Fluorescence decays, $f(t)$, record fluorescence intensities in dependence of the time, t. Multiple representations of fluorescence decays exist each having advantages and disadvantages. Often the aim of measuring a fluorescence decay is to obtain a fluorescence lifetime, τ, as a characteristic sample property. The four distinct representations to display and interpret fluorescence decays presented and discussed below. These representations can also be utilized for a time-resolved anisotropy, $r(t)$, and a FRET-induced donor decay, $\epsilon(t)$.

Exponential decays

Complex fluorescence decays are often the species fraction weighted superposition of multiple simple fluorescence decays. The fluorescence decay of a fluorophore with a single excited and ground state follows an exponential decay law. Therefore it is very educative to recall the properties exponential decays. The expected time-resolved fluorescence intensity of such a system is given by $f(t) \propto e^{-t/\tau}$. Here, τ is the fluorescence lifetime, t is the time since excitation of the fluorophore, and $f(t)$ is the fluorescence decay.

The most straight forward way of displaying $f(t)$ is the use of linear axes for the time, t, and the detected photons. Such the representation display the data very well. However, in such representation, it ‘s hard to interpret decays visually. Therefore, more frequently fluorescence decays are plotted using a logarithmic y-axis ($\ln f(t) = -t \cdot \tau$). Such plot has the advantage that the slope of such plot provides an estimator of the fluorescence lifetime. In the image below a typical experimental fluorescence decay is shown in the two representations. Hence, if the purpose of plotting experimental data is to demonstrate an exponential decay law the use of a logarithmic y-axis and a linear x-axis is very beneficial. A third alternative is to display fluorescence decays using a logarithmic time axis. Such a plot the experimental data results in a sigmoidal curve and the fluorescence lifetime can be estimated by the time t at which $f(t)$ decayed to $1/e$ of the initial value of $f(t)$.

Residuals

The number of photons per time channel follows a Poissonian distribution. The variance of a Example fit without considering the weights properly and the residuals

Anisotropies

FRET

The aim of an FRET-experiment is to recover rate constants of energy transfer from a donor, D, to an acceptor, A, fluorophore and ultimately the DA-distance.