Time-resolved fluorescence spectroscopy experiments track the emission of fluorescence light from a sample. Time-resolved fluorescence intensities are also called fluorescence decays. Analysis thereof may resolve composite samples and report on dynamics (citations). Excited fluorescent molecules stay a particular time in their excited state before emitting light and delay the detection of fluorescence. This “delay” depends on molecular properties. Hence, time-resolved fluorescence informs on the studied molecules.
Different experiments can study the delayed emission of fluorescence photons. Frequency-domain experiments use a modulated light source to excite the sample. Changes of the modulation frequency result in changes of the fluorescence intensity. These changes characterize the lifetime of the excited state. Time-domain experiments directly follow the decrease of the fluorescence intensity. A short light pulse excites the sample. Next, a detector follows a reduction of the fluorescence intensity with time. Time-domain experiments require “fast” light sources and detectors, meaning that a high-quality experimental setup needs a light source with excitation short pulse and detector with a narrow distribution of response times. Ideally, both are in the order of several picoseconds. Frequency-domain experiments have no special requirements concerning the light source and detector. On the other hand, time-domain measurements are conceptually simpler.
There are many different techniques to detect fluorescence in the time-domain. The “Techniques” issue in the series “Topics in Fluorescence” gives a comprehensive overview thereof (Topics in Fluorescence Spectroscopy Techniques, Joseph R. Lakowicz, 1999).
Among all time-domain techniques, time-correlated single photon counting (TCSPC) is the current de-facto standard. In a TCSPC experiment, a fluorescent sample is repeatedly excited by a sharp excitation pulse. A detector records for a fraction of excitation pulses, single photons relative to this excitation pulse (correlated detection). The times between excitation and detection of fluorescence measured by a clock with picosecond accuracy are integrated to generate a time-resolved histogram of the fluorescence intensity. Time-resolved fluorescence intensity histograms are also called fluorescence decays. TCSPC is sensitive, has a large dynamic range, and a high time-resolution. Additionally, the noise of recorded fluorescence decay, given by the shot-noise of the photons, is precisely known. TCSPC is used in single-molecule, fluorescence lifetime imagining (FLIM), and bulk fluorescence decay measurements in cuvettes.
A later post will discuss technical aspects of TCSPC. This post focuses on the basics of time-resolved fluorescence and its capabilities in resolving heterogeneities. Steady-state fluorescence experiments depend on the average population of fluorescent states. A fluorescence decay offers the time between excitation and detection of fluorescence as an additional dimension, which can be utilized to resolve sample heterogeneities.
The decay of the fluorescence intensity is directly proportional to the population of the fluorescent excited states. Suppose the emission rate constant of a molecule is k_F. Then the change of the probability of finding the molecule in its excited stated is:
$$dp = k_F \cdot dt$$
Therefore, the expected fluorescence decay, $f(t)$, is proportional to:
$$f_1(t) = k_F \cdot exp(-t \cdot k_F)$$
The expected fluorescence decay of a mixture of two molecules (1) and (2) with identical fluorescence properties except for their depopulation rate constants of their excited state, k_1, and k_2 is a species fraction weighted sum.
$$f_2(t) = k_F \left[x_1 \cdot exp(-t/\tau_1) + x_2 \cdot exp(-t/\tau_2)\right]$$
Time-resolved fluorescence measurements integrate the fluorescence lifetime characteristic of all ensemble members in a curve. Steady-state fluorescence measurements combine the fluorescence in a single number. A curve analysis of decay curves
can be determined the composition of the studied ensemble. Meaning that in the example above, the components, x_1 and x_2 and τ_1 and τ_2 can be estimated. Such analysis applied to a Förster resonance energy transfer (FRET) experiments on proteins and other macromolecules determine distance distributions.