Robust Agglomeration of Labeled Neurons using 3D Skeletonization

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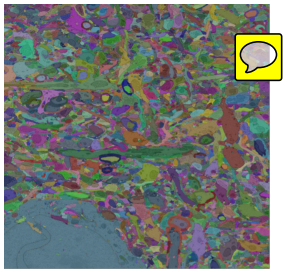
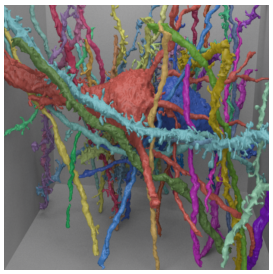




Abstract

Advancements in electron microscopy image acquisition has created massive datasets petabytes in size that are too large for complete manual segmentation. Several previous strategies for automatic segmentation of these datasets ~~re~~ work at the per-voxel level. Oftentimes, agglomeration strategies build on top of this layer to produce neuron reconstructions. Despite the success of these algorithms, there is significant room for improvement. Here, we propose a novel framework for correcting errors in existing agglomeration strategies. We create a level of abstraction on top of the existing methods by reformulating the segmentations as a graph and applying global graph-based optimization strategies. In the process we provide key insights into the use of machine-learned shape features for fixing errors.

1. Introduction

The field of connectomics concerns the wiring diagram of the brain at nanometer resolutions. Recent advancements in image acquisition using serial-section electron microscopy (sEM) has allowed neuroscientists to produce a terabyte of electron micrscopy (EM) image data every hour [12]. It is not feasible for domain experts to manually segment the vast amount of 3-D image data to model an entire brain. Neuroscientists believe that reconstructing an entire mammilian brain at fine resolution will enable new insights into the workings of the brain [16]. These observations will allow for new advancements in neuromedicine and artificial intelligence (CITE). Segmentation is part of this reconstruction process where we assign a unique label for every neuron in the EM image.

A significant amount of research focuses on automatic reconstruction of the neurons in ~~these~~ EM images because of the scope and importance of the problem. Several of these algorithms ~~attempt to~~ extract complete neurons through the 3-D volumes using only the raw image data as input. Oftentimes, convolutional neural networks predict membrane probabilities or affinities between voxels and apply simple thresholds to agglomerate the voxels into clusters [20, 29]. These *per-voxel* algorithms produce useful outputs but currently fall short of complete reconstructions with sufficient accuracy.

Researches currently address the failures of the per-voxel algorithms by training random-forest classifiers to agglomerate an oversegmentation of voxels [23] (CITE NEUROPROOF). These classifiers take the output of the per-voxel algorithms as input and generate high-level statistics such as affinity distributions between pixel regions. Presently, these methods use hand-designed features despite the evidence that machine-learned features perform better [4]. These *per-supervoxel* algorithms outperform the per-voxel algorithms but still do not provide the accuracy needed for large scale reconstruction of the brain. Additionally, these methods do not fully leverage the wealth of shape information available.

Here we present a *shape-based* strategy that builds on top of the outputs of per-supervoxel methods. Similarly we take as input the output of the per-supervoxel methods. However, we focus on the overarching shapes traversing through the label volumes to identify potential merge candidates. From these locations we extract machine-learned features and generate a probability that two segments should merge. Lastly, we construct a graphical representation of the input label volume and apply a global segmentation algorithm to produce a better reconstruction. Using graph-based optimization strategies enables us to enforce global constraints that more closely match the underlying biology of the images.

2. Related Work

A large amount of connectomics research considers the problem of extracting segmentation information at the voxel level from the raw EM images. Some methods apply computationally expensive graph partitioning techniques with a node per voxel [1] ~~following the previous work in computer vision~~ using normalized cuts for image segmentation [15, 31, 32]. ~~Meanwhile,~~ others focus on training 2-D convolutional neural networks to predict membrane probabilities per image slice [6]. More recent strategies augment these neural networks with the z-dimension creating full 3-D convolutional networks [20]. Oftentimes these networks ~~now~~ produce probabilities for the affinity between voxels rather than probabilities for a voxel belonging to a cell membrane [29]. An extensive amount of research in computer vision, applied mathematics, and statistics evaluates the loss functions and optimizers for these networks [5, 21, 22].

These ~~already neural networks~~ generate probabilities that neighboring voxels belong to the same neuron. Many algorithms work ~~at a level above these networks~~ and train random-forest classifiers to produce segmentations of the EM images where every unique neuron in the volume has a unique label [19, 23, 27, 34] (CITE NEUROPROOF). Despite the success of these classifiers, they often make mistakes based on the local information leading to errors in the segmentation. Proofreading methods create a "human-in-the-loop" framework that allows users to find errors and correct them [9, 10, 11, 35]. More recently, flood-filling networks merge the process of generating voxel affinities and then producing segmentations by training an end-to-end neural network that outputs segmentations [14]. Although these networks produce impressive segmentation accuracies, they are currently too slow for large scale connectomics reconstructions.

Many segmentation and clustering algorithms use graph partitioning techniques [1]. The multicut graph partitioning algorithm extracts segments the graph and remove edges such that there are no cycles. This closely resembles the biological constraint that neurons have a topological genus of zero. There are several useful multicut heuristics which provide good approximations with reasonable computational costs [13, 17, 18]

A sizable amount of computer vision research analyzes various hand-designed shape features for determining similarity between objects [24] and for segmentation[7]. Currently, the random-forest classifiers used in connectomics rely on hand-designed features to make merge decisions. Some of these features encode the distribution of probabilities that voxels along a segment boundary belong to the same neuron as their neighbors [23, 28]. However, there is evidence that machine-learned features outperform hand-designed ones [4].

The fields of computer graphics, mathematics, and biomedical visualization produce extensive research into the skeletonization of 3D binary volumes. Some of the research explores topologically consistent thinning algorithms and potential medical applications of these methods [25, 26]. In computer graphics, fast medial axis algorithms allow animators to quickly move characters through successive frames [2, 3]. In these works, the skeleton acts as the simplest representation of a 3-D volume. The Tree-structure Extraction Algorithm for Accurate and Robust Skeletons (TEASER) has been used by connectomics researchers to parameterize 3-D shapes [30, 33].

3. Method

Our method takes as input an ~~existing~~ oversegmentation of an EM image volume. Section 4.1.1 discusses two ~~pre-existing~~ pipelines for generating these segmentations. We evaluate our proposed method on the outputs from both of these pipelines.

3.1. Graph Creation

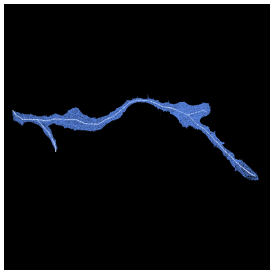
We ~~need to~~ generate nodes N and edges E to apply a graph-based optimization strategy for segmentation. In addition, these edges need non-negative weights.

3.1.1 Node Generation

The simplest node generation strategy creates one node for every unique segment label in the input volume. However, there are often a small number of labels in the volume corresponding to very small regions. It is difficult to extract useful shape features from these segments because of their small, and often random, shape. These segments are pruned from the graph and do not have corresponding nodes. For ~~the proposed~~ framework, we remove segments with fewer than 20,000 voxels (ADD PERCENT, current estimate less than 10%). Figure 1 shows two typical input segments that receive a ~~corresponding~~ node in the graph. .

3.1.2 Edge Generation

A naïve approach to generating edges ~~simply~~ produces an edge between all segments that have a single pair of neighboring voxels. Current agglomeration strategies such as NeuroProof or GALA use this criteria when deciding pairs of segments to merge into one neuron. However, for us this method will produce too many edges in our graph, many of which are easily pruneable. Many of the segments that are erroneously split have a similar structure. Consider Figure 2 which shows two such pairs. In both instances the segments around the break follow the same general shape. The segment is tubular in the close vicinity with an abrupt



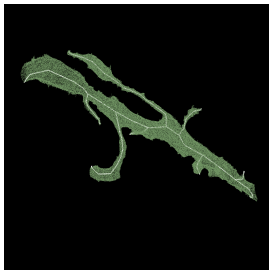
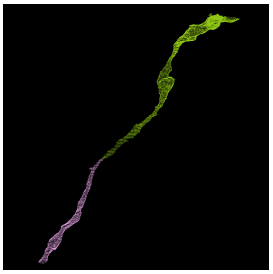


Figure 1: Two example outputs of the TEASER skeletonization algorithm.



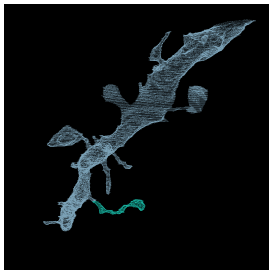


Figure 2: Two erroneously split segments that should merge together. Most segments that we want to merge have the same general structure.

break perpendicular to the elongated direction. We present the following algorithm to identify these locations.

We generate a skeleton for every segment following the intuition that a skeleton represents a simplified representation of the overall shape of an object. We use the Tree-structure Extraction Algorithm for Accurate and Robust Skeletons (TEASER) [30, 33]. These skeletons consist of a sequence of *joints* with edges between successive joints. We prune the joints that are within 50 voxels of each other to reduce unnecessary branching. For the purposes of this algorithm, joints that have only one connected neighbor are referred to as *endpoints*. Figure 1 shows the skeletons in white of two typical segments from the label volume. After skeletonization, we begin to identify segments for merge consideration with the following two-pass heuristic. In the first pass, we iterate over all endpoints e belonging to a segment S and create a set of segments $\mathbb{S}'_e$ which includes all labels that have a single voxel within t_{low} nanometers from e. This first pass often includes too many candidates that should not merge so we apply an additional pass to prune these sets. In the second pass, we consider all of the segments $\mathbb{S}'_e$ for every endpoint e. If a segment $S' \in \mathbb{S}'_e$ has an endpoint within t_{high} nanometers of e, the segment S and S' are considered for merging. We store the midpoint between the two endpoints as the "center" of the potential merge. Algorithm (ADD REF) provides pseudocode for this edge generation algorithm. The results in this paper follow from $t_{low} = 240$ and $t_{high} = 600$.

function GENERATEEDGES($skeletons$)
  for $skeleton$ in $skeletons$ **do**
    $candidates$ = set()
    for $endpoint$ in $skeleton$ **do**
      TODO ADD CODE
    end for
  end for
end function

Note that the above algorithm does not enforce any segment adjacency constraints. Figure (ADD FIGURE) shows two examples that would not be considered in previous research since they are not adjacent. Finding and merging these examples is one of the benefits of operating from per-pixel algorithms.

3.2. Edge Probabilities

The previous section outlines how to generate edges between nodes in our graph structure. Here we introduce a neural network architecture for generating probabilities that two nodes sharing an edge belong to the same neuron. The neural network takes as input only data from the input segmentation.

3.2.1 Network Architecture

The above skeletonization algorithm produces 3-D locations that require further consideration for merging. To determine which of these segments should actually merge, we train a 3-D convolutional neural network. We extract a cubic region with length 2000nm centered at these locations. These cubes will provide the local information for the neural network to predict which neighboring segments belong to the same neuron. A cubic length of 1200nm provides enough local shape context for the network without introducing an excessive amount of noise. Figure 3 shows cubes of lengths 800nm, 1200nm, and 1600nm.

We transform the extracted cubes for input into the neural network. The network takes three input channels for each voxel in the 3-D volume corresponding to the following mapping. Consider a pair of segments with labels l_1 and l_2 for every voxel v in the cube of interest. The first channel is 0.5 if $v = l_1$ and -0.5 otherwise, the second channel is 0.5 if $v = l_2$ and -0.5 otherwise, and the third channel is 0.5 if $v = l_1$ or $v = l_2$ and -0.5 otherwise. Each 3-channel volume is subsequently downsampled into an array of size $(3, 22, 22, 8)$ using nearest-neighbor interpolation. Our neural network trains on these 4-D arrays to generate probabilities that l_1 and l_2 should merge.

Our network architecture has three layers of double convolutions followed by a max pooling step [5]. As described above, the input ~~with~~ into the network is $(22, 6[illegible])$ with 3 channels. The filter size of the layers are 16, 32, and 64

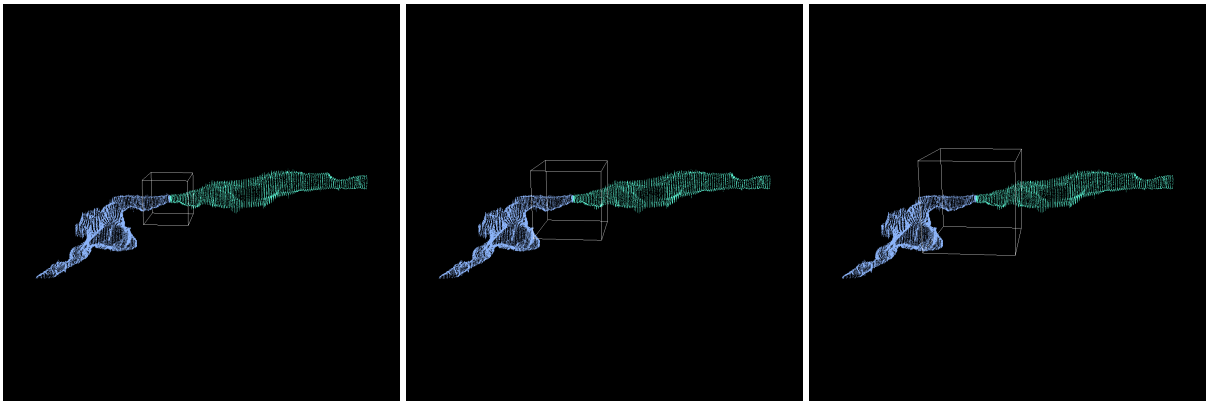


Figure 3: The white outlines show three different possible cubic sizes to input into the neural network. The left example (800nm) provides less local context than the middle example (1200nm). The right example (1600nm) extracts too large of a local region that produces noise as one of the segments leaves the bounding box only to reenter.

with size doubling deeper into the network. The first two max pooling layers are anisotropic with pooling only in x and y to match the anisotropic nature of our EM datasets. All convolutions have kernel sizes of $(3, 3, 3)$. The output size of the final max pooling layer is $(5, 5, 5)$ with 64 channels. The output is flattened into a 1-D vector with 8000 entries which enters two fully connected layers with 512 and 1 dimensions in the output respectively. All activation functions are LeakyReLU with $\alpha = 0.001$ except for the final activation which uses a sigmoid function [8, 21]. There is a dropout of 0.2 after every pooling layer and the first dense layer. There is a dropout of 0.5 after the final dense layer. The above method uses stochastic gradient descent with Nesterov's accelerated gradient [22]. This optimizer has an initial learning rate of 0.01, momentum of 0.9, and a decay rate of $5 * 10^{-8}$. Figure 4 provides an overview of the ~~proposed~~ architecture.

3.2.2 Data Augmentation

We apply ~~some~~ data augmentation to the generated examples to increase the size of the training datasets. We consider all rotations of 90 degrees along the xy-plane in addition to mirrors along the x and z axes. This produces ~~an additional~~ 16 times more training data.

3.3. Agglomeration

After constructing the ~~above~~ graph structure we ~~can~~ apply a graph-based segmentation strategy. There are many formulations of graph-based optimization strategies that provide different guarantees on their output. Neurons in the brain should be acyclic, i.e. the output shape should have a genus of zero. Current connectomics agglomeration techniques do not leverage this additional information but rather consider neighboring regions in successive order without regard to loop creation. In our graph formulation we ~~can~~ enforce this topological property by applying a multicut partition onto the graph which generates a forest on the nodes. There are several heuristics that solve the multicut problem. For our purposes we use the Kernighan-Lin algorithm [17].

4. Evaluation

4.1. Datasets

We evaluate our methods on three EM datasets. TODO: Toufiq: add description of the EM datasets (volume size, resolution, type of animal, type of image scan)

4.1.1 NeuroProof Pipeline

Our methods build on top of existing agglomeration strategies. For the purpose of this paper we used the outputs from two established pipelines. TODO: Toufiq description of neuroproof pipeline, description of FlyEM pipeline

4.2. Preprocessing

We perform significant pruning of potential merge candidates by considering only pairs of skeleton endpoints produced by Algorithm **??**. This strategy greatly reduced the number of false merge candidates that we considered. However, we evaluate the overall effectiveness of this strategy both in reducing trivial split candidates while maintaining a large majority of the candidates which should merge.

Our candidate generation strategy doesn't enforce an adjacency constraint which allows us to merge candidates that are not consider under existing frameworks. We examine the success of our pipeline in merging split candidates.

4.3. Classifier Training

DISCUSS PARAMETERS FOR CLASSIFICATION

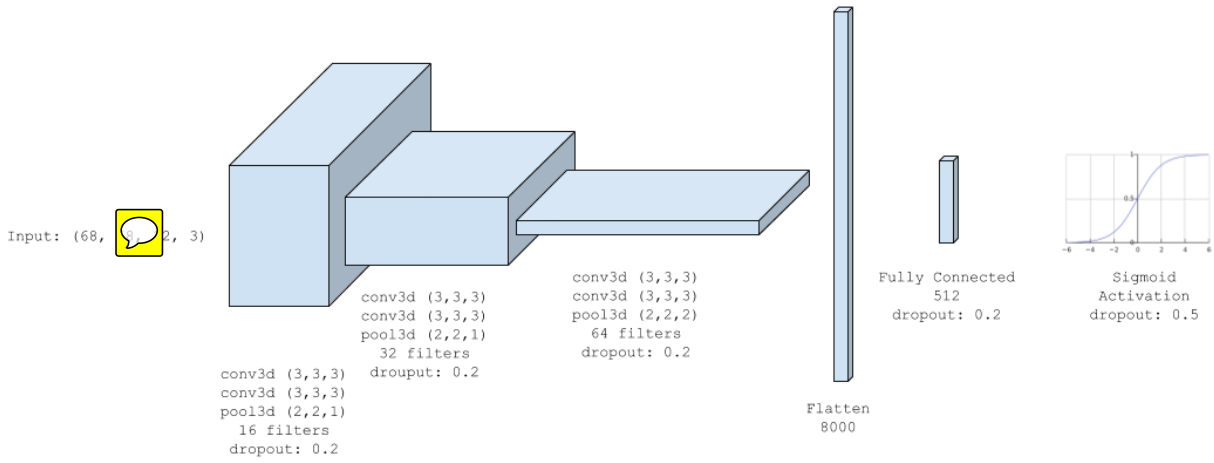


Figure 4: The architecture for the neural networks follows the *VGG* style of double convolutions followed by a max pooling operation. The number of filters doubles each layer leading to a fully connected layer and a sigmoid activation function.

4.4. Graph Optimization

We evaluate the improvement of using a graph optimization strategy over a naïve approach that performs hierarchical clustering for all segments above a certain threshold. Using a heuristic for multicut prevents the creation of cycles. Using a simple agglomeration strategy generates this many cycles.

5. Results

Here we show the results for the three main contributions for this paper: merge consideration using skeleton pruning, merge probabilities output from the trained convolutional neural network, and the final output of the pipeline.

5.1. Skeleton Pruning

5.1.1 Finding Merge Candidates

5.1.2 Finding Split Candidates

Effective pruning for merge candidates should have high precision and recall for segments that should be merged.

5.2. Neural Network Classification

5.2.1 Merge Network

Figure **??** shows the training and validation loss during the training of the neural network.

5.2.2 Split Network

5.3. Graph-based Error Correction

6. Conclusions

- Impact
- Future work

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