Fluorescence spectroscopy

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How were these pictures taken?

Basic steps of luminescence

1. Energy absorption
2. Excitation
3. Release of the accumulated extra energy in the form of light

Absorption, reminder

- the absorption process;
- the definition of absorption;
- how to measure absorption;
- the applications of absorption;

Luminescence

- **Definition**: Some objects can emit light *spontaneously*, after the *excitation* of their electrons (*cold* light).
  - Different from the heat induced light emission (incandescence).! e.g. Tungsten – lamp (*hot* light)

Luminescence

- Types (based on the type of excitation):
  - **Photoluminescence**: The high energy of optical radiation (UV light) will be transferred to light.
  - **Cathodoluminescence**: Kinetic energy of the accelerated e⁻ will be transformed to light.
  - **Electroluminescence**: The energy of the electromagnetic field will be transformed to light.
  - **Chemiluminescence**: Chemical energy will be transformed to light.
  - **Triboluminescence**: Mechanical work will be converted to light.
Chemoluminescence (firefly):

\[
\text{luciferin} + \text{ATP} \rightarrow \text{oxyluciferin} + \text{AMP} + \text{light}
\]

\[\text{light} \rightarrow \text{luciferase}\]

Triboluminescence:

Visible light and x-ray emission from peeling transparent tape:

Energy states:

\[\text{symmetric stretch mode} \quad \text{asymmetric stretch mode} \quad \text{bending mode}\]

Vibrational motions:

Energy level splitting:

\[E_{\text{sum}} = E_{\text{elect}} + E_{\text{vibr}} + E_{\text{rot}}\]

The change of the energy of electron:

\[\Delta E_{\text{sum}} = \Delta E_{\text{elect}} + \Delta E_{\text{vibr}} + \Delta E_{\text{rot}}\]

Energy level splitting:

What is S or singlet state?
Spin multiplicity within the molecules

The number of possible quantum states (n) of a system based on the spin quantum number S:

\[ M = 2S + 1 \]

Szingulett → \( S = 0; M = 1 \)

Triplett → \( S = 1; M = 3 \)

Understanding spectra – emission spectrum

Emission spectra – line and band spectra

Fluorescence

A Jablonski diagram

Understanding the transitions between energy states.
Kasha’s-rule

The emission of the fluorescence light is always starting from the lowest vibrational level of the first excited level ($S_1$).

Phosphorescence

Definition of fluorescence and phosphorescence

- $S \rightarrow S$ in the ns range
- $T \rightarrow S$ in the > ms range
**Basic fluorescence parameters**
- Fluorescence spectrum, intensity;
- Quantum efficiency
- Fluorescence lifetime
- Polarisation

**What is fluorescence spectra?**
Definition: the wavelength dependence of fluorescence emission
- Emission spectra
- Excitation spectra

**Emission spectrum**
- A graph, that shows the wavelength dependency ($\lambda_{\text{em}}$) of the emitted light intensity.
- Represents the vibrational levels within the ground state ($S_0$).
  - $\lambda_{\text{em}} = \text{constant} \rightarrow \lambda_{\text{em}} = \text{variable}$

**Excitation spectrum**
- A graph, that shows the wavelength dependency ($\lambda_{\text{ex}}$) of the absorbed light intensity.
- Represents the vibrational levels within the excited state ($S_1, S_2...$).
  - $\lambda_{\text{ex}} = \text{variable} \rightarrow \lambda_{\text{ex}} = \text{constant}$

**Mirror-image rule**
The emission spectrum is usually the mirror image of the excitation spectrum.

**Which one is longer; the excitation or the emission wavelength?**
Stokes-shift
The difference (measured in nm) between the peak of the excitation and the emission spectrum (energy loss).

Jablonski diagram

Basic fluorescence parameters
- Intensity, fluorescence spectrum
- Quantum efficiency
- Fluorescence lifetime
- Polarisation

Quantum-efficiency ($Q$)
- How efficiently will be the absorbed energy converted into the light.

$$Q = \frac{\text{number of the emitted photons}}{\text{number of the absorbed photons}}$$

Fluorescence lifetime ($\tau$)
The average length of the excited state of a fluorophore before emitting a photon.

$$F = F_0 e^{-\frac{t}{\tau}} = F_0 e^{-t/\tau}$$
if $F = F_0 / e$
then $t = \tau$
How to measure fluorescence?

Non linear arrangement !!!

The advantages

- great sensitivity and low detection limit
- fluorophores are sensitive to the environment

Summary

- Luminescence
- Fluorescence vs. Phosphorescence
- Spectra, quantum yield, lifetime
- Instrumentation
- Advantages

Thank you!