

# FLUORESCENCE CORRELATION SPECTROSCOPY: USING OF GENETIC ALGORITHM TO SEARCH RELEVANT DYNAMIC PARAMETERS

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In the FCS technique the correlation curve is fitted with an analytical expression to extract some parameters related to concentration and diffusion such as diffusion constant, triplet-state dynamics, reaction kinetics, or rate of bulk flow. Several authors have developed models, which describe the autocorrelation function of the bursts of fluorescence from single molecules diffusing in a solution through the focus of a light beam. However, these parameters are difficult to determine by an independent way (absence of suitable standards) since a confocal detection volume could not be modeled analytically (Patrasek, Biophys J. 94, 2008). Moreover, FCS in general requires calibration with one or more standard fluorescent compounds of known time diffusion.

The autocorrelation function for simple diffusion model used still contains valuable information, even though it can't be accessed via traditional FCS. Rather, the complex interactions between fluorescent tracers and the detection volume in many of these cases can be simulated quite easily or modeled by other numerical methods not amenable to standard curve fitting. In the present work, we propose an alternative approach based on genetic algorithm (GA) (John Henry Holland, 1970) to obtain relevant parameters from FCS data as an alternative method applied to autocorrelation function. The GA is a direct search method for solving optimization problems that achieve convergence in probability and based on natural selection, the process that drives biological evolution. We performed FCS using a confocal laser microscope SP2-FCS2-AOBS (Leica Microsystems, Germany) with a water immersion objective (HCX PL APO 63x / 1.2W CORR CS). The ISS VISTA software controlled shutter and fluorescence signal took place outside the scan head where a beam splitter splitted the emission light into 2 detection channels Avalanche Photodiodes (APDs). Power laser was measured with a LaserMate LD10 (Coherent, Auburn, CA) (Robert M Zucker, Owen Price. Cytometry 44:273-294, 2001).

In this study, when compared to parameters provided by classical methods, the robustness and precision of this new approach in FCS was assayed on two experimental different detection profiles of Rhodamine B in diluted aqueous solution (< 50nM) for two laser power delivered (12 and 51% of Argon laser 25mW). The effective volume determined were  $V_{\text{eff}}^{12\%} = (0.805 \pm 0.014)$  fL and  $V_{\text{eff}}^{51\%} = (0.964 \pm 0.011)$  fL. Finally, the diffusion time and the diffusion coefficient obtained for the Rhodamine B in aqueous solution were  $\tau_D = (67.0 \pm 4.7)$   $\mu\text{s}$  and  $D = (2.23 \pm 0.04) 10^{-6}$   $\text{cm}^2/\text{s}$  respectively. These values were obtained without a need of calibration with standard fluorescent compounds and they are closed to values obtained with classical physicochemical methods.