

Supporting information

# Low-Power Two-Color Stimulated Emission Depletion Microscopy for Live Cell Imaging

Jia Zhang, Xinwei Gao, Luwei Wang, Yong Guo, Yinru Zhu, Zhigang Yang, Wei Yan \* and Junle Qu \*

Key Laboratory of Optoelectronic Devices and System of Ministry of Education and Guangdong Province, College of Physics and Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China; julyzhang2021@163.com (J.Z.); 1910454012@email.szu.edu.cn (X.G.); wanglowell@szu.edu.cn (L.W.); 1800284004@email.szu.edu.cn (Y.G.); 1900453009@email.szu.edu.cn (Y.Z.); zhgyang@szu.edu.cn (Z.Y.)  
 \* Correspondence: weiyang@szu.edu.cn (W.Y.); jlqu@szu.edu.cn (J.Q.)

### Supporting information content:

Figure S1: The comparison in resolution between confocal and STED images at different depletion powers of 488 nm channel

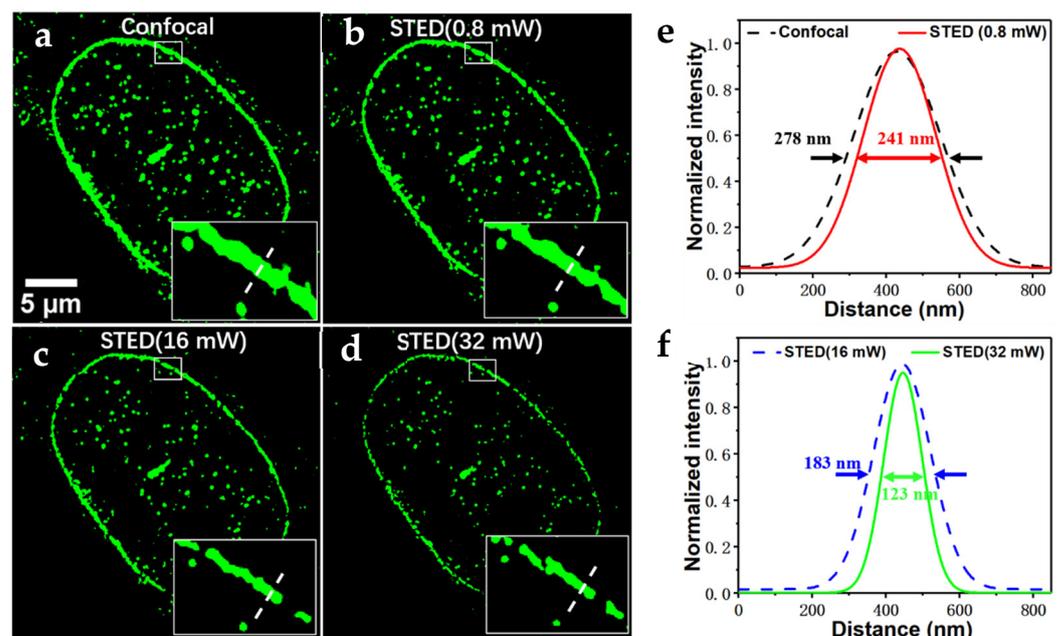
Figure S2: The images of nuclear envelope in a fixed U2OS cell in 488 nm channel

Figure S3: The comparison in resolution between confocal and STED images at different depletion powers in 635 nm channel

Figure S4: The imaging of tubulin in a fixed U2OS cell in 635 nm channel

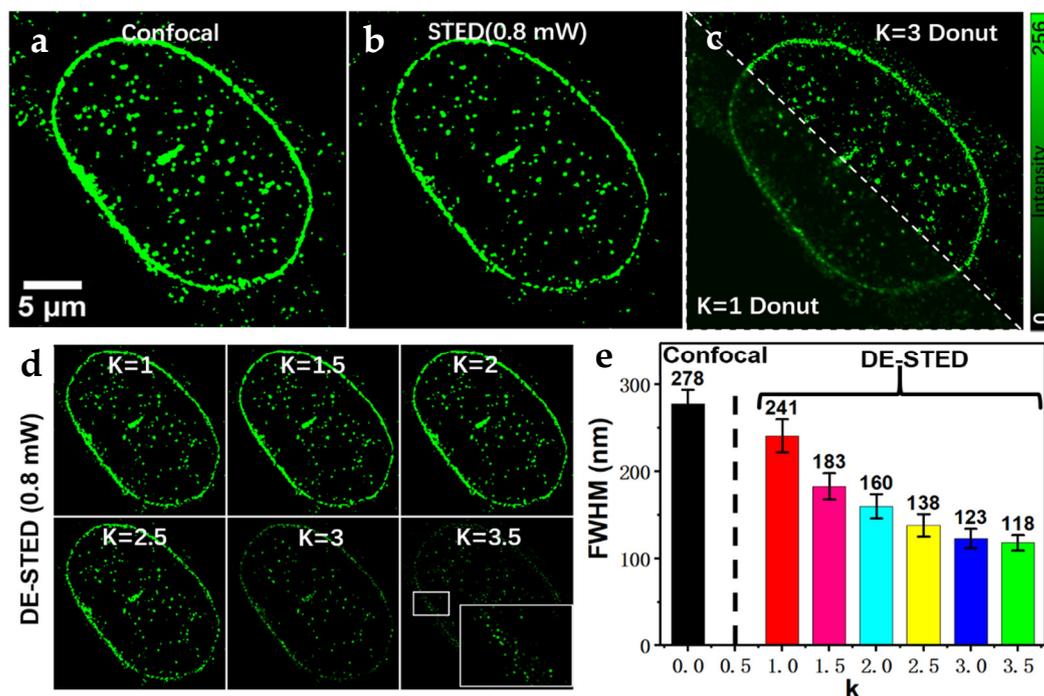
Figure S5: Long-term dynamic imaging of U2OS cell mitochondria in STED mode

Figure S6: The comparison in resolution between confocal, STED, and DE-STED images of living U2OS cell

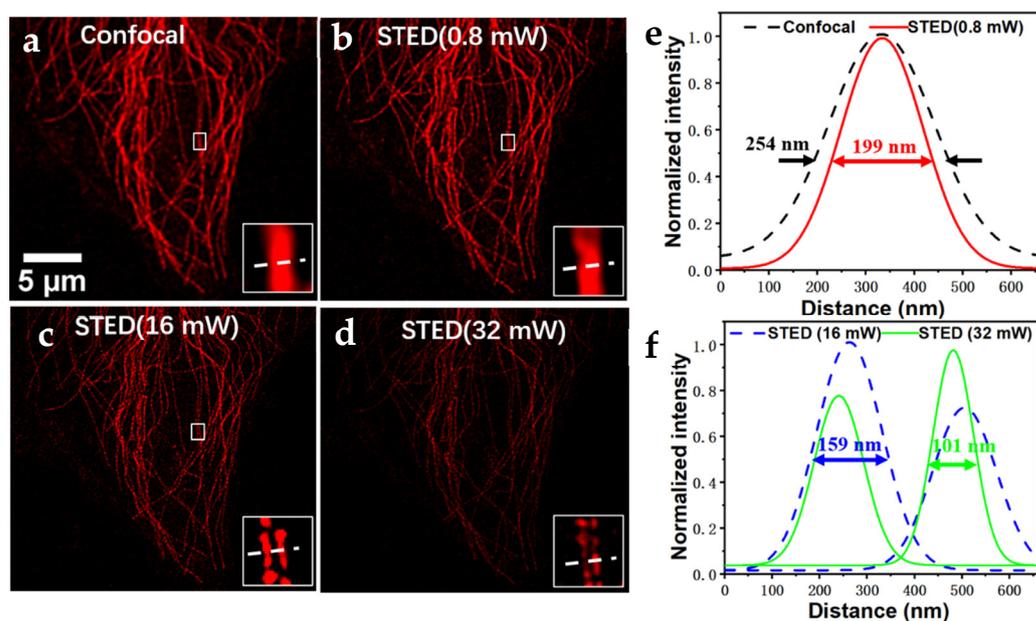


**Figure 1.** The comparison in resolution between confocal and STED images at different depletion powers of 488 nm channel. (a) Confocal image, (b)–(d) STED images obtained at depletion power of 0.8 mW, 16 mW and 32 mW. (e) Normalized intensity profiles along the white dashed lines in a and b for confocal (black curve) and STED (red curve) images; (f) Normalized intensity profiles

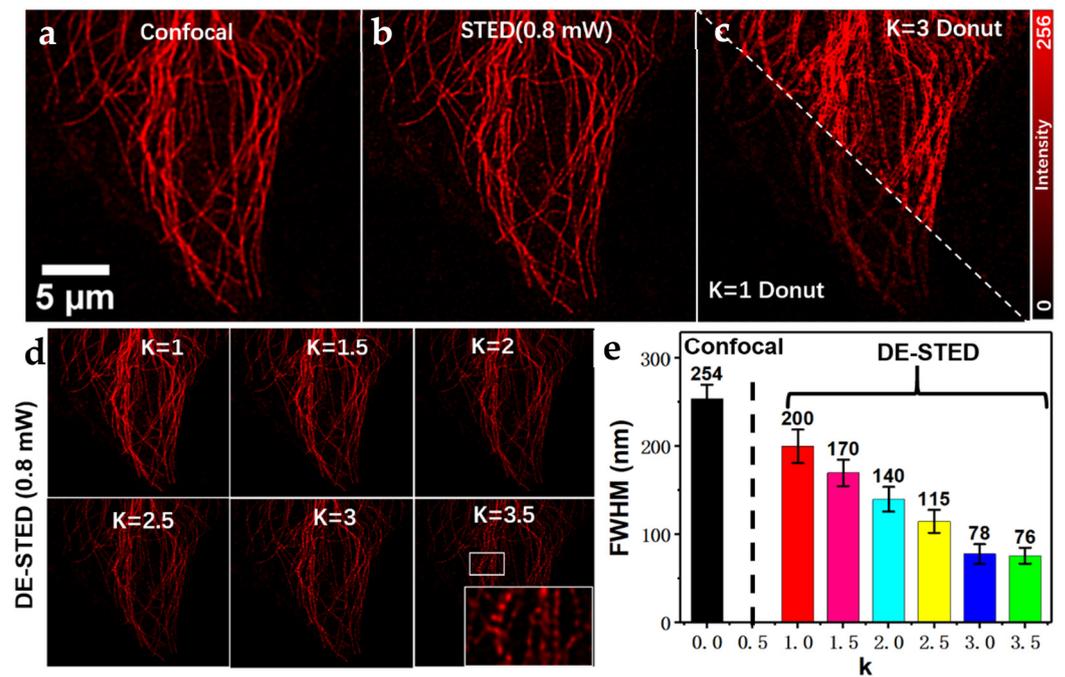
along the white dashed lines in c and d for 16 mW (blue curve) and 32 mW (green curve) images; Zoomed views of the boxed regions in white are shown in the insets. Scale bar: 5  $\mu\text{m}$ .



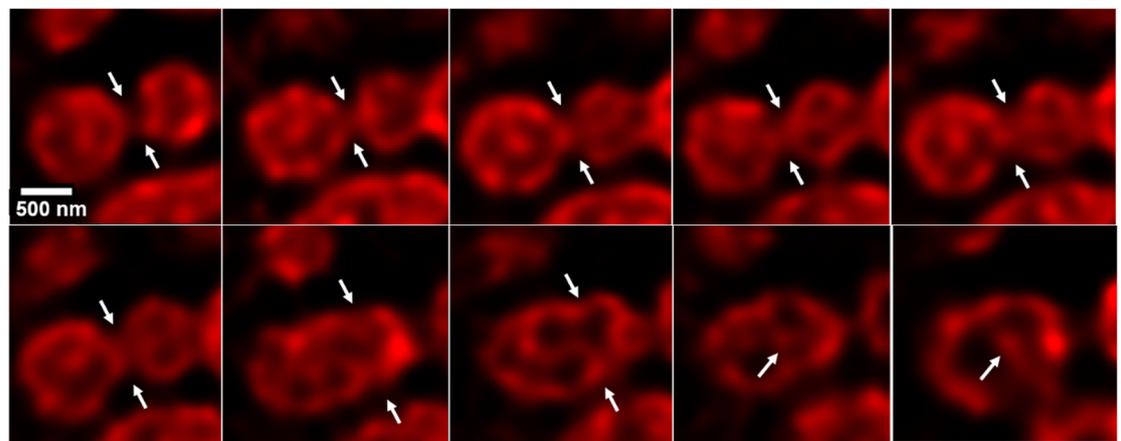
**Figure 2.** The images of nuclear envelope in a fixed U2OS cell in 488 nm channel. (a) Confocal image, (b) STED image at depletion power of 0.8 mW. (c) Donut image with k value of 1 and 3. (d) DE-STED images with the increase of k value. (e) Dependence of average FWHM of nuclear envelope on the k value. Scale bar: 5  $\mu\text{m}$ .



**Figure 3.** The comparison in resolution between confocal and STED images at different depletion powers in 635 nm channel. (a) Confocal image, (b)–(d) STED images obtained at depletion power of 0.8 mW, 16 mW and 32 mW. (e) Normalized intensity profiles along the white dashed lines in a and b for confocal (black curve) and STED (red curve) image; (f) Normalized intensity profiles along the white dashed lines in c and d for 16 mW (blue curve) and 32 mW (green curve) image; Zoomed views of the boxed regions in white are shown in the insets. Scale bar: 5  $\mu\text{m}$ .



**Figure 4.** The imaging of tubulin in a fixed U2OS cell in 635 nm channel. (a) Confocal image, (b) STED image at depletion power of 0.8 mW. (c) Donut image with k value of 1 and 3. (d) DE-STED images with the increase of k value. (e) Dependence of average FWHM of tubulin on the k value. Scale bar: 5 μm.



**Figure S5.** Long-term dynamic imaging of U2OS cell mitochondria in STED mode, the white arrow indicates a typical mitochondrial fusion process, one frame every 3 minutes. Scale bar: 500 nm.

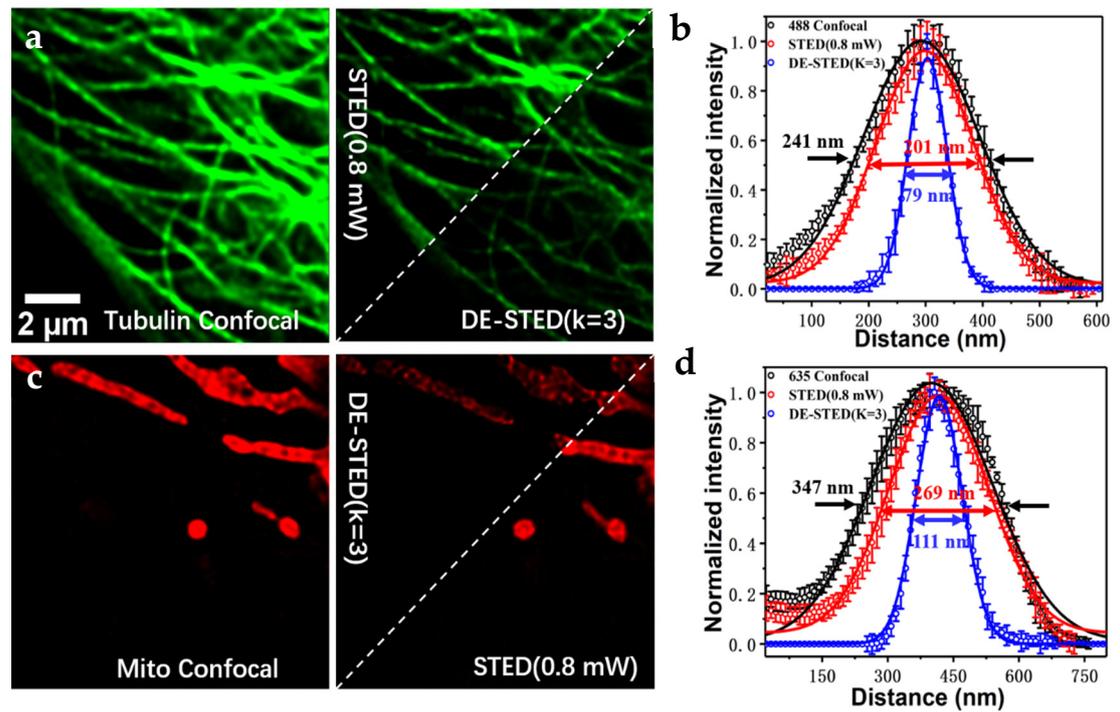


Figure 6. The comparison in resolution between confocal, STED, and DE-STED images of living U2OS cell. (a) Confocal, STED, and DE-STED(k=3) images of tubulin; (b) Mean cross section of tubulin in (a); (c) Confocal, STED, and DE-STED(k=3) images of mitochondrial; (d) Mean cross section of mitochondrial in (c). Scale bar: 2 μm.