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Single Point FCS on a Commercial Confocal Laser Scanning Microscope with Analog Detectors

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Fluorescence Correlation Spectroscopy is a technique invented in the early 1970s to measure diffusion coefficient, chemical reaction rates and photo physical processes. It is a common belief that in order to obtain single point FCS data, one needs either a sophisticated FCS instrument with photon counting detectors or avalanche photon detectors or an instrument custom made for this type of experiments. Here we show that we can obtain single point FCS data on a commercial confocal laser scanning microscope without any modifications (Nikon C1). We successfully measured the diffusion coefficient and the concentration of Rhodamine B in solution for concentrations ranging from 5 nM to 280 nM. We also determined the diffusion coefficient of two different labeled lipid analogs (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate and BODIPY TMR phosphatidylinositol (4,5) bisphosphate) incorporated in the membrane of giant unilamellar vesicles. The results obtained for these lipid analogs are in good agreement with previously published data. Finally, we highlighted the fact that the actual proportion of labeled lipid analogs incorporated in the membrane of the giant unilamellar vesicle (formed by the electroformation method) is significantly different than the proportion of these lipids in the organic solvent stock solution.