diffusion-fundamentals.org

The Open-Access Journal for the Basic Principles of Diffusion Theory, Experiment and Application

Diffusion measured with scanning fluorescence correlation spectroscopy

Zdeněk Petrášek, Susan Derenko and Petra Schwille

Biophysics group, Biotechnologisches Zentrum, Technische Universität Dresden, Tatzberg 47-51, 01307 Dresden, Germany, E-Mail: zdenek.petrasek@biotec.tu-dresden.de

Diffusion coefficient is a physical parameter that can be used to draw conclusions about the size and shape of the diffusing species and about the properties of the environment in which diffusion occurs. One of the methods to measure the diffusion coefficient of fluorescent or fluorescently labelled molecules is Fluorescence Correlation Spectroscopy (FCS). The diffusion coefficient is derived from the measured diffusion time - the characteristic time a molecule needs to diffuse through an observation volume of a known size. The diffusion time is obtained from an autocorrelation of the measured fluctuating fluorescence signal by fitting it to a model which assumes a well-defined observation volume. The small observation volume is experimentally realized by tight focusing of the excitation light combined with confocal detection of fluorescence, as known from confocal laser scanning microscopy.

Typically, the size of the observation volume is determined by calibration with molecules of a known diffusion coefficient. However, this is not always possible, for example, in applications in biological samples --- living cells and tissues. The spatial variations of optical properties of complex samples distort the measurement volume, usually enlarging its size and changing its shape. For this reason, methods are sought, that would not require independent calibration of the size of the observation volume. Several modifications of FCS have been proposed, involving spatial cross-correlation between two volumes of known geometry. The most often used of these techniques is two-focus FCS [1], where the cross-correlation between two volumes separated by a fixed distance of less than a micron is measured in addition to autocorrelations of the two signals.

Here we present a variation of FCS, where the measurement volume is scanned with a known frequency in a circle with a well defined radius R [2]. As a result of the scanning motion the autocorrelation of the measured fluorescence is modulated. The correlation values at different lag times correspond to spatial cross-correlations with distances varying between 0 and 2R. The modulation of the autocorrelation by scanning modifies the model function in such a way that both the diffusion coefficient and the volume size can be determined independently from the data fit. This is the crucial difference from a standard FCS, where the volume size must be known in order to determine the diffusion coefficient form the measured diffusion time. Possible optical distortions due to sample inhomogeneities affect the observation volume size but not the scan radius, making the

method considerably more robust in comparison with standard FCS. Since the scan frequency and radius can be varied, their values can be optimized for the expected range of diffusion coefficients.

We have originally implemented this method in a custom-built two-photon laser scanning microscope. More recently, we have completed a simplified version of this type of scanning FCS where a conventional FCS setup is converted to a scanning FCS by replacing a fixed mirror with a mirror mounted onto a two-axis piezo scanner, without adding any additional optical elements.

The method can be used to measure diffusion coefficients without any a priory knowledge about the measurement volume size, and under the presence of optical distortions. It is also applicable to diffusion studies on two-dimensional surfaces, such as biomembranes, where the size of the measurement area strongly depends on the position at which the surface intersects the probing laser beam.

References

[1] T. Dertinger, V. Pacheco, I. von der Hocht, R. Hartmann, I. Gregor and J. Enderlein, ChemPhysChem 8 (2007) 433-443.
[2] Z. Petrášek and P. Schwille, Biophys. J. 94 (2008) 1437-1448.