FRAP REFINEMENT: ANALYSIS OF TIME AND SPACE ARTEFACTS IN HETEROGENEOUS NANOSTRUCTURED MODEL SYSTEMS.

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Since in the late 1970s the method of fluorescence recovery after photobleaching (FRAP) has been developed it has been used for trafficking studies in living cells and through membranes. In a typical FRAP experiment, the fluorophores of a region are irreversibly "turned off" by illuminating the chosen area with an high energy beam (phenomenon known as photobleaching). Then the redistribution of the fluorescence due to the mobility of the marked molecules is monitored and the fluorescence signal inside the region increases until an equilibrium is reached. By analysing the curve of fluorescence recovery with a suitable theoretical model [1], quantitative information (i.e. the diffusion coefficient *D*) about the processes involved in the fluorophores diffusion can be evaluated. Unfortunately, some artefacts affects the evaluation of *D*. First, for fast molecules, diffusion during the bleaching process may play a relevant role: once photobleaching has been accomplished a larger area than the chosen one could be populated by an amount of non fluorescent molecules. When one neglects this effect, the evaluated *D* will consequently result underestimated [2].
Furthermore, due to the finite extent of the laser beam, a mismatch between the region chosen and the one effectively bleached is always induced. This phenomenon can cause a significant error when evaluating diffusion processes in structures having a size comparable or smaller than the laser beam waste (~200nm), with strongly different viscosities in the direct environment of the compartment.
We have developed new methods to analyze and quantify the errors previously described. As a model system of a heterogeneous permeable structure, we used polyelectrolyte self-assembled nanocapsules. This fuzzy network allows to control their physical properties (i.e. wall thickness, permeability, cut-off) by changing their preparation parameters [3, 4]. Furthermore this nano-organized hollow capsule is a promising candidate for an application as smart drug delivery system.

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