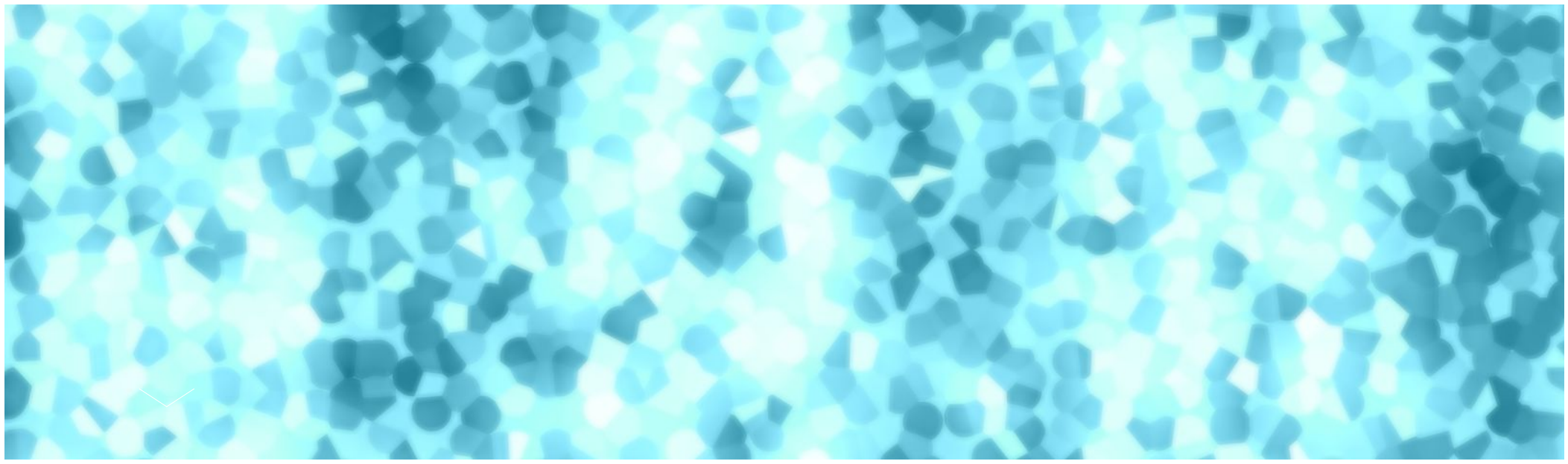

Fluorescence Spectroscopy

Principles and Theory of Fluorescence Spectroscopy

Fluorescence is a type of luminescence caused by photons exciting a molecule, raising it to an electronic excited state. It's brought about by absorption of photons in the singlet ground state promoted to a singlet-excited state. As the excited molecule returns to ground state, emits a photon of lower energy, which corresponds to a longer wavelength, than the absorbed photon.

Fluorescence spectroscopy analyzes fluorescence from a molecule based on its fluorescent properties. Fluorescence is a type of luminescence caused by photons exciting a molecule, raising it to an electronic

What is the Jablonski Diagram?

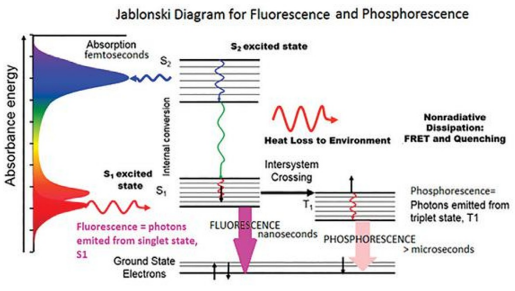

Fig. 3: The Jablonski Diagram of molecular absorbance and fluorescence

Fig. 3 shows the Jablonski diagram (Jablonski, 1933), a schematic of the transition of electronic state of a molecule during the fluorescence phenomenon. The left axis shows increasing energy, where a typical fluorescent molecule has an absorbance spectrum. This spectrum shows the energy or wavelengths, where the molecule will absorb light.

If the incident light is at a wavelength where the molecule will absorb the photon, the molecule is then excited from the electronic ground state to a higher excited state, denoted S2 here.

The electrons then go through internal conversion, affected by vibrational relaxation and heat loss to the environment. It then emits a photon from the lowest lying singlet excited state in the form of fluorescence.

In conventional fluorescence, photons are emitted at higher wavelengths than the photons that are absorbed. This diagram is extremely important to understanding fluorescence. When measuring a fluorescence spectrum, one is typically looking at the intensity at which a molecule emits, the wavelength or energy at which it emits, and also the time which the molecule spends in the excited state. This is the fluorescence lifetime, explained further in detail in coming sections.

Any number of things can affect these observables, including energy transfer to and from other molecules, quenching by other molecules, temperature, pH, local polarity, aggregation or binding. Understanding the mechanisms of these interactions can give **us** insight into what is being observed with a change in fluorescence spectra or lifetime.

There are two non-radiative deactivation processes that compete with fluorescence: internal conversion from the lowest singlet excited to the ground state and intersystem crossing from the excited singlet state to the triplet state. This last process leads to the phenomenon called phosphorescence, explained in further detail later on.

What is a fluorescence lifetime?

In simple terms, fluorescence lifetime of a molecule is the average length of time it spends in the excited state. This depends on the type of molecule and its local environment. Typically, the excited state decays in an exponential manner, as indicated in the equation below. The use of fluorescence lifetime has its advantages over that of an intensity measurement, as it is an "absolute" measurement, rather than the "relative" steady state measurement (which gives a time-averaged signal).

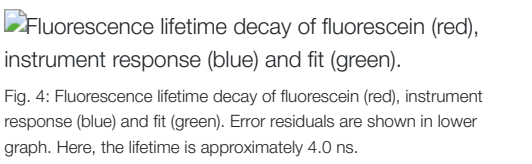
Fluorescence lifetime decay of fluorescein (red), instrument response (blue) and fit (green).

Fig. 4: Fluorescence lifetime decay of fluorescein (red), instrument response (blue) and fit (green). Error residuals are shown in lower graph. Here, the lifetime is approximately 4.0 ns.

$$I(t) = I_0 \exp^{(-t/\tau)}$$

τ is the fluorescence lifetime or the time for the intensity to decay to 1/e of its initial value.

If more than one excited state is present, sometimes because the sample under study contains a mixture of fluorescing molecules, and there are different local environments or a molecule undergoes a transformation, giving rise to different excited state species, the decay is expected to be more complex. There can be one exponential decay per excited state present. This can be represented by a sum of exponentials (see below), where α (the pre-exponential factor) is indicative of the relative concentration of each t decay to the observed overall decay.

In order to compare measurements, it is often useful to normalize the pre-exponential factors in some way. If a comparison of the concentration of each fluorescing species is required, then the normalized α may be used. If a comparison of the contribution to steady state spectrum (overall fluorescence emission) is needed, then the fractional or relative amplitude (in %) can be used. The latter is the pre-exponential factor weighted by the lifetime.

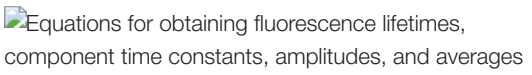
Equations for obtaining fluorescence lifetimes, component time constants, amplitudes, and averages

Fig. 5: Equations for obtaining fluorescence lifetimes, component time constants, amplitudes, and averages

At times, it can be just as acceptable to represent a complex decay by an average lifetime. However, it should be noted that this is best done by actually correctly modelling the complex decay, rather than just attempting to fit a single exponential decay to it. In most cases, the use of the amplitude average lifetime is appropriate, however, when considering quenching experiments, it is more correct to employ the intensity average fluorescence lifetime. There are published works going into the details of the relative merits of these averages. (Lakowicz, 2006) (Berezin, 2010)

What is phosphorescence?

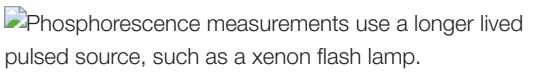

Fig. 6: Jablonski diagram for Phosphorescence emission

Phosphorescence is a process where the photon is emitted, not from a singlet-excited state, but from a forbidden triplet state. The time scale of emission is generally in the picosecond to nanosecond range, while phosphorescence typically lasts for fluorescence microseconds, milliseconds, or even longer… minutes or hours. Researchers typically use a pulsed source such as a flash lamp or LED to measure phosphorescence spectra and decays on these longer time scales. Phosphorescence measurements use a longer-lived pulsed source, such as a xenon flash lamp. The timing of the flashing lamp can be used to measure spectra at different phosphorescence lifetimes.

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