

Multi-color structured illumination microscopy for live cell imaging based on the enhanced image recombination transform algorithm: supplement

TIANYU ZHAO,^{1,2,3,6} HUIWEN HAO,^{4,6} ZHAOJUN WANG,¹ YANSHENG LIANG,¹  KUN FENG,¹ MINRU HE,^{2,3} XUE YUN,¹ PIERO R. BIANCO,⁵ YUJIE SUN,⁴ BAOLI YAO,^{2,3} AND MING LEI^{1,3,*}

¹MOE Key Laboratory for Non-equilibrium Synthesis and Modulation of Condensed Matter, School of Physics, Xi'an Jiaotong University, Xi'an 710049, China

²State Key Laboratory of Transient Optics and Photonics, Xi'an Institute of Optics and Precision Mechanics, Chinese Academy of Sciences, Xi'an, Shaanxi 710119, China

³University of Chinese Academy of Sciences, Beijing 100049, China

⁴State Key Laboratory of Membrane Biology & Biomedical Pioneer Innovation Center (BIOPIC) & School of Life Sciences, Peking University, Beijing 100871, China

⁵Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198-6025, USA

⁶Authors contributed equally to this work

*ming.lei@mail.xjtu.edu.cn

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Supplemental document

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⁶ Authors contributed equally to this work

* ming.lei@mail.xjtu.edu.cn

Resolution calibration

Three approaches were used to demonstrate the resolution enhancement of the system. First, an Argo-SIM slide (Argo-SIM, Argolight Inc., France) was used (Fig. s1a). This slide has lines spaced 30nm apart, embedded into glass (Fig. s1b). The analysis shows that the system resolves lines spaced 120nm apart but cannot resolve those spaced 90nm apart (Fig. s1c, blue trace). To further refine resolution, 46nm fluorescent beads were used. Here, the full width at half maximum (FWHM) of a single bead was measured (Fig. s1d, e). In wide-field (WF) the FWHM is 201nm, and this is improved to 106nm in SIM (Fig. s1f). Thus, the resolution is at least 106nm, yielding a two-fold resolution enhancement.

The resolution enhancement is also demonstrated in the images and analysis of mitochondria which show that the SIM system enables clear visualization of mitochondrial cristae (Fig. s2a and b). Further, in the line profile of the zoomed-in images, the peaks of the cristae are spaced 380 ± 76 nm apart in the SIM image whereas in the WF image, this spacing is far more difficult to discern and, the final peak corresponding to the outer mitochondrial membrane is not discernible (Fig. s2c-e).

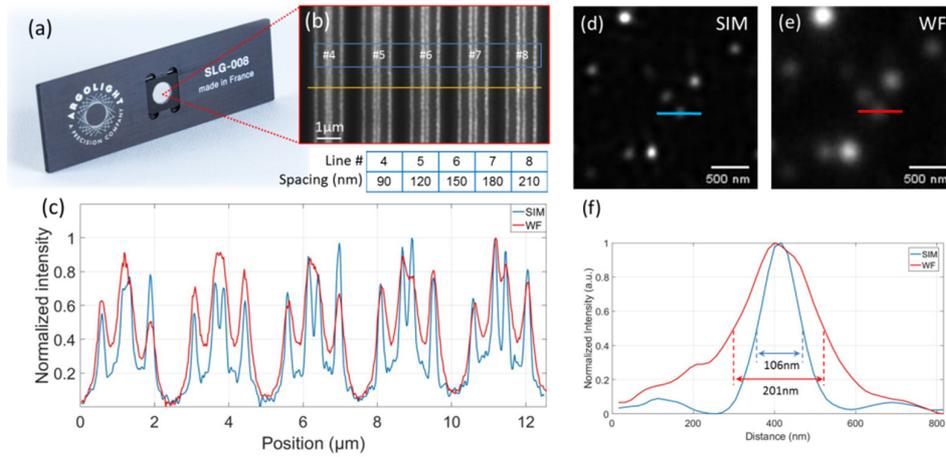


Figure S1. The resolution of the light efficient, structured illumination microscope is enhanced 2-fold relative to wide-field. (a) An Argo-SIM Fluorescent Quality Assessment slide. (b) Image of line spacing between 90 and 210 nm. (c) Intensity profile along the yellow line. Red trace, WF; Blue trace, SIM. (d)-(f) Analysis of 46 nm fluorescent beads; (d) and (e) images from SIM and WF, respectively; (f) measurement of FWHM to show resolution enhancement of the SIM system.

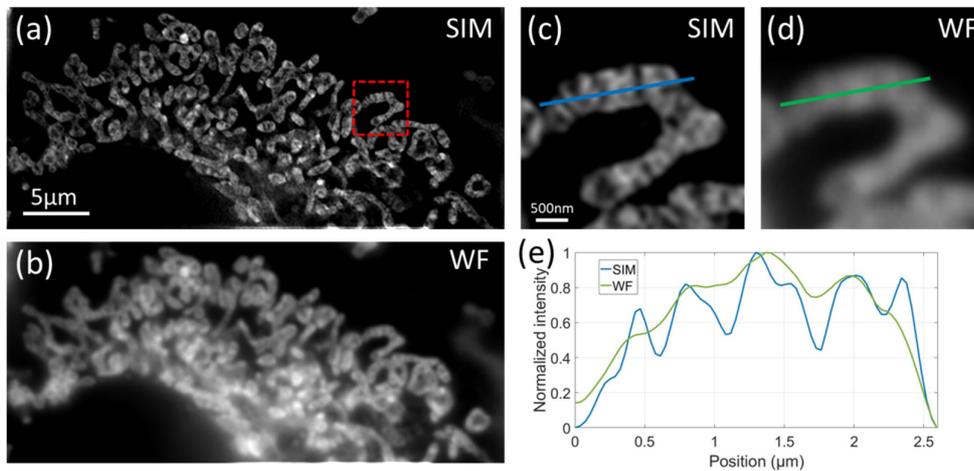


Figure S2. The SIM system enables clear visualization of mitochondrial cristae. In the images shown, mitochondrial proteins are stained with MitoTracker Green. (a), Super-resolution image obtained with the light-efficient SIM system. (b), Wide-field image of the same sample as in (a). (c) and (d), The zoomed-in images of the red dash box region in (a). (e), The intensity profile along the lines in (c) and (d). Blue trace, SIM; Green trace, WF.