PhLAM
Physique des Lasers
Atomes et Molécules

Unité Mixte de Recherche 8523 CNRS- Université de Lille

PhLAM RESEARCH SEMINAR SERIES
Jan 24th, 2025, 10:30 AM
IRCICA Building

Taking advantage of intrinsic properties of single molecule emission for enhanced super-resolution microscopy

by

Sandrine Lévêque-Fort (Institute of Molecular Sciences of Orsay , ISMO)

Single molecule localization microscopy allows one to bypass the diffraction limit and thus reach previously inaccessible observation scales in biological samples. However, to fully unravel the subcellular 3D organization of multiple proteins, different developments are still needed in particular to image in depth complex samples (embryos, spheroids, tissues). We proposed various alternatives to enhance single molecule localization by taking advantage of intrinsic information while a fluorophore is emitting in the single molecule regime. We thus developed the detection of the brightness for multitarget imaging and to enhance axial resolution we proposed two complementary strategies which offers major assets compare to usual strategies based on point spread function engineering. A first approach is based on Supercritical Angle Fluorescence (SAF) emission which corresponds to the near field components of any fluorophore placed in the vicinity of the glass/cell interface which becomes propagative and emitted at supercritical angles. It thus intrinsically presents in the sample, can represent up to 50% of the emission of the fluorophore and only requires a modification of the detection path to extract the absolute axial position of single molecule. This approach is in particular well suited to probe biological structure within the first 500 nm, and can be combined to alternative PSF engineering strategies to enhanced the capture range. We recently demonstrated that this setup could also be used for multi-target imaging, by taking advantage of dyes with close spectral properties but different brightness. For deeper observation and in particular to address complex samples such as organoids, the PSF shape is degraded by defocusing or aberrations and strongly, and its spatial analysis can degrade 3D imaging. We proposed a new localization strategy based on a time signature to retrieve fluorophores' position over the whole field of view. Wide field uniform excitation of the sample is replaced by a shifting structured excitation, typically a moving fringe pattern. This induces a time modulated emission of the illuminated fluorophores where the phase holds its position. Modulation frequencies typically >500 Hz are needed to be compatible with the short On-time of single molecule in dSTORM, which is too fast for a demodulation with sequential images for most cameras. To achieve demodulation without photon loss, we have designed an optical assembly based on a Pockels cell or galvanometrics mirrors to steer the photons in 4 sub-images acquired a single camera frame. This new localization technique called ModLoc for Modulated Localization, can provide a 2 fold improvement for the precision, but also a unique uniform sub 7nm precision for in depth imaging. Furthermore, being resistant to optical aberrations, ModLoc allows us to image in complex samples such as tissues up to several tens of microns. Multi-target imaging based on spectral demixing can be used, in particular for muscle tissues imaging.

In ModLoc, the structured excitation is generated by a conventional interference pattern, where the periodicity of the pattern requires a camera to unwrap the phase information. To avoid this step and realize a full time coding of the position compatible with the observation of live cells, we have recently proposed a new strategy, in which we introduced an original dynamic wide field structured excitation which encodes each point in the field of view with a unique modulation frequency. This permits to localize without ambiguity emitters within a large field of view but in combination with monodetectors which offers unique assets in terms of acquisition speed and functional imaging. I will present an example of implementation of TimeLoc and first results.

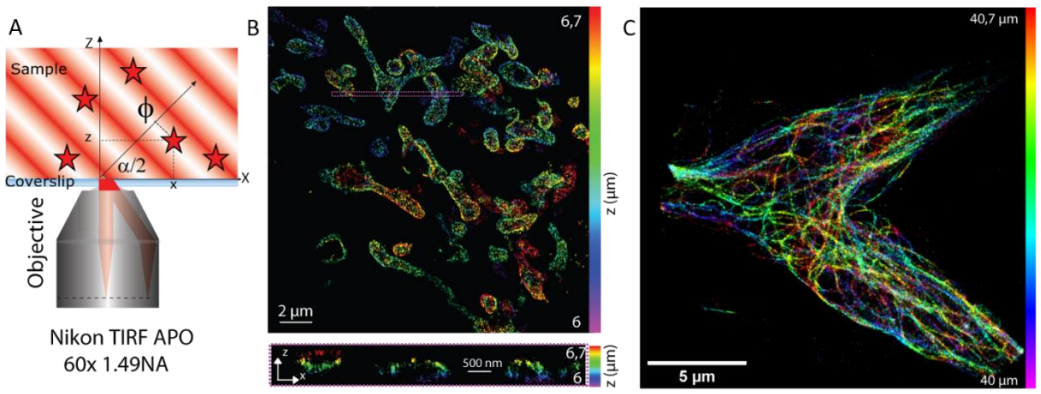

Fig. : A) Implementation of ModLoc within a single objective configuration to encode the axial position, , B-C) 3D ModLoc image in STORM (AF647) with z color coded of B)Mitochondria at 6 µm in depth in COS7 cell and C)) Tubulin network in COS7 cells in spheroids at 40µm in depth.