Deep Learning and Shapes Similarity for Joint Segmentation and Tracing single Neurons in SEM images

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ABSTRACT

Extracting the structure of single neurons is critical for understanding how they function within the neural circuits. Recent developments in microscopy techniques, and the widely recognized need for openness and standardization provide a community resource for automated reconstruction of dendritic and axonal morphology of single neurons. In order to look into the fine structure of neurons, we use the Automated Tape-collecting Ultra Microtome Scanning Electron Microscopy (ATUM-SEM) to get images sequence of serial sections of animal brain tissue that densely packed with neurons. Different from other neuron reconstruction method, we propose a method that enhances the SEM images by detecting the neuronal membranes with deep convolutional neural network (DCNN) and segments single neurons by active contour with group shape similarity. We joint the segmentation and tracing together and they interact with each other by alternate iteration that tracing aids the selection of candidate region patch for active contour segmentation while the segmentation provides the neuron geometrical features which improve the robustness of tracing. The tracing model mainly relies on the neuron geometrical features and is updated after neuron being segmented on the every next section. Our method enables the reconstruction of neurons of the drosophila mushroom body which is cut to serial sections and imaged under SEM. Our method provides an elementary step for the whole reconstruction of neuronal networks.

Keywords: Deep convolutional neural network; neuron reconstruction; active contour; membrane detection probability map, neuron tracing.

1. INTRODUCTION

Since the birth of modern neuroscience, the prevailing approach for understanding neuronal morphology has been to spend many hours, days, and weeks manually delineating complicated neuronal shapes visualized using a variety of staining techniques [1]. Until recently, experimental techniques in neuroscience have either provided detail information on a micro-scale (electron microscopy) up to serval nanometers or information averaged over relatively lager scale (fMRI, DTI, EEG). Using the high resolution Electron Microscopy (EM) imaging techniques to obtain detailed knowledge has become a realistic objective attributed to the fact that EM makes it possible to acquire volume images up to $1\ mm^3$ at nanoscopic. However, detailed knowledge of the complete connectivity pattern of all neurons [2] would be of tremendous value for the understanding of neural computation. Based on the electron microscopy, we would be able to get large amounts of images which requires enormous work load to analysize [6]. The computer-supported tools either semi-automated or automated will enlighten the burden of manual labor of EM neuronal images data analysis. In addition, automated methods for extracting neurons structures will promote the research of neuronal functions in neuromechanism perspective.

The neurons connectivity that called connectomics mainly aims at the fully volume reconstruction of neuronal circuits in such a 3D-EM. Recent years, combinations of massive manual annotation with automated analysis methods have yielded first substantial connectivity maps in the fly optical system [4] and mouse retina [5]. In the later work, [1] invented the ATUM for automated neuron tissue slices acquisition and proposed a serial of automated methods that

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using deep convolutional neural network for the reconstruction of neural circuits. In [3], Helmstaedter provided tool-set "SegEM" that gave workflow for the large scale neuronal circuits reconstruction (which also called neuronal networks reconstruction).

As the elementary step of the neuronal networks reconstruction, our method that segmenting and traceing the single neurons iteratively is different with the general method which segments the whole neurons on sections simultaneously. As depicted in Figure 1 (a), we only focus on the single neurons, and joint segmentation and tracing together. With the ATUM-SEM techniques [1], we get a sequence of neuronal SEM images of serial sections. By tracing a certain single neuron through sections sequence and segmenting the neuron in each section, we can get the neuron structure within the neuronal volume afterwards.

2. MATERIAL AND METHODS

2.1 Data acquisition

We use drosophila mushroom body neural tissue as the sample which was stained by specific chemical reagent. Mushroom body is an area of drosophila brain that possesses important functions for drosophila physiological activities. To generate drosophila mushroom body image dataset, we collected 1,797 70-nm drosophila brain slices; each slice is then recorded as a 2D grayscale image with a pixel size of about 5×5 nanometers, and finally, we can get an $8k \times 8k$ image from each slice.

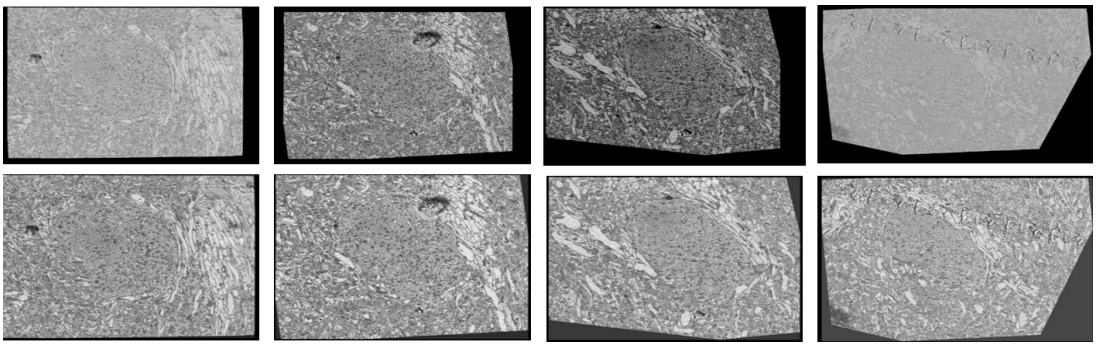
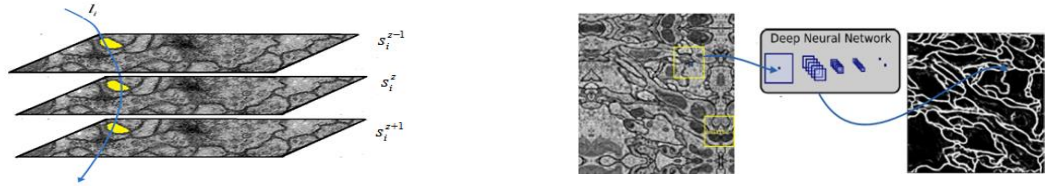


Figure 1: Examples of drosophila mushroom electron microscopy images

2.2 Deep learning based neuronal membranes detection

As the boundary of neurons, membranes are stained contrasted to intra-cellular and extra-cellular region. The detection of membrane is the basis of neuron segmentation. Our work is based on the deep convolutional neural networks [7]. The detection of neuron membrane can be converted to the task of pixel-wise classification that gives the label of each pixel or the probability of each pixel belonging to the membranes. Profiting from the ability of the DCNN, we can get much accurate membrane detection probability map (MDPM) which enhances the membranes and suppresses much of the noise.

Similar to the work of Ciresan [8,9], we use the DCNN as the pixel-wise classifier that gives the label of each pixel on the origin SEM images. Here we trained a DCNN on 3 million of labeled data which were labeled as membrane or non-membrane. The membrane detection framework is demonstrated in Figure 1 (b). For each given point on the SEM image, a square window patch centered around the point is extracted as the representation of point's local contextual information



(a) (b)

Figure 2 : (a) Demonstration of the neuron reconstruction, l_i is the trajectory of the certain i_{th} single neuron along the sections sequence, s_i^z denotes segmentation on the z_{th} section of the neuron. (b) Demonstration of the framework of the pixel-wise membrane detection, for each pixel on the SEM image, a fixed size square window patch is extracted as the input of the DCNN. The DCNN outputs the probability of the pixel classified as membrane.

In our experiments, the window size is important for the architecture of DCNNs and accordingly the accuracy of the prediction. We choose the window size by serval experiments and do not consider the model ensemble of different window sizes considering the predicting speed. Our work is based on Caffe [9], we created the deep convolution neural network of 6 weights layers including 4 convolutional layers and 2 fully connected layers. On the training stage, the DCNN outputs the label of each pixel on SEM images. The last layer is softmax : $\delta(x) = argmax(e^{w_j^t}/\sum_i^c e^{w_i^t})$, where x denotes the output of the last fully connected layer, w_i , w_j denotes the softmax layer weights. In our case, the feature is the output of the first fully connected layer. On the predict stage, we just let the DCNN output

$$Prob(x) = e^{w_M^t x} / \sum_j^c e^{w_j^t x} \quad (1)$$

where w_M denotes the weights vector that connects to the output node of membrane label.

2.3 Similarity of single neuron shapes on consecutive serial sections aids to segmentation

As described above, we use the DCNN to get the MDPM which enhance the membrane and filter out most of the non-membrane portion. Considering the property of SEM images of neuron sections, on which, the boundary of the neurons are membranes, we segment the neurons based on MDPM. Thus, image segmentation algorithms that use the boundary of objects will be more suitable for neuron segmentation. Among various techniques, the active contour model is widely used. A contour is evolved by minimizing certain energies to match the object boundary while preserving the smoothness of the contour. We need to segment the neurons on the SEM images of serial sections. The single neurons on the consecutive sections are similar in shape to some degree. Using the active contour with group similarity [11] is to our consideration. Given a sequence of SEM images $I_1, I_2, \cdots, I_n$, we try to find a set of contours $C_1, C_2, \cdots, C_n$ to segment the single neuron:

$$\min_X \sum_i^n f(C_i) + \| X \|_* \quad (2)$$

where the C_i is the coordinate vector of contour on the I_i, $\mathrm{X} = [C_1, C_2, \ldots, C_n]$, $||X||_*$ denotes the nuclear norm of matrix, $f(\cdot)$ is the energy function of active contour. The regularization term $||X||_*$ mainly constraints the dissimilarity of contour shapes. Due to the deformation of sections and the neuron self-change of morphology, the shape similarity is constrained to be within a limited number of sections. In the following, we will describe how we get the segmentation contour sequence iteratively.

2.4 Joint tracing and segmentation to single neuron reconstruction

The segmentations of single neurons is the basis of neuron reconstruction. As described above, we can get the segmentation of single neuron in a number of sections. In order to get the group of candidate patches, we need to trace the sections of the single neuron through the sequence. The SEM sequence is aligned by Chen[12], thus, the relationship of the positions of the single neuron sections is the most important information for tracing. Generally, the segmentation's centroids of the immediate neighbor sections are close with other. Using this criterion, we can get the approximate position estimation of the immediate next section by current segmentation centroid. Through the geometrical features including the size, orientation and position of the bounding box of the current segmentation, we obtain can the candidate patches of the next section. We get a small circle centered on the centroid of the current segmentation as the initial contour of the next section.

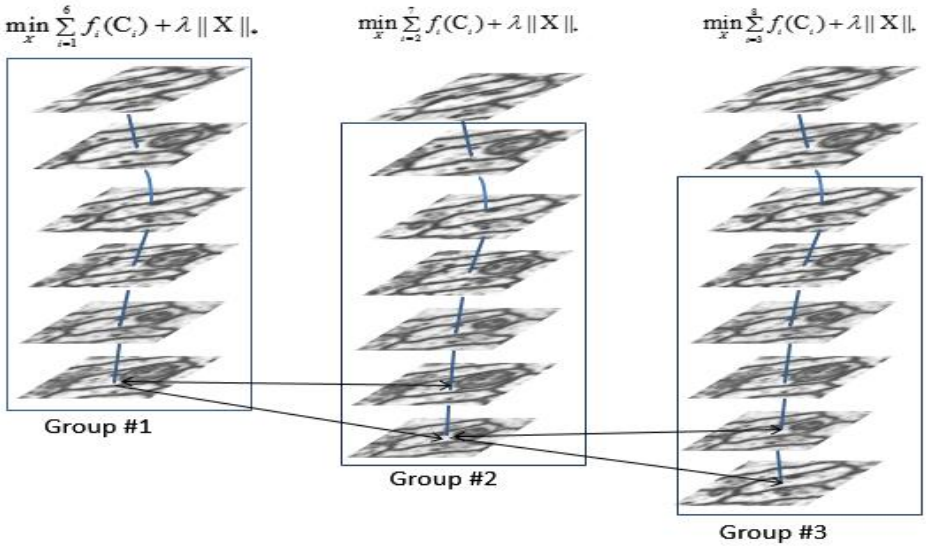


Figure 3: Framework of Joint tracking and group shape similarity segmentation

Using the active contour with group shape similarity, we get shape consistent segmentations of a fixed number of sections by iteration. In the initiation, we manually labeled the first n consecutive patches of the single neuron, where n is the fixed number we use for group similarity, and for affine transformation assumpation, n is set to 6. When we segment n patches, we can predict the position and candidate patch of the neuron section next to n_{th} patch. To trace along the sequence, we get the patches group like a slide window along the sequence, supposing that the current group contains p^i, p^{i+1}, ... , p^{i+n-1} then the next group contains p^{i+1}, $p^{i+2}, \ldots, p^{i+n}$, where p^i represents the patch on the i_{th} section, others accordingly, p^{i+n} is the predict patch on the $(n+i)_{th}$ section.

3. EXPERIMENT AND RESULTS

3.1 Evaluation

In our work, we use pixel error contrast to ground-truth as the membrane detection evaluation metric. In addition, we use the precision and success rate for quantitative analysis.

Pixe-error. One widely and directly used evaluation metric, which can be simply defined as the dissimilarity of the pixel of segmented image contrast to the ground-truth. In our experiments, the pixel error include the training error in and test error, we mostly refer to the test error as performance of membrane detection.

Mean IOU. In terms of semantic segmentation, the mean IOU represent the segmentation index of region intersection over union(IOU or IU), Let n_{ij} be the number of pixels of class i predicted to belong to class j, where there are n_{cl} different classes, and let $t_i = \sum_j n_{ij}$ be the total number of pixels of class i Mean IOU: $1/n_{cl} \sum_i n_{ii}/(t_i + \sum_j n_{ji} - n_{ii})$. We calculate the precision of each frame that can be used to analyze the stabilization of the joint tracking and segmentation.

Precision plot. In the tracking, one widely used evaluation metric is the center location error, which is defined as the average Euclidean distance between the center locations of the tracked targets and the manually labeled ground-truths. Then the average center location error over all the frames of one sequence is used to summarize the overall performance for that sequence.

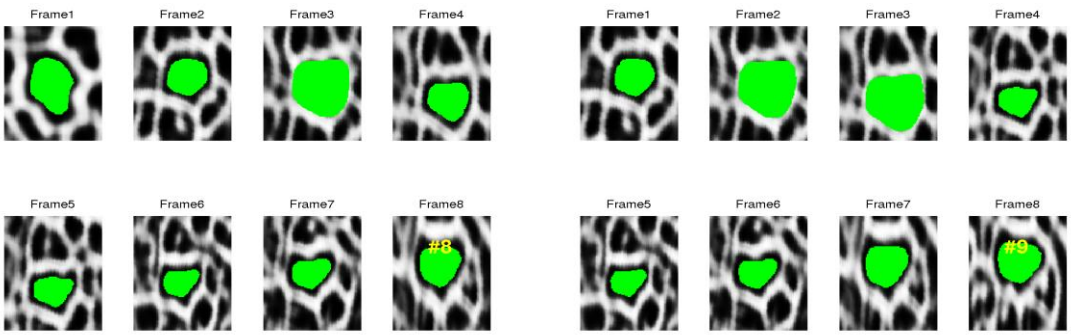
3.2 Results

We can get a binary map by thresholding on the MDPM with the median value of the MDPM. For the evaluation of membrane detection accuracy, we use the pixel error that is defined as the pixel dissimilarity between binary map and the ground truth [9]. Our results of membrane detection is in Table 1.

Table1: membrane detection pixel error

procedure	train	test
Pixel error	0.109	0.117

On the membrane detection probability map which gives the enhanced neuron profile, we segment the neuron region by active contour with group similarity. Some of the segmentation results are shown in Figure 4.



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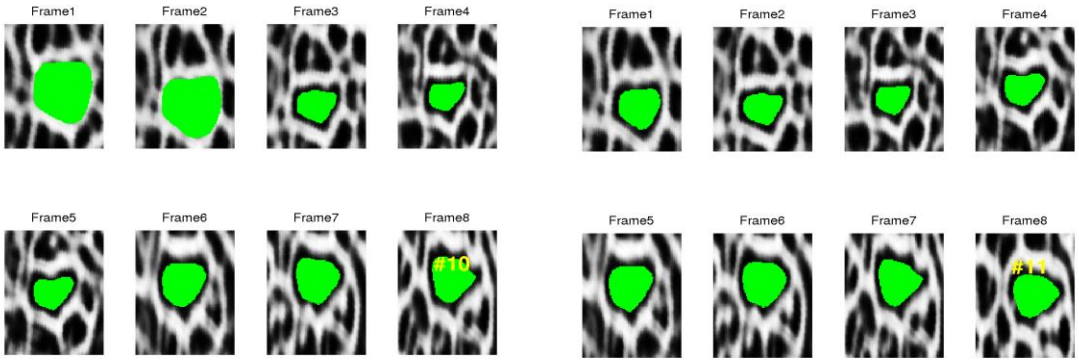


3 # 4

Figure 4: Neuron segmentation by active contour with group shape similarity. From left to right and top to bottom are the groups to be iteratively segmented with active contour

With the framework as demonstrated in Figure 3, we combined group similarity segmentation and tracking algorithm to get the joint tracking and segmentation results as in Figure 5.

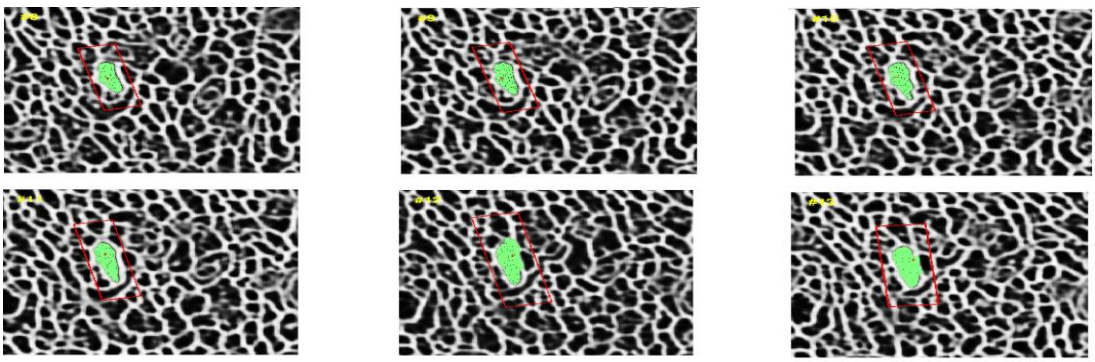
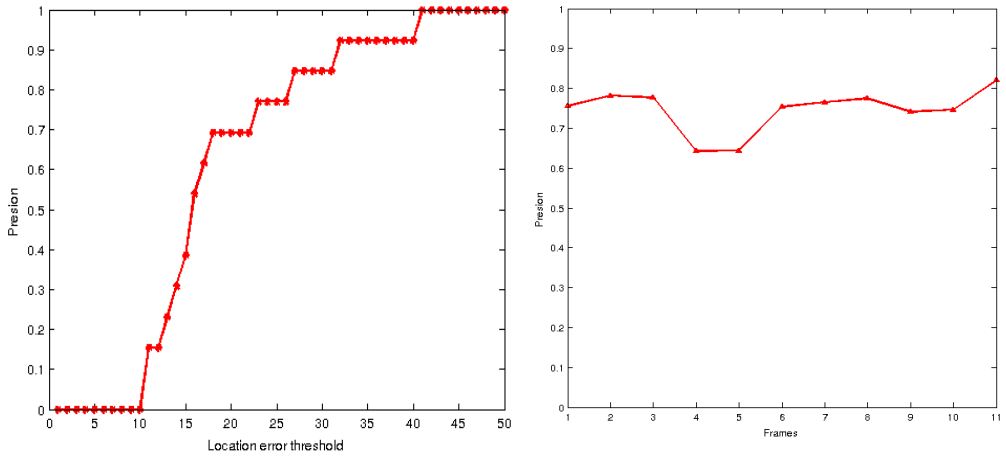


Figure 5: Some results of joint tracing and segmentation, the consecutive sections are depicted from left to right and top to bottom, the tracing speed is 5 frames per second

Our method is focused on the segmentation and tracing of single neurons. The main breakthrough of our method is that we enhance membrane by DCNN and consider the shapes similarity of the neuron sections for segmentation. The tracking **precision plot** is shown in the Figure 6 (a), and the **mean IOU** of each frame is shown in Figure 6 (b), while the average mean IOU over the frames is 0.73.



(a) (b)

Figure 6: (a) The location error-precision plot of the tracking. (b) The segmentation precision of each frame along the sequence.

4. CONCLUSION

Our method uses the DCNN as the first step to get membrane probability map. Considering the shape similarity of the single neuron sections, we use the active contour with group shapes similarity for segmentation. Our method that segmentation and tracing alternatively iterate with each other, provides a primitive step for single neuron reconstruction. In the following, we will focus on the 3D reconstruction of neurons from the segmentation and tracing of single neurons.

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