Three-dimensional total internal reflection fluorescence nanoscopy with sub-10 nm resolution

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Imaging the three-dimensional organization of biological structures down to the size of their structural proteins, ~ 4 to 10 nm, can open up exciting opportunities in the life sciences. Although this level of resolution has been reached in the (*x*,*y*) plane by DNA-PAINT and MINFLUX techniques [1-2], the issue is not yet solved for the axial counterpart. Axial resolution of fluorescence nanoscopy using a single objective lens lies in the range of 35 to 120 nm and while the 4Pi configuration allows axial resolutions well below 35 nm this comes at the cost of increased technical complexity [3]. Here, we present Supercritical Illumination Microscopy by Photometric z-Localization Encoding (SIMPLE), an easy-to-implement photometric method to determine the axial position of molecules near a dielectric interface under total internal reflection (TIR) excitation. SIMPLE consists of calibrating the detected fluorescence signal considering both the exponential decay of the excitation field (with a small non-evanescent component) and the *z*-dependant emission of single molecules that are close to the interfase, in order to retrieve the axial position of single molecules from a direct measurement of their detected fluorescence signal. In combination with DNA-PAINT, SIMPLE delivers sub-10 nm resolution in all three dimensions, enabling the direct recognition of protein assemblies at the molecular level (Figure 1).

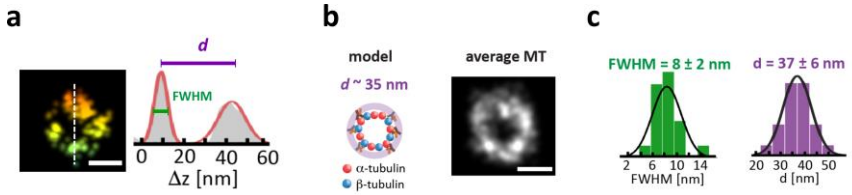

Figure 1. Axial reconstruction of immunolabelled microtubules in COS-7 cells by SIMPLE. (a) (*y*,*z*) image of a single microtubule together with its *z*-profile. (b) Average (*y*,*z*) microtubule image (n = 8) compared to its molecular model. (c) Histograms of the FWHM and peak to peak distances (microtubule diameter) calculated from *z*-profiles of single microtubules (n ~ 20). Scale bar represents 25 nm.

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