

INTENSITY DISTRIBUTION ANALYSIS IN FLUORESCENCE FLUCTUATION SPECTROSCOPY WITH A TWO-DIMENSIONAL BRIGHTNESS PROFILE

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Abstract

Fluorescence fluctuation spectroscopy is widely used in modern life sciences. Study of molecular structure and dynamics in the systems like cell membranes with diffusion limited in two dimensions needs a modification of the existing analysis methods. Here we present a modification of the photon counting histogram method, most commonly used for the intensity distribution analysis in fluorescence fluctuation spectroscopy, for the case of a two-dimensional brightness profile.

1 Introduction

Fluorescence Fluctuation Spectroscopy (FFS) methods are widely used in modern biochemical research [4]. In FFS the information about dynamics, interactions and structure of fluorescently labeled macromolecules is extracted from detected fluorescence intensity fluctuations. Fluorescence correlation spectroscopy (FCS) [3] and the photon counting histogram (PCH) [2] are the most commonly used methods allowing to extract this information. FCS resolves species by the difference in molecular weight and PCH analysis by the difference in the specific brightness. In cell membranes molecule diffusion is limited to two dimensions. This requires developing of special realizations of analysis methods. Though the two-dimensional realization of FCS is known to perform well in the study of cell membranes [5], the theory of the PCH analysis for the two-dimensional case has not been developed yet. Here we propose a modification of the PCH analysis for the case of a two-dimensional brightness profile.

2 Theory

The probability to detect k photons from a single molecule in a closed volume V_0 with brightness profile $B(r)$ can be expressed as [2]:

$$p^{(1)}(k, q, T, V_0) = \frac{1}{V_0} \int \frac{qT B^k(r)}{k!} \exp(-qTB(r)) dr, \quad (1)$$

where q is a specific brightness of molecules represented in photon counts per molecule per second (cpms) and T is a counting time interval. The probability of observing

k photons from N_{eff} molecules in an open effective volume $V_{eff} = \chi_1^2/\chi_2$, $\chi_k = \int W^k(r) dr$, is the weighted average of successive convolution $p^{(N)}(k, q, T, V_{eff})$ of N distributions (1) with itself:

$$\Pi(k, q, T, V_{eff}) = \sum_{N=0}^{\infty} p^{(N)}(k, q, T, V_0) N_{eff}^N \exp(-N_{eff})/N!, \quad (2)$$

where $p^{(0)}(k, q, T, V_0) = 0$, $k > 0$ and $p^{(0)}(0, q, T, V_0) = 1$. For M -component sample the resulting distribution is the successive convolution of distributions of all individual components. $B(r)$ is usually approximated by the 3D Gaussian [3]:

$$B(r) = \exp(-2(x^2 + y^2)/\omega_0^2 - 2z^2/z_0). \quad (3)$$

For the 3D Gaussian (1) takes the form [2]:

$$p^{(1)}(k, q, T, Q) = \frac{1}{\sqrt{\pi}Qk!} \int_0^{\infty} \gamma(k, qT \exp(-2x^2)) dx, \quad k > 0, \quad (4)$$

where $Q = V_0/V_{eff}$, $\gamma(k, x)$ is the incomplete gamma-function and

$$p^{(1)}(0, q, T, Q) = 1 - \sum_{k=1}^{\infty} p^{(1)}(k, q, T, Q). \quad (5)$$

The deviation of the true brightness profile from the 3D Gaussian approximation causes the PCH model to fail under certain conditions. To overcome this, correction factors characterizing the contribution of detected photons from the non-Gaussian part of the brightness profile into total fluorescence signal were introduced in the PCH analysis as $F_k = (\chi_k - \chi_{Gk})/\chi_{Gk}$, $k=1,2,\dots$, where G denotes the Gaussian approximation [2]. Finally, for the first order correction ($k=1$) the equation (1) takes the form:

$$p^{(1)}(k, q, T, Q) = \frac{1}{(1 + F_1)^2} (p_{3DG}^{(1)}(k, q, T, Q) + \frac{qTF\chi_{G1}}{QV_{eff}k!}). \quad (6)$$

3 Results

To perform the integration in (1) we used the modified polar coordinates $x = \omega_0\rho \cos \varphi$, $y = \omega_0\rho \sin \varphi$, $\rho \in [0; \infty)$, $\varphi \in [0; 2\pi)$. The 2D Gaussian brightness profile takes the form:

$$B(\rho) = \exp(-2\rho^2) \quad (7)$$

and V_{eff} reduces to $S_{eff} = \pi\omega_0^2$, $\chi_{2DGk} = \pi\omega_0^2/2k$. After substitution (7) into (1) and performing integration over φ the expression (1) becomes:

$$p^{(1)}(k, q, T, S_0) = \frac{2\pi\omega_0^2(qT)^k}{S_0k!} \int_0^{\infty} \exp(-2k\rho^2 - qT \exp(-2\rho^2)) \rho d\rho. \quad (8)$$

The integral in (8) can be written as

$$\int_0^{\infty} \exp(-2k\rho^2 - qT \exp(-2\rho^2)) \rho d\rho = \frac{(k-1)!}{4(qT)^k} (1 - \exp(-qT) \sum_{j=0}^{k-1} \frac{(qT)^j}{j!}). \quad (9)$$

Taking into account that

$$\gamma(k, x) = (k-1)! (1 - \exp(-x) \sum_{j=0}^{k-1} \frac{(x)^j}{j!}) \quad (10)$$

and substituting $S_0 = QS_{eff}$ in the expression (9) we obtain:

$$p^{(1)}(k, q, T, Q) = \gamma(k, qT) / (2Qk!). \quad (11)$$

To test our approach we adapted the photon counting distribution simulation scheme developed earlier for three dimensions for the 2D case [6]. At first step it is necessary to get the number of the i -th species of molecules in $S_0 = \pi\omega_0^2\rho_0^2$ by generating a Poisson random variable with the mean $N_0^{(i)} = \rho_0^2 N_{effi} / (1+F)^2$ (we set $\rho_0 = 3$) and uniformly distribute molecules in S_0 . A Poisson random variable with the mean $k_{ij} = q_i T B(r_{ij})$ is generated to get the number of detected photons from the j -th molecule of the i -th species. The number of photons generated by each molecule are summed to get the total number K_T of photons per the sampling time T . These steps are repeated m times to reach a given S/N ratio. The additional number of photons emitted from the non-Gaussian part of the brightness profile is generated by a Poisson random variable with the mean $\Lambda(T) = \sum_{i=1}^M N_{effi} q_i T F \chi_{2DG1} / (1+F)^2$ and then added to K_T .

We made simulation of the photon counting histograms for the one- and two-component

Table 1: The initial values of model parameters and their estimations

Parameter	Initial value	Estimation	Initial value	Estimation
N_{eff1}	2	1,999±0,003	1	0,9±0,1
q_{c1} , cpms	84000	83900±200	84000	88000±4000
N_{eff2}	—	—	5	4,9±0,1
q_{c2} , cpms	—	—	28000	32000±3000
F	0,4	0,398±0,002	04	0,43±0,03
χ^2	—	0,663	—	1,102

molecular systems and analyzed them using (2), (5), (6), (8). The fit parameters in the PCH analysis were obtained using the Marquardt-Levenberg nonlinear method of least squares. The quality of the fit was judged by the χ^2 -criterion and by the residual deviations between experimental and fit curves. Confidence intervals of the recovered parameters were calculated as asymptotic standard errors [1]. In simulations we set $T=50 \mu s$, $m = 10^6$ for the case of one-component system and $m = 6 * 10^6$ for the case of two-component system. Analysis results are summarized in the Table 1 and shown

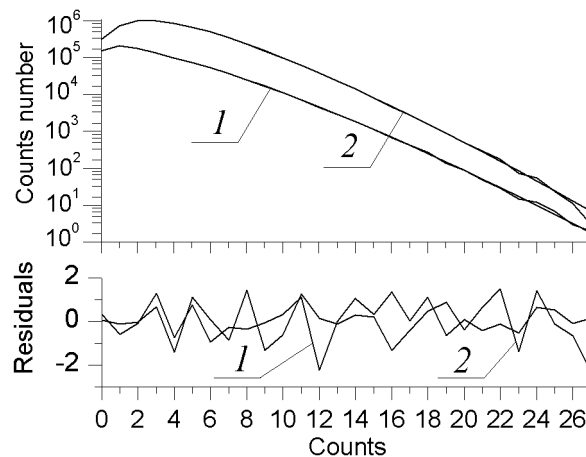


Figure 1: The fit of the simulated data for one- (1) and two-component (2) system

in the Figure 1. The obtained estimations of the model parameters are in a close proximity with initial values taken in the simulation, χ^2 -criterion values and normalized residuals indicates a good fit quality. This proves that our derivations are adequate for the considered cases.

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