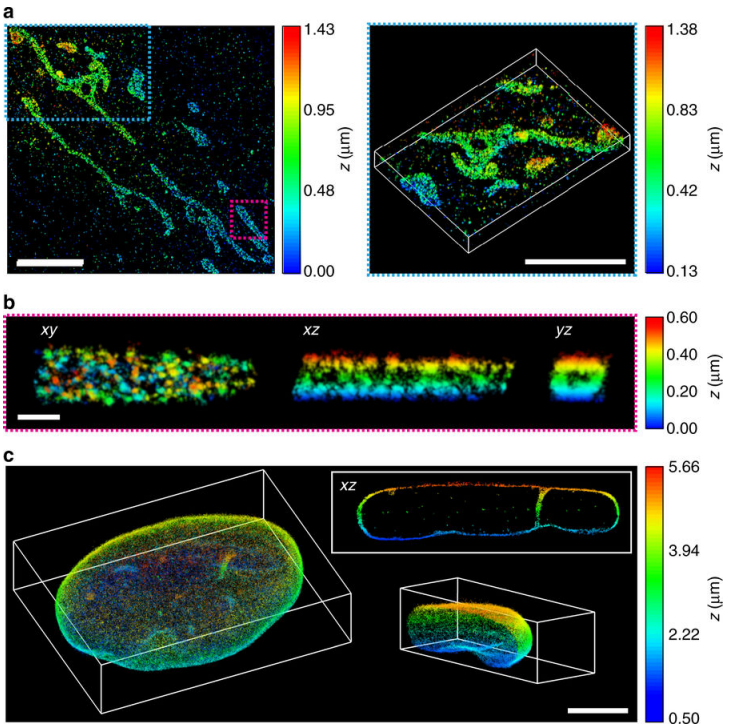
DoubleHelix

Application Note: TILT3D

We present TILT3D, an imaging platform that combines a novel, tilted light sheet illumination strategy with engineered Point Spread Function (E-PSF) technology to enable high SNR 3D super-resolution imaging in thick cells. Through the combination of the optimally engineered long-range tetrapod and high-precision extended depth double helix (DH-PSF) point spread functions, the axial positions of single emitters are uniquely encoded into their shape, thus eliminating the need for extremely thin and complicated light-sheet illumination schemes. This allows TILT3D to be built upon a standard inverted microscope with minimal custom parts,



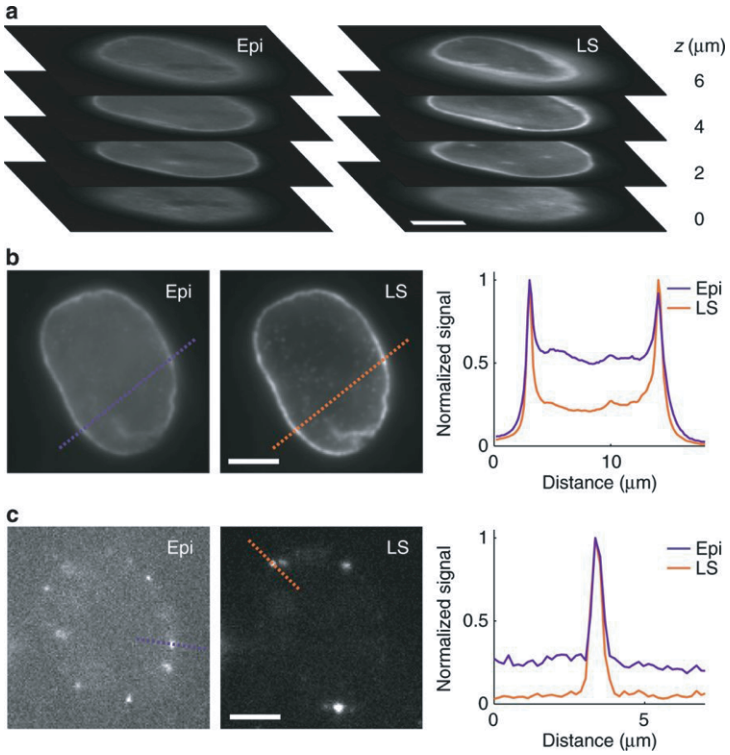
Tilted light sheet imaging with long axial range point spread functions (PSFs). a 3D super-resolution (SR) reconstructions of mitochondria (TOM20) in a HeLa cell. The double-helix (DH) PSF was used for imaging of both single molecules and fiducial beads. b xy, xz, and yz views of the mitochondrion shown in the magenta rectangle in a, revealing the hollow cylinder structure of the mitochondrial outer membrane. c 3D SR reconstruction of the entire nuclear lamina (lamin B1) in a HeLa cell. Imaging of single molecules and fiducial beads was performed with the DH-PSF and a 6-µm tetrapod PSF, respectively. The xz view shows a 1.3-µm thick y-slice through the cell, where lamin meshwork enveloping an intranuclear channel is visible. The lower right inset shows the right cap of the reconstruction. All samples imaged were immunolabeled with Alexa Fluor 647. Scale bars are 5 µm in a and c, and 500 nm in b.

"TILT3D offers a simple yet powerful tool for 3D SR imaging in whole mammalian cells using only a few axial light sheet positions."

resulting in a simple and flexible 3D super-resolution (SR) imaging solution that provides tens of nm localization precision throughout thick mammalian cells. The small (10°) tilt allows for sectioning and imaging of cells all the way down to the coverslip with a well-defined light sheet shape. Furthermore, two perpendicular objectives in close proximity are not required, which enables imaging with a high NA objective. No dipping of the objectives into the sample chamber is necessary, which reduces the risk of sample contamination.

The TILT3D Advantage

- High localization precision of single molecules in 3D over the entire axial range of a mammalian cell via a few thick light sheet slices combined with imaging with engineered PSFs in each slice.
- Light sheet illumination in combination with PSF engineering drastically improves the localization precision of single molecules and reduces photobleaching and photodamage of the sample as compared to conventional epi-illumination.
- Easy and cost-efficient to implement with a flexible design that allows for imaging close to a conventional coverslip using a high NA detection objective and easy switching between illumination modes, lasers, and PSFs.



Sectioning using light sheet illumination improves contrast in 2D imaging. **a** Comparison between epi- (Epi) and light sheet (LS) illumination at different z-positions throughout a cell nucleus. The image plane was moved using the piezoelectric objective scanner, and for LS imaging the LS plane was moved together with the image plane using the motorized mirror. The sectioning capability of the LS is clearly demonstrated, showing an improved contrast when compared with epi-illumination for all cell sections. **b** Diffraction-limited and **c** single-molecule images demonstrating that light sheet illumination improves the signal-to-background ratio up to fivefold compared to conventional epi-illumination. Graphs show line scans over the dashed lines in the images. All images show lamin B1 immunolabeled with Alexa Fluor 647 in HeLa cells. Compared images are shown with the same linear grayscale, respectively. Scale bars are 5 µm.

"TILT3D will become an important tool not only for 3D super-resolution imaging, but also for live whole-cell single-particle and single-molecule tracking."

References

For more information on TILT3D, please refer to the original paper: Gustavsson, A.-K., Petrov, P. N., Lee, M. Y., Shechtman, Y., & Moerner, W. E. (2018). 3D single-molecule super-resolution microscopy with a tilted light sheet. *Nature Communications*, *9* (1), 123.; https://doi.org/10.1038/s41467-017-02563-4

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