Fluorescence spectroscopy
Miklós Nyitrai, March 9, 2016

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):
  - Photo luminescence
    - The high energy of optical radiation (U/V light) will be transformed to light.
  - Cathodo luminescence
    - Kinetic energy of the accelerated e⁻ will be transformed to light.
  - Electro luminescence
    - The energy of the electromagnetic field will be transformed to light.
  - Chemoluminescence
    - Chemical energy will be transformed to light.
  - Triboluminescence
    - Mechanical work will be converted to light.
**Chemoluminescence (firefly)**

$$\text{Luciferin} + \text{ATP} \rightarrow \text{Oxy luciferin} + \text{AMP} + \text{Light}$$

**Triboluminescence**

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

**Energy states**

- Symmetric stretch
- Asymmetric stretch
- Bending

**Vibrational motions**

- Symmetric stretch mode
- Asymmetric stretch mode
- Bending mode

**Energy level splitting**

$$E_{\text{sum}} = E_{\text{elect}} + E_{\text{vibr}} + E_{\text{rot}}$$

The change of the energy of electron:

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

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:

\[ M = 2S + 1 \]

Singlet \( S = 0 \); \( M = 1 \)

Triplet \( S = 1 \); \( M = 3 \)

Spectrum – atoms and molecules

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

Time-scale of the changes

Definition of fluorescence and phosphorescence

S → S in the ns range

T → 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 ($S_0$).
  - $\lambda_{\text{ex}} =$ constant $\rightarrow \lambda_{\text{em}} =$ 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 states ($S_1$, $S_2$...).
  - $\lambda_{\text{ex}} =$ variable $\rightarrow \lambda_{\text{em}} =$ 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
- excited state
- S<sub>0</sub> – S<sub>1</sub>
- S<sub>1</sub> – S<sub>0</sub> (10<sup>-8</sup>s)
- S<sub>1</sub>
- excitation (10<sup>-15</sup>s)
- Stokes-shift!
- vibrational levels
- Kasha-rule!
- Mirror image!

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 (τ)
The average length of the excited state of a fluorophore before emitting a photon.

\[ F = F_0 e^{-t/\tau} \]

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

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

Thank you!