**Exploring the Nano-scale World using a custom-made Fluorescence Correlation Spectroscopy (FCS) instrument**

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**F**luorescence **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 high temporal resolution (sub-microsecond in commercially available systems, and down to picosecond time scale in custom-made instruments dedicated to the study of fast processes such as rotational diffusion of molecules and photon antibunching), diffraction-limited spatial resolution (≈ 200 nm) [2], as well as single-molecule sensitivity. Conventional FCS utilizes temporal autocorrelation analysis of fluctuations in recorded fluorescence signal caused by molecular motion through the small sample volume, often referred to as the focal volume (typically 0.2 – 1 fL) [3]. FCS enables the quantitative measurement of the concentration, translational diffusion coefficient, and interactions. 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 [4]. By implementing two detection channels, conventional FCS is extended to Fluorescence Cross-Correlation Spectroscopy (FCCS) [5]. In FCCS, dual-color excitation and detection enable the monitoring of interactions and dynamics of molecules that are labeled with spectrally distinct fluorophores [5].

Here, we present our custom-made FCS system and characterize its performance using Rhodamine 110 in aqueous solutions. We show that the sensitivity and effective volume size in our home-built FCS instrument are comparable to those in commercial instruments.

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*This work was funded by the Science Fund of the Republic of Serbia, within Promis programme, through HEMMAGINERO project.*