

## FRET MICROSCOPY: CHARACTERIZATION BY MEANS OF NANOSTRUCTURED SYSTEM ANALYSIS.

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FRET (Forster-fluorescence Resonance Energy Transfer), can be considered as a modern technique that transformed the microscope into a powerful instrument able to measure and to analyze molecular interactions within biological systems at subresolution level. In particular FRET allows to get information about molecular interactions in the range of 1-10nm, below the resolution limit of the microscope imposed by diffraction (approx. 200nm). The process is based on a resonance phenomenon between two fluorescent molecules, for which a donor transfers his energy radiationless to an acceptor close to the donor. The distance is not the only condition for FRET to occur, there must be also a large overlap between the donor emission spectrum and the acceptor absorption spectrum. Moreover the energy transfer depends also on the donor quantum yield and on the relative orientation of the donor/acceptor transition dipoles [1]. One drawback is the interpretation of FRET data because of fluorescence events that camouflage the real FRET signal like photobleaching, or autofluorescence, but in particular the Spectral-Bleed-Through (SBT). This last effect is related to the spectral overlap occurring between the fluorescent molecules of interest. In particular, donor-SBT is due to donor molecules which directly emit in the acceptor channel while the acceptor-SBT is due to acceptor molecules which are directly excited by the donor wavelength [2]. Our work is focused on the refinement of FRET data to correct from SBT effects. Our starting point was the p-FRET algorithm recently developed at Keck Cellular Center for Cellular Imaging, University of Virginia [2]. For our investigation, we developed and used for the first time a test specimen made by layer-by-layer [3] polyelectrolytes nanocapsules loaded with the FRET-couple FITC and Alexa594 bound to different layers [4] at controlled distances within the nanometric range (approx. 2.5 nm/ shell layer). Under single and two-photon excitation conditions, we determined the Forster distance ( $R_0$ , the distance at which the FRET-efficiency is 50%) as function of the donor-acceptor layer mutual distance established in the nanostructured systems by controlling their fabrication parameters.

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