

Type of presentation: Oral

LS2-O-2 Membrane Segmentation in Volumetric Electron Microscopy Data

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Introduction: Understanding the structure and function of cellular compartments—especially their 3D organization—is essential in molecular biology. Advances in microscopy now allow detailed reconstruction of cells from rich volumetric datasets. However, a key challenge is the segmentation of these large volumes. Most existing methods rely on supervised deep learning models trained on organelle-specific annotations, which limits generalizability. In contrast, we propose a membrane-centric approach. Since many cellular structures are enclosed by membranes with similar visual features, we created a dataset annotated for membranes and not for individual organelles. We then used this dataset to test several state-of-the-art segmentation methods. Our results show that a single model trained on membrane data can effectively delineate multiple compartments, suggesting a scalable strategy for broader segmentation tasks.

Methods: We used volumetric data from electron tomography of urinary bladder tissue, focusing on umbrella cells. To train the models, we manually annotated a subset of the data to serve as ground truth for segmentation. We compared five state-of-the-art methods chosen for their relevance to membrane or electron microscopy segmentation: CDeep3M [1], a cloud-enabled pipeline for 3D EM data; Ilastik [2], an interactive tool that uses classical machine learning; SAM [3], a zero-shot segmentation model from Meta AI adapted to our volumes; μ SAM [4], a SAM variant tuned for high-resolution microscopy; and nnU-Net [5], a self-configuring framework widely used in biomedical segmentation. Each method was evaluated for its ability to segment membranes in the tomographic volumes, aiming to identify models that generalize across diverse membrane-bound compartments.

Results: We evaluated all models on a held-out test set. CDeep3M and Ilastik did not provide satisfactory membrane segmentations, while SAM and its microscopy-adapted variant, μ SAM, also underperformed. In contrast, nnU-Net showed the best generalization and accuracy in the segmentation of membrane-bound compartments. Figure 1 compares the 2D results of all methods, while Figure 2 shows a 3D reconstruction from nnU-Net, highlighting its ability to capture membrane structures with high accuracy.

Discussion and conclusions: Our results show that a fully supervised, state-of-the-art model—nnU-Net—can successfully segment membranes enclosing a variety of intracellular compartments. Rather than relying on organelle-specific features, this approach focuses on learning the general visual features of membranes, which enables the detection of membrane-bound structures even if they were not explicitly included in the training set. This membrane-centric strategy reduces the need for extensive, compartment-specific annotations and provides better scalability and transferability compared to traditional supervised methods. Encouraged by these findings, our ongoing work is expanding our training datasets to include diverse electron microscopy modalities, such as FIB-SEM, as well as a wider range of voxel sizes and magnifications. Our goal is to develop a generalist membrane segmentation model that performs robustly under different imaging conditions and in different biological samples, including those not seen during training. Such a tool would support large-scale, automated analysis of complex cellular structures and provide valuable benefits to microscopy and cell biology research.

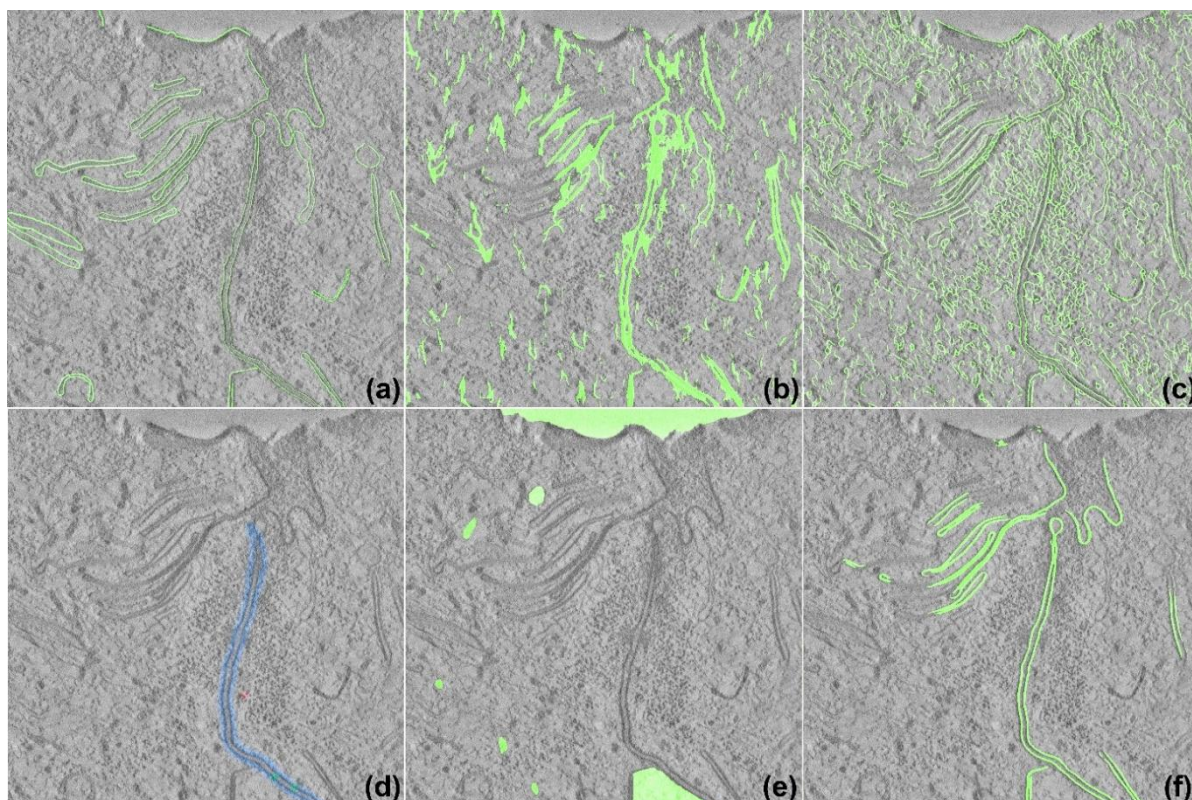


Figure 1: Comparison of segmentation results from different methods on a representative 2D slice. (a) Input electron tomography image with manually annotated ground truth, (b) CDeep3M, (c) Ilastik, (d) SAM, (e) μ SAM, (f) nnU-Net.

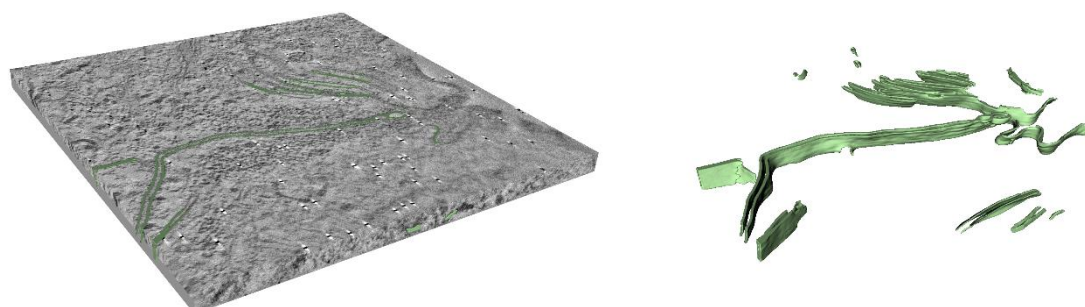


Figure 2: Example of 3D segmentation results using the best-performing method, nnU-Net. (left) Input electron tomography volume, and (right) nnU-Net segmentation output.

Acknowledgments: The work was supported by the Slovenian Research Agency research project J2-50221 – Cell visualization of unified microscopic data and procedurally generated sub-cellular structures (2024 – 2027) and Innovation Agency research core funding no. P3-0108.

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