Fluorescence decays, $f(t)$, record fluorescence intensities as a function of the time, t. Several representations exist, each with its own advantages and disadvantages. Usually the aim of measuring a decay is to obtain a fluorescence lifetime, τ, as a characteristic property of the sample. The representations discussed below apply equally to a time-resolved anisotropy, $r(t)$, and to a FRET-induced donor decay, $\epsilon(t)$.

Exponential decays

Complex fluorescence decays are often a species-fraction weighted superposition of simpler decays. A fluorophore with a single excited and ground state follows an exponential decay law, so the expected time-resolved intensity is

$$f(t) \propto e^{-t/\tau}$$

On linear axes the data are displayed faithfully, but the decay is hard to interpret by eye. Plotting the logarithm of the intensity against time linearises it:

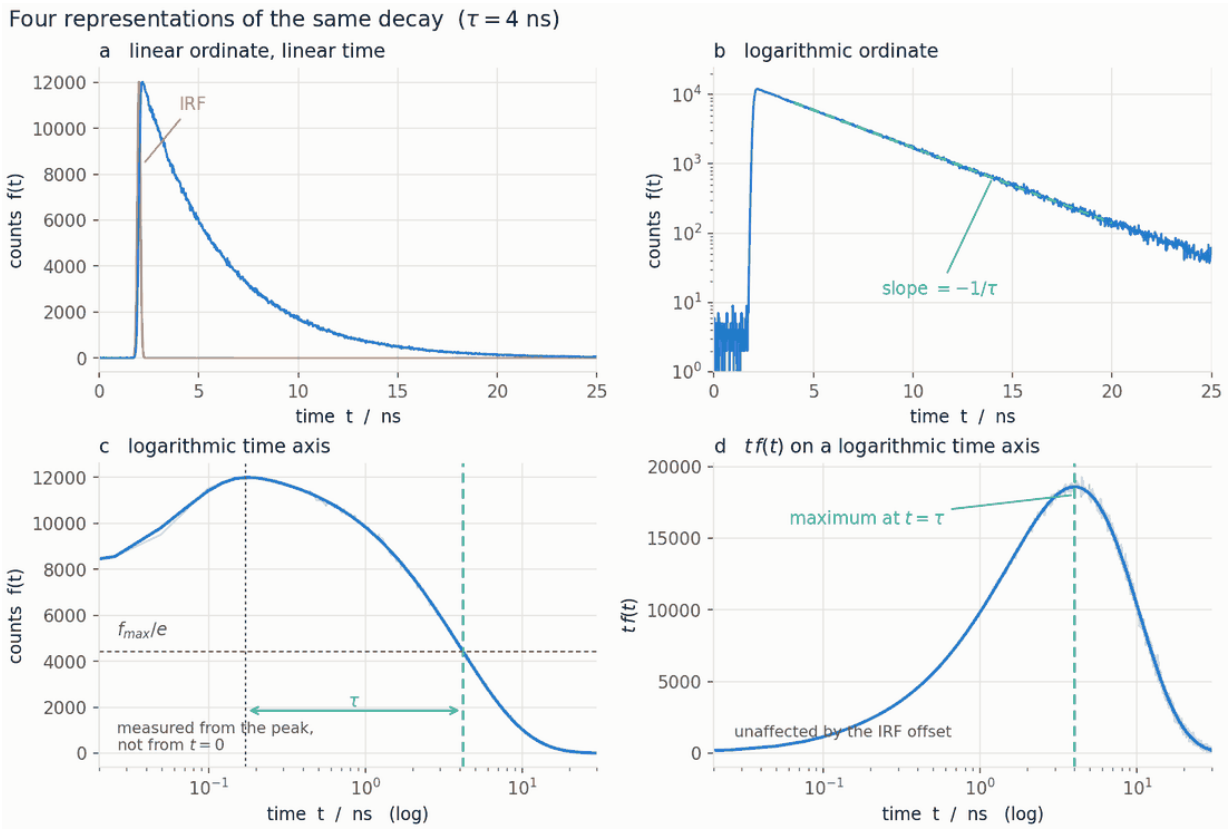
$$\ln f(t) = -t/\tau + \mathrm{const}$$

The slope of this plot is $-1/\tau$, so a single-exponential decay appears as a straight line whose negative reciprocal slope is the lifetime. Curvature indicates more than one lifetime component. A third option is a logarithmic *time* axis, which turns the decay into a sigmoidal curve. The lifetime is read off as the time over which $f(t)$ falls to $1/e$ of its maximum – but note that this must be measured *from the peak of the measured curve*, not from $t=0$. The convolution with the instrument response function shifts the maximum to a slightly later time, and reading from $t=0$ inherits that offset: in the simulation below the peak sits 0.17 ns late, so the naive reading gives 4.20 ns instead of 4.00 ns.

A fourth representation removes that problem. Plotting $t\,f(t)$ against $\log t$ preserves the area under the curve when the time axis is compressed, because $dt = t\,d\ln t$. For a single exponential this product peaks exactly at $t = \tau$, since

$$\frac{d}{dt}\left[t\,e^{-t/\tau}\right] = e^{-t/\tau}\left(1 - \frac{t}{\tau}\right) = 0 \;\Rightarrow\; t = \tau$$

A constant time offset from the instrument response enters only as a multiplicative factor and so does not move the maximum – moving the IRF between 0.5 and 3 ns leaves the peak at 4.00 ns. Both logarithmic-time representations are useful when the lifetimes present span several orders of magnitude.

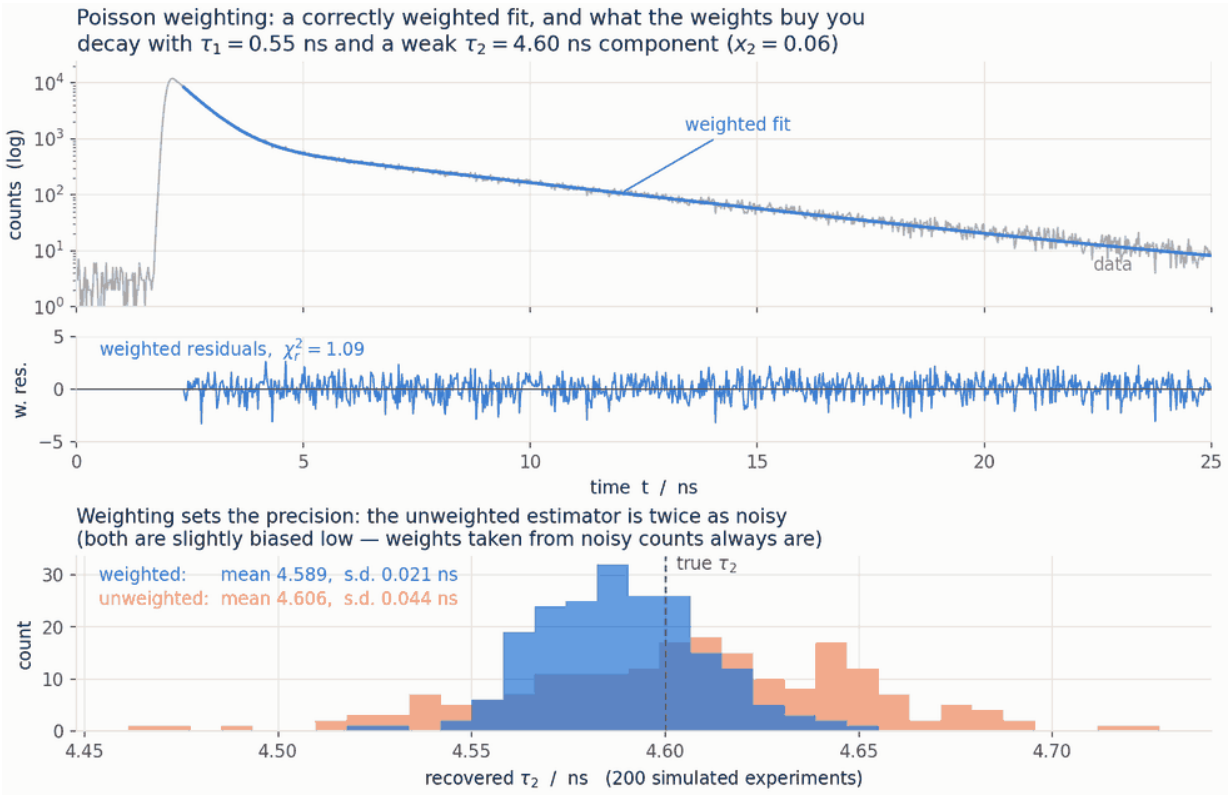


The same simulated decay (\(\tau = 4\) ns, time-correlated single photon counting with a 180 ps instrument response) in four representations. **a** linear ordinate. **b** logarithmic ordinate – a straight line of slope \(-1/\tau\). **c** logarithmic time axis – sigmoidal; the \(1/e\) time must be measured from the peak, which the IRF displaces by 0.17 ns. **d** \(t\,f(t)\) against \(\log t\) – peaks at \(t=\tau\) and is insensitive to the IRF offset. Pale traces are the simulated photon counts, solid traces the noiseless model.

Residuals

The number of photons per time channel follows a Poisson distribution, so the variance of a channel equals its expected number of counts. A fit must therefore be weighted, with \(\sigma_i = \sqrt{n_i}\); unweighted least squares treats a channel holding ten thousand photons and a channel holding ten as equally informative, which they are not.

Weighted residuals, \((n_i – f_i)/\sigma_i\), should scatter randomly about zero with unit variance, and the reduced chi-square should be close to one. Systematic structure in the residuals – a run of positive values early in the decay, for instance – indicates a model that is missing a component, not noisy data.



Top: a correctly weighted fit to a decay containing a weak long-lifetime component, with flat weighted residuals and \(\chi^2_r = 1.09\). Bottom: the recovered \(\tau_2\) over 200 simulated experiments. Weighting halves the scatter (0.021 ns against 0.044 ns), but weights taken from the observed counts also pull the estimate low – which is why Poisson maximum-likelihood fitting is preferred over weighted least squares.

Anisotropies

Time-resolved anisotropy reports on rotational motion. It is computed from the fluorescence intensities detected parallel and perpendicular to the polarisation of the excitation light,

$$r(t) = \frac{I_{VV}(t) - G\,I_{VH}(t)}{I_{VV}(t) + 2G\,I_{VH}(t)}$$

where \(G\) corrects for the polarisation-dependent detection efficiency of the instrument. For a spherical rotor the anisotropy decays exponentially, \(r(t) = r_0 e^{-t/\rho}\), with rotational correlation time \(\rho\) and fundamental anisotropy \(r_0\). As for intensity decays a logarithmic ordinate linearises the decay, and the slope is \(-1/\rho\). Departures from a single exponential reveal restricted or multi-timescale motion; for dye-labelled biomolecules this is typically fast local motion of the dye superimposed on slower tumbling of the whole molecule.

FRET

The aim of a FRET experiment is to recover the rate constant of energy transfer from a donor, D, to an acceptor, A, and ultimately the donor-acceptor distance. The FRET-induced donor decay isolates the transfer contribution by dividing the donor decay measured in the presence of the acceptor by the decay of the donor alone,

$$\epsilon(t) = f_{DA}(t) / f_{D0}(t)$$

This ratio removes photophysics common to both samples and leaves only the effect of transfer. For a single donor-acceptor distance R,

$$k_{FRET} = \frac{1}{\tau_{D0}} \left(\frac{R_0}{R} \right)^6$$

with the Foerster radius R_0. The transfer efficiency follows from the lifetimes, $E = 1 – \tau_{DA}/\tau_{D0}$, and the distance from $R = R_0 (1/E – 1)^{1/6}$. Because $\epsilon(t)$ is a ratio of two measured decays it is sensitive to distance distributions: a static distribution of distances produces a non-exponential $\epsilon(t)$ even when each individual species decays exponentially.