THREE-DIMENSIONAL TOTAL-INTERNAL REFLECTION FLUORESCENCE NANOSCOPY WITH NANOMETRIC AXIAL RESOLUTION BY PHOTOMETRIC LOCALIZATION OF SINGLE MOLECULES

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Single-molecule localization microscopy (SMLM) enables far-field imaging with lateral resolution in the range of 10 to 20 nanometres, exploiting the fact that the centre position of a single-molecule's image can be determined with much higher accuracy than the size of that image itself. However, attaining the same level of resolution in the axial (third) dimension remains challenging.

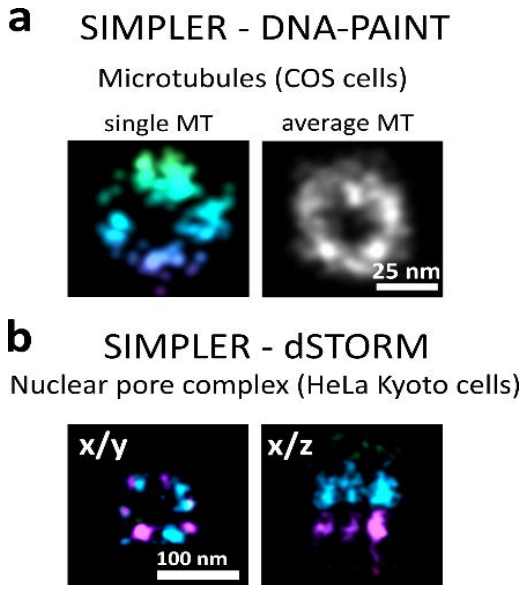

Figure 1. Example of (a) SIMPLER-DNA-PAINT and (b) SIMPLER-dSTORM imaging.

Here, we present Supercritical Illumination Microscopy Photometric z-Localization with Enhanced Resolution (SIMPLER), a photometric method to decode the axial position of single molecules in a total internal reflection fluorescence microscope [1]. Through a full theoretical modelling, including the evanescent illumination, the modulation of the angular emission and the shape of the single-molecule signals in the image plane, we demonstrate that the TIRF intensity signal can be effectively represented by just three parameters. This parameterization allows the determination of the axial position of individual molecules from a single measurement of their emission intensity. SIMPLER is fully compatible with any 2D SMLM method and requires no hardware modification to a TIRF SMLM microscope.

We will present the fundamentals of SIMPLER and performance examples of SIMPLER-dSTORM and SIMPLER-DNA PAINT reaching sub-20 nm and sub-10 nm localization precision, respectively (Fig. 1).

[1] A.M. Szalai, B. Siarry, J. Lukin *et al.* "Three-dimensional total-internal reflection fluorescence nanoscopy with nanometric axial resolution by photometric localization of single molecules." *Nat Commun* **12,** 517 (2021).