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Article

Quantitative deconvolution of autocorrelations and cross correlations from two-dimensional lifetime decay maps in fluorescence lifetime correlation spectroscopy

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ABSTRACT

Fluorescence correlation spectroscopy (FCS) is a widely used method for measuring molecular diffusion and chemical kinetics. However, when a mixture of fluorescent species is taken into account, the conventional FCS method has limitations in extracting autocorrelations for different species and cross correlations between different species. Recently developed fluorescence lifetime correlation spectroscopy (FLCS) based on time-tagged time-resolved (TTTR) photon recording, which can record the global and micro arrival time for each individual photon, has been used to discriminate different species according to fluorescence lifetime. Here, based on two-dimensional lifetime decay maps constructed from TTTR photon stream, we have developed a quantitative lifetime-deconvolution FCS model (LDFCS) to extract precise chemical rates for chemical conversions in multi-species systems. The key point of LDFCS model is separation of different species according to the global distribution of fluorescence lifetime and then deconvolution of autocorrelations and cross-correlations from the two-dimensional lifetime decay maps constructed by the micro arrival times of photon pairs at each delay time.

1. Introduction

Dynamic processes of molecules (such as diffusion and chemical reactions) can instantaneously change the numbers, structure and/or environment of molecules. If the molecule is fluorescent or labeled by a fluorescent probe, the dynamic processes bring fluctuations in fluorescence properties, such as intensity [1–4], lifetime [5–9], quantum yield [1] and/or emission wavelength [10,11]. Fluorescence correlation spectroscopy (FCS) is a method developed to study molecular diffusion and chemical kinetics based on these fluctuations (Fig. 1a) [12–18]. Most studies are on fluctuation analysis of fluorescence intensity and only several works cover fluctuation analysis of lifetime [19,20] and other fluorescence parameters. The key to obtain chemical rates is to extract cross correlations between different species, which is challenging. Based on dynamic processes accompanied changes of fluorescence property, it is possible to sort photons from different species (such as lifetime, emission wavelength and so on). Dual-color FCS is a method to sort photons for different species based on the emission energy [12,13,16], while fluorescence lifetime correlation spectroscopy (FLCS) is a method to sort photons based on fluorescence lifetime [21,22].

FLCS utilizes time-tagged time-resolved (TTTR) photon recording which can record a global time (or a macro arrive time) and a micro arrive time (relative to the corresponding excitation laser pulse and related to fluorescence lifetime) for each individual photon (Fig. 1b) [23]. Several different FLCS models have been developed to extract autocorrelations for different species and cross correlations between different species. For example, Kapusta et al. [24] have developed lifetime-filtered FCS, in which autocorrelations and cross correlations are calculated by weighting each photon according to the micro arrival time. Lifetime-filtered FCS requires prior knowledge of exact fluorescence decay curves for every species to calculate the weighting factors. Tahara and coworkers [25–27] have developed two-dimensional (2D) FLCS by global fitting of two-dimensional lifetime decay maps constructed from the micro arrival times of photon pairs [25–27]. 2D FLCS can get timescale of chemical conversions between species. In this

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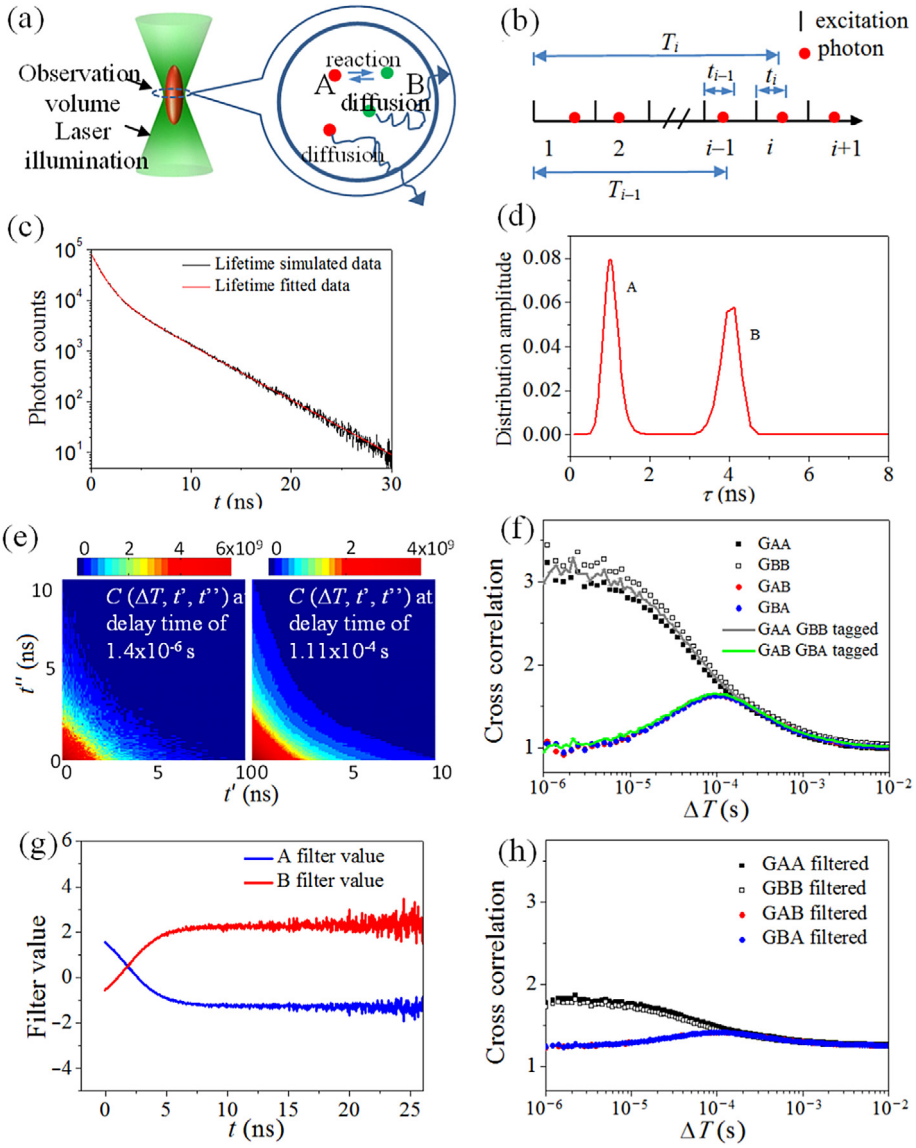

Fig. 1. (a) Illustration of molecule diffusion and chemical conversions in a confocal volume; (b) illustration of TTTR photon recording, in which both macro arrival time T_i and micro arrival time t_i are recorded. Simulation results of a two-species system with $\tau_A = 1$ ns, $\tau_B = 4$ ns, $k_{AB} = k_{BA} = 1 \times 10^4$ s^{-1}, $D_A = D_B = 1 \times 10^{-6}$ cm^2/s, $C_A = C_B = 1$ nmol/L, total collected photon number of $\sim 3.9 \times 10^6$; (c) lifetime decay histogram; (d) fitted lifetime distribution; (e) 2D decay maps, i.e., $C(\Delta T, t_m, t_n)$ at two different delay times ($\Delta T = (1.25–1.55) \times 10^{-6}$ s and $(1.04–1.20) \times 10^{-4}$ s); (f) LDFCS-deconvoluted autocorrelations and cross correlations; (g) filter value and (h) autocorrelations and cross correlations obtained by lifetime-filtered FCS.

work, we have developed a lifetime-deconvolution FCS (LDFCS) model based on the same two-dimensional lifetime decay maps as in 2D FLCS. In this LDFCS model, autocorrelations and cross correlations have been quantitatively deconvoluted and accurate chemical rates have been precisely fitted.

2. Principle of the model

FCS method records temporal fluctuations of fluorescence intensity $I(T)$ as a function of time T in the observation volume. The fluorescence intensity correlation, $C(\Delta T)$, can be calculated by [1]

$$C(\Delta T) = \langle I(T)I(T+\Delta T)\rangle = \sum_{ij}\langle I_i(T)I_j(T+\Delta T)\rangle \sum_{ij} C_{ij}(\Delta T), \quad (1)$$

where ΔT is the delay time and the angle brackets denote the time average, i, j denote different species, $C_{ij}(\Delta T)$ is the correlation between species i and j. Assuming that a species, for example species i, has a lifetime distribution of $\{\alpha_{p,i}\}$ at fluorescence lifetime space $\{\tau_p\}$, then the fluorescence decay of the species can be written as [28–30]

$$I_i(t) = I_{i,0}\sum_p a_{p,i}\exp(-t/\tau_p), \quad (2)$$

where $\sum_i\sum_p \int_0^\infty a_{p,i}\exp(-t/\tau_p)\mathrm{d}t = \sum_i\sum_p a_{p,i}\tau_p = 1$, $I_{i,0}$ is the integrated fluorescence intensity for species i.

And fluorescence intensity at global time T and micro arrival time t of species i can be written as

$$I_i(T,t) = I_{0,i}(T)\frac{\sum_p a_{p,i}\exp(-t/\tau_p)}{\sum_p\int_{t=0}^{t=\infty} a_{p,i}\exp(-t/\tau_p)\mathrm{d}t} = I_{0,i}(T)\sum_p a_{p,i}\exp(-t/\tau_p). \quad (3)$$

Then the correlation $C(\Delta T)$ can be expanded into two dimensions according to the micro arrival time of the photon pairs, i.e.,