

Supercritical Angle Fluorescence emission: from microscopy to nanoscopy

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abstract

The near field components of a fluorophore placed in the vicinity of the glass/cell interface can become propagative and emitted at supercritical angles. Supercritical Angle Fluorescence (SAF) emission sharply decays with the fluorophore/surface distance z over a characteristic length of about 150 nm, thus providing an efficient way to perform wide field axial filtering in dense samples. This optical Fourier filtering can be implemented by amplitude or phase modulation of the SAF emission and allows to probe simultaneously intracellular and membrane events for live imaging.

The intrinsic SAF emission can also be exploited to improve axial resolution of super-resolution technics such as STED or dSTORM/PALM. The seminar will mainly focus on the combination of Single Molecule Localization Microscopy (SMLM) with SAF detection, which provides absolute axial localization of molecule, thus improving 3D co-localization at the nanoscale. This 3D absolute method, called "Direct Optical Nanoscopy with Axially Localized Detection" (DONALD), gives an axial localization precision of 15 nm within an axial range of ~150 nm above the coverslip, while preserving typical lateral localization precision (~10 nm). Axial position can be accessed up to the first 600 nm within the sample, but with lower localization precision. To extend the depth capture up, SAF detection can be coupled to complementary PSF engineering technic such as astigmatism. This original combination called DAISY preserves lateral and axial localization precision while extending the depth capture range to 1.2 μm , and addresses several limitations encountered when using astigmatism only, such as axial drift and chromatism. DAISY technology is at the core of **abbelight** 3D nanoscope, on demo at Emory Integrated Cellular Imaging Core (17-18 December).

about the speaker

Sandrine Lévêque-Fort is a CNRS Researcher Director at the Institute of Molecular Science in Orsay (ISMO). She developed different strategies to improve fluorescence microscopy such as original configuration for wide field FLIM, but also the use of plasmonics substrates to engineer fluorescence emission. Since 2009, her research focus is on supercritical angle fluorescence microscopy and super-resolution microscopy techniques.

about abbelight

Abbelight is the result of 10 years of research on new detection methods in fluorescence microscopy at the ISMO-Paris-Saclay University and Langevin Institute (ESPCI, Paris). Firmly rooted in academia, **abbelight** aims to make 3D fluorescence nanoscopy accessible to all research laboratories, by providing advanced imaging solutions tailored to answer biological questions.

