Revealing 3D nanometer podosome architecture thanks to DONALD method

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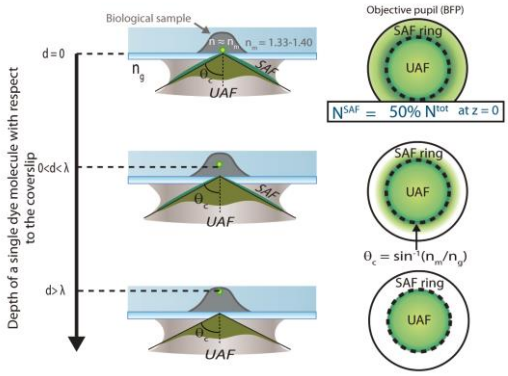

Figure 1 Principle of SAF emission

Single Molecule Localization Microscopy (SMLM) such as PhotoActivated Localization Microscopy or (direct) STochastic Optical Reconstruction Microscopy ((d)STORM) allows the capture of images with a spatial resolution higher than that imposed by the diffraction limit. To retrieve fluorophore axial position with nanometer accuracy, several strategies such as Point Spread Function (PSF) engineering methods or interferometric PALM (iPALM) have been developed and combined to SMLM. Unfortunately, all these strategies imply an increased experimental complexity while they only provide the relative axial positions of the fluorophores with respect to an arbitrary focal plane. Hence, we have decided to take advantage of the forbidden light, also called Supercritical Angle Fluorescence (SAF) [1,2] at the single molecule level. Indeed, when a fluorophore is located in the vicinity of the coverslip interface, the SAF number of photons N^{SAF} decreases exponentially with the distance from the coverslip (Fig. 1), it is straightforward to localize the absolute axial position of each fluorophore by measuring their N^{SAF} thanks to a simple optical module. This new 3D absolute method, that we called "Direct Optical Nanoscopy with Axially Localized Detection" [3], provides an isotropic 3D localization precision of ~15-20 nm within an axial range of ~150 nm above the coverslip. Axial position can also be measured within the first 600 nm within the sample, but with a lower localization precision.

This unique absolute property can be extremely useful in the case of multiplexing SMLM experiments and biological studies of cell membrane, motility or adhesion complexes such as focal adhesion and podosome. Podosomes are adhesion structures involved in degradation of the extracellular matrix and formed by macrophages and monocyte/macrophage-derived cells. If iPALM [4] have been used successfully used to reveal focal adhesion organization, structure of the podosomes is still unknown due to its axial extension (~600nm). After an introduction of the principle of SMLM methods and a detailed description of DONALD technique, we will present the 3D organization of podosome ring proteins around the F-actin core revealed by our nanoscope (Fig. 2).

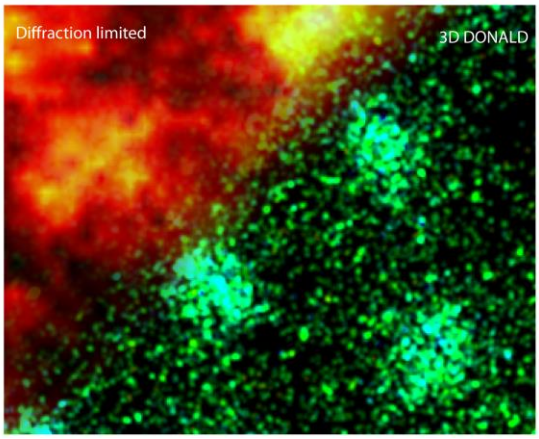

Figure 2 : Actin network of podosomes

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[3] N. Bourg et al., Direct Optical Nanoscopy with Axially Localized Detection (DONALD), Nat. Photonics, 2015, in press
[4] Kanchanawong, P., et al., Nanoscale architecture of integrin-based cell adhesions. Nature, 2010. 468(7323): p. 580-4