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Kinetic Modeling of Multivariate Spectroscopic Images

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1 Introduction

In this presentation we report the use of kinetic modeling of temporal hyperspectral fluorescence image data to extract kinetic information and rate constants for reactions of interest to biologists and computer modelers. In traditional kinetic modeling algorithms, the initial concentrations of all species in the postulated model must be known; however, in hyperspectral fluorescence images of biological specimens it is impossible to know the initial concentrations of all species.

2 Theory

Two modeling techniques are reported for kinetic fitting of systems with unknown initial concentrations: direct non-linear (DNL) fitting and separable least-squares (SLS).[1] In the DNL approach, all parameters including rate constants and initial concentrations are estimated with a non-linear solver. In the separable least-squares approach, the inherently linear parameters (concentrations) and non-linear parameters (rate constants) are separated and solved in succession. Two methods for estimating these have been developed. In the first approach, DNL, the rate constants and initial concentrations are both estimated using a non-linear solver. In the second approach, SLS, the linear parameters (e.g., initial concentrations) and non-linear parameters (e.g., rate constants) are separated and estimated sequentially. This approach is only applicable to systems in which the inherently linear and non-linear model parameters are separable such as the first-order decay models used in this investigation. In both of these techniques as in soft modeling, only the relative intra-specie concentrations can be obtained. However, with spectral normalization of the data, relative inter-specie concentrations can also be determined given the assumption of identical response for all species. The normalization step was also required to prevent intensity ambiguities in the fitting process, which are commonly observed in soft modeling techniques.[2]

2.1 Direct non-linear estimation of initial concentrations

In this method the non-linear optimizer 'lsqnonlin' from the Matlab® toolbox is used to estimate the rate constants and initial concentrations for a given mechanism. This optimizer uses a trust-region method[3] with preconditioned conjugate gradients.[4] In this optimization routine the residuals shown in equation 1 are minimized by adjusting the rate constants and initial concentrations.

$$\mathbf{E} = \mathbf{D} - \mathbf{C}\mathbf{S}^{\mathbf{T}} \tag{1}$$

2.2 Separable least-squares estimation of initial concentrations

A key feature in the SLS approach is the separation of intrinsically linear parameters (initial concentrations) and non-linear parameters (rate constants) according to equation 2, which shows a set of observations in $\mathbf{y}$ that are described by a set of linear parameters $c_1, c_2, \ldots, c_n$ and a set of basis functions $F_1, F_2, \ldots, F_n$ which are dependent on the non-linear parameters $k_1, k_2, \ldots, k_n$ and t.

$$\mathbf{y} = c_1 F_1(k_1, t) + c_2 F_2(k_2, t) + \ldots + c_n F_n(k_n, t) \tag{2}$$

In this particular application, the technique has been applied to the mechanism of first-order decay. The non-linear optimizer 'fminsearch' from the Matlab® optimization toolbox is used to estimate the rate constants for the mechanism of first-order decay and the linear parameters are estimated by equation 3, where $\mathbf{c} = [c_1, c_2, \ldots, c_n]$, $\mathbf{k} = [k_1, k_2, \ldots, k_n]$, and $\mathbf{F} = [F_1, F_2, \ldots, F_n]$.

$$\mathbf{c} = \mathbf{F}(\mathbf{k}, t) \setminus \mathbf{y} \tag{3}$$

The principles of this approach can be applied to any system in which the intrinsically linear and non-linear parameters are separable.[1]

3 Material and methods

The two DNL and SLS methods are demonstrated and compared for the resolution of photo-bleaching in multicomponent glass beads and in temporal hyperspectral fluorescence images of fixed Human pulmonary type II epithelial A549 cells transiently transfected with IKKα proteins tagged with Green Fluorescent Protein (GFP) and MAVS proteins tagged with Yellow Fluorescent Protein (YFP).

All image data was acquired using a hyperspectral fluorescence confocal microscope recently developed by Sandia National Laboratories which has the ability to acquire 512-point emission spectra for each pixel in an image at up to 18 time points in rapid succession[5, 6].

4 Results and discussion

Due to complexities in cell images and the presence of Poisson noise, successful implementation of kinetic fitting required S/N based thresholding, pixel selection, fitting of multiple exponential decays for some fluorescent species, and automatic fitting of temporal baseline offsets. In order to reduce computation times, we also formed super-pixels by averaging multiple spectrally correlated pixels in the image.

Results of fitting the reaction model shown in equation 4 to photo-bleaching of fixed transfected A549 cells are shown in Figures 1, 2, and 3. Figure 1 is an RGB image showing the estimated concentration map at t=0 of YFP, GFP and auto-fluorescence species represented by blue, green, and red respectively. Figure 2 shows the estimated resolved pure-component spectra, and Figure 3 shows the time-dependent concentration profiles for a single selected super-pixel generated from the data from all 18 time-resolved hyperspectral images.

Reaction model: (4)

$$YFP \xrightarrow{k_1} YFP_{bleached}$$

$$GFP \xrightarrow{k_2} GFP_{bleached}$$

$$Autofluor \xrightarrow{k_3, k_4} Autofluor_{bleached}$$

Plus offsets for *YFP*, *GFP*, *Autofluor* (non-reactive forms)

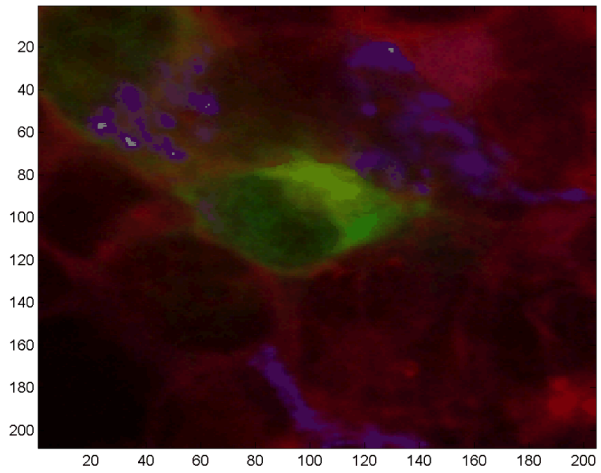

Figure 1 – RGB image showing the estimated concentration map at t=0 of YFP, GFP and auto-fluorescence species represented by blue, green, and red, respectively

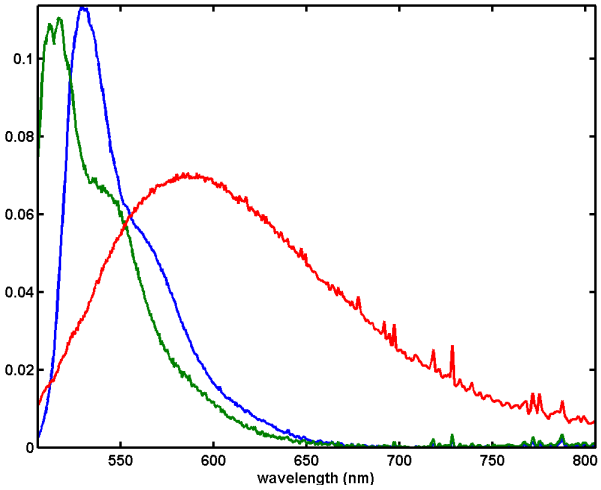

Figure 2 – Estimated resolved pure-component spectra of GFP, YFP and autofluorescence (green, blue and red, respectively).

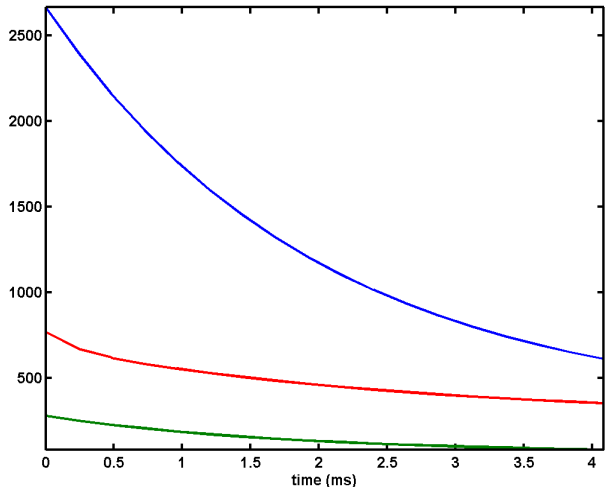

Figure 3 – Time-dependent concentration profiles for a selected super-pixel in Figure 1. Modeled photobleach decay curves correspond to GFP (green), YFP (blue), and autofluorescence (red).

5 Conclusion

We find that the SLS method offers significant improvements in computational speed and robustness compared to the DNL method. Due to complexities in cell images and the presence of Poisson noise, successful implementation requires S/N based thresholding, pixel selection, fitting of multiple exponential decays for same fluorescent species, and automatic fitting of temporal baseline offsets.

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