Fluorescence correlation spectroscopy under two photon excitation for the study of diffusion and reactivity of bacteriophages inside biofilms.

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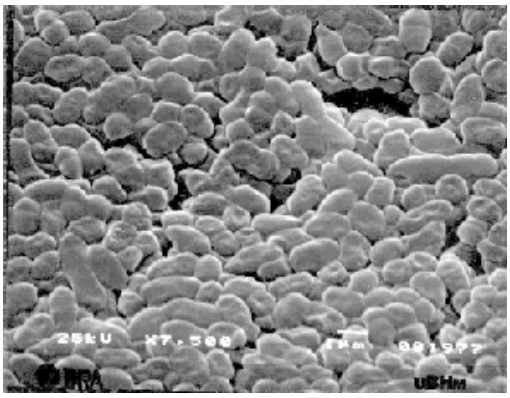
Fluorescence correlation spectroscopy (FCS) has emerged as an ultrasensitive technique for monitoring molecular dynamics operating at the single molecule level [1]. In association with two-photon excitation (TPE), this fluorescence emission analysis has opened new opportunities for the study of biological systems. Indeed, due to the very weak concentration of fluorophores used and the small volumes probed, FCS allows non invasive in vivo studies. We have recently demonstrated that FCS under TPE can be applied successfully to characterize the penetration and the diffusion capabilities of fluorescent nanoprobes inside microbial biofilms [2]. The formation of biofilms (defined as microbial communities attached to a surface) is an extremely common phenomenon associated with significant problems in medical, industrial and environmental areas. These problems include the possibility for biofilms to trap toxicants or hostile microorganisms such as virus, depending on their steric and physicochemical interactions with the adherent cells and the organic matrix [3].



Our method of FCS under TPE has been applied in this context to analyse the diffusion and reactivity of the phage C2 (a prolate type bacteriophage with a long and non contractile tail) into two type of *lactococcus lactis* biofilms selected for their different sensitivity for the bio contaminant (Figure). The study was extended to a *Stenotrophonas maltophilia* biofilm in order to characterize the role of the exopolymer matrix in the phage retention into the microbial structure.

For these experiments, we have optimized the biofilms production using flow cells in order to reproduce the natural formation of these structures. Controls were also performed to ascertain the bacteria viability during the experiments of microscopy.
We will present fluorescence intensity vectors and autocorrelation curves of the phage C2 through the biofilms by comparison with the curves obtained for the free molecule and discuss the results according to the structure and affinity of the biofilm toward the bacteriophage.

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