Methods xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Methods

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

Scanning fluorescence correlation spectroscopy comes full circle

German Gunther, David M. Jameson, Joao Aguilar, Susana A. Sánchez *

Laboratorio de Cinética y Fotoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile
Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, USA
Universidad de Concepción, Facultad de Ciencias Químicas, Departamento de Polímeros, Concepción, Chile

ARTICLE INFO

Article history:
Received 31 October 2017
Received in revised form 23 January 2018
Accepted 30 January 2018
Available online xxxx

Keywords:
FCS
Circular scanning FCS

ABSTRACT

In this article, we review the application of fluorescence correlation spectroscopy (FCS) methods to studies on live cells. We begin with a brief overview of the theory underlying FCS, highlighting the type of information obtainable. We then focus on circular scanning FCS. Specifically, we discuss instrumentation and data analysis and offer some considerations regarding sample preparation. Two examples from the literature are discussed in detail. First, we show how this method, coupled with the photon counting histogram analysis, can provide information on yeast ribosomal structures in live cells. The combination of scanning FCS with dual channel detection in the study of lipid domains in live cells is also illustrated.
© 2018 Elsevier Inc. All rights reserved.

Contents

1. From point to circular scanning FCS . . . 00
   1.1. Nano-historical overview . . . 00
   1.2. Fluorescence correlation Spectroscopy: fluctuations in one spot . . . 00
   1.3. Circular scanning FCS: increasing spatial resolution . . . 00
   1.4. Circular scanning FCS: how slow should the laser scan . . . 00
   1.5. Circular scanning FCS: data acquisition and analysis . . . 00
   1.6. *Circular scanning FCS (csFCS): data analysis* [19,26,29,35] . . . 00
2. Instrumentation . . . 00
3. General experimental considerations and controls . . . 00
   3.1. Labeling and the appropriate dye . . . 00
      3.1.1. Fluorescent antibodies . . . 00
      3.1.2. Fluorescent proteins . . . 00
      3.1.3. Biomolecular fluorescence complementation . . . 00
      3.1.4. Halo-Tags . . . 00
   3.2. Concentration range and crowdedness . . . 00
   3.3. Autofluorescence . . . 00
4. Selected applications . . . 00
   4.1. Ribosomal structure and the power of PCH analysis for *in vivo* studies [24] . . . 00
      4.1.1. Experimental approach (Fig. 6a) . . . 00
      4.1.2. Instrumental setup: (Fig. 6b) . . . 00
      4.1.3. Sample preparation . . . 00
      4.1.4. Troubleshooting . . . 00
      4.1.5. Data acquisition and analysis . . . 00
   4.2. Lipid domains *in vivo*: mixing scanning FCS and Laurdan GP. [25] . . . 00
      4.2.1. Experimental approach (Fig. 7a) . . . 00
      4.2.2. Instrumental setup: (Fig. 7b) . . . 00
      4.2.3. Sample preparation . . . 00

* Corresponding author at: Universidad de Concepción, Facultad de Ciencias Químicas, Departamento de Polímeros, Concepción, Chile.
E-mail address: susanchez@udec.cl (S.A. Sánchez).

https://doi.org/10.1016/j.ymeth.2018.01.023
1046-2023/© 2018 Elsevier Inc. All rights reserved.

4.2.4. Troubleshooting: four controls were tested . . . 00
4.2.5. Data acquisition and analysis . . . 00
Acknowledgements . . . 00
References . . . 00

1. From point to circular scanning FCS

1.1. Nano-historical overview

Fluctuation methods have been utilized for more than a century, dating back to the theoretical work of Albert Einstein in 1905 [1] and Marian Ritter von Smolan Smoluchowski (1906) [2] on Brownian diffusion and the experimental studies by Theodore Svedberg on colloids in 1911 [3]. Application of fluctuation analysis to light scattering began in the 1960s, and in the early 1970s Elson, Magde and Webb developed fluorescence correlation spectroscopy (FCS) and used it to study binding reactions and chemical kinetics [4,5]. Independently, in 1974, Ehrenberg and Rigler developed the method to study rotational diffusion of macromolecules [6]. In 1990, Denk and Webb [7] demonstrated microscopy based on two-photon excitation and in 1995 Berland, So and Gratton put the two technologies together, namely two-photon excitation microscopy and Fluctuation Correlation Spectroscopy (FCS), and demonstrated the potential of this methodology for intracellular measurements [8]. Elliott Elson, one of the pioneers of FCS, has written a series of historical overviews of the method well worth the attention of anyone with a sustaining interest in FCS [9–11].

Scanning FCS refers to an extension of FCS where the focal volume is moved relative to the sample to measure correlation functions in both space and time. Scanning FCS was introduced in 1976 by Weissman [12], who used a circular rotating sample cell (keeping the excitation volume fixed) to expose several statistically independent volumes to the illumination beam providing, in addition, a way of separating the desired fluctuation from the noise. In 1986 Petersen [13] published FCS measurements in 3T3-cells using a custom designed linear translating stage having a position detector allowing control of the sample position with a precision of ~20 nm.

1.2. Fluorescence correlation Spectroscopy: fluctuations in one spot

The basic principles of FCS have been discussed in many publications [11,14]. Initially, microscopy and FCS were realized using a single point of excitation and observation as illustrated in Fig. 1 [14]. Some instruments use standard confocal pinhole methods to achieve the very small observation volume while others use two-photon principles which are intrinsically confocal [15]. In point FCS, the small illumination volume is kept immobile and the signal fluctuations detected will arise from the instrumental noise and from the stochastic Brownian motion of molecules moving randomly in and out of the illumination volume as a function of time. As the figure indicates, if the concentration of fluorophores is sufficiently low, then fluctuations in the signal are evident. Analysis of these fluctuations provides information on the motility (diffusion coefficients), concentration (number of particles) and association state (molecular brightness) of the particles responsible of the fluctuations observed. To extract this information, data can be analyzed by using the Autocorrelation Function (ACF) or the Photon Counting Histogram (PCH).

Consider the data stream depicted in Fig. 2. The average fluorescence intensity in the data stream is termed $\langle F(t) \rangle$, while the variation of any point from the average is termed $\delta F(t)$. To calculate the autocorrelation function, $G(\tau)$, the intensity at some time, t, is multiplied by the intensity at a later time, $t + \tau$, as illustrated in Fig. 2a. The average of this product, carried out for many values of τ, is then divided by the square of the average fluorescence intensity to generate the value $G(\tau)$.

$$G(\tau) = \frac{< \delta F(t) \times \delta F(t+\tau) >}{< F(t)>^2} \quad (1)$$

By carrying out this calculation over many τ values, an entire autocorrelation curve is constructed, as shown in Fig. 2b. Two parameters can be recovered from the ACF analysis: the diffusion coefficient (D_{coef}) and the average number of particles in the observation volume ($\bar{N}$) given by the inverse of G (0) and multiplied by a constant (γ) that depends on the illumination profile.

For PCH analysis [16,17] the probability of detecting photons per sampling time is calculated from the histogram of the detected photons, comparing the theoretical with the experimental distribution. The occupation number of particles freely diffusing in

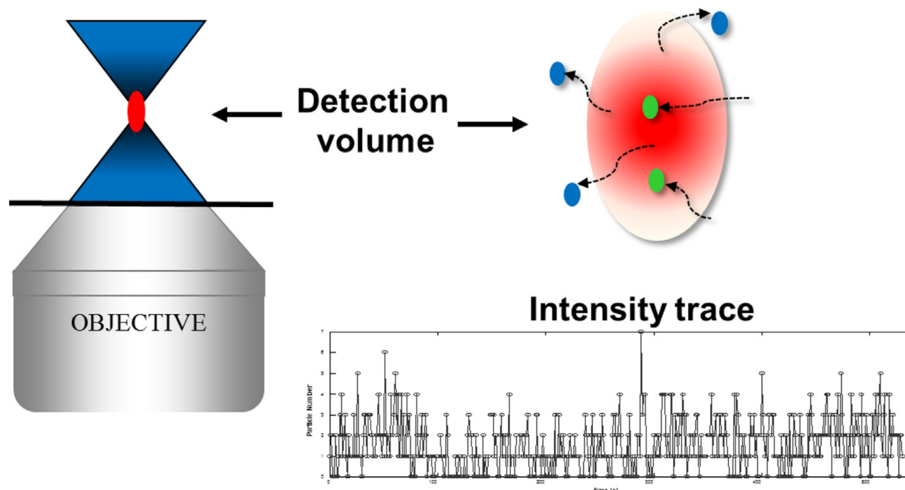

Fig. 1. Fluctuations are measured in a small volume (femtoliter range). The small illumination volume is generated either by the use of pinholes or directly by using two-photon excitation.

Download English Version:

https://daneshyari.com/en/article/8340054

Download Persian Version:

https://daneshyari.com/article/8340054

Daneshyari.com