

## 3D single-molecule super-resolution microscopy with a tilted light sheet



Tilted light sheet microscopy with 3D point spread functions (TILT3D) combines tilted light sheet illumination with long axial range point spread functions (PSFs) for low-background, 3D single-molecule super-resolution imaging with tens of nm precision throughout thick mammalian cells.



The tilted light sheet (LS) sections thick mammalian cells and improves contrast up to five-fold relative to epi-illumination (Epi). The phase of the light emitted from single molecules and fiducial beads in the sample is modulated by the Double-helix and the Tetrapod phase masks to encode information about the 3D position of the emitters.

3D single-molecule super-resolution reconstructions of (a) mitochondria and (b) the nuclear lamina in HeLa cells. (c) 1.3  $\mu$ m thick y-slice through the cell in (b) showing a lamina channel. Scale bars are 5  $\mu$ m.

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