Journal of Luminescence 132 (2012) 2059–2064

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Mean fluorescence lifetime and its error

Eva Fišerová [a,1], Martin Kubala [b,*,1]

[a] Department of Mathematical Analysis and Applications of Mathematics, Faculty of Science, Palacky University in Olomouc, tr. 17. listopadu 12, CZE-77146 Olomouc, Czech Republic

[b] Department of Biophysics, Faculty of Science, Palacky University in Olomouc, tr. 17. listopadu 12, CZE-77146 Olomouc, Czech Republic

ARTICLE INFO

Article history:
Received 24 November 2011
Received in revised form
2 March 2012
Accepted 8 March 2012
Available online 18 March 2012

Keywords:
Fluorescence
Mean lifetime
Error
Confidence interval
TCSPC
Histogram

ABSTRACT

Mean excited-state lifetime is one of the fundamental fluorescence characteristics and enters as an important parameter into numerous calculations characterizing molecular interactions, such as e.g. FRET or fluorescence quenching. Our experiments demonstrated that the intensity-weighted mean fluorescence lifetime is very robust characteristic, in contrast to the amplitude-weighted one, which value is dependent on the data quality and particularly on the used fitting model. For the first time, we also report the procedure for the error estimation for both the intensity- and amplitude-weighted mean fluorescence lifetimes. Furthermore, we present a method for estimation of the mean fluorescence lifetime directly from the fluorescence-decay curve recorded by TCSPC (Time-Correlated Single-Photon Counting) method. For its simplicity and low computational demands, it could be a useful tool in the high-throughput applications, such as FACS, FLIM-FRET or HPLC detectors.

1. Introduction

Due to the extreme sensitivity, non-invasivity and availability of wide range of techniques, fluorescence spectroscopy became a very useful tool for monitoring of molecular features and interactions in modern biology. However, the fluorescence intensity, which is the easiest-to-get characteristic, is also the least reproducible one, because its value is dependent on the instrument setup (and consequently on the instrument stability) or precise knowledge of the fluorophore concentration. The latter becomes important in applications, which are based on the comparison of the fluorescence from two samples, e.g. FRET (Förster Resonance Energy Transfer), where one compares the fluorescence of a donor in the absence or the presence of an acceptor. Unfortunately, in many samples the information about the concentration is estimated with an error, which largely exceeds an error of spectroscopic measurement, in some cases it is even unavailable (e.g. in microscopy). For these reasons, fluorescence characteristics that are independent on the fluorophore concentration are preferable.

Kinetic of the fluorescence decay is independent on the fluorophore concentration, and its measurement has been implemented also in fluorescence microscopes (FLIM—Fluorescence Lifetime IMaging, or FLIM-FRET—Fluorescence Lifetime IMaging used for monitoring of Förster Resonance Energy Transfer) [1], flow-cytometers [2] or chromatography detectors [3]. Although it is not straightforward to interpret fluorescence kinetic parameters they are used in more complex calculations of molecular features. For example, in the dynamic fluorescence quenching the fluorescence lifetime delimits the time-window, within which the collisions between the fluorophore and quencher molecules cause effective fluorescence quenching. Hence, the parameter, which can be used for calculation of diffusion coefficients [4] or fluorophore steric accessibility [5] is given by

$$k_Q = K_{SV}/\tau_0, \tag{1}$$

where K_{SV} is the well-known Stern–Volmer quenching constant, τ_0 is the fluorescence lifetime in the absence of the quencher and k_Q is denoted as bimolecular quenching constant. Similarly, in FRET experiments the formula

$$E = 1-\tau_{DA}/\tau_D, \tag{2}$$

where τ_{DA} denotes the donor fluorescence lifetime in the presence of acceptor and τ_D the lifetime in its absence, is used for estimation of the energy transfer efficiency (E), which is further used for the calculation of the donor–acceptor distance [6].

In the simplest case, the fluorescence decay of a homogenous population of single fluorophore following the δ-pulse excitation can be described by an exponential function. However, many commonly used organic fluorophores [7], fluorescent proteins [1] or semiconductor quantum dots [8] display more complex kinetic

* Corresponding author. Tel.: +420 585634179; fax: +420 585634002.
E-mail address: mkubala@prfnw.upol.cz (M. Kubala).
[1] Both authors contributed equally to this work.

0022-2313/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jlumin.2012.03.038

and the decay is usually fitted by a sum of exponentials:

$$I(t)=\sum_i A_i e^{-t/\tau_i} \tag{3}$$

In such a case, the mean fluorescence lifetime is inserted into formulae, as given e.g. in Eqs. (1) or (2) and it is calculated either as intensity-weighted

$$\tau_I=\frac{\sum_i A_i\tau_i^2}{\sum_i A_i\tau_i} \tag{4}$$

(because the term $A_i\tau_i$ reflects the contribution of the i-th component to the steady-state fluorescence intensity) or amplitude-weighted one

$$\tau_A=\frac{\sum_i A_i\tau_i}{\sum_i A_i} \tag{5}$$

The intensity-weighting is used in the case of dynamic quenching, while for the FRET applications the amplitude-weighting is recommended [9,10].

As mentioned above, the fluorescence lifetime is frequently used in the complex mathematical formulae for the estimation of important molecular parameters, and therefore accuracy of its estimation becomes an important issue. Numerous methods were proposed for the estimation of the accuracy of individual decay parameters A_i and τ_i, such as asymptotic standard errors (ASEs) [11], support plane analysis (SPA) [12] or Monte Carlo simulations [13]. However, we found no description of the mean fluorescence lifetime error calculation, nor it is involved in the commercially available software for the fluorescence-decay fitting. In this paper, we provide a theoretical description of the error calculation for both the intensity- and amplitude-weighted mean fluorescence lifetime, and furthermore, we analyzed influence of the various experimental parameters, particularly the temporal resolution of the measurement, statistics quality, and the goodness of fit.

2. Materials and methods

2.1. Fluorescence experiments

Fluorescence decays of 20 μM solutions of human serum albumin (HSA) in 10 mM Tris, 100 mM NaCl, pH 7.5, were measured on a TCSPC (Time-Correlated Single-Photon Counting) fluorometer PicoHarp300 (Picoquant, Germany), using pulsed LED centered at 298 nm as the source of excitation light, with repetition frequency 10 MHz. The instrument response function (IRF) was obtained using Ludox solution as a scatterer, estimated FWHM (IRF) was 0.45 ns. Emission was detected under magic-angle conditions at 350 nm through the UG3 filter (Carl Zeiss, Jena), the emission bandpass was 16 nm in all cases. The data were acquired at 295 K (bath controlled). Data were recorded into a histogram on a time-scale 0–100 ns. In one series of experiments, the data were acquired for 600 s, and the time-width of one channel was 4 ps, 8 ps, 16 ps, 32 ps, 64 ps, 128 ps or 256 ps. In another series, the time-width of one channel was kept constant with the value of 32 ps, and the data were acquired until 100 (19,000), 300 (64,000), 600 (132,000), 1000 (221,000), 3000 (672,000), 6000 (1,380,000), 10,000 (2,280,000) or 30,000 (6,980,000) counts in the peak-channel were achieved (the numbers in parenthesis give total numbers of counts in the decay). Fluorescence decays were fitted using the FluoFit 4.2.1 software (PicoQuant) as a sum of exponentials:

$$I(t)=IRF\otimes\sum_i A_i e^{-t/\tau_i} \tag{6}$$

2.2. Bootstrap simulations

Using the Monte-Carlo simulations modifying the noise by random duplication of points in the original data set, 1000 fits were performed. The results were plotted in the table, which enabled estimation of correlation between individual decay parameters.

3. Theory

3.1. Estimation of mean lifetime error after fitting of the fluorescence decay by the multi-exponential model

Mathematically, errors for both the intensity- and amplitude-weighted mean fluorescence lifetimes are characterized by standard error of measurement. We propose two methods for their estimation based on the bootstrap [14]. The first method is purely bootstrap, the second one is a combination of both parametric and bootstrap approach. However, both the intensity- and amplitude-weighted mean fluorescence lifetime has asymmetric distribution, and thus we also propose confidence intervals for both the intensity- and amplitude-weighted mean fluorescence lifetime that based on the method of bootstrap percentiles.

Bootstrap methods are based on the so-called a bootstrap sample [14]. Suppose we have the data set x with m observed values. A bootstrap sample is a random sample of size m drawn with replacement from data set x. The bootstrap data set consists of members of the original data set, some appearing zero times, some appearing once, some appearing twice, etc. The bootstrap algorithm for estimating the standard error of measurement for the intensity- and the amplitude-weighted mean fluorescence lifetime is the following three steps procedure (14):

1. select B independent bootstrap samples $x_1,\ldots, x_B$, each consisting of m data values drawn with replacement from data set x,
2. using Eqs. (4) and (5) evaluate the bootstrap replication $\tau_I(x_b)$, $\tau_A(x_b)$ corresponding to each bootstrap sample x_b, $b=1,\ldots, B$,
3. estimate the standard errors SEM(τ_I), SEM(τ_A) by the sample standard deviation of B replications.

$$SEM(\tau_I)=\sqrt{\frac{\sum_{b=1}^{B}[\tau_I(x_b)-\overline{\tau}_I]^2}{B-1}},\overline{\tau}_I=\frac{1}{B}\sum_{b=1}^{B}\tau_I(x_b) \tag{7}$$

$$SEM(\tau_A)=\sqrt{\frac{\sum_{b=1}^{B}[\tau_A(x_b)-\overline{\tau}_A]^2}{B-1}},\overline{\tau}_A=\frac{1}{B}\sum_{b=1}^{B}\tau_A(x_b) \tag{8}$$

The second method for estimation of standard errors respects the structure of the estimator. Firstly, we will consider intensity-weighted mean fluorescence lifetime calculated from s-exponential model. Let us denote

$$d_I=\left(\frac{\partial\tau_I}{\partial A_1},\frac{\partial\tau_I}{\partial\tau_1},\frac{\partial\tau_I}{\partial A_2},\frac{\partial\tau_I}{\partial\tau_2},\ldots,\frac{\partial\tau_I}{\partial A_s},\frac{\partial\tau_I}{\partial\tau_s}\right)^T \tag{9}$$

the vector of all partial derivatives of intensity-weighted mean fluorescence lifetime. Here the symbol T stands for transposition