Mitochondrial Cristae Revealed with Focused Light

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Supplemental materials

1. Immunofluorescence labelling:

PtK2 (kangaroo rat) cells were grown on cover slips. Cells were fixed with 8 % (w/v) formaldehyde in PBS (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) for 10 min at 37 °C, extracted with 0.1 % (w/v) SDS in PBS, and blocked with 5 % (w/v) BSA in PBS. Subsequently, cells were incubated with a monoclonal mouse antiserum directed against the alpha subunit of the mitochondrial F_1F_oATPase (Molecular Probes, Eugene, OR, USA). The primary antibodies were detected with secondary antibodies (sheep anti-mouse; Jackson ImmunoResearch Laboratories, West Grove; PA; USA) custom labelled with the fluorophore KK114. After several washing steps with PBS, the samples were prepared for all-optical isoSTED-imaging.

2. Depletion of mitofilin:

RNAi experiments were performed in Ptk2 cells as described using the short hairpin RNAi construct pAVU6mitofilin.[1]

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3. IsoSTED imaging:

For isoSTED imaging, the PBS buffer was exchanged by a dilution series with TDE (2,2'-thiodiethanol) in PBS,[2] finally resulting in an embedding medium of 97 % (v/v) TDE in PBS. The sample was covered with a second cover slip that was sparsely coated with fluorescent beads (Crimson fluorescent microspheres, specified diameter: 100 nm; Molecular Probes, Eugene, OR, USA) to facilitate the initial alignment of the isoSTED nanoscope. Excitation at a wavelength of 635 nm was performed with a pulsed semiconductor laser (LDH-P-C 635b with PDL 800-B, PicoQuant, Berlin, Germany) delivering <100 ps excitation pulses which were synchronized with the ~1 ns long pulses of the STED laser. For STED we used a frequency doubled fiber laser (ELP-5-775-DG, IPG Photonics Corporation, Oxford, MA, USA) operating at 20 MHz and at a wavelength of 775 nm. The time-averaged STED power in the sample was 100 mW. Detection of the emission of the fluorophore KK114 was carried out in the 660–700 nm wavelength range using a photon-counting avalanche photodiode (APD) (PerkinElmer, Waltham, MA, USA). The performance of the isoSTED nanoscope was assessed by imaging fluorescent beads (Crimson fluorescent microspheres, specified diameter: 40 nm and 20nm, Molecular Probes, Eugene, OR, USA), showing an effective point spread function (PSF) diameter of about 30 nm (Figure S1a). Images of mitochondria were non-linearly deconvolved by applying 12-24 iterations of a Richardson-Lucy algorithm[3] (Figure S1b) utilizing an estimated PSF with 30 nm width to account for the blurring effects of the imaging system.

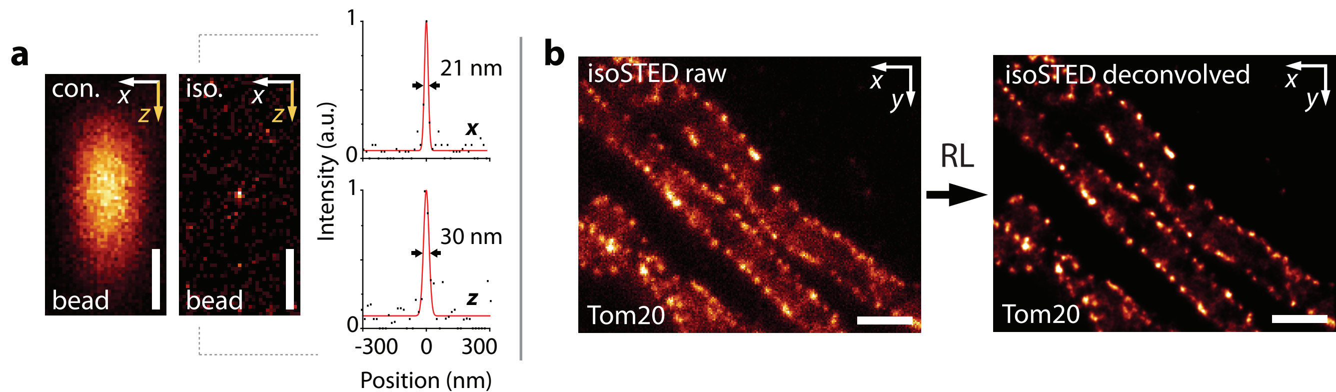

Figure S1. IsoSTED PSF and image deconvolution. (a) Panels show *xz* images of fluorescent microspheres, recorded in confocal (left) and isoSTED mode (right, bead diameter <20 nm). The FWHM of the effective PSF was seen to be in the 30 nm range. (b) STED recordings of mitochondria (raw data, left) and deconvolved. (right). Scale bars: 250 nm (a) and 500 nm (b).

References

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