biosensors

Supporting information

Ultralow Laser Power Three-Dimensional Superresolution Microscopy Based on Digitally Enhanced STED

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S1. Image acquisition

Detailed parameters of the image acquired process are as follows:

1. Image of fluorescent beads on the lateral profile: (1) acquisition mode: xyz, (2) scan direction: unidirectional, (3) scan speed: 400Hz, (4) format: 512×512, (5) pixel size (micron): 0.015, (6) frame average: 1, (7) line average: 1, (8) pixel dwell time (microsecond): 1.44.
2. Image of fluorescent beads on 3D: (1) acquisition mode: xzy, (2) scan direction: unidirectional, (3) scan speed: 400Hz, (4) format: 1024×1024, (5) pixel size (micron): 0.018, (6) frame average: 1, (7) line average: 1, (8) pixel dwell time (microsecond): 0.721.
3. Imaging of the lateral nuclear pore complex: (1) acquisition mode: xyz, (2) scan direction: unidirectional, (3) scan speed: 400Hz, (4) format: 1024×1024, (5) pixel size (micron): 0.020, (6) frame average: 1, (7) line average: 1, (8) pixel dwell time (microsecond): 0.721.
4. Image of nuclear pore complexes in 3D: (1) acquisition mode: xzy, (2) scan direction: unidirectional, (3) scan speed: 600Hz, (4) format: 1024×1024, (5) pixel size (micron): 0.021, (6) frame average: 1, (7) line average: 1, (8) pixel dwell time (microsecond): 0.48.

S2. Deconvolution

Deconvolution is performed with the Huygens software (Scientific Volume Imaging). Detailed parameters of the deconvolution process are as follows: (1) background: automatic estimation, (2) estimate mode: lowest, (3) area radius (micron): 0.7, (4) deconvolution algorithm: CMLE, (5) maximum iterations: 10, (6) signal-to-noise ratio: 7, (7) quality threshold: 0.05, (8) iteration mode: optimized, (9) brick layout: auto and (10) PSFs per brick: auto.

S3. Mathematical formula of 3D DE-STED imaging

The PSFs of the donut and the 3D DE-STED images are calculated with the formulas:

$$\mathrm{PSF}_{\text{Donut Lateral}} = \mathrm{PSF}_{\text{Confocal Lateral}} - \mathrm{PSF}_{\text{STED Lateral}} \tag{S1}$$

$$\mathrm{PSF}_{\text{Donut Axial}} = \mathrm{PSF}_{\text{Confocal Axial}} - \mathrm{PSF}_{\text{STED Axial}} \tag{S2}$$

where $\mathrm{PSF}_{\text{Confocal Lateral}}$, $\mathrm{PSF}_{\text{STED Lateral}}$, and $\mathrm{PSF}_{\text{Donut Lateral}}$ are the PSFs of confocal, STED, and donut images on the lateral side; $\mathrm{PSF}_{\text{Confocal Axial}}$, $\mathrm{PSF}_{\text{STED Axial}}$, $\mathrm{PSF}_{\text{Donut Axial}}$ are the PSFs of confocal, STED, and donut images on the axial side.

$$\mathrm{PSF}_{\text{DE−STED Lateral}} = \mathrm{PSF}_{\text{Confocal Lateral}} - \mathrm{K} \times \mathrm{PSF}_{\text{Donut Lateral}} \tag{S3}$$

$$\mathrm{PSF}_{\text{DE−STED Axial}} = \mathrm{PSF}_{\text{Confocal Axial}} - \mathrm{K} \times \mathrm{PSF}_{\text{Donut Axial}} \tag{S4}$$

where $PSF_{DE-STED\ Lateral}$ is the PSF of 3D DE-STED in the lateral direction, $PSF_{DE-STED\ Axial}$ is the PSF of 3D DE-STED in the axial direction, and K is the enhancement coefficient.

S4. Calculation of the SNR and PSNR

The SNR and PSNR of the DE-STED images are calculated with the formulas:

$$MSE = \frac{1}{mn}\sum_{i=0}^{m-1}\sum_{j=0}^{n-1}\|f(x,y) - F(x,y)\|^2 \quad \text{(S5)}$$

$$SNR = 10 \cdot log_{10}\left[\frac{\sum_{x=1}^{N_x}\sum_{y=1}^{N_y}(f(x,y))^2}{\sum_{x=1}^{N_x}\sum_{y=1}^{N_y}(f(x,y)-F(x,y))^2}\right] \quad \text{(S6)}$$

$$PSNR = 10 \cdot log_{10}\left[\frac{\sum_{x=1}^{N_x}\sum_{y=1}^{N_y}(255)^2}{\sum_{x=1}^{N_x}\sum_{y=1}^{N_y}(f(x,y)-F(x,y))^2}\right] = 10 \cdot log_{10}\left[\frac{255^2}{MSE}\right] \quad \text{(S7)}$$

where m and n represent the number of pixels in the X-and Y-directions; F(x, y) donates the original confocal image; f(x, y) donates the DE-STED image; and MSE is the mean squared error.

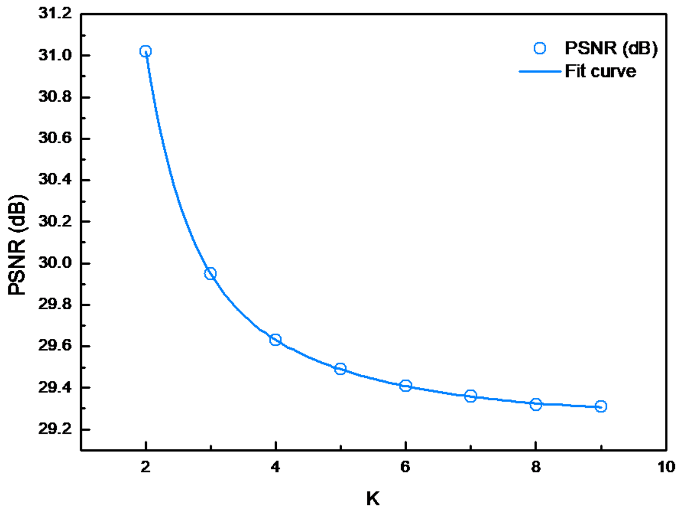

Figure S1. The curves of the relationship between PSNR and the K value of fluorescent beads 3D DE-STED image.