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 exploiting 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.

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