LINE-RESCANNED STED MICROSCOPY IS GENTLER AND FASTER THAN POINT-DESCANNED STED MICROSCOPY

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ABSTRACT: Stimulated emission depletion (STED) [1] substantially improves the resolution of point-scanning confocal microscopy, at the cost of reduced emission signal and massively increased illumination dose. The resulting tradeoffs between resolution, speed, signal-to-noise ratio, and photodamage can be frustrating, especially for live samples. We describe a gentler, much faster alternative technique based on a combination of STED with line-scanning confocal microscopy [2], analog structured illumination microscopy [3], and multi-image fusion [4].

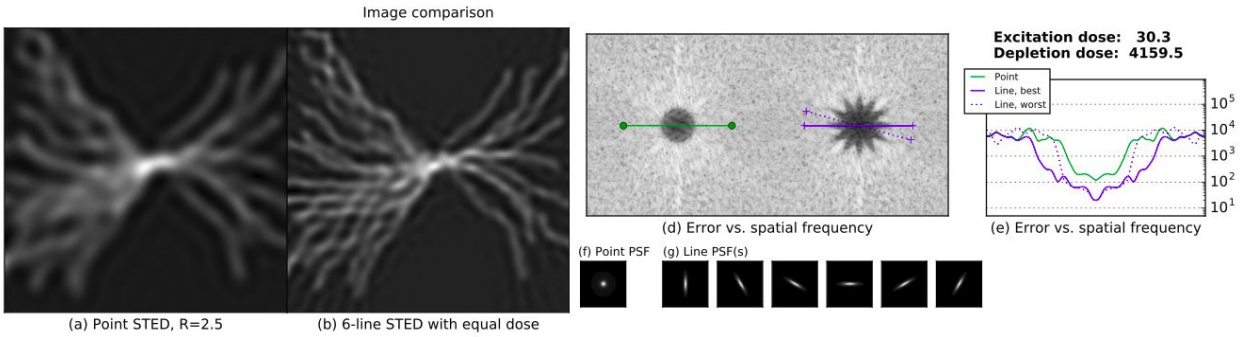

Figure 1: Line STED gives higher image quality than point STED for the same photodose. (a) Simulated point-STED image. (b) Simulated line-STED image using the same excitation and depletion dose as (a). (d) Reconstruction error vs. spatial frequency for (a) (point STED, left panel) and (b) (line STED, right panel); darker pixels indicate lower error. Values along the lines drawn in (d) are plotted in (e). Green line in (d) and (e) shows point STED reconstruction error vs. spatial frequency, blue lines show line-STED reconstruction error vs. spatial frequency along a worst-case direction (dotted blue), and a best-case direction (solid blue). (f) Point-STED PSF used for simulated imaging and deconvolution in (a). (g) Line-STED PSF(s) used for simulated imaging and deconvolution-based image fusion in (b), one for each scan direction.

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