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Quantitative measurement of concentration and diffusion properties of molecules using fluorescence correlation spectroscopy

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Fluorescence **C**orrelation **S**pectroscopy (FCS) is a powerful and non-invasive technique for quantitative characterization of the concentration, mobility, and interactions of fluorescent/fluorescently labeled molecules *in vitro* and *in vivo* [1]. By exploiting the capabilities of a confocal microscope and time-correlated single photon counting (TCSPC), FCS offers extremely high temporal resolution (down to picosecond time scale), diffraction-limited spatial resolution (≈ 200 nm), as well as single-molecule sensitivity. Conventional Fluorescence Correlation Spectroscopy (FCS) utilizes temporal autocorrelation analysis of fluctuations in the recorded fluorescence signal caused by the motion of molecules (concentrations in nM range) through the small sample volume, often referred to as the focal volume (typically 0.2 – 1 fL) [2]. Furthermore, FCS can provide insights into local microenvironments, such as viscosity or pH, or about any other molecular process related to alterations in the fluorescence signal. By labeling the drug or the target molecule with a fluorophore, FCS can quantify the binding affinity, association/dissociation kinetics of drug-target interactions [3].

Here, we present our custom-made Fluorescence Correlation Spectroscopy (FCS) system, which utilizes a laser diode operating at 488 nm to effectively excite fluorescent dyes such as Alexa 488, FITC, Atto 488, and Rhodamine 110. We conducted a comprehensive characterization of the system's performance by studying Rhodamine 110 in both aqueous solutions and sucrose solutions with varying mass percentages. In the case of a 2 nM aqueous solution of Rhodamine 110, we obtained a diffusion time of 30 µs. Further analysis of the obtained results clearly demonstrates that our home-built FCS system achieves single-molecule sensitivity and possesses a focal volume that is comparable to that of commercial instruments.

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