Supplementary Information : Pinhole Engineering based Enhanced Resolution (PEER) for Fluorescence Lifetime Imaging Microscopy

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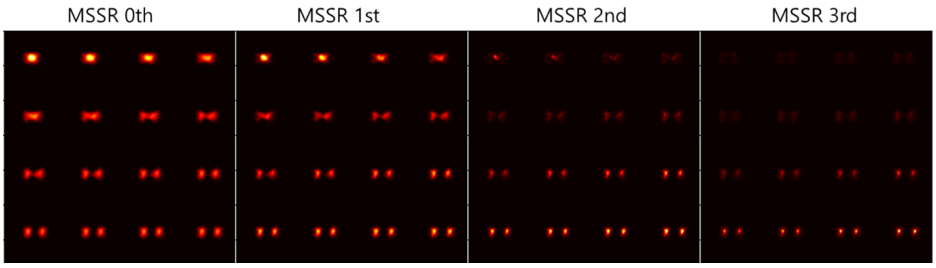
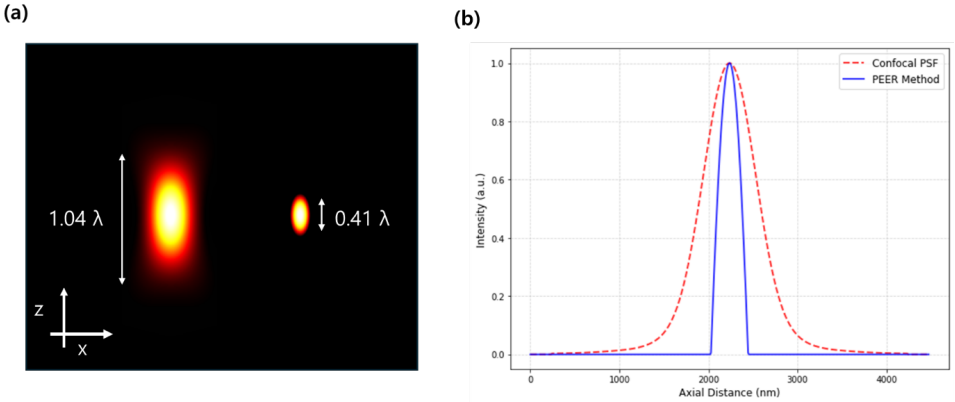
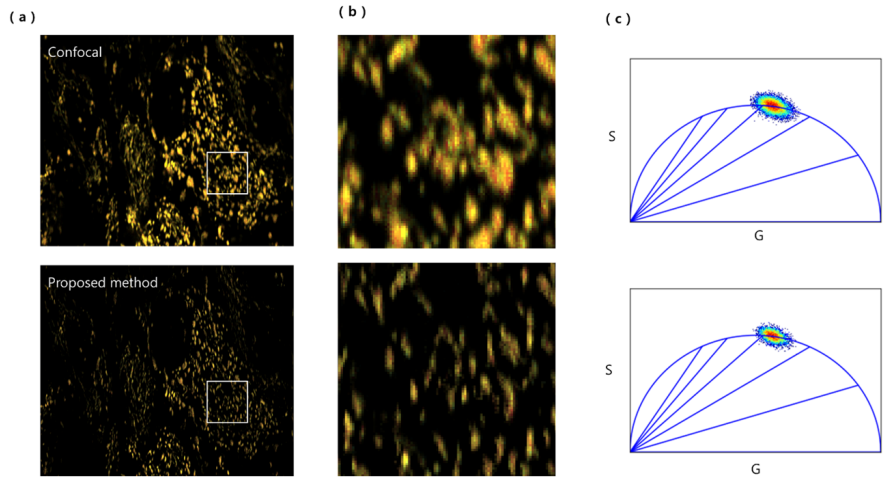
Supplementary Figures



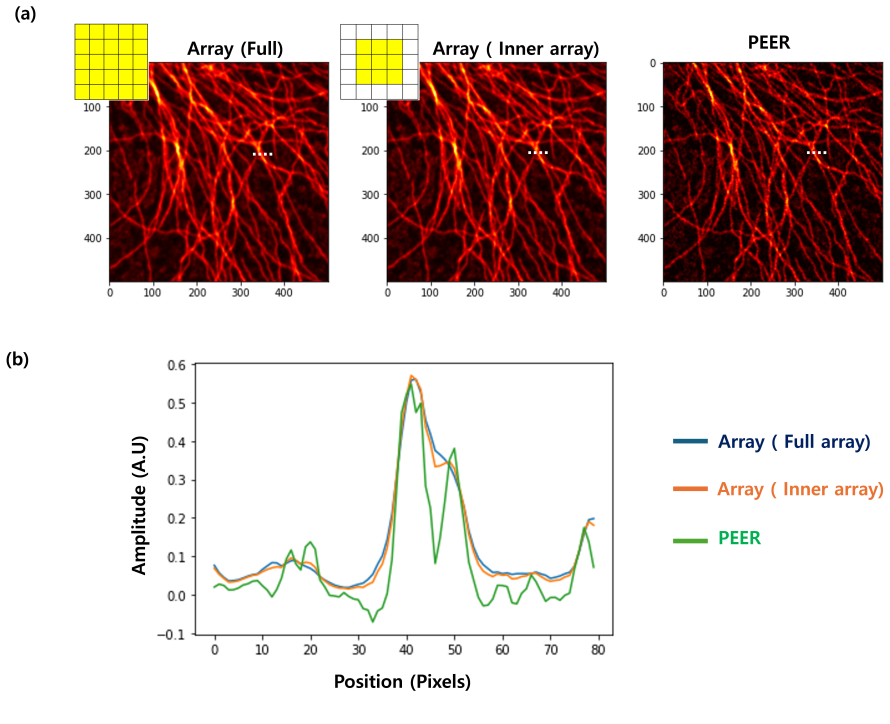
Supplementary Figure 1. Comparison of MSSR imaging of different orders of a synthetic image with emitter pairs spaced from 120 nm to 270 nm.



Supplementary Figure 2. Simulated axial PSF distributions generated using an open-source Python function [1]. (a) The left panel shows the axial PSF from a conventional confocal microscope (0.5 A.U.), while the right panel displays the axial PSF obtained with the PEER method. (b) Axial cross-sectional profiles comparing the confocal PSF (red dots) and the PEER PSF (blue line).



Supplementary Figure 3. (a) Comparison of confocal imaging (top) and the PEER method (bottom) of mitochondria in the ovarian cancer cell line OVCA-429 transduced with lentivirus as previously described using a mitochondrial GFP reporter (mito-PAGFP, Addgene Plasmid #23348) [2]. (b) Magnified view of mitochondria. (c) Phasor distributions for mitochondria, demonstrating fluorescence lifetime characteristics. Scale bars: 1 µm.



Supplementary Figure 4. Efficient implementation of the PEER method using a single-photon array detector. (a) Side-by-side comparison of 'open' confocal, 'closed' confocal, and PEER images of microtubules. (b) Line profile along the dashed line. The dataset was obtained from previously published work [3].

Supplementary Methods

0.1 Sample Preparation

Ovarian cancer cells expressing green fluorescent protein (GFP) in the mitochondria: The ovarian cancer cell line OVCA-429, transduced using a mitochondrial GFP reporter [2], was cultured in RPMI media (Corning™ RPMI 1640 Medium (Mod.) 1X with L-Glutamine, cat. no: 10040CV) supplemented with 10% fetal bovine serum (FBS) (Corning cat. no. 35011CV) and 100 IU/mL penicillin and 100 /mL streptomycin (Corning, cat. no. MT-30-001-CI) in a humidified 5% CO_2 balanced-air atmosphere at 37 °C. Next, cells were plated onto glass coverslips (Neuvitro Corp, 1.5H high performance coverslips 18 mm PLL coated, cat. no. GG-18-1.5H-PLL) and incubated for 24 hours. Cells were washed 3× with phosphate-buffered saline (PBS) and fixed using 4% paraformaldehyde (EMS, Formaldehyde Aqueous Solution EM Grade) for 10 minutes. Cells were then washed again 3× with PBS. Finally, coverslips with fixed cells were removed and mounted onto a glass slide with mounting medium (Abberior, Abberior mount, Liquid antifade, cat. no. mm-2009-2x15ML) and sealed with clear nail polish.

Supplementary References

References

[1] Christoph Gohlke. psf, 2024. Version 2024.5.24.

[2] Randy J Giedt, Paolo Fumene Feruglio, Divya Pathania, Katherine S Yang, Aoife Kilcoyne, Claudio Vinegoni, Timothy J Mitchison, and Ralph Weissleder. Computational imaging reveals mitochondrial morphology as a biomarker of cancer phenotype and drug response. *Scientific reports*, 6(1):32985, 2016.

[3] Marco Castello, Giorgio Tortarolo, Mauro Buttafava, Takahiro Deguchi, Federica Villa, Sami Koho, Luca Pesce, Michele Oneto, Simone Pelicci, Luca Lanzanó, et al. A robust and versatile platform for image scanning microscopy enabling super-resolution flim. *Nature methods*, 16(2):175–178, 2019.