ESONN'2021 European School On Nanosciences and Nanotechnologies

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N°47: Molecular mobility and oligomerization measurements in cells by fluorescence fluctuation techniques

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Individual fluorescent molecules diffusing in living cells, or solutions are yet too small and too rapid to be followed up by the optical imaging techniques, however their signal fluctuations may be studied in a small confocal volume (0.2fl). The autocorrelation of this signal unravels, among others, the translational and rotational dynamics of the molecules, their concentration, molecular brightness and oligomer stoichiometry. For this purpose, we will use the method of Fluorescence correlation spectroscopy (FCS)[1] and its derivatives, such as Number & Brightness (N&B) or Raster image correlation (RICS). In case of fluorescently labelled enzyme (kinase) and optogenetic stimulation these methods may shed light on cell signalling pathways[2] in a non-invasive and reversible manner.

The molecular quantification with FCS relies on the knowledge of the geometry of the confocal volume that is prone to distortions due to optical aberrations. We will take advantage of the new method of adaptive confocal microscopy[3] and FCS in order to measure and compensate these aberrations in different optical conditions. This unique technology is developed in collaboration with Physics lab (LIPhy) and will be compared to standard FCS results in solutions and in living cells.

The students will operate the state of the art adaptive confocal laser-scanning microscope in order to compensate optical aberrations, to calibrate the confocal volume and to measure the dynamic parameters of optogenetic enzyme (**Fig**).

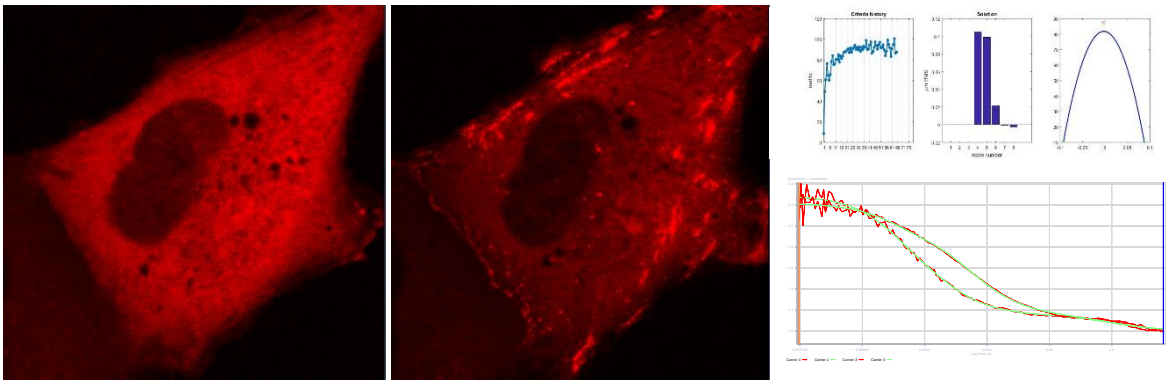

Figure. Relocation of Cry2-mCherry-kinase (left) on the focal adhesions after Cry2 photo-induced oligomerization (middle). Slowing down of Cry2-mCherry diffusion upon 488 nm photostimulation (right)

Prerequisites: basic knowledge in confocal & epifluorescence microscopy

1. Haustein E, Schwille P. Fluorescence correlation spectroscopy: novel variations of an established technique. Annu Rev Biophys Biomol Struct. 2007; 36:151-69.
2. Kerjouan, A., et al. (2021). "Control of SRC molecular dynamics encodes distinct cytoskeletal responses by specifying signaling pathway usage." J Cell Sci **134**(2).
3. Booth, M. J. (2007). "Adaptive optics in microscopy." Philos Trans A Math Phys Eng Sci 365(1861): 2829-2843.