

PERFORMANCES AND LIMITS OF FCS AND FRAP UNDER TWO PHOTON EXCITATION FOR THE MOLECULAR DIFFUSION STUDY INSIDE MICROBIAL BIOFILMS AND POLYMER INCLUSION MEMBRANES

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KEYWORDS: FCS, FRAP, Two-photon excitation, Biofilms, Polymer inclusion membranes.

Biological processes which occur on the submicrometer scale such as diffusion, molecular complex formation, molecular interaction can be analyzed by indirect approaches of fluorescence microscopy like FCS and FRAP. Both methods are closely related; they allow monitoring molecular dynamics (mobility, transport and diffusion) by the analysis of fluorescence changes in small open regions of both artificial and biological systems. However, depending on the properties of the system, FRAP or FCS can be more convenient to apply. Indeed, there is no limitation of fluorophore dye concentration and diffusion times using FRAP method but photodamages of the system are often unavoidable. Low laser powers needed for FCS measurements minimize the perturbations of the sample but the method requires very low fluorophore concentrations and precludes slow diffusion process study.

FCS and FRAP under two photon excitation are complementary used to study molecular mobility inside biofilms and polymer inclusion membranes (PIM) respectively. A biofilm can be described as a community of adhering microorganisms, generally embedded in an extracellular polymeric substance (EPS) matrix which controls the diffusion of molecules (oxygen, nutrients, antimicrobial...) [1]. The PIMs studied are composed of a polymer matrix (cellulose triacetate (CTA)) in which a metal ion carrier Lasalocid A and a plasticizer 2-nitrophenyl octyl ether (NPOE) are included. Depending on Lasalocid A concentration, plasticizer liquid phase domains are formed in the core of the PIM facilitating metal ion transport by diffusion of the metal-carrier complex [2].

We have established that FCS allows non invasive *in vivo* studies of antimicrobial molecule diffusion through biofilm structure by comparison with FRAP measurements. By contrast, for lasalocid A behaviour inside CTA membranes, FRAP is well suited due to the constraints on the fluorophores concentration.

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