

Organelle Interactions visualized by 3D Correlative Microscopy

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Eukaryotic cells are compartmentalised into a complex array of membrane-bound organelles. The exchange of contents between organelles is tightly regulated, and relies on vesicular transport or direct membrane contacts between organelles. Misregulation of organelle contact sites have been recently associated with neurological disorders, numerous metabolic disorders including diabetes, and cancer [1]. Due to the transient and confined nature of organelle contact sites, unveiling their regulation requires an approach to analyse the spatio-temporal localization of molecules together with the ultrastructure. However, we don't yet have a comprehensive method with high spatial and temporal resolution to study subcellular organelle interactions.

Volume correlative light and electron microscopy (3D-CLEM) approaches offer a unique potential to explore molecular characteristics together with high-resolution ultrastructural details across cellular volumes [2]. In our recent work, we have shown that the trafficking, fusion and fission dynamics of single endo-lysosomal organelles can be analyzed, and correlated to their ultrastructure with 3D-CLEM [3]. Building on this work, here we describe the utilization of integrated confocal fluorescence microscopy for increased throughput and correlation efficiency in 3D-CLEM. Our approach aids high-precision registration between 3 dimensional (3D) fluorescence microscopy (FM) and electron microscopy (EM). We can notably assure a sub-100 nm correlation efficiency between 3D-FM and 3D-EM datasets, and correlate each and every organelle imaged in FM to the volume-EM data with high-precision. This greatly benefits correlative analyses, enables imaging several organelles of interest directly in volume-EM, and obviates the need for post-correlation in big datasets.

Extending the 3D-CLEM pipeline with live-cell imaging, we link rare (e.g. membrane contact sites) and transient (e.g. organelle interactions) cellular events to the underlying 3D ultrastructure. We present targeted EM imaging of live-cell imaged lysosomes for analysis of their motile characteristics within the ultrastructural context. We relate motility of single lysosomes to their morphology, and the contact sites they have with endoplasmic reticulum (ER). 3D-CLEM pipeline opens up powerful possibilities to study temporal regulation of cellular processes with respect to the 3D ultrastructure.

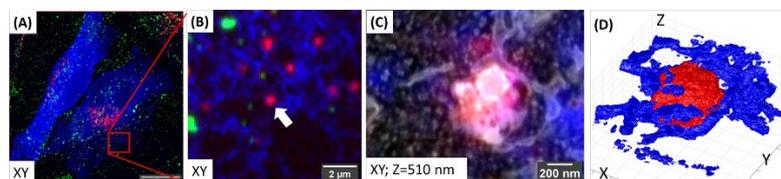


Figure 1: (A)(B) show FM images of ER and lysosomes. (C) 3D-CLEM overlay of a single lysosome. (D) 3D segmentation showing ER-lysosome interactions.

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