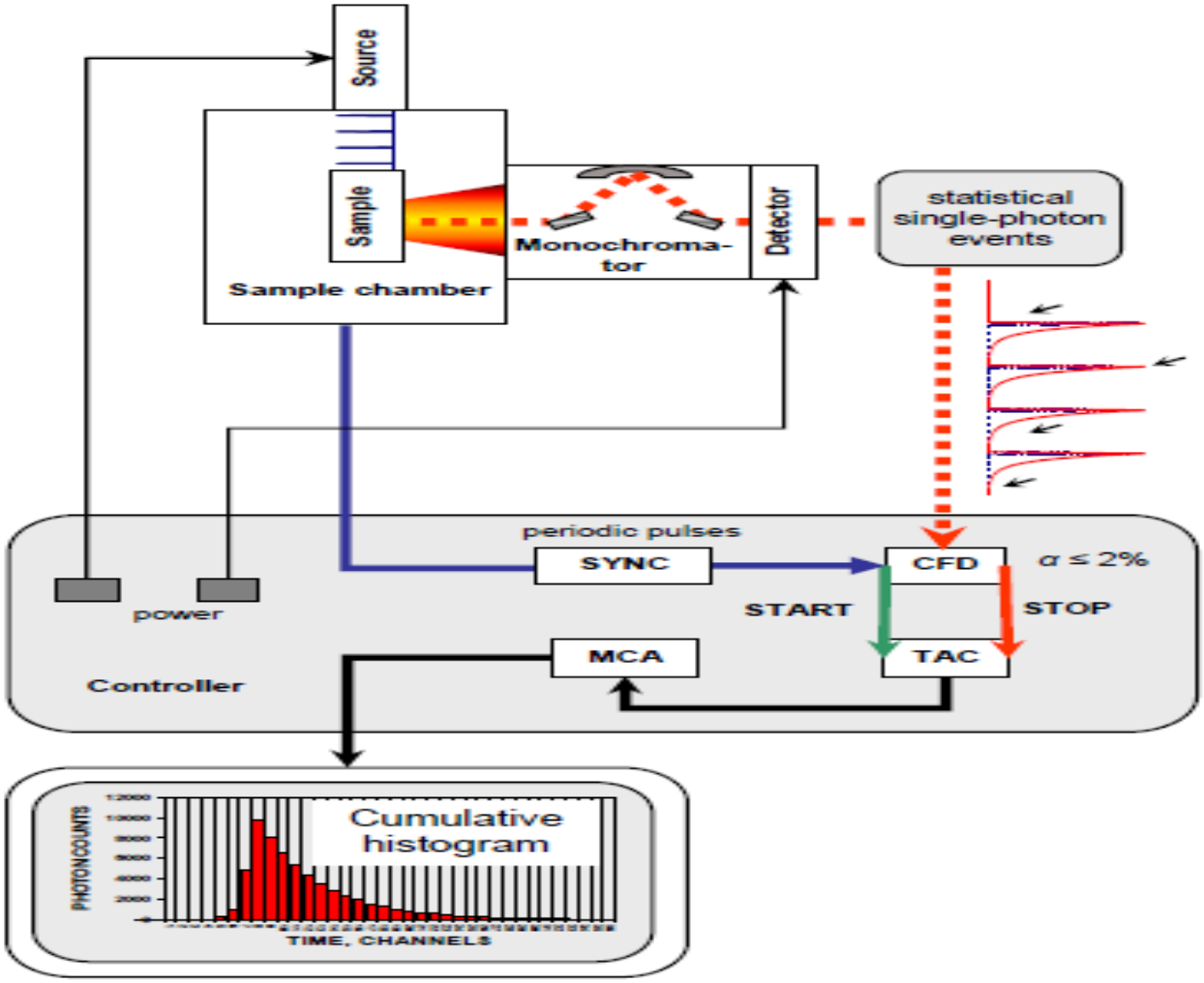
- The fluorescence lifetime is defined as the time in which the initial fluorescence intensity of a fluorophore decays to **1/e** of the initial intensity

$$I(t) = I_0 e^{-\frac{t}{\tau}}$$



[6]

Time-Correlated Single-Photon Counting (TCSPC) measurements



Quenching

- External molecules added to the fluorescent system can quench fluorescence intensity and therefore quantum yield
- Dynamic quenching: quenchers will decrease the fluorescence while entering in collision with the fluorophore
- Static quenching: quenchers form a nonfluorescent complex with the fluorophore

$$I/I_0 = 1 + K_{SV}[Q] = 1 + K_q\tau_0$$

- I_0 and I - fluorescence intensities observed in the absence and presence, respectively, of quencher
- [Q] - the quencher concentration
- K_{SV} - the Stern-Volmer quenching constant
- K_q -bimolecular quenching rate constant
- τ_0-excited state lifetime in the absence of quencher

FRET- Förster energy transfer

- Fret or energy transfer at a distance occurs between two molecules, a donor (the excited fluorophore), and an acceptor (a chromophore or fluorophore).
- Energy is transferred by resonance.

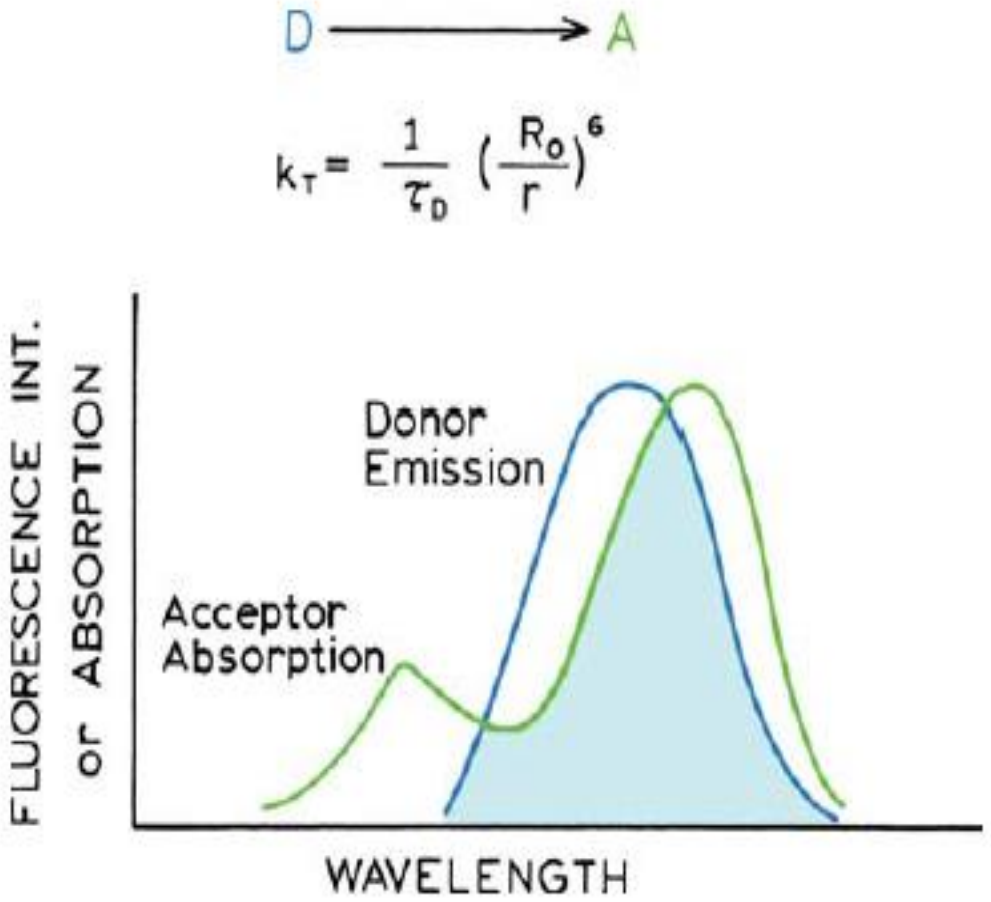
FRET

K_T- rate of energy transfer

R_0- Förster distance

r-distance between D and A

τ_D- lifetime of the donor in the absence of energy transfer

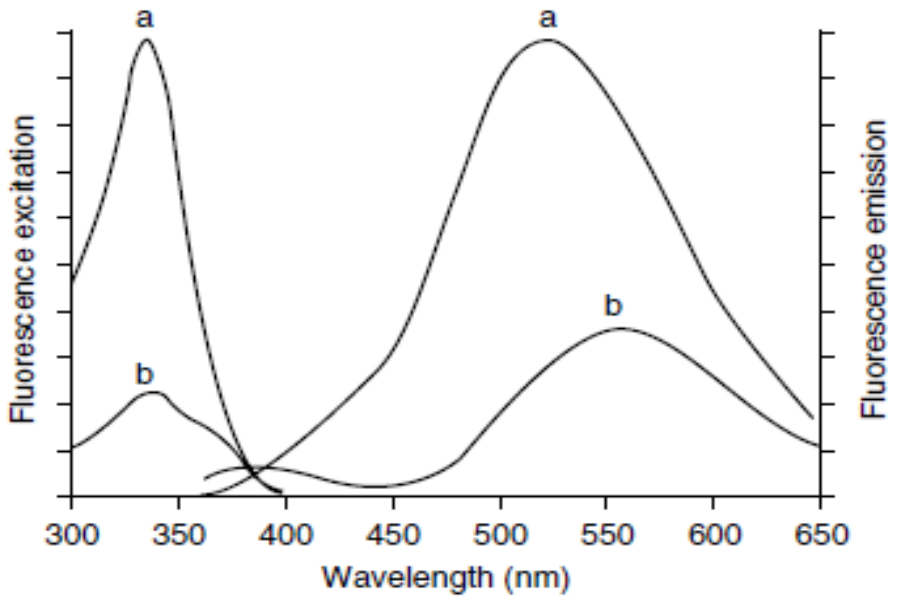


Lackowicz, J. R., Principles of Fluorescence Spectroscopy, Springer, 2006

Applications

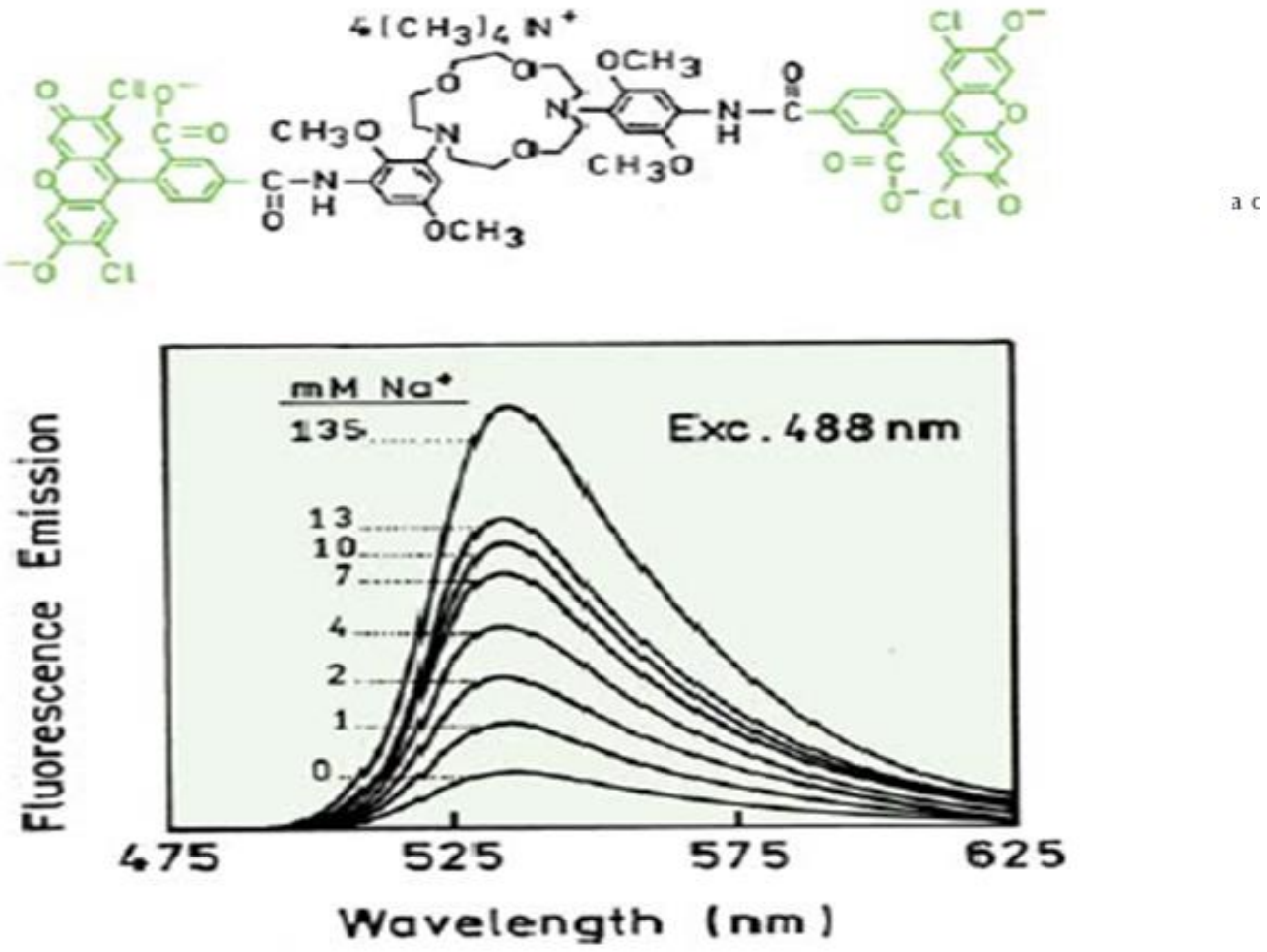
- Biology
- Ion detectors
- Medicine
- Nanoscience
- Molecules labeling
- DNA

Ion detectors



Fluorescence excitation (detected at 505 nm) and emission (excited at 340 nm) spectra of SBFI in pH 7.0 buffer containing 135 mM (a) or zero (b) Na^+.

[2]

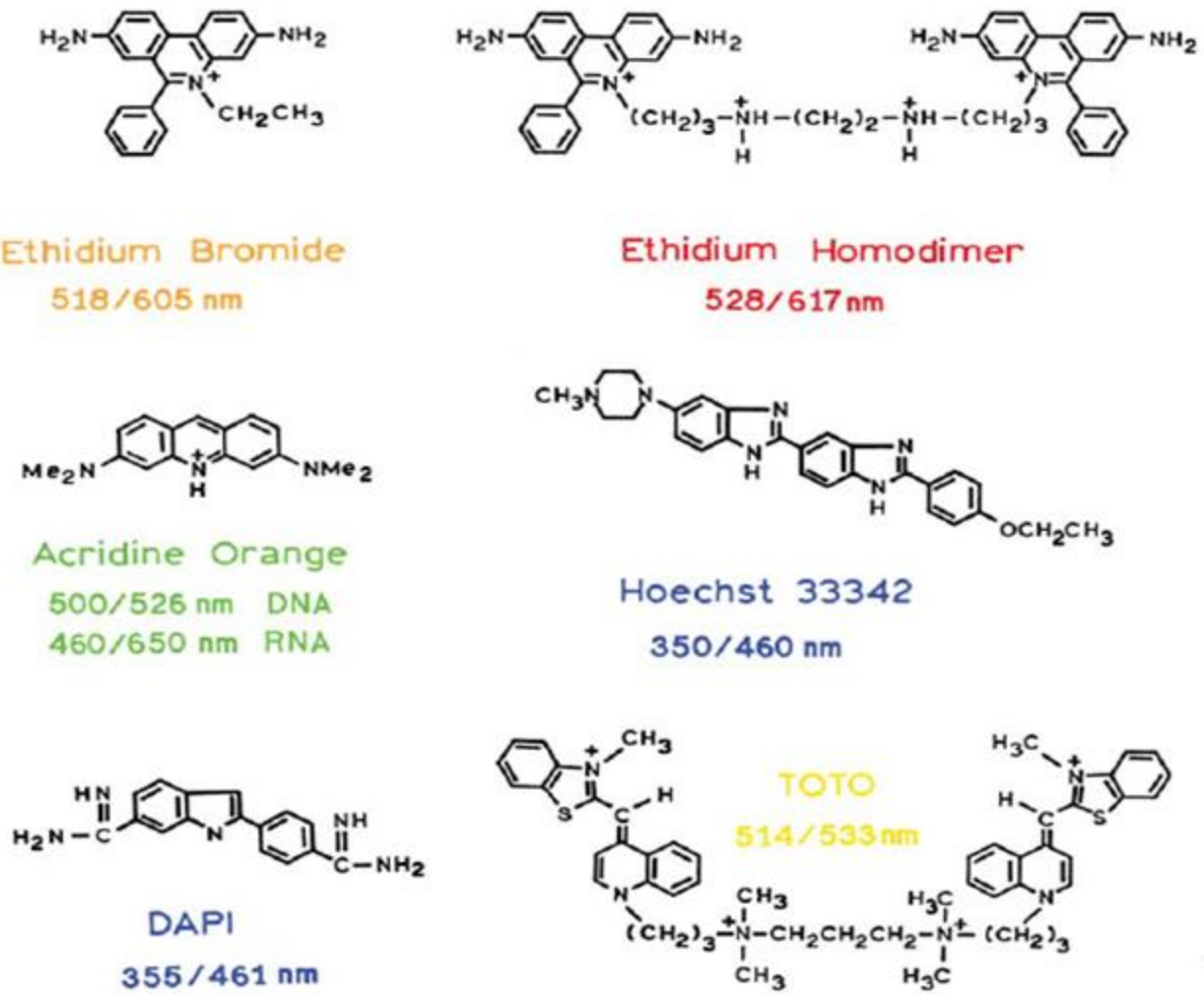


Effects of sodium on the emission of Sodium Green.

Lackowicz, J. R., Principles of Fluorescence Spectroscopy, Springer, 2006

DNA

- Deoxyribonucleic acid is weakly or nonfluorescent
- Several dyes bind spontaneously to DNA—such as acridines, ethidium bromide, and other planar cationic species
- Staining of cells with dyes that bind to DNA is used to visualize and identify chromosomes



Representative DNA probes. Excitation and emission wavelengths refer to DNA-bound dye.

Lackowicz, J. R., Principles of Fluorescence Spectroscopy, Springer, 2006

Conclusions

- Fluorescence spectroscopy
- Lifetime measurements
- Concentration measurements
- Multiple applications

References

1. Lackowicz, J. R., Principles of Fluorescence Spectroscopy, Springer, 2006
2. Wang, X. F., Herman, B., Fluorescence lifetime spectroscopy and imaginig. Principles and applications in biomedical diagnostics, CRC Press, Taylor and Francis Group, 2015
3. Albani, J. R., Principals and applications of fluorescence spectroscopy, Blackwell, 2007
4. http://www.horiba.com/scientific/products/fluorescence-spectroscopy/tutorialswebinars/
5. http://www.horiba.com/scientific/products/fluorescence-spectroscopy/application-notes/
6. http://micro.magnet.fsu.edu/primer/techniques/fluorescence/fluorescenceintro.html
7. Fisherman, D., An introduction to fluorescence spectroscopy, University of California, http://www.chem.uci.edu/~dmitryf/manuals/Fundamentals/Fluorescence%20Spectroscopy.pdf
8. Yildiz, A., Lecture notes, University Of California, http://research.physics.berkeley.edu/yildiz/Teaching/PHYS250/Lecture_PDFs/Lecture_3.pdf