Title
Detecting correlated brightness changes between two species using dual channel two photon fluctuation correlation spectroscopy

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Authors
Eid, JS
Gratton, E

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John S Eid and Enrico Gratton.

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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. The two photon effect localizes the laser excitation to a three dimensional volume of a femto-liter. Using two channels allows additional information to be obtained from FCS: the cross-correlation. Different criteria to split the signal into the two channels can be used (i.e. dichroic, polarizer, beam splitter). The cross-correlation, at its most basic level, contains information that quantifies the presence of static correlation between two species (e.g. a dichroic can be used to discern if two differently colored fluorophores are physically associated with each other). In addition, kinetic information about a process causing a constant rate of change in the correlation between two species can also be gathered. We present simulations that elucidate the strengths and limitations of this method. The usefulness of this technique in determining rates of change in the conformations of bio-molecules of interest will be demonstrated. Supported by the National Institutes of Health, PHS 5 P41 RRO3155.