IMAGING OF PROTEINS' ORGANIZATION IN 3D USING SINGLE MOLECULE ORIENTATION AND LOCALIZATION MICROSCOPY (SMOLM)

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Imaging molecular orientation at the nanoscale in live cells and tissues is fundamental for the understanding of proteins' organization, which is driven by their structural and conformational properties. Measuring the orientation of fluorescent molecules is a way to approach this problem, providing that the label is rigidly attached to the protein of interest. Despite the great progresses in fluorescence imaging down to nanometric scales with Single Molecule Localization Microscopy (SMLM), orientation imaging is still challenging, especially in dense molecular structures. It is in particular delicate to disentangle the information about single molecules' 3D orientations and their 3D spatial localization, since spatial and orientational parameters are entangled in the single molecule point spread function (PSF) image formation.
We will present methods in polarized fluorescence microscopy that are able to report both orientational and spatial information from single molecules in a non-ambiguous way. These approaches, based on polarization splitting [1] or on the manipulation of phase and polarization at the pupil plane [2], give access to orientation parameters in combination with high spatial localization precision. We will present the potential and limits of these approaches for the imaging of the nanoscale organization of proteins in cells in dense environment, and discuss their prospects for applications in nanophysics that exploit scattering of complex 3D fields by metal nanoparticles.

[1] C. Rimoli, C. Valades Cruz, V. Curcio, M. Mavrakis, S. Brasselet. 4polar-STORM polarized super-resolution imaging of actin filament organization in cells. Nat. Communications 13, 301 (2022).
[2] V. Curcio, L. A. Aleman-Castaneda, T. G. Brown, S. Brasselet, M. A. Alonso, Birefringent Fourier filtering for single molecule Coordinate and Height super-resolution Imaging with Dithering and Orientation (CHIDO). Nat. Communications 11 (1) (2020)