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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. Flexibility results from using different criteria to split the signal into the two channels (i.e. dichroic, polarizer, beam splitter). We have investigated more closely the meaning of the crosscorrelation curve and its relation to the autocorrelation curves. In addition technical issues involving the positioning of the Avalanche Photo Diode (APD) detectors affecting the two autocorrelation curves from the two channels and the cross-correlation curve was explored. A biological system was examined to determine the usefulness of this technique as a biological microscopy tool. Supported by the National Institutes of Health, PHS 5 P41 RRO3155.