A fluorescence lifetime estimation method for incomplete decay

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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 resolve 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, O_2, Ca^{2+}, 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 TCSPC module measures the time delay between the excited laser pulse and the detected photon [3]. This procedure is repeated and a fluorescence histogram is generated for extracting lifetimes. Usually the period of laser pulses is set to be at least four to five times of the average lifetime [4]. When a fluorescence lifetime is comparable to the period of laser pulses, the incomplete decay caused by a pulse will superimpose to the subsequent pulses and distort the fluorescence histogram leading to wrong lifetime estimations [4].

As concluded in Ref. [4], incomplete decay effects only affect multi-exponential fitting. The incomplete decay model was first proposed by Barber *et al.* in 2005 [5]. They discussed how incomplete decay effects corrupted the global fitting results, but did not provide correction methods. Most researchers have been using commercial software to analyse incomplete decays [6, 7], but no detailed information on how to realize this method has been released. This may be due to lack of efficient correction methods.

Here we assume that there are P exponential decays, and the fluorescence intensity can be expressed as

$$I_0(t) = K \cdot \sum_{j=1}^{P} \left(f_{Dj} e^{-t/\tau_j} \right), \qquad (1)$$

where $f_{D1} + f_{D2} + \cdots f_{DP} = 1$ with f_{Dj} being the amplitudes and τ_j the corresponding fluorescence lifetimes, $j = 1, \cdots, P$.

Leung *et al.* proposed a correction method based on the estimated τ_j and f_{Dj}, $(j = 1, 2)$ using a fitting method [4] for the amplitude weighted fluorescence lifetime, $\tau_{AVE} \equiv \sum_{j=1}^{P} f_{Dj}\tau_j$. However, the incomplete decay also affects the estimations of f_{Dj} making it unable to completely correct the incomplete decay effects. Moreover, it is only applicable to bi-exponential decays and can only correct τ_{AVE}, not $\tau_j, j = 1, \cdots, P$.

In this article, a new incomplete decay signal model is proposed based on the model we previously proposed [8]. The proposed method 1) does not involve f_{Dj}, 2) is a non-fitting method that suitable for embedded system realizations for real-time applications, 3) is suitable for multi-exponential decays to estimate the amplitude weighted fluorescence lifetime and 4) is able to correctly estimate each lifetime $\tau_j, j = 1, \cdots, P$.

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

$$I(t) = \lim_{n \to \infty} \left(I_0(t) + I_0(t + T) + \cdots I_0(t + D \cdot T) \right). \qquad (2)$$

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

$$I(t) = K \left(\frac{e^{-t/\tau_1}}{1 - e^{-t_0/\tau_1}} f_{D1} + \frac{e^{-t/\tau_2}}{1 - e^{-t_0/\tau_2}} f_{D2} + \cdots + \frac{e^{-t/\tau_P}}{1 - e^{-t_0/\tau_P}} f_{DP} \right). \quad (3)$$

The photon count in the *m-th* bin in the histogram is

$$y(m) = \int_{(m-1)h}^{mh} I(t)dt = K \cdot \sum_{j=1}^{P} \left(\tau_j f_{Dj} \frac{e^{h/\tau_j} - 1}{1 - e^{-T/\tau_j}} e^{-mh/\tau_j} \right),$$

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

$$
\begin{bmatrix} y(1) \\ y(2) \\ \vdots \\ y(M) \end{bmatrix} =
\begin{bmatrix}
Q_1 & Q_2 & \cdots & Q_P \\
Q_1 e^{h/\tau_1} & Q_2 e^{h/\tau_2} & \cdots & Q_P e^{h/\tau_P} \\
\vdots & \vdots & \ddots & \vdots \\
Q_1 e^{Mh/\tau_1} & Q_2 e^{Mh/\tau_2} & \cdots & Q_P e^{Mh/\tau_P}
\end{bmatrix}
\begin{bmatrix} K \cdot f_{D1} \\ K \cdot f_{D2} \\ \vdots \\ K \cdot f_{DP} \end{bmatrix} +
\begin{bmatrix} n(1) \\ n(2) \\ \vdots \\ n(M) \end{bmatrix},
$$
(4)

where $Q_j = \frac{e^{h/\tau_j} - 1}{1 - e^{-T/\tau_j}} \tau_j$ and $n(m)$ is additive shot noise. We can simplify this equation as

$$Y = AS + N. \qquad (5)$$

And the covariance matrix of (5) is

$$R_Y = E[YY^H] = AR_s A^H + \Sigma, \qquad (6)$$

where $R_S = E[SS^H]$ and $(\cdot)^H$ represents the Hermitian transpose.

Compare Eq. (6) with the signal model we previously proposed [8], the MUSIC algorithm can be also applied here to estimating lifetimes. To use MUSIC, we need to apply a theorem: Let the eigenvalues λ_m (listed in the descending order) and the corresponding eigenvectors u_m ($m = 1, 2, \cdots, M$) be the solutions of $R_S u = \lambda \Sigma_0 u$. If R_S is a full rank matrix, then each column of A is orthogonal to the matrix $U_n = [u_{P+1} \quad u_{P+1} \quad \cdots \quad u_M]$. The proof can be found in Ref [11]. Based on this theorem, once we obtain the noise subspace U_n, we define

$$F_{search}(\tau) = 1/\|U_n^H A(\tau)\|^2, \qquad (7)$$

where $A(\tau) = \frac{e^{h/\tau} - 1}{1 - e^{-T/\tau}} \tau \cdot [1 \quad e^{h/\tau} \quad \cdots \quad e^{(M-1)h/\tau}]$.

The P largest peaks found in $F_{search}(\tau)$ are corresponding to τ_j, $(j = 1, 2, \cdots, P)$.

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, $\tau_1 = 2$ns, $\tau_2 = 5$ns, $f_{D1} = f_{D2} = 0.5$, 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).
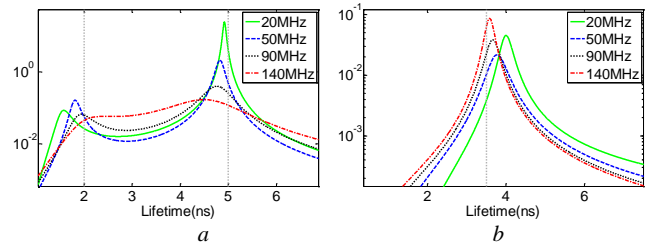
Fig.1 *Spectra of the proposed method*
a Spectra for two estimated fluorescence lifetimes, τ_1 and τ_2
b Spectra for the average fluorescence lifetime, τ_{AVE}

Monte Carlo Simulations: Based on the single-run simulations, Monte Carlo simulations were carried out to demonstrate the statistical

performance of the proposed algorithm. We defined the estimation error of the average lifetime similar to Ref [10] as $E \equiv \frac{\tau_{AVE\_EST} - \tau_{AVE}}{\tau_{AVE}}$.

The results are plotted in Fig. 2(a). Here, $\tau_1 = 10$ns, $2\text{ns} \leq \tau_2 \leq 20$ns, $0.1 \leq f_{D1} \leq 0.9$, 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 $\tau_2 \leq 3$ns or $\tau_2 \geq 16$ns ($f_{D1} < 0.5$). At other circumstances, the estimation error is negligible ($E < 5\%$). Overall, our method is nearly independent of f_{D1} as long as $3\text{ns} < \tau_2 < 16$ns. On the other hand, the existing correction method [10] has the fractional error depending on f_{D1} and has a smaller dynamic range ($6\text{ns} < \tau_2 < 14$ns) for $E < 5\%$. 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). $\tau_1 = 10$ns, $2\text{ns} \leq \tau_2 \leq 20$ns, and $f_{D1} = 0.5$. 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 $5\text{ns} < \tau_2 < 20$ns.
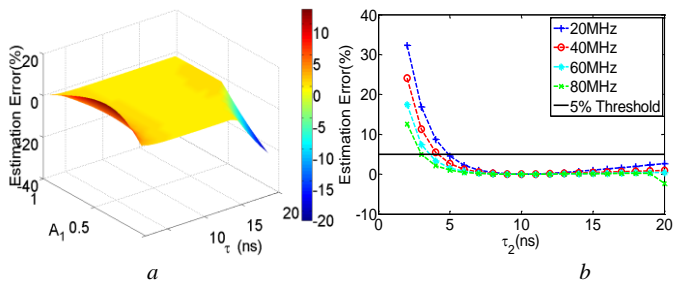
Fig.2 *Performances of the proposed method*
a Estimation error (%) of the τ_{AVE} for bi-exponential decays
b Estimation error (%) of the τ_{AVE} for different LPRs

Finally, Monte Carlo simulations were carried out to demonstrate how the proposed algorithm can estimate all lifetime components. $\tau_1 = 3$ns and $\tau_2 = 6$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.
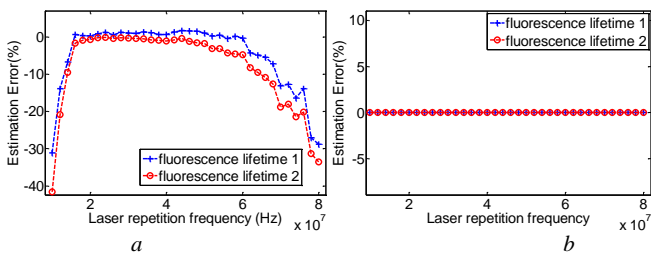
Fig.3 *The estimation error (%) of τ_1 and τ_2*
a with shot noise
b without shot noise

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, τ_{AVE}, the proposed method can correctly estimate every fluorescence lifetime component, $\tau_j, j = 1, \cdots, P$. 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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