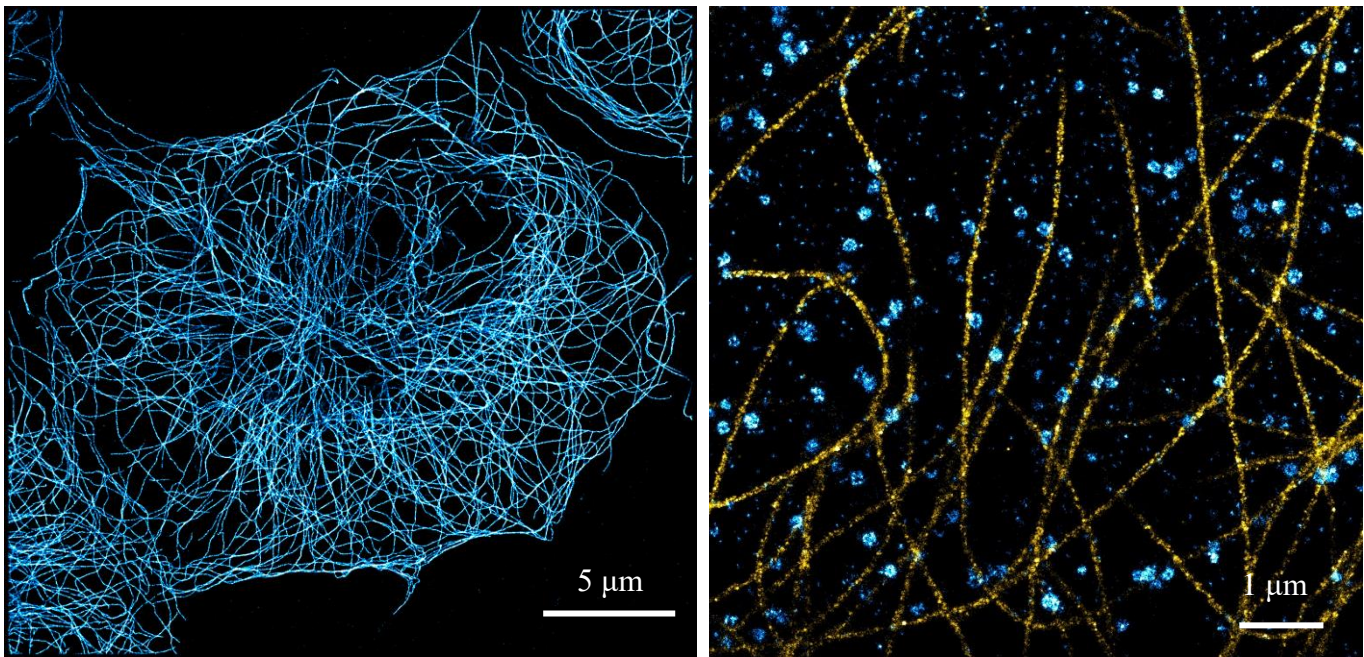


## New approaches to multicolor 3D STORM Microscopy

Super-resolution microscopy methods have recently allowed us to visualize biological structures using fluorescence microscopy down to the nanometer scale. In particular, STORM [1] – a type of localization microscopy that relies on fluorescent dyes imaged in an adapted chemical environment – is a popular technique due to the use of standard dyes and standard inverted microscopes. However, multicolor 3D imaging is limited by the few number of protocols that yield equally good images with the different dyes & channels and can work away (up to 10-15 micrometers) from the coverglass.



**Figure 1: (Left): Large field of view STORM image of microtubules (Credit: H. Boukhatem/ N. Olivier - LOB) (Right): 2-color STORM of Clathrin and tubulin using SafeRedSTORM (Credit: Abbelight)**

In this collaborative project between the Laboratory for Optics and Biosciences at Ecole Polytechnique and the company Abbelight, we want to develop new protocols [2,3] for multicolor 3D-STORM. In particular we want to

1. Improve the SNR of a single-wavelength multicolor imaging by spectral unmixing approach (SafeRedSTORM [4])
2. Improve multicolor imaging using multiple excitation wavelengths (560/640/750)

Abbelight is a french company located in Cachan that focuses on the design and development of products in the nanoscopy area with particular attention for Single Molecule Localization Microscopy techniques. SMLM lacks straightforward multi-color capability, which is crucial to tackle most biological questions. This issue is elegantly addressed by implementing a spectral demixing strategy allowing 3D simultaneous multi-color nanoscopy. However optimization of

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both sample preparation and image analysis is still a requirement for quantitative multicolor nanoscopy. Valentina Coarsi is Head of R&D in Imaging at Abbelight and will co-supervise this project.

The Laboratory of Optics and Biosciences (LOB) is affiliated with the French Institute of Health and Medical Research (INSERM), the French National Centre for Scientific Research (CNRS), and Ecole Polytechnique/IPP. The Laboratory brings together scientists with expertise in molecular and cellular biology, in optics and in femtosecond dynamics with the aim of developing synergies beyond the themes specific to each specialty, and promoting the emergence of new concepts and tools, in particular for the understanding of the functioning of proteins and their organization within cells and tissues. Nicolas Olivier is CNRS researcher (CR) and he will co-supervise this project.

This project, which will mostly take place at Ecole Polytechnique is primarily experimental and combines aspects of sample preparation (cell biology, immunochemistry, and chemistry), microscopy, and image/data analysis (ImageJ /Python/ Matlab). It is well suited for a student with a background in Biophysics or Physics interested in interdisciplinary work.

#### References:

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