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Object Segmentation on Cryo-electron Tomography Data

Lan Dang[1, 2], Steven J. Ludtke[2]

[1]Graduate Program in Quantitative and Computational Biosciences, Baylor College of Medicine, Houston, TX, US.
[2]Verna Marrs and McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, US.
* Corresponding author: dlan@bcm.edu

Cells are the structural and functional unit of life. Their functions are reflected through their ultrastructure: the distribution of their organelles and the assembly of the macromolecules inside the cells. In contrast to traditional imaging techniques where purification or fluorescent labeling is required, cryo-electron tomography (Cryo-ET) is suitable for studying the ultrastructure of cells in near native states. While this allows the study of spatial organization and interaction between structures *in situ*, cryo-ET data analysis is difficult due to an array of factors: low signal-to-noise ratio, missing wedge artifacts, and the conformational and compositional variability of the specimens [1]. These limitations require manual annotation to highlight the spatial organization of subcellular components. However, this step is time and labor-intensive, creating a bottleneck in Cryo-ET data processing pipeline.

In recent years, several computational programs have been introduced to make the tomogram annotation process more efficient [2],[3],[4]. These programs learn different set of features from tomograms to separate the macromolecules of interest from background, resulting in a heat map where the intensity corresponds to the probability that the pixel/voxel doesn't belong to the background. Despite providing great visualization benefit, this is insufficient to study the morphology and spatial interactions between cellular components since it lacks consideration for organelles as particular objects. For quantification on organelles or cellular components, each non-background pixel/ voxel needs to be associated with an individual identity. Furthermore, organelles annotation can help identifying specific particles associated with specific organelles, i.e., provides information about the relative location and polarity of individual macromolecules with respect to different classes of organelles. In this study, we introduce a workflow to segment and multiclass categorize cryo-electron tomography images using deep-learning.

The workflow consists of three steps: pixel-wise segmentation, clustering, and object classification. In the first step, we built a deep neural network that takes in only a few manual segmentation examples specified by users and label each pixel as objects or background. A diverse training set, including examples from multiple imaging conditions, will result in a more robust network that can be generalized to various tomograms. Then, predicted object pixels will be automatically grouped into clusters based on connectivity and cluster convexity. Finally, subregions of the image that contain point clusters will be classified into different object types through a convolutional neural network (CNN). This CNN is pre-trained using available cellular Cryo-ET data and can be easily finetuned with a few examples provided by users depending on application. In conclusion, the workflow supports not only visualization of individual organelles but also quantitative downstream analysis on spatial organization and structure, aiding studies of cellular ultrastructure.

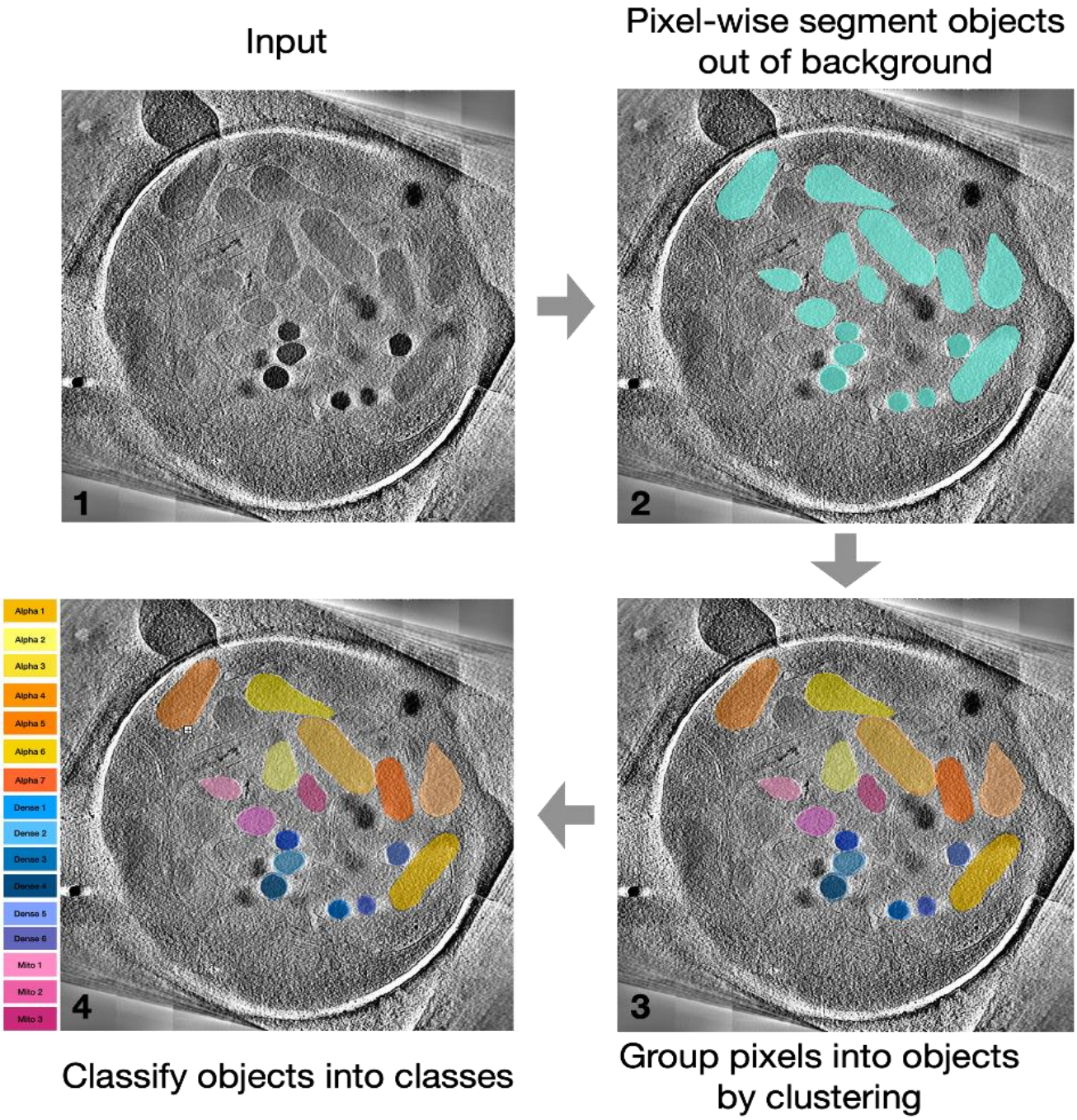

Figure 1. Expected output of each step of the object segmentation on an example tomogram slice. (1), a binary map of object/background (2) clusters of objects pixel into convex clusters (3), labeled convex clusters with correct object labels (4).

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