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Application of scanning fluorescence correlation spectroscopy for determination of particle shape

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Journal

BIOPHYSICAL JOURNAL, 78(1)

ISSN

0006-3495

Authors

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Publication Date

2000

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Peer reviewed

Katarína Kis-Petiková, Yan Chen, Joachim D Müller, and Enrico Gratton.

Application of scanning fluorescence correlation spectroscopy for determination of particle shape.

44th Annual Meeting of the Biophysical Society, New Orleans, Louisiana, 2000. *Biophys J.* 2000; 78(1 Pt 2), 2603-Pos.

Abstract

Two-photon fluorescence correlation spectroscopy (FCS) has been proved as a powerful experimental technique for the study of fluctuations in the fluorescence signal of samples with low concentrations. From the measured autocorrelation function, the diffusion coefficient, chemical reaction rates or number of labeled particles can be determined. In the scanning FCS (S-FCS) technique the exciting beam is scanned periodically across the sample. By scanning through independent subvolumes, sensitivity of the measurement can be improved significantly. In addition, the autocorrelation function obtained from S-FCS in principle contains more information concerning the shape of diffusing molecules, when particles are of the size comparable to the laser beam size and scanning radius. Besides of time correlation, analyzed in FCS measurement, a spatial correlation at short distances can be analyzed. In the presented work, computer simulations of FCS and scanning FCS measurements of different shape and size of particles were performed. Optimal scanning parameters, as diameter of the circular path, and angular frequency, were found to differentiate between particle shapes and labeling. Model systems were measured and analyzed by both methods, and advantages of scanning were demonstrated. Supported by the NIH, PHS 5 P41 RR03155.