

Low-level Neuron Segmentation in Sub-micron **Resolution Images of the Complete Mouse Brain**

Summary

- Motivation: To develop an automated neuron segmentation algorithm to accelerate construction of mouse neuromorphological atlases.
- Method: Generated an image segmentation dataset from point-based neuron traces and compared a multi-layer perceptron model to a baseline model and a state of the art convolutional neural network.
- **Results:** The multilayer perceptron model performed best on a dataset involving 46 $(100 \mu m)^3$ fully traced subvolumes.

Introduction

Imaging advances have made it possible to assemble atlases of neuromorphology, but manual tracing remains a bottleneck [5]. Neurons are well resolved (Fig. 1), but image inhomogeneities render simple automated segmentation solutions, such as intensity thresholding, ineffective. On the other hand, the large scale of the data ($\sim 15 \text{ TB}$ per channel), makes segmentation efficiency crucial.

Many existing "ground-truth" neuron traces involve points in space, and edges connecting them, making them incompatible with state of the art algorithms that operate on voxels, such as convolutional neural networks. Here, we convert point-based neuron traces to neuron segmentation masks, then implement a multi-layer perceptron model that performs well in the task of neuron segmentation.



Figure 1: Coronal view of an image volume in the Mouselight dataset. Neuron traces are overlaid in color, and a cutout of a fluorescently labeled neuron is enlarged to illustrate resolution.

Thomas L. Athey ¹, Jeremias Sulam ¹, Joshua T. Vogelstein ¹, Ulrich Mueller ², Michael I. Miller ¹ ¹Department of Biomedical Engineering, Johns Hopkins University, ²Department of Neuroscience, Johns Hopkins University

Materials

Our work uses data from the Mouselight Project at HHMI Janelia [5]. The dataset involves 50 $(100\mu m)^3$, or $330 \times 330 \times 100$ voxel subvolumes taken from two different mouse brains. All neuronal processes contained in the subvolumes were traced. The image segmentation algorithms compared were:

- **1** Logistic Classifier (LC): a baseline segmentation model, whose input is the voxel's intensity [4].
- **2** TRAILMAP (TM): state of the art segmentation model [2].
- **3** Multi-Layer Perceptron (MLP): our proposed segmentation model, whose input is the $7 \times 7 \times 7$ neighborhood centered at the voxel, with 1 hidden layer of size 100 [4].

Rationale for MLP Model

We start with the popular assumption that voxel labels are increasingly dependent the closer the voxels are. In particular, we make a local Markov assumption that the class membership of voxel i (denoted C_i), once conditioned on all voxels in a local neighborhood, is independent of the class membership of voxels outside the neighborhood.

We use a multi-layer perceptron (MLP) to approximate the posterior distribution $P(C_i|N_i)$ where N_i is the set of intensities in the neighborhood of voxel *i*. We chose MLPs due to their flexibility and scalability. Finally, we estimate the parameters θ by optimizing cross-entropy loss, which is equivalent to a maximum likelihood procedure in our binary classification setting.

After 4 subvolumes were removed due to trace mis-

alignment, the subvolumes were separated randomly into a training, validation, and test sets of size 38, 4 and 4 respectively. The traces were converted into image segmentations as follows:

• For edges: fill using the Bresenham algorithm [1]. **3** Fill in voxels within $1\mu m$ (a reasonable radius of axons in mice [3]) of previously filled voxels.

LC and MLP were both trained on 76000 random voxels from the training set. Training data for both of these models was centered and scaled to unit variance. TM was initialized with the original weights and the weights that performed best on the validation set over 70 training epochs were saved.



Methods

• For points: fill in the nearest voxel.



Figure 2: Receiver operating characteristic curves for the three classifiers on the test set.

Figure 3: Example in the test set (maximum intensity projection), with segmentations given by translucent red masks. Left: Original image, Center: Ground truth, Right: MLP segmentation after removal of components with volume $< (2\mu m)^3$.

As shown in Fig. 2, our *MLP* model performed better than both the baseline intensity classifier, LC, and the TRAILMAP model. The fact that TRAILMAP did not even perform better than the baseline classifier suggests that either the model was not trained for long enough, or there was not enough training data. TRAILMAP may be the best classifier if these limitations are addressed, but the high AUC of the MLP model (0.96) indicates that there is not much room for improvement in this binary classification task. As shown in Fig. 3, false positive regions still exist, and true positive neuron processes are sometimes split into sections. So, future work will focus on these higher level structures to complete the automatic reconstruction of neurons.

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Conclusion

References

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Contact Information

Email: tathey1@jhmi.edu