A fit-free method for the analysis of fluorescence decays

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Time resolved florescence spectroscopy and microscopy are widely used in biological studies and material science in order to extract fluorescence lifetimes of the species involved and derive structural information, sense local environment or detect conformational changes. For this purpose, florescence decay profiles measured by single photon counting method are analyzed classically using multi-exponential fitting, i.e. a sum of weighed exponential that models the contribution of different species (amplitude) with their respective lifetime.

We propose here a fit-free approach based on multivariate curve resolution (MCR) to analyze global decay profiles. This approach requires first the data matrix to be split in two: one submatrix $\mathbf{M_1}$ corresponds to the measurements for short time (where the effect of convolution with the IRF is significant) whereas the second ($\mathbf{M_2}$) corresponds to the measurements for long time (where the effect of convolution with IRF can be ignored and which follows a multi-exponential decaying behavior). On $\mathbf{M_2}$ a data slicing procedure is used [1]. This method is based on the fact that exponential decay functions, when translated in time, keep the same shape only the amplitude change. The data are thus sliced in different slabs (submatrices of the same dimension taken at different time lags) giving several "time shifted" decay profiles. Finally, a multiset is construct combining $\mathbf{M_1}$ and slabs of $\mathbf{M_2}$, on which MCR using trilineairty constraint on the slices [2] is applied (we call this approach MCR slicing).

We validate our approach by decomposing (1) a data set composing of ten different mixtures of three commercial dyes measured at six different emission wavelengths and 2) a fluorescent protein where the fluorescence decays have been measured using different excitation and emission wavelengths.

The main benefits of MCR-slicing are, by contrast to iterative fitting algorithms the following ones: i) solution obtained is unique, ii) there is no need for initial guess of the parameters, iii) the approach is non-iterative and iv) parameter correlation is avoided.

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