MULTICOLOR 3D MINFLUX NANOSCOPY FOR BIOLOGICAL IMAGING

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The ultimate goal of biological super-resolution fluorescence microscopy is to provide three-dimensional resolution at the size scale of a fluorescent marker. Due to the limited number of photons that fluorescent molecules can emit before bleaching, the resolution of STED or PALM/STORM remained constrained to roughly 10-20 nanometers in experiments. By probing the position of switchable fluorophores with a minimum of excitation light, MINFLUX renders the emitted photons more informative while leaving the photon budget untouched [1]. Compared to standard camera-based localization schemes, fewer photons are thus required to deduce the position of the molecule with a certain precision.

Here we show that MINFLUX nanoscopy can provide resolutions in the range of 1 to 3 nm for structures in fixed and living cells [2]. This progress has been facilitated by approaching each fluorophore iteratively with the probing-donut minimum, making the resolution essentially uniform and isotropic over scalable fields of view. By exploting a minimum of excitation light confined in all dimensions, we further demonstrate MINFLUX imaging of the nucleoporin Nup96 in mammalian cells with nanometer-scale resolution in three dimensions and two color channels. We show first 3D two-color MINFLUX acquisitions of dense protein distributions in human mitochondria. The unprecedented 3D resolution of the images paves the way for new quantitative analysis approaches that we exploit for studying the distribution of proteins within the heterooligomeric MICOS protein complex.

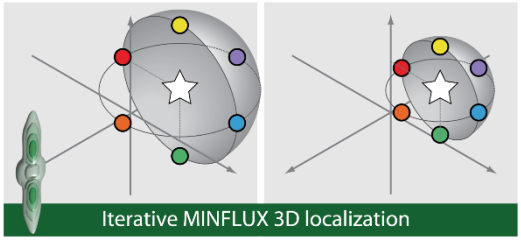

Figure 1: Schematic of the iterative 3D MINFLUX localization scheme using a a 3D donut-shaped excitation beam.

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