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Three-Dimensional (3D) image segmentation and quantitative analysis of mitochondrial morphology under stress

Background: Confocal Laser Scanning Microscopy (CLSM) enables the understanding of cellular organelles such as mitochondria at nanoscale resolution. Quantitative analysis of images from CLSM is laborious and time-consuming when working with larger datasets. While several deep learning-based segmentation models have been developed to tackle this, most of them are for 2-Dimensional (2D) images. In our work, we focus on 3D segmentation, owing to spatio-temporal information, that describes the structure, dynamics and distribution of organelles in space. We work with single cell CLSM images from the moss species Physcomitrium patens. The channel with mitochondrial signals is used for segmentation to understand mitochondrial stress response. These mitochondria have a range of shapes from circular, tubular, rod-shaped, branched and elongated, and no open-source data with ground truth labels in 3D is available for such shapes.
Methods: Owing to the non-convex shapes of mitochondria, we trained Omnipose –a deep learning-based image segmentation model with U-Net based architecture –from scratch for performing 3D volumetric segmentation. We manually annotated the ground truth labels for 24 volumetric images. Omnipose model was trained for 2500 epochs, with a learning rate of 0.01 and Adam optimizer. To ensure the reliability of segmentation results, we calculated the Average Precision (AP) metric from omnipose's prediction and compared it with the AP range of 3 manual annotations.
The second part of our work is on quantitative analysis of morphological features calculated from the segmentation results. Mitochondrial morphology gives information about the functional state of mitochondria. Shape features such as solidity, sphericity, aspect ratio, and size features such as volume and surface area were calculated, as these features are quantitative representation of various morphologies. We performed Principal Component Analysis (PCA) to reduce the dimensionality of features extracted from the 3D mitochondrial segmentation masks. This was followed by Linear Discriminant Analysis (LDA) to improve class separation of different stress conditions.
Results: The AP of Omnipose prediction falls in the range of AP values observed between 3 human annotations. Our LDA results segregate the mitochondrial population of strong oxidative and toxic stressors from other stress and control groups. This result is in accordance with the noticeable changes in mitochondrial morphology of these 2 stress groups when the images were visually inspected. To summarize, we performed 3D volumetric segmentation of mitochondria from CLSM images and performed quantitative analysis of morphological features. Our approach reveals that strong oxidative and toxic stressors cause prominent change in mitochondrial morphology.

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