Estimating fluorescence lifetimes using the extended Kalman filter

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The extended Kalman filter (EKF) has been widely used in communication, signal processing and navigation control. In this paper, we applied EKF, for the first time to our knowledge, to simultaneously estimate fluorescence lifetimes and instrument response functions (IRF) for time-domain fluorescence lifetime imaging microscopy (FLIM) systems (we focus on gating and time-correlated single-photon counting techniques in this work). Monte Carlo simulations were performed to test its performances in comparison with previously reported methods. Simulation results show that the proposed algorithm can achieve comparable or better results than the others. More importantly, with EKF there is no need to measure the IRF of the FLIM system.

Introduction: Fluorescence lifetime imaging microscopy (FLIM) has been showing great potential in biomedical imaging, material sciences and chemical analysis. It can be used to reveal protein-protein interaction networks in living cells [1].

Time-correlated single-photon counting (TCSPC) has been the gold standard technique (for its excellent timing performance) widely used for FLIM applications [2, 3]. In this paper we will focus on TCSPC FLIM systems, where the measured fluorescence response is the convolution of the fluorescence emission density and the instrument response function (IRF). The IRF is a mapping function depending on the light pulse, detection mechanisms, front-end electronics and processing units [4].

Most FLIM systems either use tail-fitting techniques (ignoring the IRF) or need to measure the IRF before FLIM experiments are conducted [5, 6]. However, the former approaches produce biased estimations, whereas the latter requires extra efforts for measuring the IRF and solving deconvolution problems [7, 8].

The extended Kalman filter (EKF) is the nonlinear version of the Kalman filter [9]. The EKF has proven to be a useful method for obtaining good estimates of parameters in communication, ranging and navigation systems [9, 10]. Godavarty *et al.* used EKF to analyze the image data obtained from a three-dimensional fluorescence lifetime tomography system [11], but they did not consider the IRF in their work.

In this paper, we proposed a new EKF based FLIM algorithm, capable of estimating the lifetime and IRF simultaneously.

Theory: We assume that the fluorescence density function is single-exponential $g(t) = Ae^{-t/\tau}$. The measured fluorescence decay $y(t)$ is the convolution of $g(t)$ and the IRF, $IRF(t)$, in a TCSPC-FLIM experiment:

$$y(t) = g(t) * IRF(t) + v(t), \quad 0 \leq t \leq T, \quad (1)$$

where $v(t)$ is the additive noise following a Poisson distribution [1].

Assume that $IRF(t) = 0$, $t \notin [0, Lh]$ (h is the resolution of the TCSPC), and the measurement window T (note that $Lh < T$) is divided to M bins. Eq. (1) can be discretized as

$$y_k = g_k * IRF_k + v_k = Ae^{-\frac{kh}{\tau}} * IRF_k + v_k = \sum_{i=k-(L-1)h}^k Ae^{-\frac{ih}{\tau}} IRF_{k-i} + v_k, \quad (2)$$

where $IRF_k = IRF(kh)$, $k = 0, \dots, L-1$, v_k is the noise in the k -th time bin, and IRF_k and y_k are shown in Fig. 1(a).

Fig. 1 (a) Histograms of IRF_k and y_k and (b) EKF processing flow.

In this paper, we use EKF to estimate τ and IRF_k , $k = 0, \dots, L-1$. The state transition and observation models can be established first following

the EKF theory [9, 10]. The estimated results can be updated using each extra measured information, y_k , $k = 0, \dots, M-1$, showed in Fig. 1(b). We define that $\tau[k]$ and $IRF_0[k], \dots, IRF_{L-1}[k]$, $k \geq L$ are the lifetime and IRF parameters to be estimated when y_k is obtained, and according to the EKF theory the nonlinear state transition and observation models can be defined as [9]

$$s[k] = f(s[k-1]) + u[k], \quad (3)$$

$$y[k] = b(s[k]) + v[k], \quad (4)$$

where $s[k] = \{\tau[k], IRF_0[k], \dots, IRF_{L-1}[k]\}^T$ is the state vector corresponding to y_k , $u[k] = \{u_\tau[k], u_0[k], \dots, u_{L-1}[k]\}^T$ is the noise that is assumed to be zero-mean multivariate Gaussian noise with a covariance matrix Q , and $v[k]$ is the observation noise which is assumed to be zero-mean noise with a variance C . The function $f(\bullet)$ can be used to compute the predicted state at the k -th time bin from previous estimate, and similarly the function $b(\bullet)$ can be used to compute the predicted measurement from the predicted state.

We assume that

$$\tau[k] = \tau[k-1] + u_\tau[k],$$

$$IRF_{L-1}[k] = \alpha * IRF_{L-1}[k-1] + u_{L-1}[k],$$

where $\alpha \leq 1$ is the fading factor of IRF_{L-1} . And using $y_1, y_2, \dots, y_{L-1}$ obtained from Eq. (2), the relationships between IRF_i , IRF_{i+1} , and IRF_{i-L} , $i = 0, \dots, L-1$ can be derived. And $f(\bullet)$ can be represented as

$$f(s[k-1]) = \begin{bmatrix} \tau[k-1] \\ (y_1 - IRF_1[k-1])e^{\frac{h}{\tau[k-1]}} \\ (y_{i+1} - IRF_{i+1}[k-1])e^{\frac{h}{\tau[k-1]}} - IRF_{i-1}[k-1]e^{\frac{h}{\tau[k-1]}} \Big|_{i=1, \dots, L-1} \\ \alpha IRF_{L-1}[k-1] \end{bmatrix}. \quad (5)$$

From Eq. (2) and Eq. (4), $b(\bullet)$ can be represented as

$$b(s[k]) = Ae^{-\frac{kh}{\tau[k]}} * IRF[k] = A \sum_{i=k-(L-1)h}^k e^{-\frac{ih}{\tau[k]}} IRF_{k-i}, \quad (6)$$

According to the EKF theory [9], the predicted state $\hat{s}[k|k-1]$, i.e. the predicted k -th state based on the $(k-1)$ -th state, the predicted covariance $\Omega[k|k-1]$, the Kalman gain $G[k]$, the updated state $\hat{s}[k|k]$ (the updated k -th state based on the k -th measurement) and the updated covariance $\Omega[k|k]$ can be derived respectively as

$$\hat{s}[k|k-1] = a(\hat{s}[k-1|k-1]),$$

$$\Omega[k|k-1] = F[k-1]\Omega[k-1|k-1]F^T[k-1] + Q,$$

$$G[k] = \Omega[k|k-1]B^T[k](C + B[k]\Omega[k|k-1]B^T[k])^{-1}, \quad (7)$$

$$\hat{s}[k|k] = \hat{s}[k|k-1] + G[k](y[k] - b(\hat{s}[k|k-1])),$$

$$\Omega[k|k] = (I - G[k]B[k])\Omega[k|k-1],$$

where

$$F[k-1] = \frac{\partial f}{\partial s[k-1]} \Big|_{s[k-1] = \hat{s}[k-1|k-1]} = \begin{bmatrix} 1 & 0 & 0 & \dots \\ 0 & 0 & -e^{\frac{h}{\tau[k-1]}} & \dots \\ 0 & 0 & \ddots & \ddots \\ \vdots & \vdots & \ddots & \ddots \\ 0 & 0 & \dots & \alpha \end{bmatrix},$$

$$B[k] = \frac{\partial b}{\partial s[k]} \Big|_{s[k] = \hat{s}[k|k-1]} = A \left[\sum_m \frac{mh IRF_{k-m}[k]}{\tau[k]^2} e^{-\frac{mh}{\tau[k]}}, e^{-\frac{kh}{\tau[k]}}, \dots, e^{-\frac{(k-L+1)h}{\tau[k]}} \right]^T \Big|_{s[k] = \hat{s}[k|k-1]}.$$

In order to estimate τ , the initial values of $\hat{s}[0|0]$, Q and C must be assigned. In general, a bigger Q will provide faster convergence but less precision, but the estimation results are not sensitive to the initial values. In this paper the data is normalized before applying EKF. The initial trial

function for the IRF is $\delta(t)$, and the other initial values are $\hat{s}[0|0] = [T/2, 1, 0, \dots, 0]$, $Q = [1, 0, \dots, 0, 0.1]$, and $C = 1$.

Monte Carlo Simulations: Monte Carlo simulations were performed to investigate the statistical performances of the proposed EKF algorithm.

The estimated IRFs are plotted in Fig. 2. Here, $L = 61$ and $\alpha = 0.05$ (it is an optimal value obtained from simulations). The number of photons, denoted as N_c hereafter, is 1024, $T = 10$ ns, and $M = 512$. We chose different IRFs using Poisson density functions with different full width at half maximum (FWHM, denoted as Θ hereafter). Fig. 1(a) shows the estimated IRFs (IRF_E) and the original IRF (IRF_O) with different τ ($\tau = 0.5$ ns and 4ns) and different Θ ($\Theta_1 = 70$ ps, $\Theta_2 = 250$ ps, $\Theta_3 = 400$ ps). It is clear that all the estimated IRFs are close to the original IRFs, meaning that the proposed algorithm is robust to extract the IRF profiles.

Fig. 2 Comparison of the original and estimated IRFs with (a) $\tau = 0.5$ ns and (b) $\tau = 4$ ns.

Similar to Ref [12], we define the normalized bias and normalized precision of the lifetime estimations as $E = \Delta\tau/\tau = |\tau_{EST} - \tau|/\tau$ and $F = N_c^{0.5}\sigma_\tau/\tau$. Fig. 3 shows the converge performance of the proposed algorithm. Here, $L = 31$, and the other simulation parameters are the same as those in Fig. 2. Fig. 3(a) shows E curves, and Fig. 3(b) shows F curves. From Fig. 3, E converges to zero after $k > 200$, and F converges after $k > 300$. Figure 4 shows how Θ affects the proposed algorithm. Here, $M = 15$ for IEM, $M = 1024$ for CMM, and the other simulation parameters are the same as above.

Fig. 3 Converge performances of (a) E and (b) F .

Fig. 4 E and (b) F plots as a function of Θ/τ .

Fig. 5 (a) E and (b) F plots as a function of τ for different methods.

To investigate the dynamic range of the proposed algorithm, Monte Carlo simulations were performed. The results are showed in Fig. 5. Here, Θ is 300ps, and the other parameters are the same as those in Fig. 4. From Fig. 4 and Fig. 5, IEM performs the worst among all, showing that it is

sensitive to the IRF. Both IEM and CMM produce significant biases when Θ/τ increases showing the need to calibrate the IRF, whereas EKF produces negligible biases and its photon efficiency is reasonably good considering its capability to estimate the IRF at the same time.

Conclusion: In this paper, we proposed a new lifetime estimation algorithm based on EKF. Compared with the previously reported methods that need to measure the IRF beforehand, the proposed method are able estimate the lifetime and IRF simultaneously without the prior information about IRF. Simulations also indicate that the proposed method is insensitive to the measurement window and has a large dynamic range.

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