Optical Spectroscopy:
The study of absorption and emission of light in nature

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PHYS 403 Spring 2019
Optical Spectroscopy in Astronomy

- By looking through a standard telescope, you are observing the night sky at the visible light spectrum.
Gamma Rays
Black hole binaries in disks of plasma are sources of gamma rays.
X-ray sources include stars, supernova, gaseous shells ejected during a violent explosion of a dying star, and synchrotron radiation
Infrared

Sourced from stars
Microwave

Cosmic microwave background radiation emitted from the big bang and inflation
cold intergalactic hydrogen mostly found in the milky way emits radio waves.
Luminescence: Emission of light from any substance

- **Fluorescence**: transition from excited state to ground state is fast (~ns – ms range)
- **Phosphorescence**: transition from excited state to ground state is slow (~s – ks range)

Perrin-Jablonski energy diagram
Types of Fluorescent Molecules

Synthetic Organic:
Fluorescein

Semiconductor Nanocrystal:
Cadmium Selen

Crystals:
Ruby and assorted minerals
From mineralman.net

Naturally Occurring:

Fluorescent Proteins:
Green Fluorescent Protein

Fluorescent Nanodiamonds

Image from Zrazhevskiy et al. 2010

Nano Lett., 2010, 10 (9), pp 3692-3699. DOI: 10.1021/nl1021909
Fluorescence ($S_1 - S_0$)

Solvent Effect:

Organic dye:

Ruby:

http://micro.magnet.fsu.edu

http://www.bio.davidson.edu
Time-Dependent Fluorescence: Fluorescence Lifetime

Fluorescence Lifetime: The average amount of time a molecule stays in excited state

Probability of being in the excited state

\[ P(T) \]

\[ T_0, T_0+\Delta t, T_0+2\Delta t, T_0+3\Delta t, T_0+4\Delta t, \ldots \]

\[ \text{time, } T = T_0 + M \Delta t \]

\[ k_i = \text{rate constant for leaving excited state while emitting a photon} \]

\[ k_i = \text{rate constant for leaving excited state through other means (ie. Dynamic quenching, Energy Transfer, etc)} \]

Fluorescence Lifetime:

\[ \tau = \sum \frac{1}{k_i} \]

Lifetime is sensitive to other decaying pathways present!

Measuring the Depletion of the excited state

\[
\left[ \# x^* \right] = \left[ \# x_o^* \right] e^{-(k_F + k_i)t}
\]

\[
\left[ \# x^* \right](k_F) = \text{Intensity that you measure}
\]

\[K_F\] is rate constant of fluorescence

Intensity measured is proportional to the \# of molecules in the excited state!
Measuring Lifetime: Time Domain

What do you need?

- Collect signal fast enough
- Fitting
Measuring Lifetime: Frequency Domain

\[ E(t) = E_o + E_\omega \cos(\omega_E t + \varphi_E) \]

\[ F(t) = F_o + F_\omega \cos(\omega_E t + \varphi_E - \varphi) \]

\[ \tan(\varphi) = \omega_E \tau_\varphi \]

\[ M = \frac{F_\omega / F_o}{E_\omega / E_o} = \frac{1}{\sqrt{1 + (\omega \tau_{Mod})^2}} \]

What do you need?

- Intensity modulators
- Synchronization
Applications of Fluorescence in Biology
Fluorescence Lifetime Imaging on Live Cells

Top of cell: Intensity

Top of cell: Lifetimes

Optical Sections - Rendered by Lifetime

Intensity @ 4.5µm

Images courtesy of John Eichorst
Center of the distribution can be determined in ~1.5 nm accuracy if \( N \) is more than \( 10^4 \)