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Fluorescence correlation spectroscopy of molecular motions and kinetics

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Abstract

The foundations for fluorescence correlation spectroscopy (FCS) were already laid in the early 1970s, but this technique did not become widely used until single-molecule detection was established almost 20 years later with the use of diffraction-limited confocal volume element. The analysis of molecular noise from the GHz- to the Hz-region facilitates measurements over a large dynamic range covering photophysics, conformational transitions and interactions as well as transport properties of fluorescent biomolecules. From the Poissonian nature of the noise spectrum the absolute number of molecules is obtainable. Originally used for the analysis of molecular interactions in solutions, the strength of FCS lies also in its applicability to molecular processes at either the surface or interior of single cells. Examples for the analysis of surface kinetics including on and off rates of ligand–receptor interactions will be given. The possibility of obtaining this type of information by FCS will be of particular interest for cell-based drug screening.

Keywords: Fluorescence spectroscopy; Photophysics; FCS; FRAP; FRET; Parallel detection; Autocorrelation; PCH; TIR

Contents

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1. Introduction to FCS and confocal single-molecule detection

1.1. Diffusion

Fluorescence correlation spectroscopy (FCS) is an analysis method that can measure the dynamics of molecular processes from observations of spontaneous microscopic fluctuations in molecular concentration. These measurements are commonly performed in thermal equilibrium where spontaneous fluctuations of molecules result from Brownian motion. In order to derive phenomenological parameters, e.g., diffusion coefficients and chemical rate constants, it is necessary to perform a statistical analysis on these fluctuations. In FCS, this statistical analysis is performed by a correlation function, which directly gives information about the diffusion time, bound/free ratio of molecules, triplet time and triplet fraction, indirectly yielding information about concentration, binding constants and on/off constants.

Single-molecule detection sensitivity is enabled by a small open volume element generated by a Gaussian laser beam. A high signal-to-noise ratio (SNR) is guaranteed, because a pinhole is inserted into the image plane of the objective, rejecting fluorescent and scattered laser light. In the early 1970s, Elson et al [1–4] as well as Ehrenberg and Rigler [5] derived the principle theory behind FCS. Thereafter, the confocal volume element was introduced [6] and FCS has evolved according to its application.

To understand the principles of FCS, at least qualitatively, consider a small confocal volume of observation filled with laser light that excites molecules (Fig. 1). The subsequent fluorescence is recorded and displayed as intensity, I, which fluctuates since the molecules move due to Brownian motion. The fluctuation in time, δI, will be distributed around a mean intensity, $\langle I \rangle$. A molecule entering the excitation volume element by Brownian motion emits photons and, depending on the random path of the molecule, it may remain a while, emitting more photons (maybe even crossing the whole detection volume element), or, alternately, it may quickly exit the volume element having emitted only a single

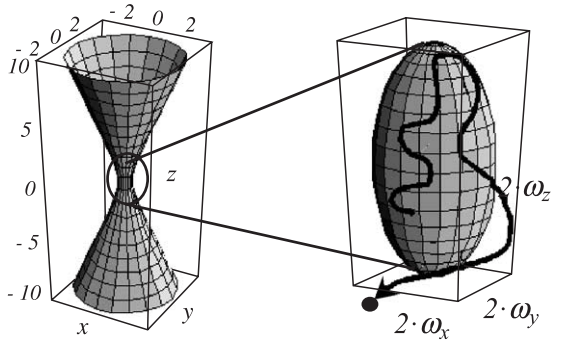

Fig. 1. Left: Gaussian laser excitation profile. Right: Confocal volume of observation. The dimension of the observation volume element is defined by the half-axis in length (ω_z) and width (ω_{xy}). The ratio $R=\omega_z/\omega_{xy}$ between the two axes is often around 5 and ω_{xy} is typically below 1 μm.