

Proofreading Workflow for the Semantic Segmentation of Mitochondria in Serial Section Electron Microscopy Image Volumes

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Background

The acquisition of volume electron microscopy images provides an opportunity to study at high-resolution, in unbiased manner, the numbers, geometries, and placement of subcellular organelles within cells. Manual segmentation of the resultant large populations of organelles is intractable, so auto-segmentation using deep learning (DL) approaches have been applied to the task. DL approaches are not error-free and require efficient proofreading. We describe here a method for rapid proofreading for segmentations of mitochondria.

Methods

Anisotropic serial blockface scanning EM (SBEM) image volumes of $98\text{ }\mu\text{m} \times 78\text{ }\mu\text{m} \times 68\text{ }\mu\text{m}$ of the medial nucleus of the trapezoid body (MNTB) of a 6-day old mouse were collected. Mitochondria probability maps were then obtained using CDeep3M2, a containerized implementation of the DeepEM3D U-Net, trained to segment voxels of 3D SBEM volumes containing mitochondria.

We developed a proofreading process in which voxels with high probability of being membrane ($> 80\%$) are masked out of the mitochondria probability maps. Distinct objects of connected voxels containing mitochondria with at least 50% probability are labeled. Each connected component was then subjected to marker assisted binary watershed across a set of increasing probability thresholds from 50% to 94%, creating a tree of mitochondrion instance segmentation results for further evaluation using both automated and human influenced proofreading.

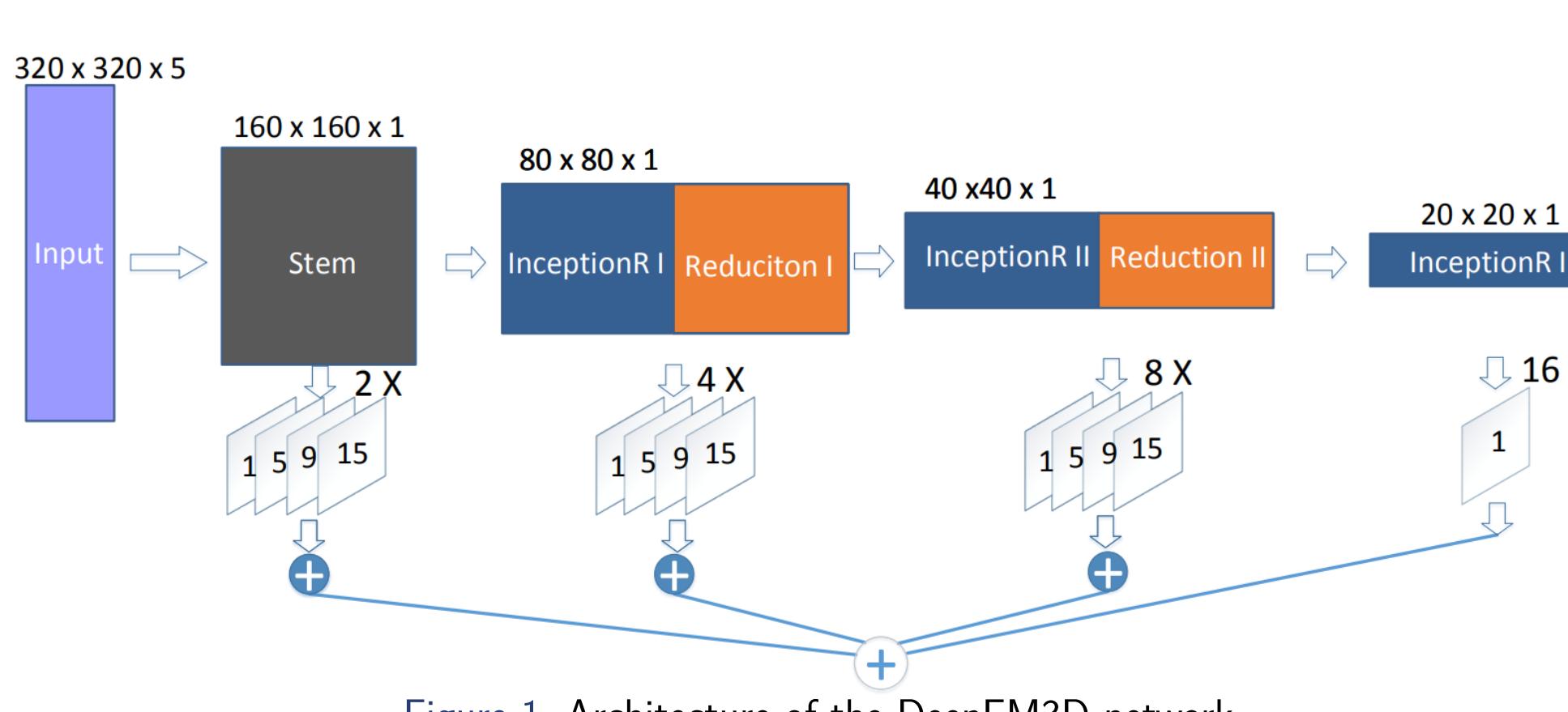


Figure 1. Architecture of the DeepEM3D network.

Proofreading Workflow

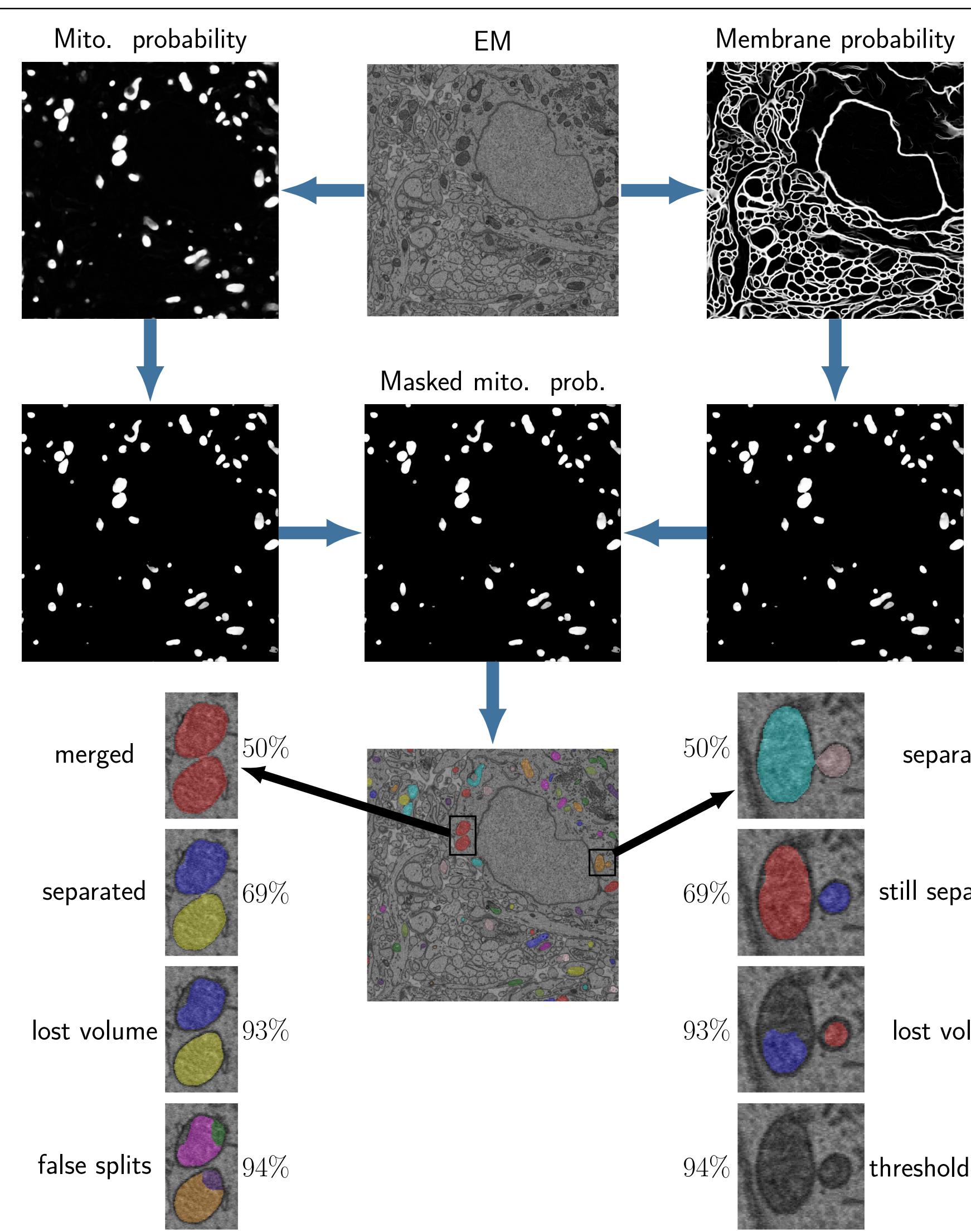


Figure 2. Proofreading workflow for the instance segmentation of mitochondria from SBEM image volumes.

No branching - 53% of the connected components

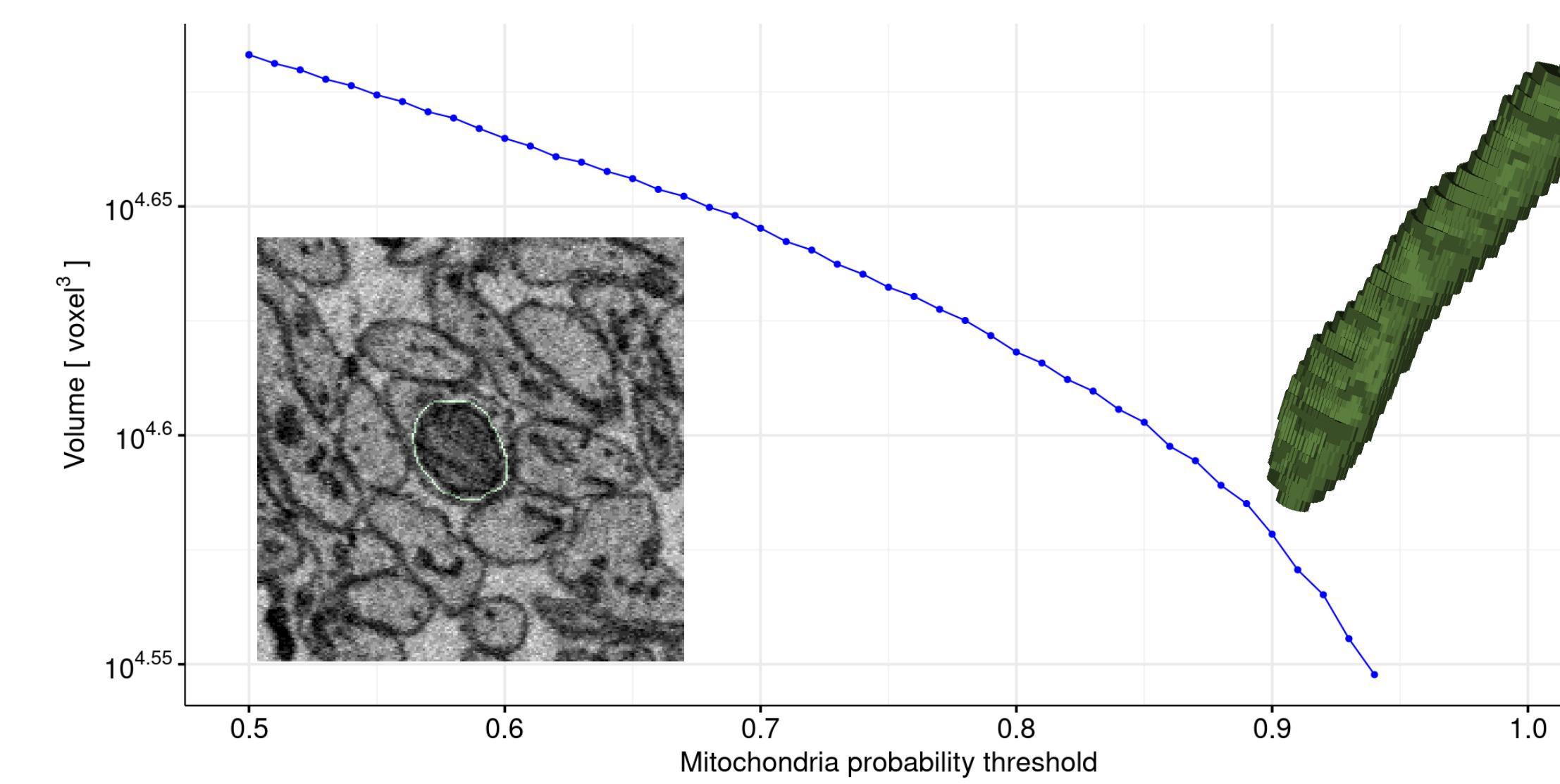


Figure 3. Example connected component which never branched and is a true mitochondrion with 99% probability. Connected components following this behavior can be automatically characterized as mitochondrion.

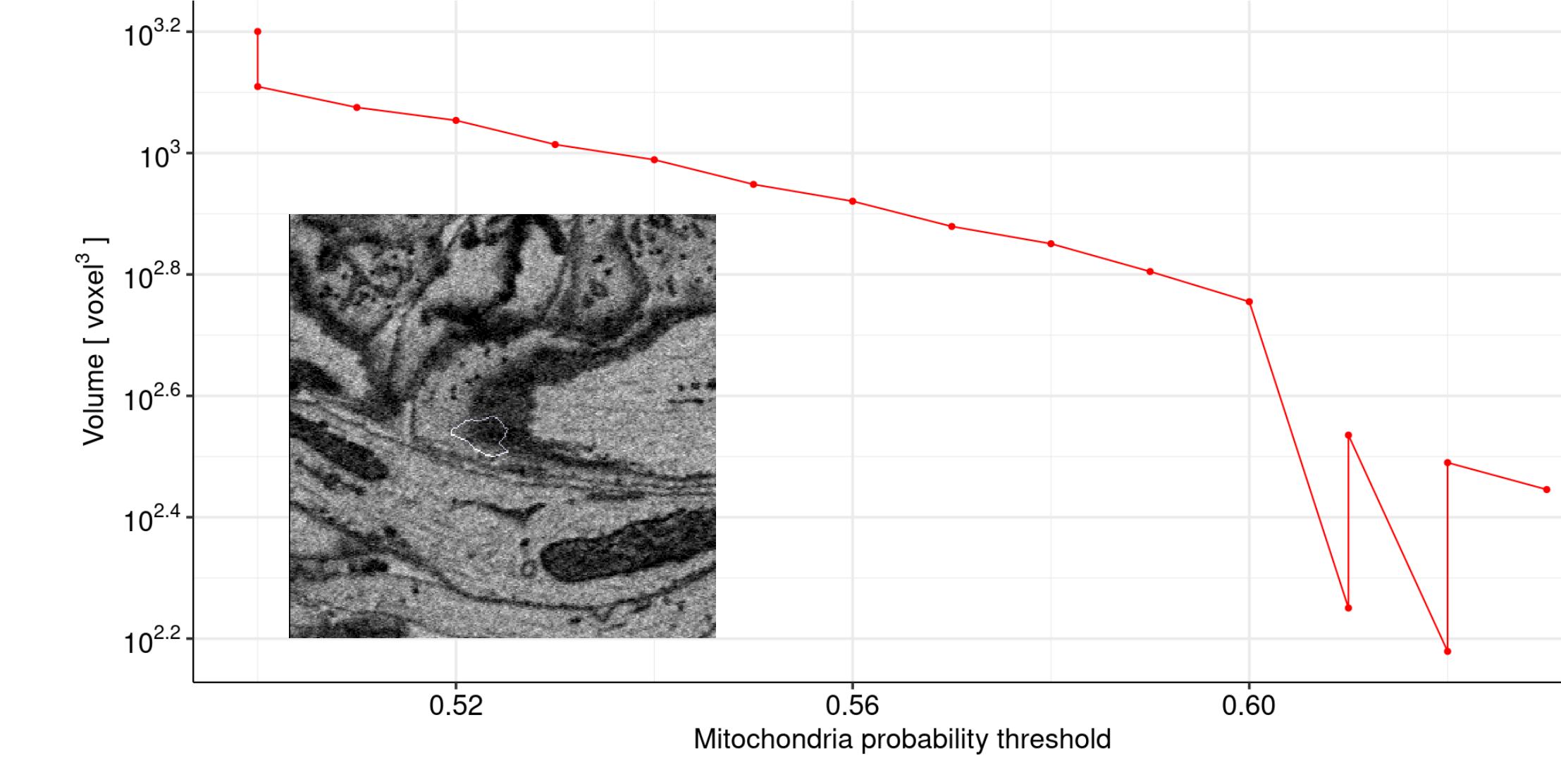


Figure 4. Example connected component which never branched, is clearly not a mitochondrion, and thresholded out at around 64% probability. Connected components following this behavior can be automatically characterized as not a mitochondrion.

Single branch - 22% of the connected components

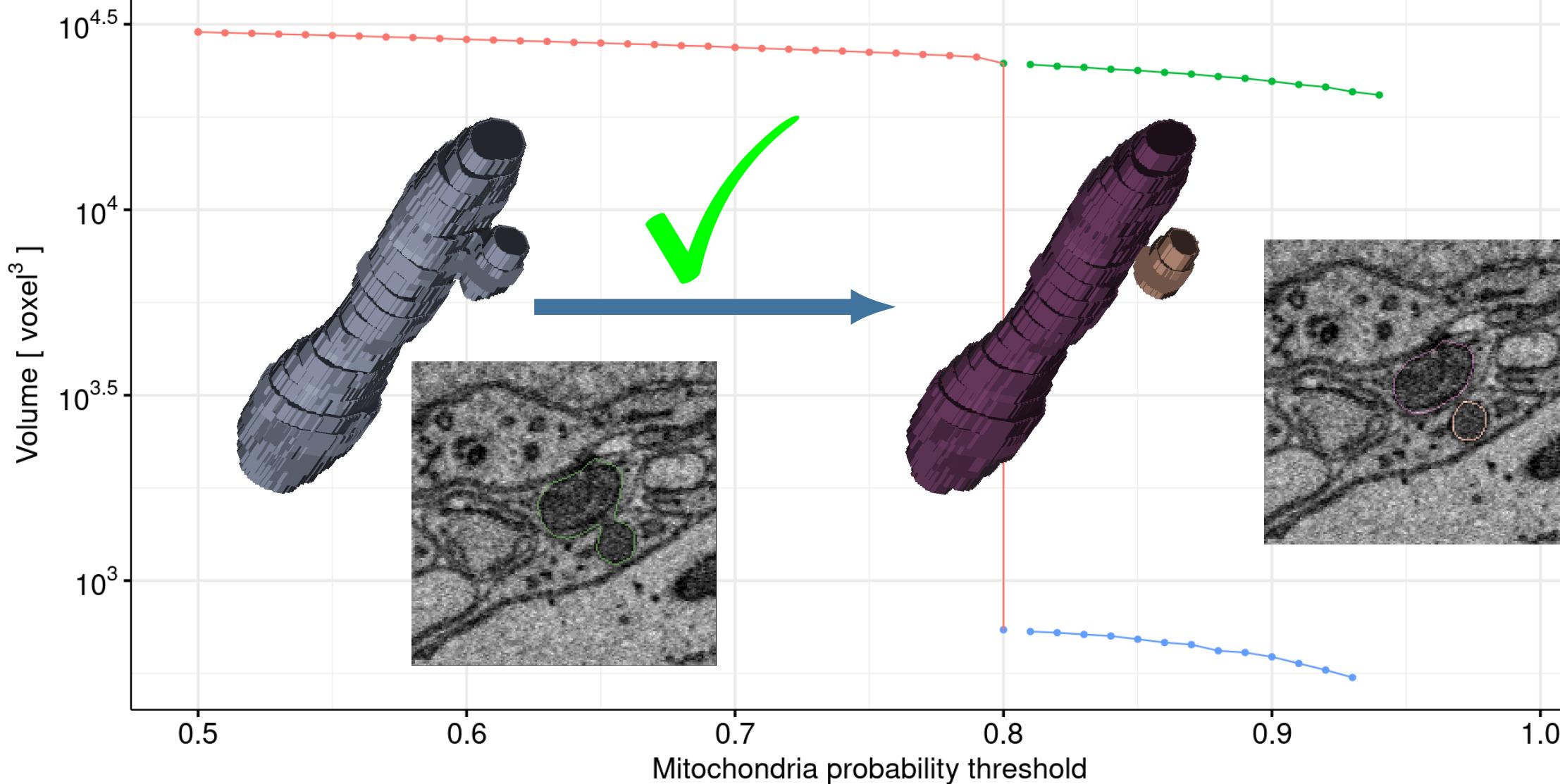


Figure 5. Example connected component which only branched one time (at 80%) into two distinct mitochondria, both of which thresholded out well above 90% probability. Since both objects continue to demonstrate stable decreases in volume with increasing threshold, they can automatically be characterized as two distinct mitochondria.

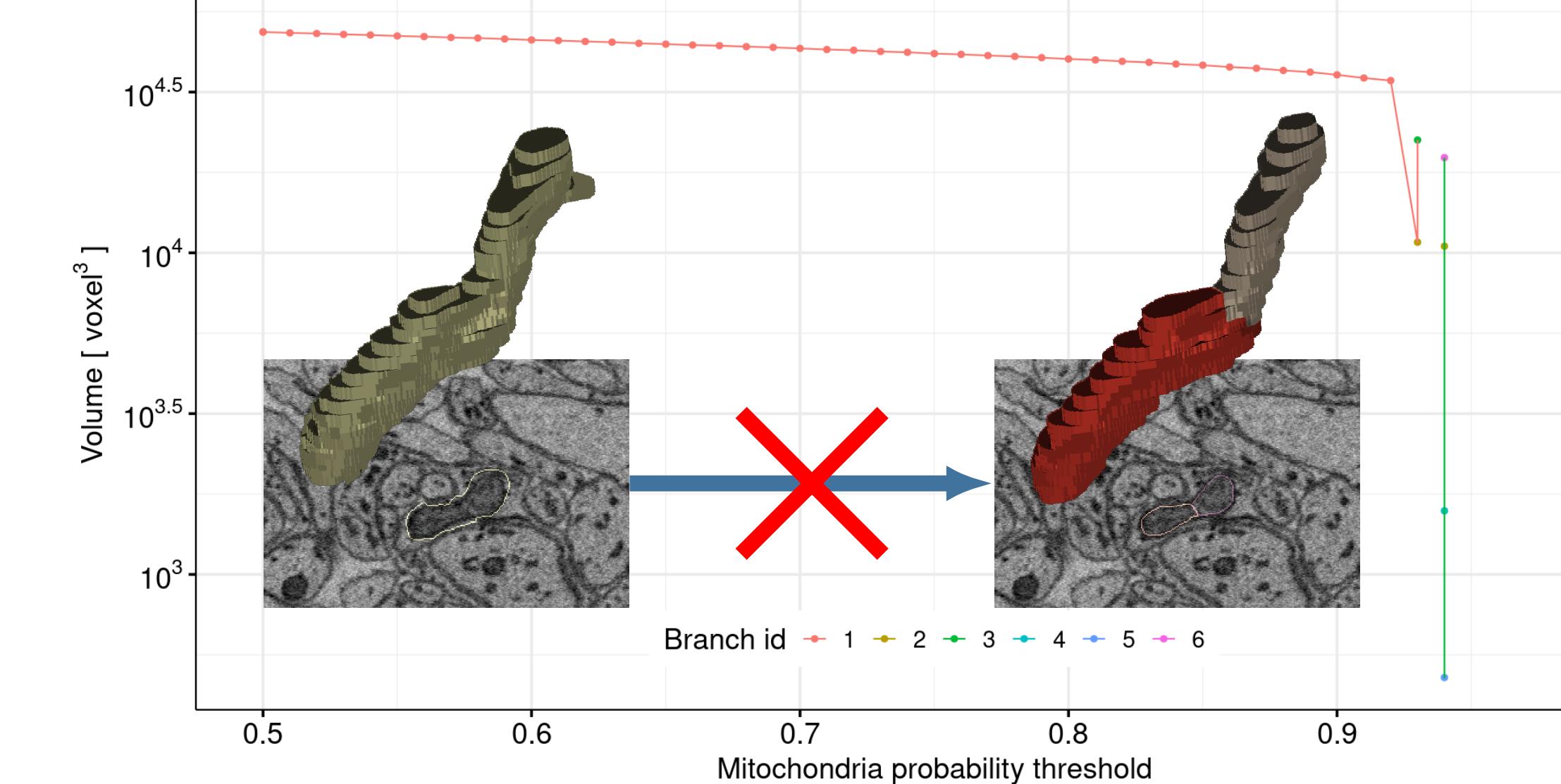


Figure 6. Example connected component which falsely splits at 92% probability and continued to falsely split as threshold increased. Consecutive false splits demonstrated in this example can be detected, aiding in the automated classification as a single mitochondrion.

Many branches - 25% of the connected components

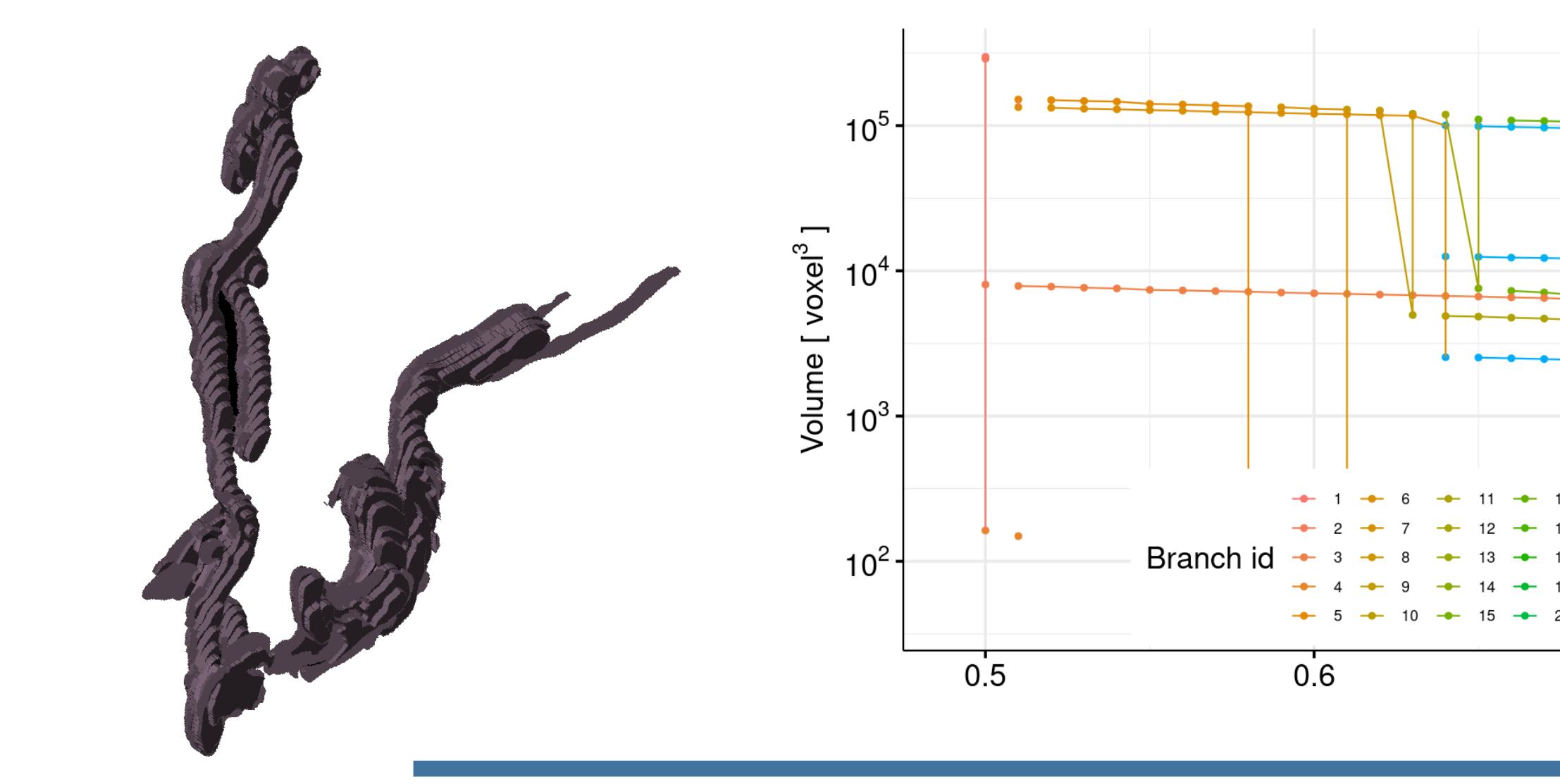


Figure 7. Complex example connected component which falsely splits into many branches, 55 in this case. Complex branched behavior such as this requires human proofreading via the proofreading application we developed. The 3D representation on the left is the original connected component while the representation on the right is the final proofread instance segmentation demonstrating 9 distinct mitochondria.

Human proofreading validated 0 and 1 branch examples, required for complex branching patterns

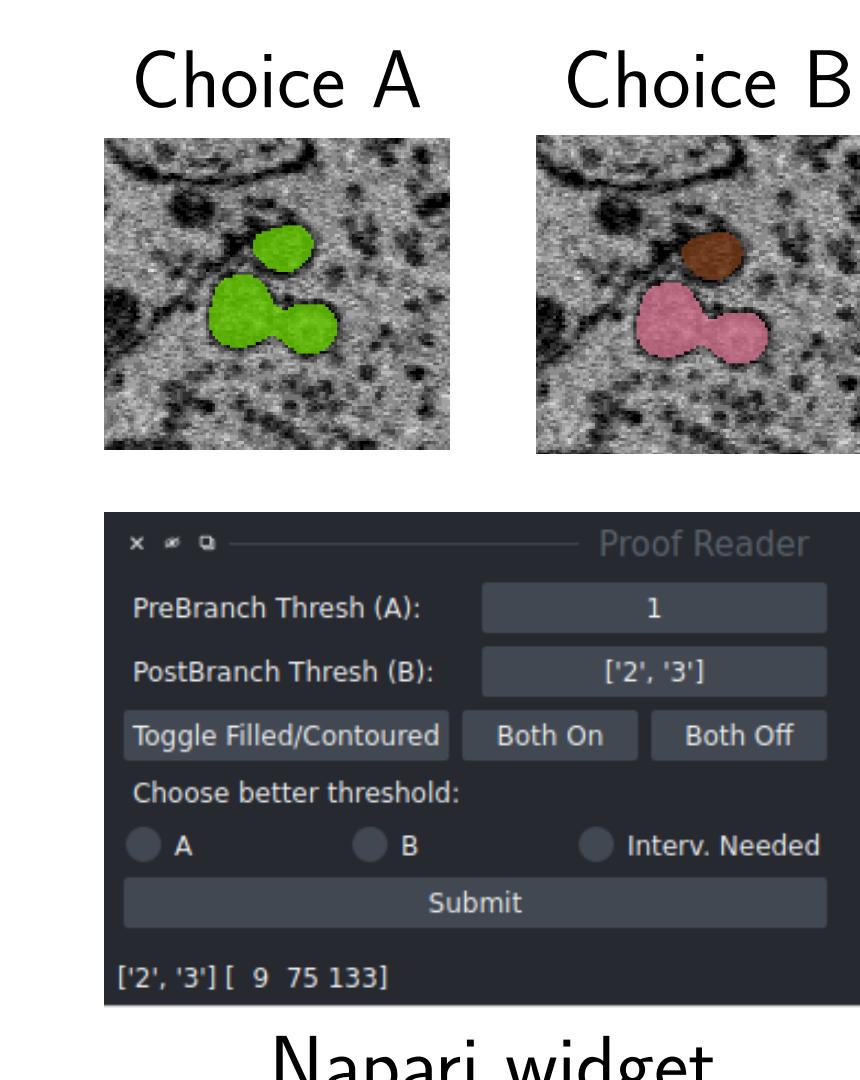


Figure 8. Proofreading application interface showing a single proofreading choice (A or B) and the branched results as a function of increasing thresholds.

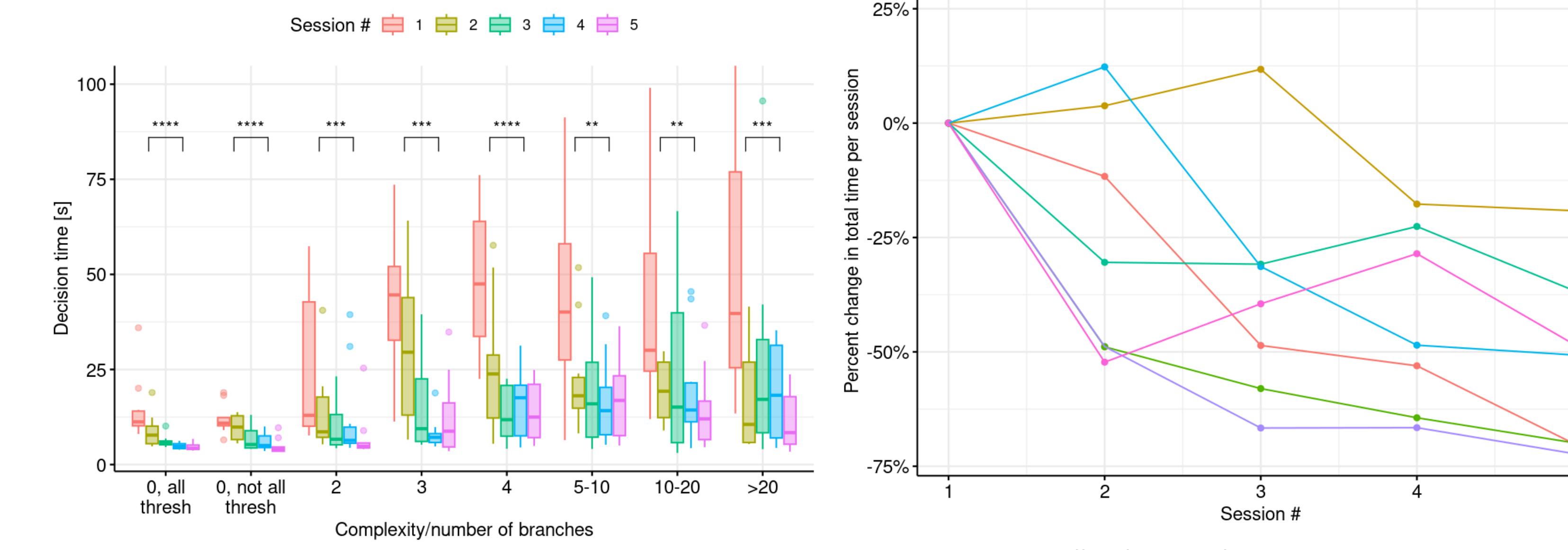
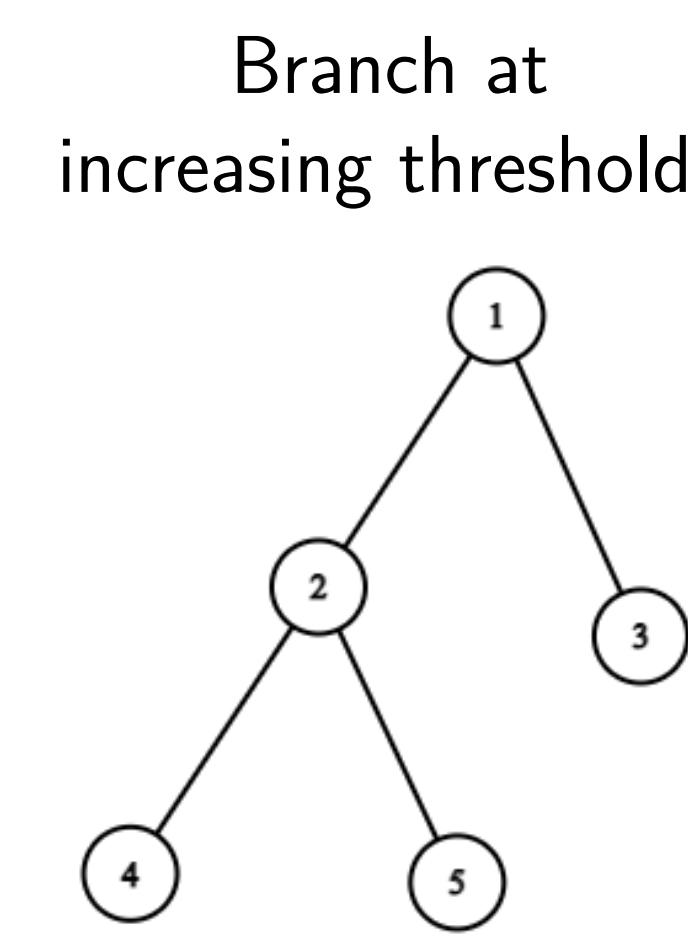


Figure 9. Proofreading task decision time as a function of complexity and session for a single user in training.

Segmented mitochondria in an astrocyte

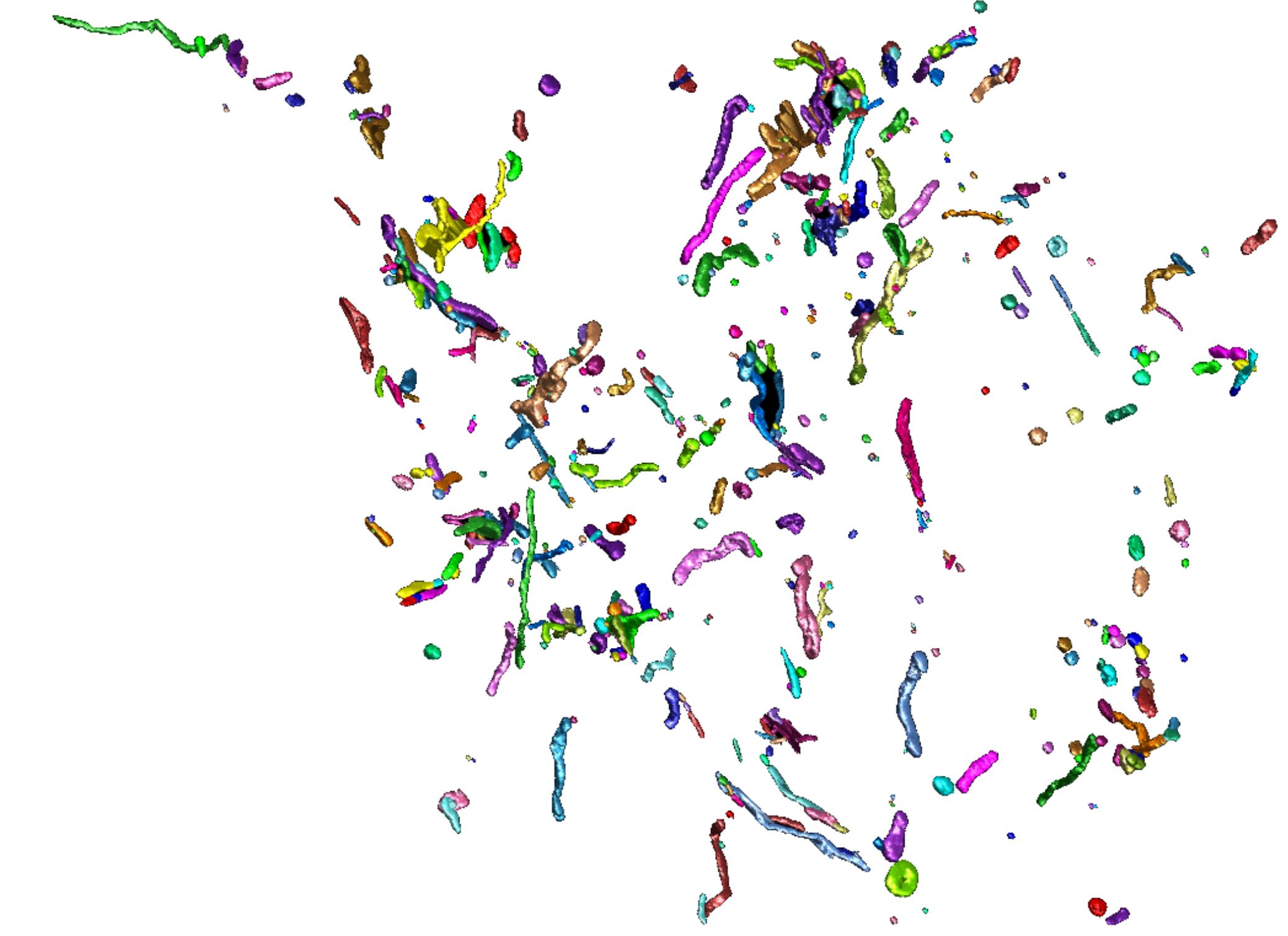


Figure 10. Mitochondrion within an astrocyte including its cell body and fine processes.

Results

- 53% of the 19,400 connected components **never branch** when probability thresholds increase with 16% at all thresholds and 37% at lower thresholds.
- 22% branch **only one time** prior to a threshold of 90% or continuously branch right before they threshold out.
- Extremely complex branching patterns require human proofreading but lead to successful instance segmentation of mitochondria with decision times as low as 1 second per decision.
- Decision time improves (decreases) with increased exposure to proofreading tasks for all proofreaders we trained.

Conclusions

- Taking advantage of the decreasing volume of connected components with increasing threshold in binary watershed leads to several distinct patterns and the development of at least partially automated instance segmentation techniques.
- Up to 75% of connected components have one or less branches, allowing for **automated** identification of the proper proofreading decisions in most cases.
- **Significant** reduction in human proofreading requirements leads to reasonable proofreading times.
- The addition of the syGlass Virtual Reality environment will **significantly enhance** the 3D representation of the EM and labels, greatly improving throughput for human proofreading when required.

References

- [1] M. G. Haberl, C. Churas, L. Tindall, D. Boassa, S. Phan, E. A. Bushong, M. Madany, R. Akay, T. J. Deerinck, S. T. Peltier, and M. H. Ellisman. CDeep3M—Plug-and-Play cloud-based deep learning for image segmentation. *Nature Methods*, 15(9):677–680, 2018.
- [2] T. Zeng, B. Wu, and S. Ji. DeepEM3D: approaching human-level performance on 3D anisotropic EM image segmentation. *Bioinformatics*, 33(16):2555–2562, 2017.