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Dual channel two photon FCS study in a cell

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John S Eid, Joachim D Müller, Yan Chen, and Enrico Gratton.

Dual channel two photon fcs study in a cell.

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Abstract

Fluctuation correlation spectroscopy (FCS) is a non-perturbative fluorescence microscopy technique used to obtain kinetic information by analysis of the stochastic fluctuations in a molecule's fluorescence. A localized femto-liter volume is achieved by two photon excitation of the sample. This inherent three dimensional sectioning provides a distinct advantage when performing measurements in a cell since photo-damage and unwanted auto-fluorescence signal is kept to a minimum. In order to study biologically relevant processes the interaction between two or more molecules needs to be investigated. Dual channel FCS yields this information through the cross-correlation of the signal from two different fluorescent markers. The effect of cellular conditions on the interaction between two molecules can be studied by measuring the change in the cross-correlation signal as a function of the systematic modification of the cellular environment. Simple free dye experiments in cells, as well as experiments involving fluorescently labeled bio-molecules were performed to demonstrate the feasibility of this approach to cellular investigation. Supported by the National Institutes of Health, RR03155.