Facilitating access to 3D nanoscopy for biological research

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After a decade of development, the application of localization-based super-resolution imaging methods (SMLM) to biological research continues to be hampered by the complexity of their implementation [1]. In this regard, putting super-resolution microscopy within reach of non-specialists is an indispensable step to fully realize the potential of SMLM methods. To respond to this need, we developed a complete solution for 3D-SMLM, from acquisition to analysis, which improves performances, experimental repeatability, and reliability of the data, and greatly simplifies access to SMLM for non-specialists.

At the core of our system is a dual-view optical setup, which exploits the patented DONALD technology [2]. Based on supercritical angle fluorescence detection (SAF), our setup permits isotropic ~10 nm localization precision up to 1 μm above the glass coverslip; moreover, it is insensitive to axial drift, facilitating deep multifocal imaging, and it greatly reduces chromatic axial aberrations allowing the reliable implementation of multi-color imaging strategies.

In order to simplify experimental execution, and improve reproducibility of dSTORM [3] experiments, we optimized a multicolor chemical buffer, which delivers users from time-consuming trial-and-error attempts.

Finally, we developed a comprehensive and user-friendly software suite that allows users to perform imaging, processing, real-time visualization (with instantaneous lateral drift correction), and analysis of the super-resolution experiment within the same environment, which streamlines experimental execution and improves data quality.

The simple installation of the optical module, together with our software package and dSTORM buffer are sufficient to upgrade any commercial inverted microscope into a powerful 3D-nanoscope, thus simplifying access to for non-specialists.

We describe here a schema of the conversion of a microscope into a 3D-nanoscope, and a detailed imaging workflow. We highlight the performances of our system with examples of multicolor 3D reconstructions of biological samples.

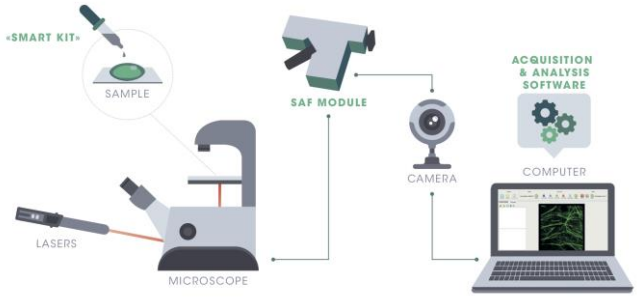

Figure 1 Upgrade of microscope into 3D nanoscope

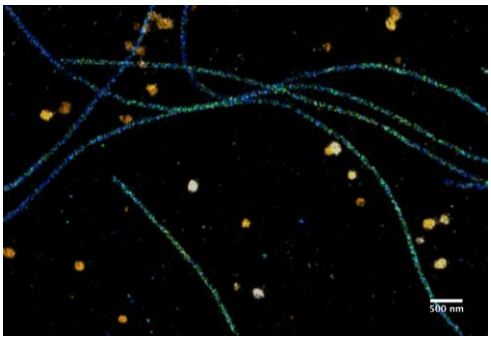

Figure 2 Two-color 3D nanoscopy image (Tubulin-AF555, Clathrin-AF647)

[1] von Diezmann et al., *Chemical Reviews* **117**, 7244-7275 (2017).

[2] Bourg, N. *et al.*, *Nature Photonics* **9**, 587-593 (2015).

[3] Van de linde et al., Nature Protocols, (2011)