

Quantitative Fluorescence Microscopy Techniques: From living cells to single molecules

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Fluorescence microscopy has become an invaluable tool to study protein dynamics in living organisms. The emergence of fluorescent proteins (FPs) has opened the way to the visualization in space and time intracellular proteins thanks to a non-invasive one-to-one tagging obtained by fusing FP with proteins of interest by recombinant DNA techniques. Moreover, a large palette of FP mutants, which display emission spectra all over the visible range as well as improved fluorescence quantum yield and sensitivity to different chemical environments, have been obtained by molecular engineering. Through these developments, quantitative imaging by means of multicolour labelling and advanced fluorescence measurements is now currently used to quantify biomolecular interactions in living cells with a spatial resolution far below the diffraction limit.

In this lecture, I will present the different approaches developed in the lab to monitor biomolecular interaction in living cells. In particular, I will show how Fluorescence Lifetime Imaging Microscopy (FLIM) and Fluorescence Correlation Spectroscopy (FCS) can be used to decipher protein-protein interactions. However, these techniques, performed at the ensemble level, do not allow measuring the kinetic constants of a biomolecular reaction. To overcome this limitation, we developed a widefield microscope having single molecule sensitivity. With the help of Förster Resonance Energy Transfer (FRET) I will show that it is possible to follow, over time, the interaction between a single protein and a single oligonucleotide allowing to obtain information about the reaction kinetic. In addition, the ability to detect individual fluorophores can also be used in a cellular context to visualize cellular compartments with a spatial resolution of 30 nm overcoming thus the resolution of conventional optical microscopy.