Convolution

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A fluorescence decay D(t) of a single fluorophore in homogeneous solution can be usually described as a single-exponential function in accordance with a 1^{st} order kinetic scheme:

$$D(t) = a\exp\left(-\frac{t}{\tau}\right) \qquad (1)$$

where τ is the lifetime defined in Eq. 3.

If the sample contains more than one fluorophore or the fluorophore is contained in a heterogeneous environment, the fluorescence decay can be better described as a sum of exponential functions:

$$D(t) = \sum_{i=1}^{n} a_i \exp\left(-\frac{t}{\tau_i}\right) \qquad (2)$$

where τ_i are fluorescence lifetimes of various fluorescent forms and a_i are corresponding pre-exponential factors. The interpretation of a_i depends on the nature of the sample: if the emission comes from a single fluorophore in different conformational forms or environments, the a_i factors are proportional to respective populations. If multiple lifetimes result from the presence of several fluorophores in the sample, the a_i pre-exponential factors will depend not only on their populations, but also on radiative probability constants and molar extinction coefficients of respective fluorophores.

The decay curves described by equations 4 and 5 will only be observed if the sample is excited by an infinitely narrow pulse. In most cases the temporal width of the excitation pulse cannot be neglected and the observed decay $D_{obs}(t)$ will be distorted by convolution with an instrument response function L(t) in accordance with the following equation:

$$D_{obs}(t) = \int_0^t L(t-s)D(s)ds \qquad (3)$$

The instrument response function (IRF) L(t) can be determined experimentally by using a scatterer solution instead of a sample. The IRF accounts for the shape of the excitation pulse and for the temporal response of the detection system. Figure X shows an example of the traces obtained in a typical fluorescence decay experiment.

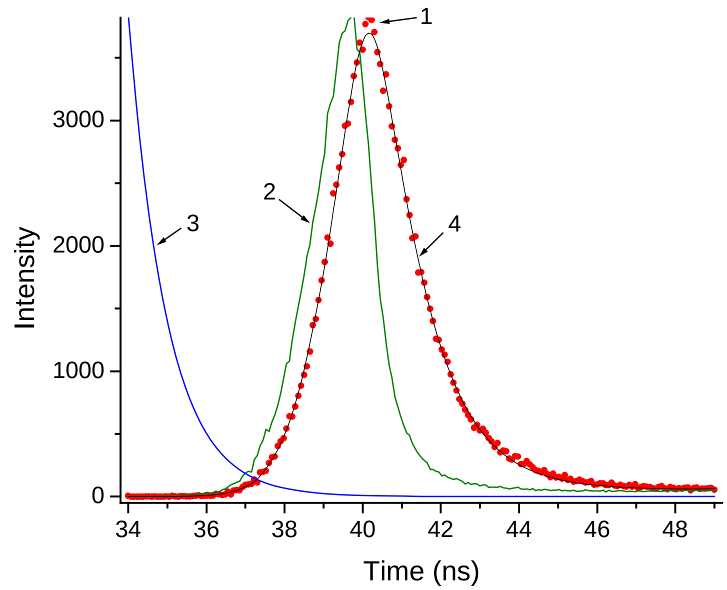

Fig. 1. Illustration of the convolution effect as described in Eq. 6: 1 - experimental decay $D_{obs}(t)$; 2 - instrument response function L(t); 3 – undistorted exponential decay D(t); 4 – the best fit to experimental decay, i.e. convolution of curves 2 and 3.

Once the $D_{obs}(t)$ and L(t) have been measured, the analysis software performs iterative reconvolution according to Eq. 6 varying the fit parameters a_i and τ_i until the best fit to the experimental decay is obtained.

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