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Fluorescence correlation spectroscopy: the technique and its applications

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Abstract

Fluorescence correlation spectroscopy (FCS) is an experimental technique using statistical analysis of the fluctuations of fluorescence in a system in order to decipher dynamic molecular events, such as diffusion or conformational fluctuations of biomolecules. First introduced by Magde *et al* to measure the diffusion and binding of ethidium bromide onto double-stranded DNA, the technique has been undergoing a renaissance since 1993 with the implementation of confocal microscopy FCS. Since then, a flurry of experiments has implemented FCS to characterize the photochemistry of dyes, the translational and rotational mobilities of fluorescent molecules, as well as to monitor conformational fluctuations of green fluorescent proteins and DNA molecules.

In this review, we present the analytical formalism of an FCS measurement, as well as practical considerations for the design of an FCS setup and experiment. We then review the recent applications of FCS in analytical chemistry, biophysics and cell biology, specifically emphasizing the advantages and pitfalls of the technique

compared to alternative spectroscopic tools. We also discuss recent extensions of FCS in single-molecule spectroscopy, offering alternative data processing of fluorescence signals to glean more information on the kinetic processes.

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