# Semi-automated Neuron Boundary Detection and Slice Traversal Algorithm for Segmentation of Neurons from Electron Microscopy Images

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## ABSTRACT

Neuroscientists are developing new imaging techniques and generating large volumes of data in an effort to understand the complex structure of the nervous system. To aid in the analysis, new segmentation techniques for identifying neurons in these feature rich datasets are required. However, the extremely anisotropic resolution of the data makes segmentation and tracking across slices difficult. This paper presents a complete method for segmenting neurons in electron microscopy images and visualizing them in three-dimensions. First, we present an advanced method for identifying neuron membranes, necessary for whole neuron segmentation, using a machine learning approach. Next, neurons are segmented in each two-dimensional section and connected using correlation of regions between sections. These techniques, combined with a visual user interface, enable users to quickly segment whole neurons in large volumes.

## 1. INTRODUCTION

Neural circuit reconstruction is an important method for studying neural circuit connectivity and its behavioural implications. The differences between neuronal classes, patterns and connections are central to the study of the nervous system and critical for understanding how circuits process information. Electron microscopy (EM) is a useful method for determining the anatomy of individual neurons and their connectivity because it has a resolution that is high enough to identify features, such as synaptic contacts and gap junctions. These features define connectivity, and therefore are required for neural circuit reconstruction. Manual analysis of this data is extremely time consuming. Early work in mapping the complete nervous system of the relatively simple *C. elegans*, took many years [14]. Several researchers have undertaken extensive EM imaging projects in order to create detailed maps of neuronal structure and connectivity [5, 3, 13]. In comparison, newer imaging techniques are producing much larger volumes of very complex organisms, with thousands of neurons and millions of synapses [4, 2]. Thus, automating the reconstruction process is critical in the understanding of these large systems. Recent research using new imaging techniques and automated segmentation methods are enabling more rapid reconstructions of neural circuits.

## 2. METHODS

The overall method proposed in this paper for reconstructing whole neurons consists of two steps. First, neuron membranes are segmented in 2D and neuronal cross-sections are identified. Second, the regions are linked across all the sections to form 3D renderings. The initial neuron segmentation used for each 2D section incorporates previous work which uses a series of artificial neural networks (ANNs) to detect neuron membranes. This paper presents a method for improving the detection by incorporating learned membranes from sequential sections into another ANN. Also drawing from previous work, we incorporate an optimal path algorithm to connect similar regions through the volume to form complete 3D segmentations. This paper combines all of the above techniques into an interactive tool, called the Neuron Reconstruction Viewer (NeRV), that lets the user view large datasets, evaluate the segmentations, and make corrections to both the 2D membrane detection and the joining of regions through the sections to segment a neuron in 3D.

### 2.1 2D Membrane Detection

The method developed here for neuron membrane detection extends previous work, which uses a series of ANN classifiers and image stencil neighborhood feature vectors to detect

neuron membranes in 2D images [7]. In that paper, membrane detection was limited to features within a 2D section. This work has been extended to train on information from neighboring sections, using the confidence from sequential sections. Given the anisotropic nature of the data, sequential sections have very poor membrane correspondence. To account for this, classified results representing the membrane probability image are registered and a 3D stencil that spans 3 sections is formed for training. Finally, tensor voting, a method for closing remaining gaps, is used. This provides significantly improved segmentation results over the original method [7].

*Serial Neural Network Architecture.* In previous work, a serial classifier architecture was implemented that used a series of classifiers, each operating on input from the previous classifier, to incrementally gain knowledge of a large neighborhood [7, 11]. This architecture is novel for two reasons. First, the data used for training requires no preprocessing with filter banks or statistics, and the classifier is trained directly on sampled image intensities. Second, by applying several classifiers in series, each classifier uses the classification context provided by the previous network to improve membrane detection accuracy. To initialize this architecture, the first classifier is trained only on image intensities. Each remaining classifier in the series then uses an input vector containing samples from the original image appended with the values from the output of the previous classifier, yielding a larger feature vector. While the desired output labels remain the same, each classifier is dependent on the information from the previous network and therefore must be trained sequentially. The output from each network is used to generate an image that represents the membrane probability map at that stage.

*Sequential Section ANN Architecture.* Sequential sections from EM data often contain similar structures that we would like to use to improve the quality of the 2D segmentation. One way to do this would be with a stencil that spans multiple sections. However, the membrane locations between sections have poor correspondence. This is partly because of the anisotropic nature of the data, which often results in large movement of membranes between sections, and membranes sometimes do not run perpendicular to the cutting plane causing membranes to have low contrast and appear fuzzy. Membranes in sequential sections are near each other, but they do not correspond well enough to use them directly in a 3D stencil that would span multiple sections. To correct for this, we propose a novel approach which aligns sequential membrane probability map images using a correlation-based nonlinear registration. We prefer to register only the membrane probability images because the classification process has removed many of the internal structures that would make an extremely fine-scale nonlinear registration on raw image data difficult. More specifically, after the membrane detection is complete for each section using the serial ANN architecture, images are registered to each other and used, as input, for the ANN. The registration method proposed is a B-spline deformable registration [6]. Once registered, a 3D stencil that spans 3 adjacent sections samples the classification results from the previous stage and provides information to be used in the final classification step. The final feature vector used to train one last ANN contains the original intensities from the raw image and values from the registered learned membranes sampled using the 3D stencil.

*Tensor Voting.* Tensor voting [10] is the final algorithm used in our method for closing small gaps in the membrane detection. In tensor voting, a structure tensor casts a vote in a regional area around itself where the voting field is determined by the orientation and the stick-ness of the tensor as determined by the ratio of the eigenvalues. Tensor voting strengthens salient curvilinear structures, while removing noise and blobby artifacts [9].

## 2.2 Neuron Region Linking

For this paper, neuron identification across a stack of EM images is formulated as an optimal path problem with a graph data structure [8]. The vertices of the graph are defined as the regions obtained by 2D segmentation of the individual sections. Edges in the graph represent possible linkages between regions in neighboring sections. Linking together the neuron regions in the graph is performed using Dijkstra's shortest path algorithm. The resulting path through the graph is used to reconstruct the neuron in 3D.

## 2.3 Neuron Reconstruction Viewer

The automatic methods described up until this point all work fairly well on their own, but in the end, require the ability for viewing and editing of the segmentation results. The Neuron Reconstruction Viewer (NeRV) (shown in Figure 1) attempts to bridge these two requirements by providing an interface to large volumes of EM images and viewing of neuron segmentations, with the option to make corrections, which will, in the long term improve the segmentation. Primarily, NeRV is an interface for the user to view the raw image data and the 3D reconstruction. Interacting with the image data and the rendered neuron provides insight for the scientist on the arrangement of the neurons within the data. The pane on the left, in Figure 1, is mainly a slice viewer. The user can view the membrane detection, the region segmentation, and the raw data all in one viewer. Spheres highlight the paths neurons take through the volume. The keyboard arrow keys or the slider in the middle lets the user scroll through the sections. The pane on the right, is a 3D viewer of the reconstructed neuron. Raw image data can be turned off and on in this view, and users can select other sections simply by clicking on the area of the neuron.

# 3. RESULTS

Two EM datasets are segmented using the proposed methods. The first dataset is a stack of 400 sections from the ventral nerve cord of the *C. elegans* worm. The second dataset is a stack of 400 sections from the mouse neuropil. These datasets contain very different types of neural cells. The *C. elegans* data has a resolution of 6nm×6nm×33nm and each 2D section is 4008×2672 pixels, whereas the mouse neuropil data has a pixel resolution of 10nm×10nm×50nm and each 2D section is 4096×4096 pixels. Figure 2(a) shows images from each of these datasets. Note that the membranes in the mouse and worm images, are very different, varying

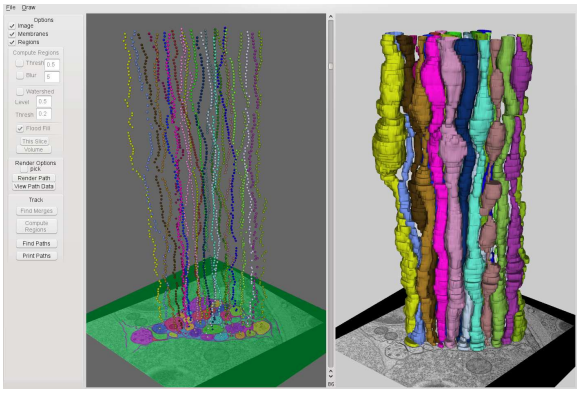


**Figure 1: [color] Screen capture of NeRV displaying the automatic segmentation results on the *C. elegans* ventral nerve cord for a portion of the data.**

in their signal to noise ratio and membrane thickness and contrast.

## 3.1 C. elegans Ventral Nerve Cord

The nematode *C. elegans* is an important organism for neural circuit reconstruction because it is the only organism for which the connectivity has been determined [14, 13]. To segment the membranes in this dataset and create a 3D reconstruction, we first had to align all the ssTEM images into a volume. We performed a ridged alignment using a brute-force search for the unknown rotation and translation between adjacent pairs of sections [12]. For validation, we had experts segment 40 selected images from the first 400 sections. Each expert placed a one pixel wide line along the membranes of the neurons, which we dilated using a 5 pixel wide structuring element, to cover most of the membrane pixels. For the training data, 30 images were randomly selected for training and the remaining 10 were used for validation. From those images, 1 million samples were randomly selected from the manually marked images. Because of the relatively small percentage of positive examples (representing membrane pixels), these 1 million samples were chosen to contain $\frac{1}{3}$ positive and $\frac{2}{3}$ negative examples.

Figure 2(a), left column, shows one section from the *C. elegans* dataset. The final membrane detection with the proposed method is shown in Figure 2(e). The sequential section ANN uses information about membranes also detected in neighboring sections to improve the current segmentation. The tensor voting uses, as input, the final classification and closes remaining gaps.

Figure 3(a) shows the 3D reconstruction of 10 neurons through the first 300 sections of the *C. elegans* ventral nerve cord. Building this reconstruction was a two part processes. First, we identified 6 significant breaks in the image volume where there was missing data due to lost or badly imaged sections. To completely reconstruct these paths through the whole 300 sections, NeRV was used to manually merge neurons in sequential sections, forming complete reconstructions through the whole volume.

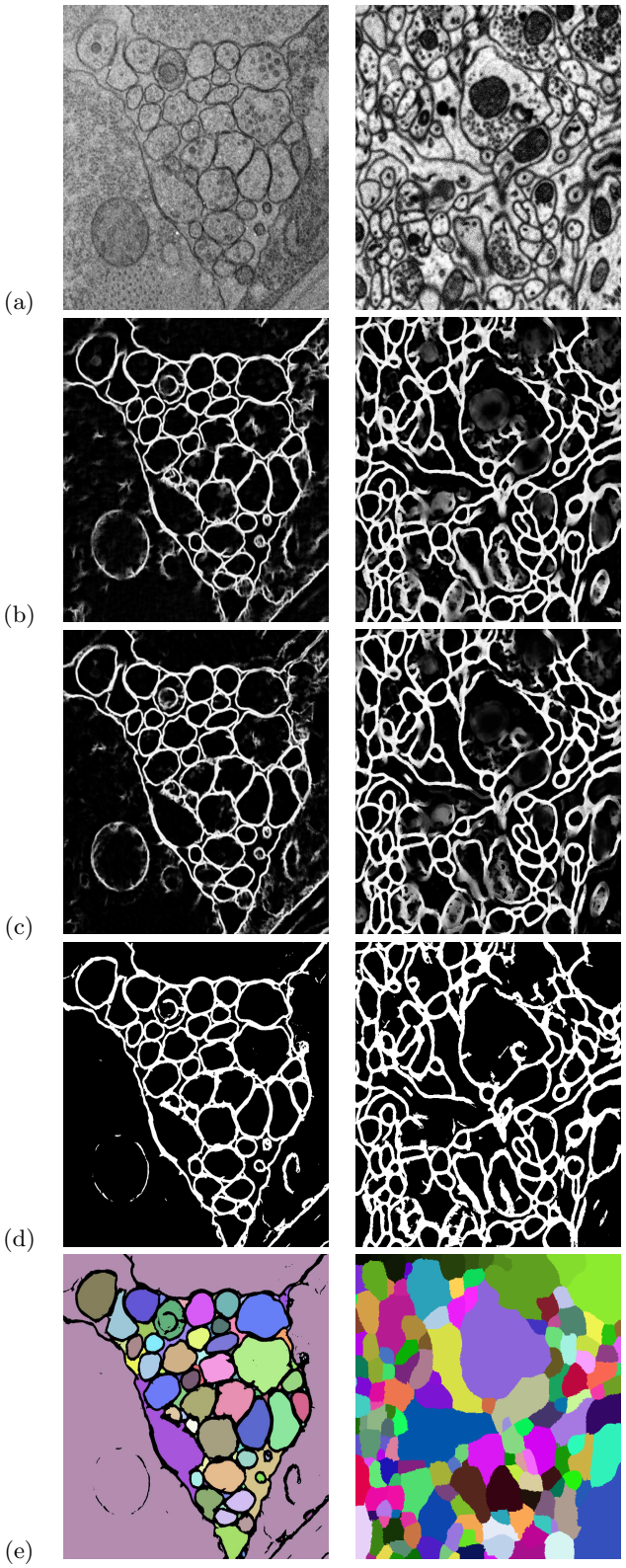
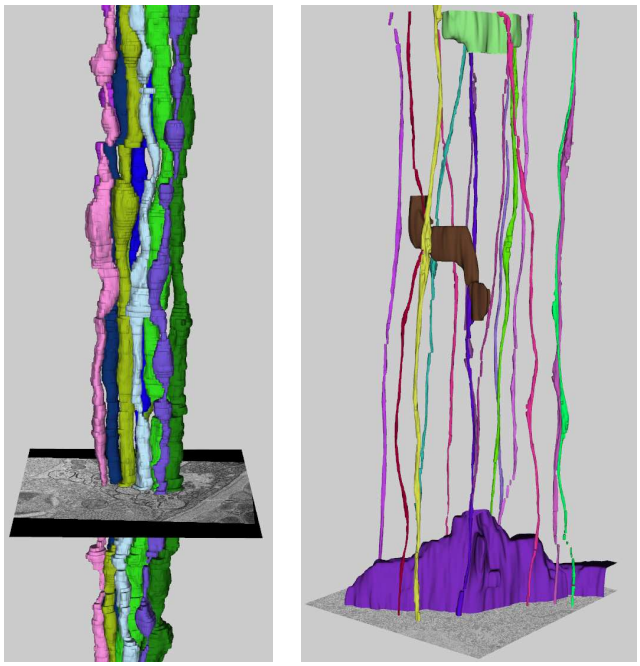



**Figure 2: [color] Output of the method on test images. (a) is the raw image, (b) is the output from the final stage of the series ANN (Section 2.1), (c) is the output from the sequential section ANN (Section 2.1), (d) is the output after tensor voting (Section 2.1), and (e) are the final segmentations of the neuron regions from a flood fill (left) and watershed segmentation algorithm (right).**



(a) (b)

**Figure 3: [color] (a) 10 neurons spanning 300 sections of the ventral nerve cord of the *C. elegans*. (b) 14 fully automatically segmented parallel fibers spanning 400 sections of the mouse neuropil. The larger 3 structures, Purkinje cells, were segmented manually using the NeRV interface.**

## 3.2 Mouse Neuropil

Understanding the connectivity, types of connections, and roles of different cells in the mouse neuropil is an increasingly more common area of study. The entire neuropil dataset is 4096×4096×400. To train and validate our neural networks, a subset of this data (700×700×270) was manually segmented using Amira [1] by an expert. From that set, 42 images were randomly selected and used for training in our classifier. The training set contained 4.5 million examples. To decrease training time, the ANN was trained first on 1 million examples for 50 iterations. The weights from this network were used to initialize the ANN for the 4.5 million training examples. The ANN contained one hidden layer of 10 nodes.

Figure 2, right column, shows the segmentation results on one section form the neuropil. Final reconstruction of the volume on the entire dataset turned this task into a large data challenge, since the actual size of the full volume is much larger than the training data. NeRV easily handles the size of this data because it only loaded into memory what was requested by the user. Finally, users can easily select the neurons they want to view in the volume. The final 3D visualization of this dataset can be seen in Figure 3(b).

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# 6. REFERENCES

[1] Visage imaging, amira.

[2] J. Anderson, B. Jones, J.-H. Yang, M. Shaw, C. Watt, P. Koshevoy, J. Spaltenstein, E. Jurrus, K. U.V., R. Whitaker, D. Mastronarde, T. Tasdizen, and R. Marc. A computational framework for ultrastructural mapping of neural circuitry. *PLoS Biology*, 7(3):e74, 2009.

[3] K. L. Briggman and W. Denk. Towards neural circuit reconstruction with volume electron microscopy techniques. *Current Opinion in Neurobiology*, 16(5):562–570, October 2006.

[4] K. L. Briggman and W. Denk. Towards neural circuit reconstruction with volume electron microscopy techniques. *Current Opinion in Neurobiology*, 16(5):562–570, October 2006.

[5] J. C. Fiala and K. M. Harris. Extending unbiased stereology of brain ultrastructure to three-dimensional volumes. *J Am Med Inform Assoc.*, 8(1):1–16, 2001.

[6] L. Ibanez, W. Schroeder, L. Ng, and J. Cates. *The ITK Software Guide*. Kitware, Inc. ISBN 1-930934-15-7, http://www.itk.org/ItkSoftwareGuide.pdf, second edition, 2005.

[7] E. Jurrus, A. Paiva, S. Watanabe, J. Anderson, B. Jones, R. Whitaker, E. Jorgensen, R. Marc, and T. Tasdizen. Detection of neuron membranes in electron microscopy images using a serial neural network architecture. *Medical Image Analysis*, 14(6):770–783, 2010. DOI: 10.1016/j.media.2010.06.002.

[8] E. Jurrus, R. Whitaker, B. Jones, R. Marc, and T. Tasdizen. An optimal-path approach for neural circuit reconstruction. In *Proceedings of the 5th IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, pages 1609–1612, 2008.

[9] Z. Leng, J. R. Korenberg, B. Roysam, and T. Tasdizen. Automatic markup of neural cell membranes using boosted decision stumps. In *Proceedings of the 6th IEEE International Symposium on Biomedical Imaging*, 2011.

[10] G. Medioni, M.-S. Lee, and C.-K. Tang. *Computational Framework for Segmentation and Grouping*. Elsevier Science Inc., New York, NY, USA, 2000.

[11] A. Paiva, E. Jurrus, and T. Tasdizen. Using sequential context for image analysis. In *Pattern Recognition (ICPR), 2010 20th International Conference on*, pages 2800 –2803, aug. 2010.

[12] T. Tasdizen, P. Koshevoy, B. C. Grimm, J. R. Anderson, B. W. Jones, C. B. Watt, R. T. Whitaker, and R. E. Marc. Automatic mosaicking and volume assembly for high-throughput serial-section transmission electron microscopy. *Journal of Neuroscience Methods*, 193(1):132 – 144, 2010.

[13] L. R. Varshney, B. L. Chen, E. Paniagua, D. H. Hall, and D. B. Chklovskii. Structural properties of the <italic>caenorhabditis elegans</italic> neuronal network. *PLoS Comput Biol*, 7(2):e1001066, 02 2011.

[14] J. White, E. Southgate, J. Thomson, and F. Brenner. The structure of the nervous system of the nematode caenorhabditis elegans. *Phil. Trans. Roy. Soc. London Ser. B Biol. Sci.*, 314:1–340, 1986.