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A fluorescence lifetime estimation method for incomplete decay

Hongqi Yu and David Day-Uei Li

A new incomplete decay signal model is proposed to describe the incomplete decay effects in a time-correlated single-photon counting (TCSPC) based fluorescence lifetime imaging (FLIM) system. Based on this model, we modified a Multiple Signal Classification (MUSIC) algorithm to eliminate the incomplete decay effects. Monte Carlo simulations were carried out to demonstrate the performances of the proposed approach. Simulations show that the proposed method is insensitive to the laser pulse rate and has a larger lifetime dynamic range compared with previously reported approaches. As far as we know, this new method is the first non-fitting method that can correct incomplete decay effects for multi-exponential decays.

Introduction: Fluorescence lifetime imaging microscopy (FLIM) is a powerful tool that has been widely used in material sciences, biology, chemical analysis, diagnosis, etc. The fluorescence lifetime is the average time that the excited molecule stays at the excited state before dropping back to the ground state. It is sensitive to the microenvironment, but independent of the illumination intensity and probe concentration. Therefore it can be a robust indicator to probe physiological parameters such as pH, O2, Ca2+, viscosity, refractive index, glucose, etc. [1, 2].

When the detector in a time-correlated single-photon counting (TCSPC) FLIM system captures a photon emitted from fluorophores, the wrong lifetime estimations [4]. The subsequent pulses and distort the fluorescence histogram leading to laser pulses, the incomplete decay caused by a pulse will superimpose to lifetime [4]. When a fluorescence lifetime is comparable to the period of fluorescence histogram is generated for extracting lifetimes. Usually the TCSPC module measures the time delay between the excited laser pulse exponential fitting. The incomplete decay model was first proposed by Barber et al. [6, 7], but no detailed information on how to methods. Most researchers have been using commercial software to not involve

Theory: According to the signal models proposed previously [5, 9, 10], the fluorescence intensity including incomplete decay effects is

\[ I(t) = \lim_{m \to \infty} \left( l_0(t) + I_0(t + T) + \cdots + I_0(t + D \cdot T) \right). \]

where \( T \) is the period of laser pulses and \( D \) is the number of the previous tails added to the intensity. Consider \( D \to \infty \), then

\[ I(t) = k \left( e^{-t/\tau_1} f_{\tau_1} + e^{-t/\tau_2} f_{\tau_2} + \cdots + e^{-t/\tau_p} f_{\tau_p} \right). \]

The photon count in the \( m \)-th bin in the histogram is

\[ y(m) = \int_{(m-1)h}^{mh} I(t) \, dt = k \cdot \sum_{i=1}^{p} \left( f_{\tau_i} \cdot e^{-t/\tau_i} \right) \cdot (m-1)h, \]

where \( h \) is the bin width, \( m = 1, 2, \cdots, M \) and \( M \) is the number of time bins in the histogram. To decrease the computation burden and data transport threshold, we can rearrange the histogram to have a smaller number of bins [8]. We can arrange \( y(m) \) as follows

\[ y(1) = Q_1, y(2) = Q_2, \cdots, y(p) = Q_p, \]

\[ y(M) = Q_1 \cdot e^{h/\tau_1} + Q_2 \cdot e^{h/\tau_2} + \cdots + Q_p \cdot e^{h/\tau_p}, \]

Therefore it can be a robust indicator to probe physiological parameters such as pH, O2, Ca2+, viscosity, refractive index, glucose, etc. [1, 2].

In this article, a new incomplete decay signal model is proposed based on the estimated \( \tau_1 \) and \( f_{\tau_1} \). It is shown in Ref. [6]. The corresponding eigenvalues in the descending order and the corresponding eigenvectors \( u_m \) \( (m = 1, 2, \cdots, M) \) are the solutions of \( R_y \cdot u = \lambda_s \cdot u \). If \( R_y \) is a full rank matrix, then each column of \( A \) is orthogonal to the matrix \( U_u = [u_{p+1} \cdot u_{p+2} \cdots \cdot u_M] \). The proof can be found in Ref [11]. Based on this theorem, once we obtain the noise subspace \( U_n \), we define

\[ F_{\text{search}}(\tau) = 1/\|U_n^H \cdot A(\tau)\|^2, \]

where \( A(\tau) = e^{h/\tau_1} \cdot e^{h/\tau_2} \cdots e^{h/(M-1)h}\). The P largest peaks found in \( F_{\text{search}}(\tau) \) are corresponding to \( \tau_i \), \( (i = 1, 2, \cdots, p) \).

Single-run Simulations: To demonstrate the proposed method, the spectra according to Eq.(7) at different laser pulse rates (LPR) are plotted in Fig. 1(a). The Poisson noise is included to the synthesized data. In the simulations, \( \tau_1 = 2ns, \tau_2 = 5ns, f_{\tau_1} = f_{\tau_2} = 0.5, \) and \( M = 1024 \). The photons number at the peak is 2000. The equivalent signal to noise ratio (SNR) is 30.9dB. The bias for each curve is due to the Poisson noise.

To estimate the average fluorescence lifetime, the same simulations are carried out with \( P \) being set to be 1. The spectra for average fluorescence lifetime at different LPRs are shown in Fig. 1(b).

![Fig.1 Spectra of the proposed method](image)
The proposed algorithm can estimate all lifetime components. We defined the estimation error of the average lifetime similar to Ref [10] as \( \varepsilon = \frac{\text{true value} - \text{estimated value}}{\text{true value}} \times 100\% \). The results are plotted in Fig. 2(a). Here, \( \tau_1 = 10\text{ns}, 2\text{ns} \leq \tau_2 \leq 20\text{ns}, 0.1 \leq f_{\text{LO}} \leq 0.9, \) and LPR = 80MHz. Other simulation parameters are the same as those in Fig. 1. Fig. 2(a) shows that the estimation error is significant when \( \tau_2 \leq 3\text{ns} \) or \( \tau_2 \geq 16\text{ns} \) (\( f_{\text{LO}} < 0.5 \)). At other circumstances, the estimation error is negligible (\( \varepsilon < 5\% \)). Overall, our method is nearly independent of \( f_{\text{LO}} \) as long as \( 3\text{ns} < \tau_2 < 16\text{ns} \). On the other hand, the existing correction method [10] has the fractional error depending on \( f_{\text{LO}} \) and has a smaller dynamic range (6ns \( \leq \tau_2 < 14\text{ns} \) for \( \varepsilon < 5\% \). Not only can the proposed method provide a better range for the average lifetime, it can correctly estimate all lifetime components.

To demonstrate how the LPR affects the proposed algorithm, Monte Carlo simulations were carried out. The results are shown in Fig. 2(b). \( \tau_1 = 10\text{ns}, 2\text{ns} \leq \tau_2 \leq 20\text{ns}, \) and \( f_{\text{LO}} = 0.5 \). The LPRs = 20MHz, 40MHz, 60MHz and 80MHz, respectively. Other simulation parameters are kept the same as those in Fig. 1. Simulations show that the proposed method is nearly independent of LPRs when \( 5\text{ns} < \tau_2 < 20\text{ns} \).

Fig. 2 Performances of the proposed method
a Estimation error (%) of the \( \tau_{\text{avg}} \) for bi-exponential decays
b Estimation error (%) of the \( \tau_{\text{avg}} \) for different LPRs

Finally, Monte Carlo simulations were carried out to demonstrate how the proposed algorithm can estimate all lifetime components. \( \tau_1 = 3\text{ns} \) and \( \tau_2 = 6\text{ns} \). Other simulation parameters are the same as those in Fig. 3. Fig. 3(a) shows that the proposed method has the ability to accurately resolve bi-exponential decays when the LPR is between 10MHz to 60MHz. The performance will deteriorate when the LPR is lower than 10MHz or higher than 60MHz. The reason is that at these circumstances, the algorithm needs a higher SNR to obtain accurate estimations. Fig. 3(b) shows similar simulations but without shot noise. It shows that the performance is almost the same for all the LPRs, indicating that the proposed algorithm can accurately estimate individual lifetime if the photon count is large enough.

Fig. 3 The estimation error (%) of \( \tau_1 \) and \( \tau_2 \)
a with shot noise
b without shot noise

Conclusion: In this paper, we proposed a new incomplete decay model. Based on this model, a modified MUSIC algorithm for fluorescence lifetime estimations was presented for resolving fluorescence decays with incomplete decay effects. Compared with the previously reported methods that are only able to resolve the average lifetime, \( \tau_{\text{avg}} \), the proposed method can correctly estimate every fluorescence lifetime component, \( \tau_j, j = 1, \ldots, P \). Simulations also indicate that the proposed method is independent of LPRs and has a larger dynamic range than the previously reported method.

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Hongqi Yu (School of Electronic Science and Engineering, National University of Defense Technology, Changsha, People’s Republic of China)
E-mail: 13755132901@163.com

David Day-Uei Li (Centre for Biophotonics, University of Strathclyde, Glasgow, United Kingdom)

References