Reliable SMLM cell imaging demonstrated on Abbelight SAFe imaging platform.

N. BOURG[1]

[1] ISMO, Orsay.

Abstract

Biological imaging has successfully pierced the nanoscale in recent years; resolving the intricate interaction of multiple targets in a system naturally represents the next step in the evolution of fluorescence nanoscopy. Among nanoscopy methods, Single molecule localization microscopy (SMLM) gives the best 3D spatial resolution and can offer inherently the largest FOV because of its origin from standard widefield imaging.

Several years ago, Abbelight has co-developped with the group of Dr. Sandrine Lévêque-Fort, a new method of large uniform illumination called ASTER for Adaptable Scanning for Tunable Excitation Regions1. Thanks to ASTER which is integrated into Abbelight SAFe imaging platform, 150 x 150 µm² SMLM images, with the same spatial resolution along this FOV, is now accessible.

Figure 1 a ASTER STORM imaging of COS-7 cells labeled for microtubules and an AF647-coupled secondary antibody, FOV size 200 µm × 200 µm, 20,000 frames at 20 fps. Extracted from [1]

Two dimensional SMLM images is now straightforward to acquire. However biological samples are three dimensional. Therefore, Abbelight SAFe imaging platform has developed and integrated different 3D super-localization strategies to get the best XYZ spatial resolution. Furthermore, Abbelight has recently integrated into its NEO SAFe Software, its own version C Spline fitting algorithm which now offers to achieve the CRLB (Cramer-Rao Lower Bound, the best localization precision which can be achieved).

Figure 2 3D Large FOV SMLM Images (Z color coded, 1 um imaging depth)

With this workshop, we would like to demonstrate the versatility of our SAFe

imaging platform in acquiring large FOV 3D SMLM images. We will image several type of sample: COS7 cells, hippocampal rat neurons and others.

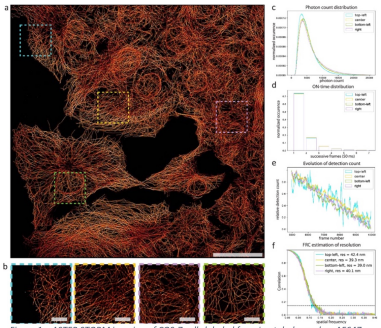

Figure 1 a ASTER STORM imaging of COS-7 cells labeled for microtubules and an AF647-coupled secondary antibody, FOV size 200 µm × 200 µm, 20,000 frames at 20 fps. Extracted from [1]

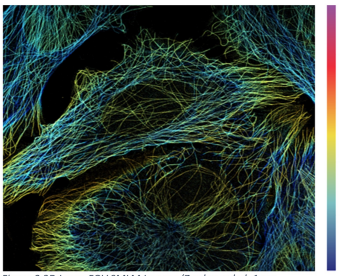

Figure 2 3D Large FOV SMLM Images (Z color coded, 1 um imaging depth)

Schedule

15 min Introduction: SMLM, ASTER and 3D Strategies integrated into Abbelight setup (Presentation)

15 min Acquisition a large FOV 2D Image in STORM and DNA-PAINT

5 min Calibration of the 3D using C Spline fitting

10 min Acquisition of 3D SMLM images

15 min Summary of the workshop & Questions

References

1. A. Mau et al, Fast widefield scan provides tunable and uniform illumination optimizing super-resolution microscopy on large fields, Nat. Communication, 2021