

3. Fluorescence and Phosphorescence

Luciérnaga (Bicho de luz)



Las luciérnagas son una presencia familiar en las calurosas noches de verano. Cada luciérnaga hace brillar su luz según el patrón específico de su subespecie.

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1- Steady State Emission Spectrum

Measurement: **Emission Intensity vs λ**

- *Emission Spectra*: λ_{exc} , $\lambda_{em}^1 - \lambda_{em}^2$
- *Excitation Spectra*: $\lambda_{exc}^1 - \lambda_{exc}^2$, λ_{em}

Information from: maximum position, shape of the spectrum and intensity

- Chemical Structure of the fluorophore
- Fluorophore Concentration
- External Conditions: T^a , pH, I, presence of additives

$$F_{\lambda}^S \text{ or } I_{\lambda}^S \text{ (a.u.)} = KC \text{ (inner filter effect and additive)}$$

K: instrumental factor, I_0 , detector sensitivity, Substance

SPECTRA CORRECTION

- Quantum Yield (Φ)

$\phi_F = \text{emitted photons/photons absorbed} = (ABC)_{em} / A_{\lambda_{exc}}$

$(ABC)_{em}$ corrected emission

1- Steady State Emission Spectrum

Microenvironment Polarity

$\pi \rightarrow \pi^*$: increasing polarity \rightarrow red shift (higher λ)
Bathochromic Shift

$n \rightarrow \pi^*$: increasing polarity \rightarrow blue shift (lower λ)
Hypsochromic Shift

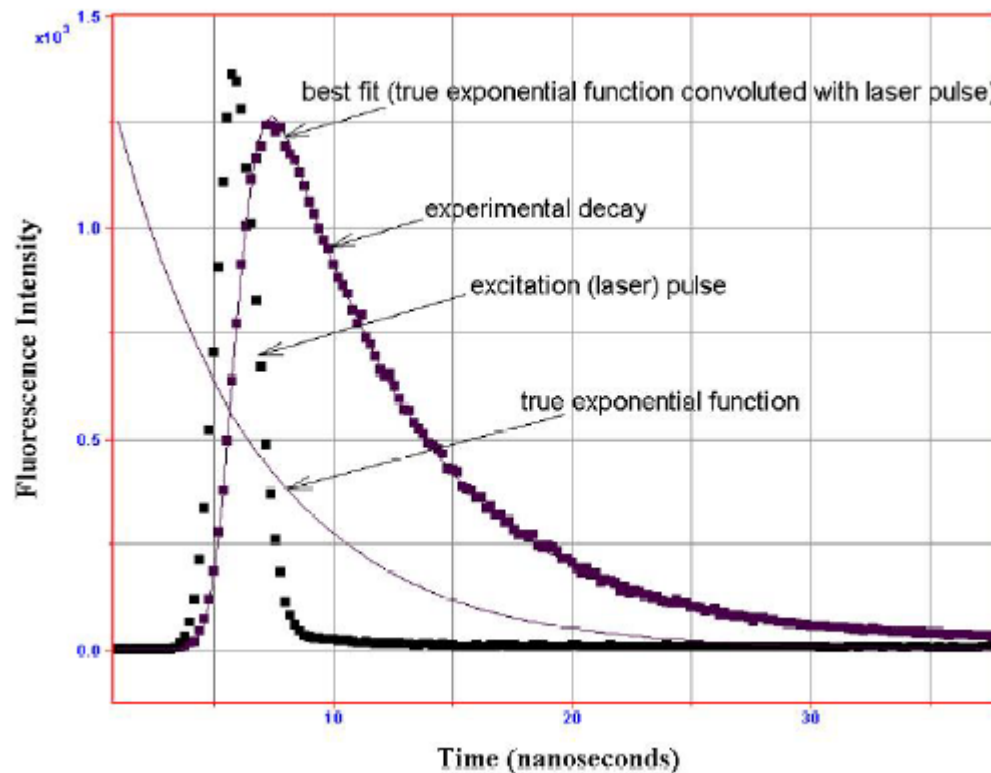
2-Time Resolved Emission Spectrum

Measurement: Emission Intensity vs time

Photons are measured after excitation.

Fitting of the decay allows to get lifetimes of the emitting compound (τ_F).

-Technique: Single Photon Counting



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3. Phosphorescence

CONDITIONS:

- High rigidity
- Oxygen absence
- Presence of heavy atoms

PARAMETERS FOR GETTING AN SPECTRUM

- λ_{EXC} , λ_{EM}
- Number of pulses
- Delay Time (ms)

4. Fluorescence Applications

A- Quantitative: Concentration determination

- * high sensitivity (10^{-7}M)
- * high selectivity
- Detection Systems (HPLC)
- Chemical Reactions Kinetics
- Binding Constants Determination
- Dissociation Constants
- Partition Coefficients
- Aggregation Equilibrium Constant
- Degradation Constant

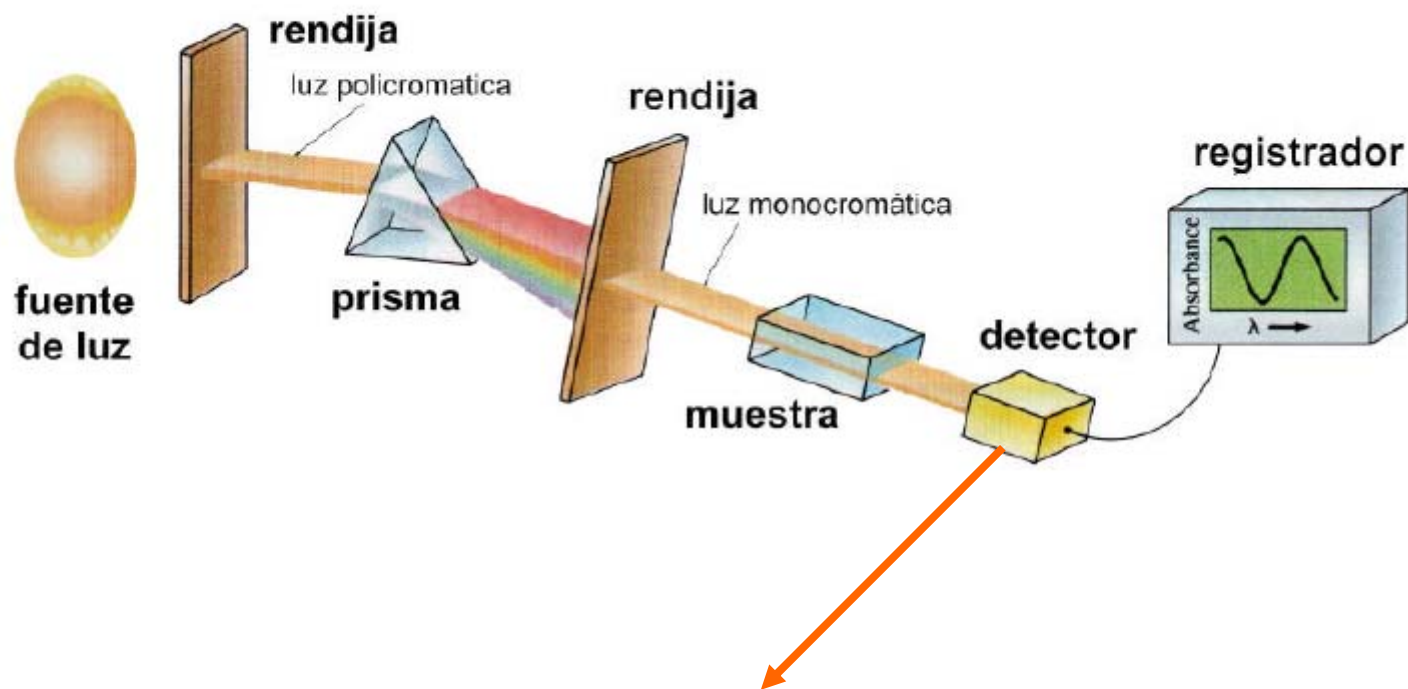
B- Qualitative:

- Brief structural information
- Polarity of microenvironments
- Viscosity of microenvironments
- Rigidity of the medium
- Conformational Changes
- Interactions with other molecules
- Distances between molecules or chromophores.....

Everything able to modify the electronic levels distribution

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5. Spectrophotometer and Spectrofluorimeter



SPECTROFLUORIMETER

90° MONOCHROMATOR + SLIT + DETECTOR

6. BIBLIOGRAPHY

1. **Principles of Fluorescence Spectroscopy**. Joseph Lakowicz. Plenum Press, New York (3rd. edición)