PhD project

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Polarized single molecule imaging of rotational dynamics at the nanoscale

A great interest in studying the behaviour of fluorescent single molecules in biological samples by optical microscopy, is to be able to report their spatial localization in 3D within 10's nm precision, providing super resolution imaging and capabilities for single molecule tracking in live environments. The MOSAIC team at Institut Fresnel has pioneered methods in polarized optical microscopy to bring, in addition to spatial localization, the information of the orientation of single fluorescent molecules in 3D, at scales below the diffraction limit. The principle is based on the manipulation of the phase and light polarization at the pupil detection plane of a microscope, followed by the analysis the polarized images of the single emitters. This approach gives unique insight into the nanoscale structural and orientational organization of biomolecules.

So far these methods involved measurements over 10-100's ms integration time scales, averaging rotational dynamics. An important, missed information, is the rotational time of single molecules, which is a signature of the local viscosity and steric constraint for their local environment. Such information is key for the understanding of biomolecular packing and aggregation, occurring in particular in the formation of condensates that are membrane-less compartments built from phase separation, involved in many biological processes. The key to access rotational dynamics is the joint control of the excitation and detection polarizations in a microscope, in addition to monitoring single emitters' images, therefore providing insight into their rotational time as compared to fluorescence life time.

The goal of this PhD project is to expand polarized imaging to the investigation of the translational and rotational dynamics of single emitters in constraint environments, based on polarization control in a microscope. The project will cover (1) the theoretical and computing modelling of the effect of rotational diffusion of single molecules in polarized single molecule imaging, (2) the implementation of dedicated microscopy instrumentation to probe single molecule rotational dynamics, based on polarization control, and (3) the application to nanoscale probing of the local environment in biological model systems *in vitro* and in cells. The samples will be developed together with collaborators from biology laboratories.

Reference: *S. Brasselet, M.A. Alonso, Polarization microscopy: from ensemble structural imaging to single molecule 3D orientation and localization microscopy. Minireview. Optica 10 (11), 1486-1510 (2023) doi.org/10.1364/OPTICA.502119*