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Segmentation, Modeling and Quantification of Electron Cryotomographic Datasets

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Electron microscopy (EM) has long been a powerful tool for delineating the ultrastructure of cells and tissues beyond what can be seen by light microscopy, and the advent of cryo-EM has provided the opportunity to update our understanding of cellular/cytoplasmic structure with unprecedented preservation and clarity. Electron cryotomography (ECT), in particular, has become a method-of-choice for studying the three-dimensional (3-D) spatial configuration of molecules within their near-native cellular context [1], and in some cases is able to dissect the molecular architecture of individual macromolecular complexes *in vivo*. With the automation of tomographic data collection [2, 3] and advances in microscope stability [4], the throughput of this once "slow" imaging method has increased dramatically, making analysis of the large amounts of data generated a serious issue.

A tomographic reconstruction of only a few square microns of mammalian cytoplasm can contain hundreds of different molecular species and subcellular objects (Fig 1A), making segmentation and modeling of information contained within a single tomogram cumbersome and very time consuming (Fig. 1B). Unfortunately, there is no way around this step if we are to be quantitative in our description of the thousands of tomograms a single lab can produce every year. As throughput for data collection increases and the questions being asked by ECT extend to larger length-scales, performing this analysis becomes a seemingly impossible task. Additionally, depending on the quality and quantity of the data, along with the type of objects to be segmented/modeled, different approaches provide different advantages and disadvantages. Luckily, there is a variety of software packages, scripts and algorithms being developed to aid in this process [5, 6], but there is no single solution capable of doing everything and different workflows must be developed to handle different types of datasets.

Here we describe the approaches taken in our lab to deal with the problem of 3-D segmentation, modeling and quantification using freely and commercially available software. We explore the different kinds of cellular spaces and objects typically encountered in a cytoplasmic environment and the methods that have proven useful in our investigation. We discuss how to optimize data for specific approaches, as well as the problems with current approaches that need to be overcome to allow more efficient and objective quantitative investigation of cell structure by ECT.

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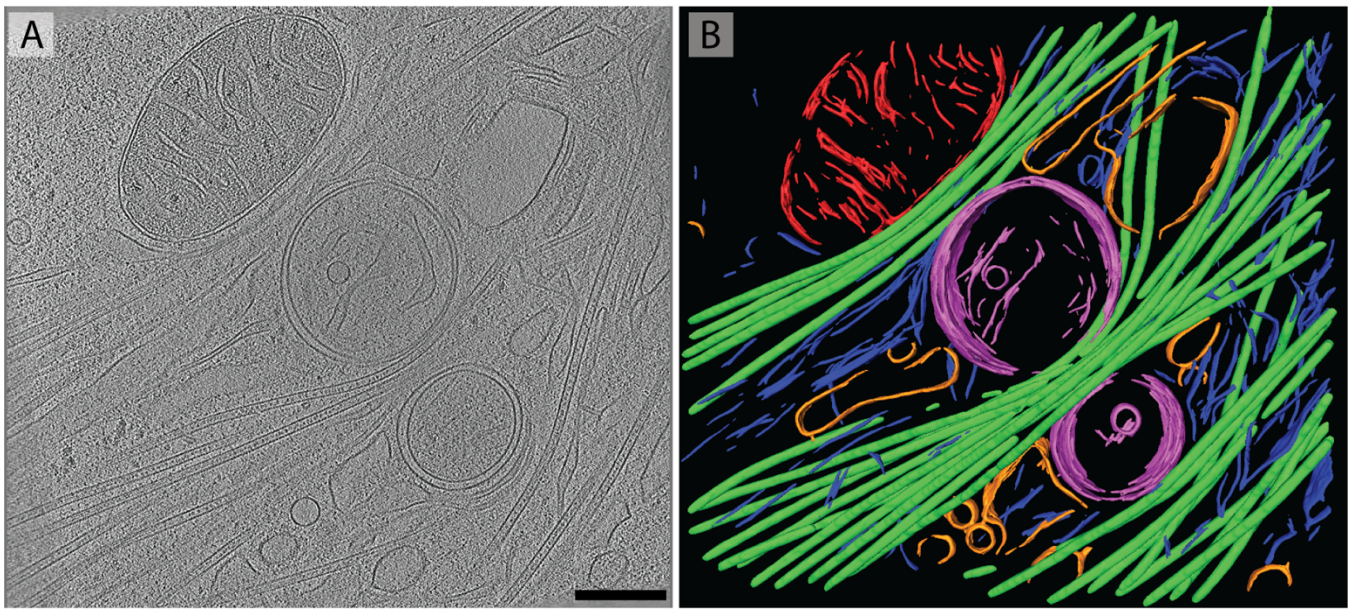

Figure 1. A) 10 nm-thick slice through a tomographic reconstruction of neuronal cytoplasm. B) Semi-automated segmentation of microtubules (green), mitochondrion (red), endoplasmic reticulum (blue), putative autophagosomes (purple) and unidentified vesicular objects (orange), using the convolutional neural network approach developed in [6]. Post-segmentation clean-up, surface model rendering and colorization was performed in the commercially available software Amira (ThermoFisher). Scale bar in (A) represents 200 nm.

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