Single protein localization and molecular mobility measurements in cells by fluorescence microscopy techniques.

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Capture of the blinking of molecular fluorescence is a powerful tool to bypass the Abbe's limit of optical resolution and to study the localization of single molecules in fixed cells. Alternatively, in case of diffusing molecules *in vivo*, the analysis of fluorescence fluctuations may be used to unravel the molecular translational dynamics. Here two methods of fluorescence blinking measurements will be illustrated using the superresolution stochastic optical reconstruction microscopy (STORM) and fluorescence correlation spectroscopy (FCS) [**1**].

The aim of the present practical is, first, to apply the methods of correlation spectroscopy in solutions and living GFP-expressing cells to locally quantify and map the dynamics and concentrations of tagged proteins. FCS monitors the single confocal volume (~ $0.2 \cdot 10^{-15}$ l) and it is efficient in the nM to µM range. It is suited to the very fast dynamics and in some cases allows differentiation between transport mechanisms, diffusion type or transient interactions. However, no imaging is possible with this technique and the study of slow fractions is challenging due to inherent cellular movements.

The quantification with FCS relies on the knowledge of the geometry of the confocal volume *in vivo* that is prone to distortions due to optical aberrations. We will take advantage of the Adaptive Optics in confocal microscopy and FCS in order to measure and compensate these aberrations. This unique technology is developed in IAB in collaboration with Physics laboratory (LIPhy) and will be compared to standard confocal FCS tools.

The localisation of the proteins in fixed cells will be performed in the second time by STORM using the fluorescently labelled antibody with Alexa647 [**2**]. A strong continuous excitation at 640 nm and optimised buffers result in fluorescence blinking of this label, and the barycentre of each fluorescent spot can be determined with a high resolution (<50 nm). Adhesion structures and the cytoskeleton in fixed adherent cell will thus be imaged (**Fig. 1**).

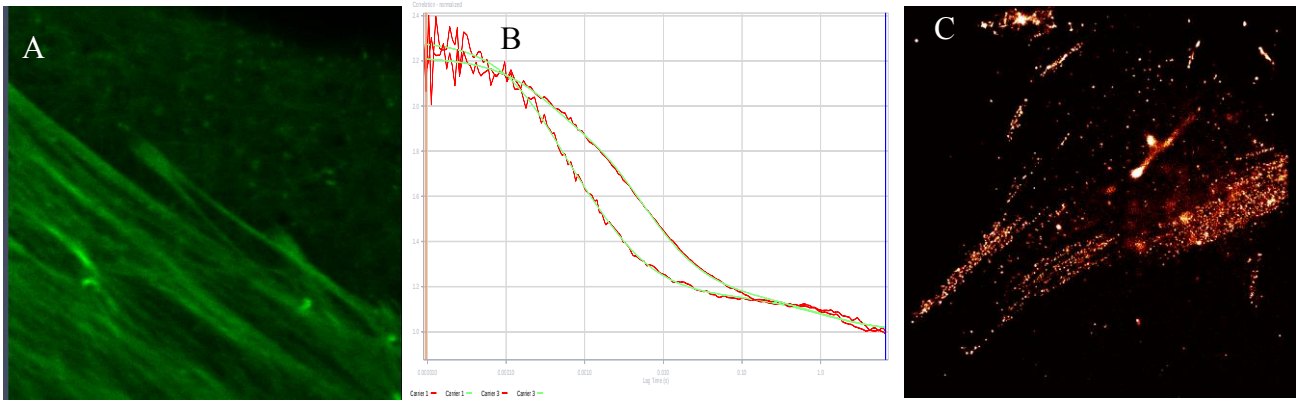

Figure 1. A. Confocal image of cell actin. **B**. FCS curves of proteins with different mobilities. **C**. STORM image of the cytoskeleton in fixed cell.

The work plan of the practical:

- Short theoretical reminder of the used F-techniques
- FCS calibration and measurements in solution and living cells (Confocor 3, Zeiss)
- Adaptive Optics adjustments for quantitative FCS (ConfoBright, AlpAO, Nikon)
- Sample preparation for STORM experiments (buffer, cell chamber)
- Superresolution image acquisition and reconstruction (iMIC FEI-TILL, RainSTORM)

Prerequisites: basic knowledge in fluorescence microscopy

(1) Digman MA, Gratton E. (2011) Lessons in fluctuation correlation spectroscopy. Annu Rev Phys Chem 62:645-68.
(2) Fornasiero E.F. Opazo F. (2015) Super-resolution imaging for cell biologists. Bioessays No.37 : 436-45