ESONN' 2020 European School On Nanosciences and Nanotechnologies

Virtual practicals & on-line tutorials

N°47: Single protein localization and molecular mobility measurements in cells by fluorescence fluctuation techniques

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Analysis of molecular fluorescence blinking is a powerful tool to bypass the Abbe's limit of optical resolution and to precisely locate the immobile single molecules. Alternatively, in case of probe diffusion in living cells, the analysis of their fluorescence fluctuations in a small confocal volume unravels the translational or rotational dynamics. Here such methods based on fluorescence fluctuations will be illustrated using the superresolution stochastic optical reconstruction microscopy (STORM) and fluorescence correlation spectroscopy (FCS).

Taking into account the particular conditions of the remote ESONN school this year, we propose the interactive online practical session, including the record of the specimen preparation, instrument calibration and data acquisition and analysis as well as the on-line discussion of the advantages and pitfalls of each technique.

The 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 and FCS in order to measure and compensate these aberrations. This unique technology is developed in collaboration with Physics laboratory (LIPhy) and will be compared to standard FCS results in solutions and in living cells.

The super-resolved (SR) imaging of single proteins will then be performed by STORM using the AF647 fluorescently labelled antibody. The principle steps of the fixed cell preparation, labelling and mounting in blinking buffer will be shown. A sequence of images under strong continuous excitation at 640 nm will be acquired and the SR image will be reconstructed. The choice of reconstruction parameters, the drift compensation and other tricks will be discussed.

The work plan of the practical (~2.5 h):

- Short on-line reminder of the used fluorescence fluctuation techniques
- Video records:
  - FCS optimization with adaptive optics in solution
  - Measurement of protein mobilities in living cells
  - Preparation for STORM experiments (buffer, cells, chamber)
  - Acquisition of raw data and the reconstruction of the SR image.
- On-line discussions and conclusions

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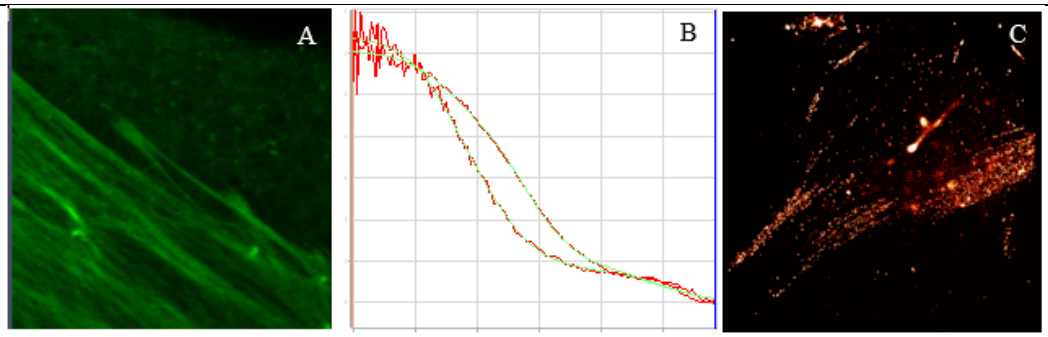

A. Confocal image of cell cytoskeleton. **B**. FCS curves of proteins with different mobilities. **C**. STORM image of the cytoskeleton in fixed cell.

Prerequisites: basic knowledge in confocal & epifluorescence microscopy