Visualization and Quantification of Mitochondrial Dynamics with Lattice Light-sheet Microscopy

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Mitochondria has been elaborately studied in few past decades but its dynamics such as fusion-fission events and mitophagy still poses serious challenges for high-resolution live cell imaging. To investigate such highly three-dimensional (3D) dynamics, we developed multi-color lattice light-sheet microscopy with excellent optical sectioning, sub-second volumetric imaging speed, and low photobleaching and phototoxicity [1]. Demonstrating such 3D images, however, is an extremely challenging step. To display a 3D dataset onto a 2D screen, 3D maximum intensity projection is the most common volume rendering method, but such a dimensionality reduction method causes misunderstanding of multi-layer geometric structures. Furthermore, these kinds of data have to be pre-processed before any step of quantitative analysis can be performed; that is, segmentation has to be applied to create a high-quality binary image.

In the presented work we aimed at segmenting images acquired within a second by lattice light-sheet microscopy into chromosome, mitochondria, and cytosol to obtain better visualization and quantification of mitochondrial dynamics. Since the three organelles have different signal-to-noise ratio (SNR), we separated the three preprocessing methods. First, in the Cherry channel, smoothing the low-SNR data by a bilateral filter and matching contrast to a common reference frame prevented chromosome images from over-segmentation by adaptive thresholding. Secondly, in the GFP channel, a top-hat filter suppressed background noise and enhanced the contrast between mitochondria to improve mitochondrial segmentation by adaptive thresholding. Finally, thanks to the powerful optical sectioning, we also found high-SNR cytosolic autofluorescence in the GFP channel, and thus, using a single global thresholding could segment the box-filtering images into cell volume.

In contrast to maximum intensity projection, segmentation rendered the 3D data with a clear hierarchy on the planar display (Fig. 1). Besides, we quantified these segments including calculating the change of cell volume and evaluating the geometric features of the mitochondria and chromosome during cytokinesis, and further can track mitochondrial fusion and fission to understand its biological mechanisms.

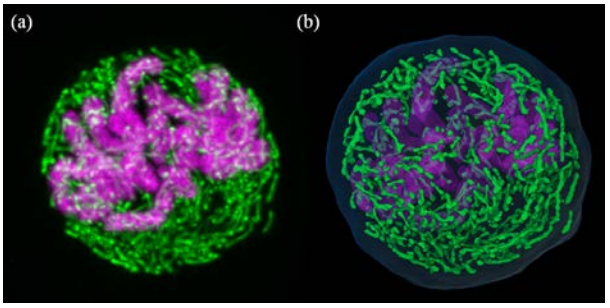

Figure 1: 3D data rendering through (a) Maximum intensity projection (b) Segmentation

[1] Chen, Bi-Chang, et al. "Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution." *Science* 346.6208 (2014): 1257998.