SMLM with light sheet illumination for deep cell imaging

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Single molecule localization microscopy methods like PALM [1] and STORM [2] allow to overcome the optical diffraction limit by a factor of ~10. However, these methods are limited to imaging a single focal plane in the sample because they generally use widefield or TIRF illumination. In TIRF, only the first hundred nanometers above the coverslip are imaged while in widefield deeper focal plane imaging is permitted but the out of focus planes are photobleached preventing their imaging in high resolution. In Single Plane Illumination microscopy (SPIM) [3], a single focal plane is illuminated by a thin light sheet, thereby leaving the out of focus planes free of photobleaching. Scanning the light sheet then enables volumetric imaging of the cell with a confocal resolution.

Taking advantage of both methods, and following previous work, we aimed to perform SMLM using a thin light sheet illumination in order to achieve volumetric high resolution microscopy of entire cells [4, 5, 6]. We present the development of a SPIM system for SMLM, which allows to perform high resolution imaging deeper in the cells. The microscope is based on a commercial body using 2 high numerical aperture and high magnification objective lenses oriented at 120° from each other. This configuration allows to create a very thin light sheet and to optimize photon collection [7]. We will show preliminary applications of this system to imaging nuclear pores and mitochondria at high resolution up to ~25 µm in depth.

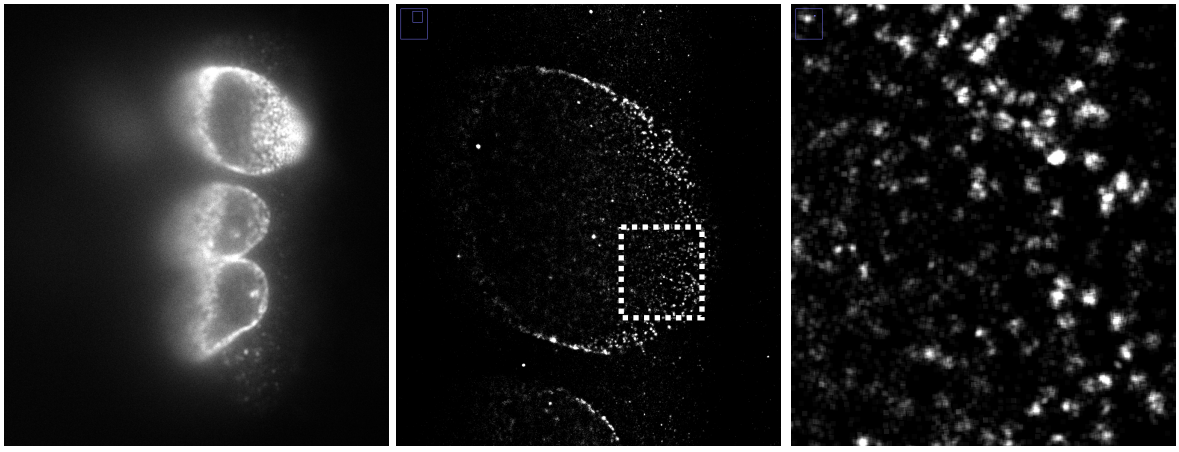

Figure 1: Images of nuclear pores with the oblique SPIM microscope. **a)** Low resolution image of three nuclei stained with NUP96-SNAP A647. **b)** High resolution image of the top nucleus. **c)** Zoomed view of the region in the white dashed rectangle in (b) showing single nuclear pore complexes.

(1) E.Betzig, et al., "*Imaging Intracellular Fluorescent Proteins at Nanometer Resolution »,* **Science** (2006)
(2) M.Rust, et al., "*Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)*", **Nat Meth** (2006)
(3) Jan Huisken, et al., "*Optical Sectioning Deep Inside Live Embryos by Selective Plane Illumination Microscopy*", **Science** (2004)
(4) A-K Gustavsson et al., "*3D single-molecule super-resolution microscopy with a tilted light sheet"*, **Nat Comm** (2018)
(5) F.Cella.Zanacchi et al., "*Live-cell 3D super-resolution imaging in thick biological samples"*, **Nat Meth** (2011)
(6) W.R.Legant et al., "*High-density three-dimensional localization microscopy across large volumes"*, **Nat Meth** (2016)
(7) Theer P, et al., "*pi-SPIM: high NA high resolution isotropic light-sheet imaging in cell culture dishes"*, **Sci Rep** (2016)