

# MITOCHONDRIAL DYNAMICS QUANTITATIVELY REVEALED BY STED NANOSCOPY WITH AN ENHANCED SQUARAIN VARIANT PROBE

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**Abstract:** Mitochondria play a critical role in generating energy to support the entire lifecycle of biological cells, yet it is still unclear on how their morphological structures evolve and regulate their functionality. Conventional fluorescent microscopy can only provide ~300 nm resolution, which is insufficient to visualize the mitochondria cristae. A variety of super-

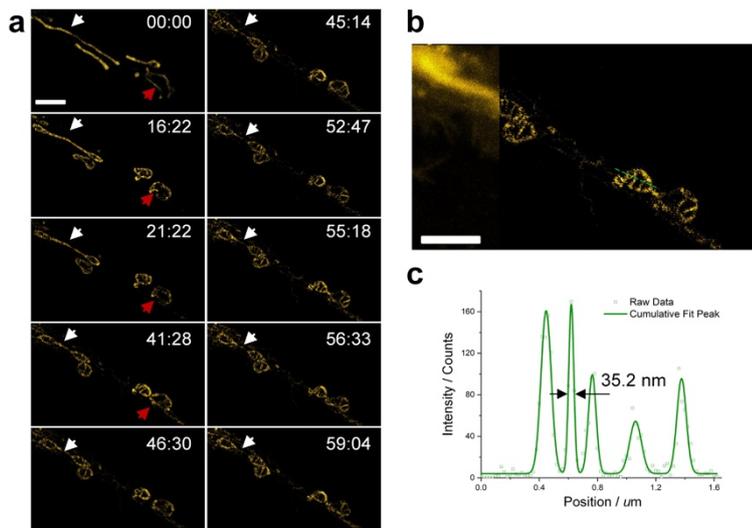


Figure 1 (a) Time-lapse STED imaging of the mitochondria. (b) The comparison of confocal (left) and STED (right) imaging. The intensity profile across the green dash line in (b) are shown in (c).

form of cristae during mitochondria fusion and fission can be clearly resolved. Our study demonstrates the emerging ability of optical STED nanoscopy to investigate intracellular physiological processes on the nanoscale in real time.

resolution techniques have been applied to visualizing the sub-mitochondrial structures. For example, Jans et al. discovered the mitochondria inner membrane organizing system (MINOS) with STED<sup>1</sup>. With 3D-STED, Schmidt et al. imaged the mitochondria cristae with isotropic resolution<sup>2</sup>. Here, we developed an enhanced squaraine variant dye (MitoESq-635), to study the dynamic structures of mitochondrial cristae in live cells. The low saturation intensity and high photostability make it ideal for long-term, high-resolution STED nanoscopy. We demonstrate the time-lapse imaging of mitochondrial inner membrane over 50 minutes in living HeLa cell with 35.2 nm resolution for the first time. The

[1] Jans, D. C., Wurm, C. A., Riedel, D., Wenzel, D., Stage, F., Deckers, M., ... & Jakobs, S. (2013). STED super-resolution microscopy reveals an array of MINOS clusters along human mitochondria. *Proceedings of the National Academy of Sciences*, 110(22), 8936-8941.

[2] Schmidt, R., Wurm, C. A., Punge, A., Egner, A., Jakobs, S., & Hell, S. W. (2009). Mitochondrial cristae revealed with focused light. *Nano letters*, 9(6), 2508-2510.