Appendix 3 – Convolution

Determining the fluorescence decay rate from dye solutions becomes more difficult at high concentrations. We are using a photodetector with approximately 0.8 ns time resolution, and as the effective fluorescence decay time we are trying to measure approaches this value, the real fluorescence data becomes masked by the detector.

The experimental observable is determined both by the detector time-resolution and the time scale of the fluorescence relaxation. More generally, an experimental measurement is an integral over the instrumental response R(t) – the detector time resolution – and the response from the sample S(t) – here the fluorescence decay. The observed signal – the time-resolved fluorescence intensity – is termed a convolution integral:

$$I(t) = \int R(t - t')S(t')dt' \qquad (1)$$

This essentially is the fluorescence relaxation summed over the profile of the detector response. When the fluorescence decay is much faster than the detector response, S(t) approaches a delta function and all we see is the detector. When the detector is much faster than the fluorescence relaxation, R(t) approaches a delta function and our signal looks purely like the fluorescence. When the two exist on similar time scales, we can still extract information on our decay by comparing our fluorescence data with our instrument response. Suppose we model our detector time resolution by a Gaussian function with width σ,

$$R(t) = A_1 \exp\left(-\frac{t^2}{2\sigma^2}\right) \qquad (2)$$

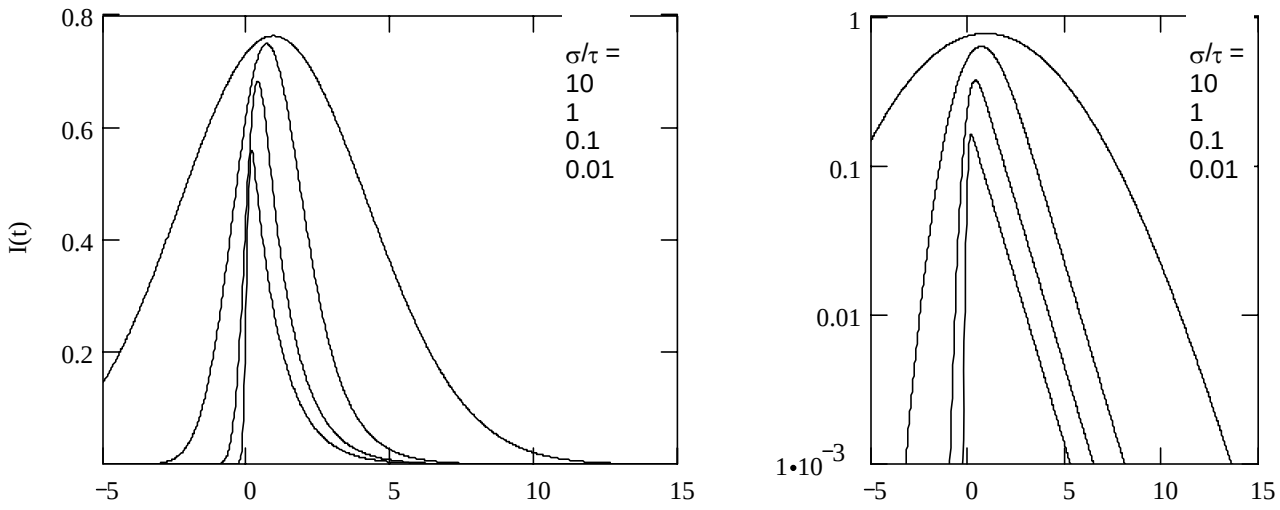
and our fluorescence decay by an exponential with decay time τ,

$$S(t) = A_2 \exp\left(-\frac{t}{\tau}\right) \qquad (3)$$

Evaluating our convolution integral (and dropping constants), we obtain

$$I(t) = \exp\left(\frac{\sigma^2}{2\tau^2} - \frac{t}{\tau}\right)\left(1 - \mathrm{erf}\left(\frac{\sigma^2 - \tau t}{\sqrt{2}\sigma\tau}\right)\right) \qquad (4)$$

Here, erf(x) is the error function, which is a standard function in most mathematical software packages. (erf(0)=0; erf(∞)=1).



What does this mean for our experiment? Suppose we are trying to resolve a fluorescence decay with an effective decay constant of τ with a detector response width of σ. The linear and semi-log figures above show our calculated convolution integral for values of σ/τ from 0.1 to 3, compared to the exponential decay (dashed line). For small σ, we just observe the exponential relaxation, but as σ gets larger, the observed signal approaches the detector response and we cannot extract a value.

In another comparison, we can show the exponential fluorescence decay that we are trying to recover (dashed line) from the observed signal (solid) measured with a detector response (dotted) of $\sigma = 3\tau$, τ, and 0.3τ. Clearly one cannot get a meaningful measurement for $\sigma=3\tau$, but by measuring the relaxation in the tail one can extract the decay for the other cases. (Notice that one can also measure the shift of the peak of the signal relative to the peak of the detector response to give some idea of the decay time).