Three-color live cell super-resolution imaging of sub-mitochondrial regions

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Sub-mitochondrial regions are beyond the diffraction limit and were only resolvable using electron microscopy before the advent of optical nanoscopy. Resolving and simultaneous imaging of multiple mitochondrial compartments in a living cell is an important first step towards understanding the nanomachinery of mitochondria, indispensable powerplants of eukaryotic cells[1]. Among the existing nanoscopy techniques, structured illumination microscopy (SIM) is most suitable for live cell imaging[2]. Here, we used 3D SIM to simultaneously image three sub-mitochondrial compartments in the same living cell (MCC13, Merkel carcinoma from human skin cell line) using spectrally separated probes. The mitochondrial compartments targeted were: a) mitochondrial outer membrane (by GFP-tom20[3] (Gtom)), b) mitochondrial matrix (by CellLight Mitochondria-RFP BacMam 2.0 (BM)), and c) mitochondrial intermembrane space (by MitoTracker Deep Red (MT)). Each of these sub-mitochondrial structures was first imaged individually and then simultaneously using 3D SIM.

In this work, we also discuss several challenges and advantages associated with simultaneous labelling and super-resolution imaging of multiple mitochondrial regions. The challenges are reduced labelling efficiency, morphological artifacts and different imaging parameters. These sub-cellular changes made it necessary to re-optimize the staining protocols for simultaneous labelling of mitochondria with multiple colors. By optimizing the labelling strategy and imaging conditions we showed simultaneous three-color super-resolution imaging of mitochondria in living cells. Combining mitochondrial labels also enabled recognizing effects and artefacts resulting from individual probes not recognizable using single labelling only. By combining 3D SIM with deconvolution microscopy, long-term time-lapse imaging of mitochondria was achieved while maintaining the ability to have a closer look through the utilization of 3D SIM at specific time-points.

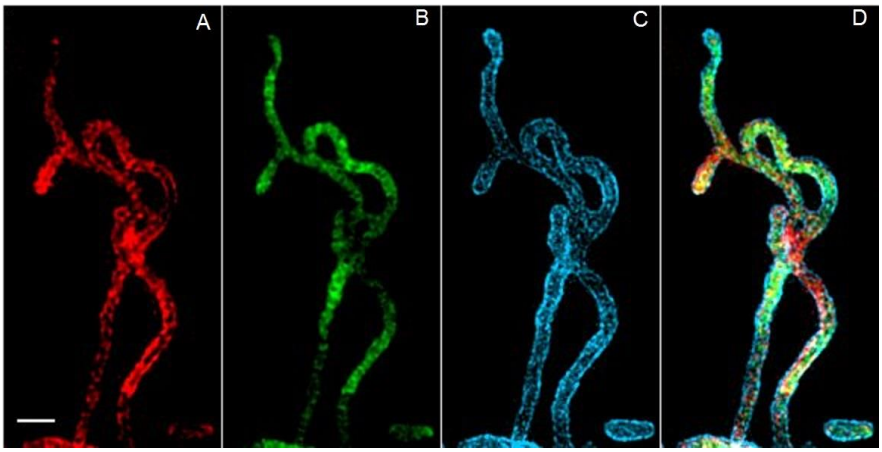

Figure 1: Mitochondria in a living MCC13 cell labelled with three different mitochondrial probes: A: MitoTracker Deep Red, B: CellLight Mitochondria-RFP BacMam 2.0, C: plasmid mEmerald-TOMM20-N-10, and D: previous combined. Projected 3D SIM images. Scale bar 1 µm.

1. Jakobs, S. and C.A. Wurm, *Super-resolution microscopy of mitochondria.* Curr Opin Chem Biol, 2014. **20**: p. 9-15.
2. Waldchen, S., et al., *Light-induced cell damage in live-cell super-resolution microscopy.* Sci Rep, 2015. **5**: p. 15348.
3. mEmerald-TOMM20-N-10 was a gift from Michael Davidson (Addgene plasmid # 54282)