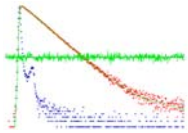


Time-resolved emission spectra / decay associated spectra

The use of time-resolved fluorescence enables more of the fluorescence signature of a compound to be elucidated. The outcome is a 3-D surface that can be "sliced" to allow the temporal evolution of the fluorescence to be monitored, as well as the resolution of spectrally overlapping species. This can be used to provide information concerning fluorophore mixtures and solvent relaxation. It is also possible to discriminate against scattered excitation light and impurity fluorescence.

Methodology

The acquisition of time-resolved emission spectra (TRES) is dependent on the presence of a monochromator on the emission channel of the time-resolved fluorometer. A typical measurement procedure involves incrementing the monochromator in fixed wavelength steps, with time-resolved decays acquired for either fixed time intervals or to a predetermined peak count at each wavelength. To obtain intensity information, the option of fixed time interval should be chosen. Acquisition of the instrumental response (IRF or prompt) allows further analysis using reconvolution.

In this note three examples on the use of this technique will be briefly explored. Note graphics are stylised for clarity.

- **Temporal resolution of spectra**

The first step is the acquisition of the wavelength dependent time resolved decays. Typically this is automated and shown schematically in Fig. 1.

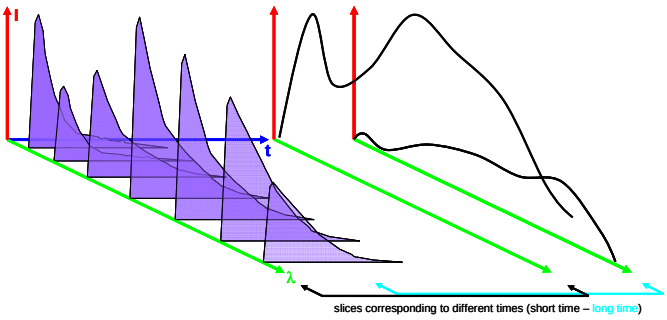


Fig. 1. Scheme showing spectra slices to give TRES

It is possible to take "slices" of data in the intensity – wavelength plane to obtain spectra at different times (t) during the decay. It is possible to sum adjacent time channels to obtain more data points in the spectral slices. Using this method the spectra of shorter-lived species will be predominantly found at shorter times, while only longer-lived species should be found at longer times. Thus, this simple approach can be employed to distinguish the spectra of species with differing decay times. This approach can be limited by the time-resolution per point, the differences between lifetimes and amount of the species to be resolved.

- **Evolution of the fluorescence signal**

As well as application in resolving different fluorescing species, TRES can be employed to follow solvent induced effects, such as solvent relaxation. The use of polar solvents can induce a red shift in the fluorescence, which is dependent on the change of the fluorophore dipole moment upon excitation. This is because of the interaction between the dipole of the fluorophore and that of the surrounding solvent molecules. Briefly the higher the solvent polarity, the greater the red shift and the timescale of this solvent relaxation process is largely determined by the mobility (local viscosity) of the solvent envelope surrounding the fluorophore. The reorientation of the dipoles and an estimate for the timescale of this process can be obtained using TRES. This is shown schematically in Fig. 2.

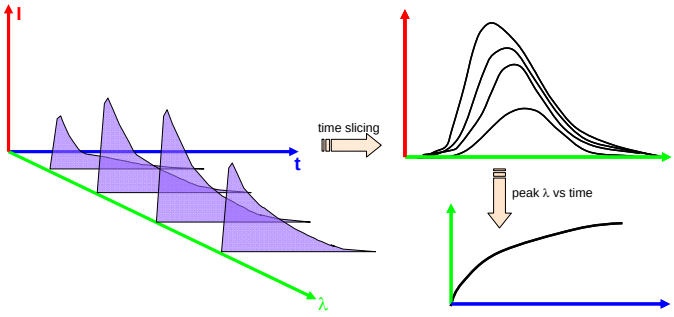
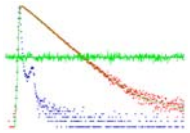


Fig. 2. Scheme for TRES used to follow solvent relaxation

In the above figure the decays are measured as in the first example, although it should be remembered that this process can occur on short timescales so a high temporal resolution is necessary. Slicing through the time axis allows the spectra to be obtained. From this the peak wavelength can be determined and plotting this (commonly converted to wavenumber) against time produces a curve, from which the time constant for this process can be obtained.



- **Decay associated spectra**

This example of the use of a TRES measurement requires a slightly more complex form of analysis, but the outcome allows spectra to be associated with specific decay times. The summation of the individual spectra also allows the steady state spectrum to be obtained. Again this technique is useful in resolving mixtures of fluorescing species with overlapping spectra and data is acquired in a similar manner to the previous examples. Since reconvolution analysis can be employed the measurement of the instrumental response is advisable. A representation of this procedure is given in Fig. 3.

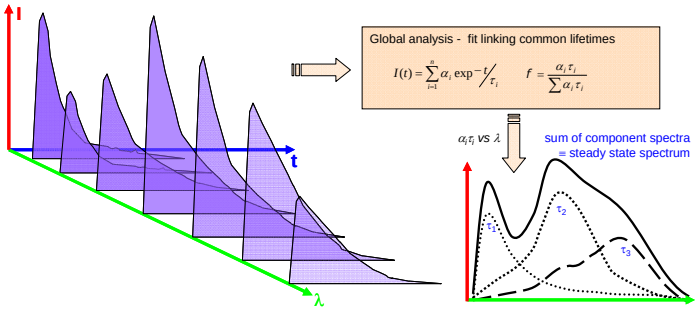


Fig. 3. Scheme for obtaining decay associated spectra

After the decay data and IRF have been acquired the whole dataset can be analysed globally. This iterates common lifetimes for all the fluorescence decays. However the associated pre-exponential factors (α_i – see Fig. 3.) will vary from decay to decay, depending on the amount of that species present. Plotting this value against wavelength, weighted by the lifetime, will produce an intensity-wavelength graph which represents the spectrum associated to a particular lifetime. Summation of the individual spectra plotted in this manner should return the equivalent of the steady state spectrum.

Applications

- resolving fluorophore mixtures
- monitoring species formed during a photochemical reaction
- solvent relaxation
- discrimination against scattered excitation
- discrimination against background / impurity fluorescence