3D Reconstruction of Neurons in Electron Microscopy Images

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Abstract— **With the prevalence of brain-related diseases like Alzheimer in an increasing ageing population, Connectomics, the study of connections between neurons of the human brain, has emerged as a novel and challenging research topic. Accurate and fully automatic algorithms are needed to deal with the increasing amount of data from the brain images. This paper presents an automatic 3D neuron reconstruction technique where neurons within each slice image are first segmented and then linked across multiple slices within the publicly available Electron Microscopy dataset (SNEMI3D). First, random Forest classifier is adapted on top of superpixels for the neuron segmentation within each slice image. The maximum overlap between two consecutive images is then calculated for neuron linking, where the adjacency matrix of two different labeling of the segments is used to distinguish neuron merging and splitting. Experiments over the SNEMI3D dataset show that the proposed technique is efficient and accurate.**

I. INTRODUCTION

Discovering the wiring of the brain is a challenging process that targets a comprehensive 3D map of neuron connections within an organism's nervous system. Different methods have been proposed in recent years but accurate and efficient reconstruction of 3D neuron structures is still an open problem. In particular, the segmentation of the narrow neuron within each 2D slice as well as linkage of neurons across multiple slices are still prone to different types of error. In practice, a proof reading procedure is usually needed to spot and correct the errors in neuron segmentation and linkage that are introduced by CAD systems.

Various approaches have been proposed for neuron segmentation and linkage across layers. For example, level set appoch is used in various methods [1]. Macke et al. [2] used the level-set propagation of the probabilistic field between slices. They assume that the objects are continuous across adjacent images but neurons may not be always perpendicular to the imaging surface. Jurrus et al. [3] proposed an interactive method for axon tracing in the Electron Microscope (EM) data. They used iterative Kalman-snakes to estimate the contour of the axon in the next slice given the contour of the previous slice.

Vazquez-Reina et al. [4] used the sequence of watershed transforms to provide multiple segmentation hypotheses for each slice. They built a graph with nodes of all segmentation hypotheses and links of the overlap segments in consecutive slices. By considering global constraints on the node and link energies on the graph, the MAP-MRF is solved to segment the whole slices simultaneously. This method just handles the continuation of neurons and instead of merging and splitting. Funke et al. [5] followed the previous method and provided multiple hypotheses by changing the prior probability of pixels being membrane. In the next stage, using continuity constraints on the selecting the segments and their links, the optimization problem is solved which was relaxed by Integer Linear Programming method.

We propose a novel 3D neuron reconstruction technique. The proposed technique has three novelties. First, superpixel technique is adopted to first group similar pixels into membrane and non-membrane, hence providing prior knowledge on a typical two class classification problem. Second, a novel neuron linkage technique is proposed where neuron splitting information is exploited in two ways of Top-Down and Bottom-Up to find the merging neurons in different slices of the dataset. Third, the segmentation and linkage stages are automatic and the user interaction is limited.

II. METHOD

Figure 1 shows the pipeline of the proposed technique. A superpixel method is first applied from which a set of features are extracted. Random Forest Classifier is then adapted to provide the probability map of the images for segmentation. A novel linkage method is proposed to handle neuron splitting and merging across slices, which further lead to a 3D neuron reconstruction map as to be described in the following sections.

A. Segmentation

Segmentation of the EM data plays an important role in the 3D neuron reconstruction. A small neuron segmentation error may lead to a large neuron linkage error. This segmentation problem is actually a two-class classification problem: a pixel either belongs to the membrane or not. Neurons can be segmented through connected component analysis once each image pixel is classified.

Since the classification of each image pixel is a computationally intensive process, a superpixel algorithm is used to reduce the classification complexity. To create superpixels, the simple linear iterative clustering (SLIC) [6] method is used.In this regard, the complexity of the images decreases from pixels to superpixels.

Random forest classifier [7] is exploited to compute the probability map of each slice image as illustrated in Fig. 2b. The features of each superpixel are computed by the average of features of pixels in the superpixel. For each image

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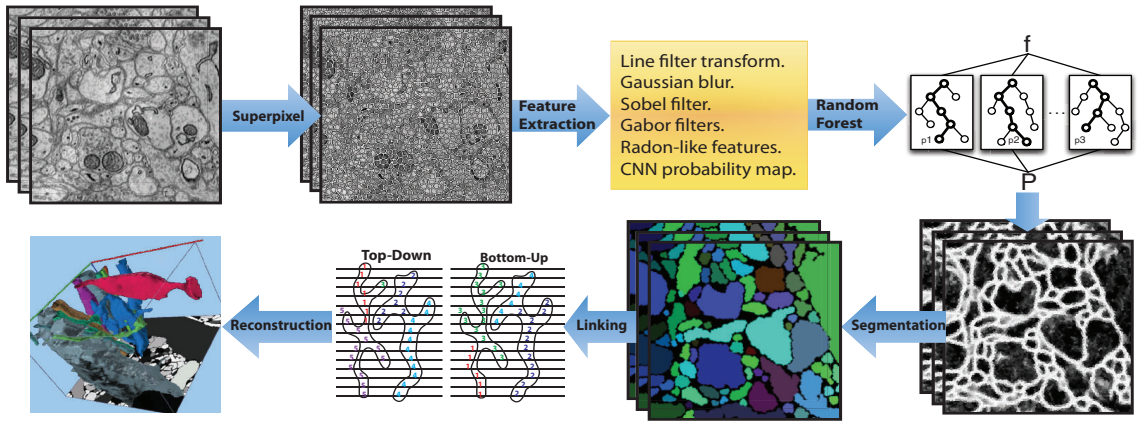

Fig. 1. The flowchart of the proposed method.

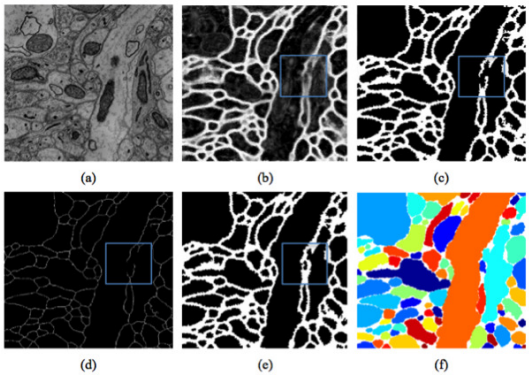

Fig. 2. (a) The input image. (b) The probability map of the image using RF. (c) The thresholded probability map and the discontinuity in the membrane. (d) Skeleton of probability map and the position of end points. (e) The out put after post processing. (f) The segmentation results.

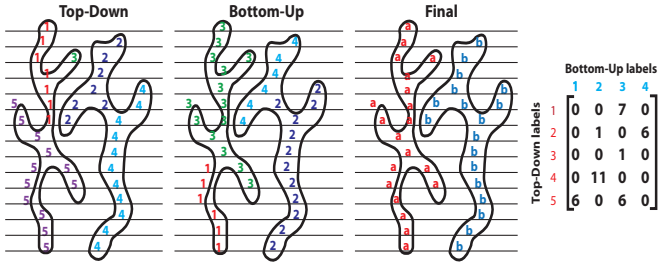

Fig. 3. Top-Down and Bottom-Up approach in linking neuron processes in two synthetic neurons and The sparse overlapping segments matrix.

pixel, the exploited features consist of rotation of membrane projections, Gaussian blurred, gradients, Gabor and tensor structures [8]. Additionally, radon-like features [9], which are proved to be representative in segmenting neuronal processes and 2D probability map provided by Ciresan et al. [10] are used. In total, 98 features per superpixel are used.

For training of the Random Forest, the features of the membrane superpixels in training set are used as positive and the rest as negative samples. The superpixels whose majority of their pixels belong to the membrane, assumed as positive class and the rest are negative ones. To generate each of 200 trees, 20 random features are selected. The neuron segmentation is obtained by thresholding the output of the Random Forest Classifier (i.e., the probability map of the slice image) as illustrated in Fig. 2c. Note that the segmentation could introduce error when the edge of some segments are not close curves as shown in Fig. 2c. To overcome this problem, the end points of all the predicted edges are discovered by finding their skeleton as shown in Fig. 2d. A patch centered on each end point is thresholded again within the probability map to connect the disjoint membrane. The process is continued till no end point is remained in the patch as shown in Fig. 2e.

B. Linking Stage

By segmenting each image in the stack, the linking propagates the label of each neuron across slices. The major challenge is to overcome the appearance, disappearance, splitting and merging of neurons. The straightforward approach is to make use of the overlap regions between two segments in consecutive image slices. However, it is arduous to label two merging neurons from the beginning of their appearance due to the similar textures of most of neurons and large number of neurons in one image. We propose a novel neuron linking technique that converts the neuron merging process into a neuron splitting process and so the whole linking process into a splitting process only. The rational is that the detection of the split is much easier than detection of merge because the split of neurons does not happen by a sudden change of position from the previous image. The conversion is achieved through a bottom-up and top-down labeling process where the merges/splits in the top-down manner is the same as splits/merges in the bottom-up manner. Figure 3 illustrate the labeling process with two synthetic neurons.

$$\text{Top-Down } \underline{\text{split}} \approx \text{Bottom-Up merge}$$
$$\text{Top-Down } \overline{\text{merge}} \approx \text{Bottom-up } \underline{\text{split}}$$

Assume TDs and BUs are the set of labels of the segments that are calculated in Top-Down and Bottom-Up approach respectively. Each set has different number of segments because of the assumed overlap in two directions. We therefore build a sparse adjacency matrix (Overlapped Segments

OS) that contains the number of segments with different labels in TDs and BUs orders, respectively. The size of the OS is n×m, where n and m are the number of the labels in TDs and BUs respectively. Algorithm 1 shows how to label the segments using overlaps in the Top-Down order. In algorithm 1, all the segments in the first image

Input: properties of segments: position, area
Output: Segments' labels
Assign a unique label to each of the segments in slice 1;
foreach *segment* $s_j^z, (z > 1)$ **do**
  if $s_i^{z-1} \cap s_j^z > threshold$ **then**
    Assign the label of s_i^{z-1} to s_j^z
  end
  if s_j^z *has no label* **then**
    Assign the unique label to s_j^z
  end
end

Algorithm 1: Labeling in Top-Down approach

are assigned a unique label. If a segment s_i in the previous slice has an overlap with segment s_j in the current slice more than a threshold, the label s_i is assigned to segment s_j. Finally, new labels are assigned to the segments with no labels in the current image. These segments represent the appearance of neurons in the micro-tube. On the other hand, if one segment does not have any overlap with any segments in previous slice, it represents the disappearance from the micro-tube. To compute the Bottom-Up labels, we use the same algorithm but in reverse fashion. In the next stage, the sparse overlapping segments matrix (OS) is build as:

$$OS_{ij} = \sum_i \sum_j \psi(i,j) \tag{1}$$

$$\psi(i,j) = \begin{cases} 1 & |s_{l_i}^{TD} \cap s_{l_j}^{BU}| \\ 0 & otherwise \end{cases} \tag{2}$$

Algorithm 2 defines the method in which the information of two way linking are concatenated to produce one unique labeling of neurons in the slices. In each row of the OS

Input: segments' label, position and area.
Output: Labels of neurons
Create the Overlapping Segments matrix (OS) of the labels in Top-Down and Bottom-Up order.
foreach *row* i *in* OS **do**
  Find the non-zero columns j.
  foreach j **do**
    Find the non-zero elements row, (k).
  end
  Assign a unique label for all i,j and k. Change all the values in rows and columns i,j and k to zero.
end

Algorithm 2: Total Labeling Algorithm

matrix, one non-zero element OS_{ij} represents two different labels of a set of segments which belong to one neuron. These neurons have labels l_i and l_j in Top-Down and Bottom-Up approach, respectively. Therefore, a unique label should be assigned to these segments. Additionally, the j^{th} column's non-zero elements represent the segments of this

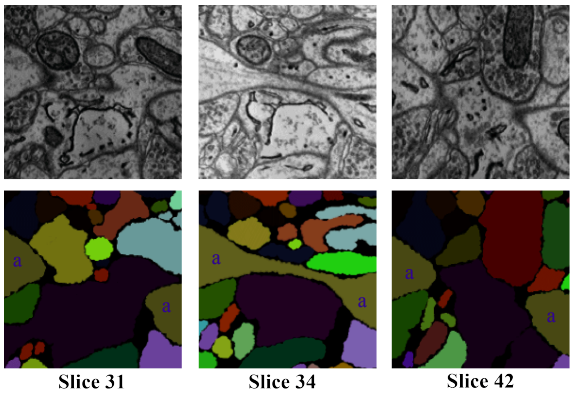

Fig. 4. Successful split and merge in the dataset.

specific neuron. Thus, the same unique label of i^{th} row should be assigned to them. By considering all non-zero elements of the OS matrix in computing each row, they get the same label, which belong to same neuron process. Finally, these elements change to zero and algorithm runs for other rows in OS matrix as well.

III. EXPERIMENTS AND RESULTS

A. SNEMI3D Dataset

The SNEMI3D dataset consists of two 100-slice sets for training and testing. Each of them covers approximately 6×6×3 micrometers of mouse cortex. The serial section Scanning Electron Microscopy (ssSEM) by resolution of 6×6×30 nanometer per pixel is captured from the micro-cube. This is an anisotropic dataset, which has high resolution in x and y-axis but low resolution in z direction. For the training dataset, the ground truth consists of a unique label for each neuron process in the micro-cube. The organizer of the challenge evaluates the results in test dataset for the participants and announce them in the challenge website.[1]

B. 3D Reconstruction

The proposed algorithm is applied on the SNEMI3D dataset. Figure 4 illustrates the successful merge and split in the dataset where two segments in slice 31 labeled with "a", which are far from each other are merged successfully in slice 34 with the same label and split again in slice 42 without changing the label.

After segmenting the slices and linking them by the proposed novel method, the 3D visualization is produced using Vaa3D[11] software, which is shown in Fig. 5. The proposed method successfully solves the problem of the splitting and merging neurons. For example, as can be seen in Fig. 5, neuron B in the Top-Down order of linking has two labels of green and red and in Bottom-Up order, this neuron gets two labels of green and gray. By computing the total linking, the whole neuron gets a unique label.

Additionally, the proposed linkage method can help improve the neuron segmentation. As can be seen in Fig. 5,

[1]http://www.biomedicalimaging.org/2013/program/isbi-challenges/

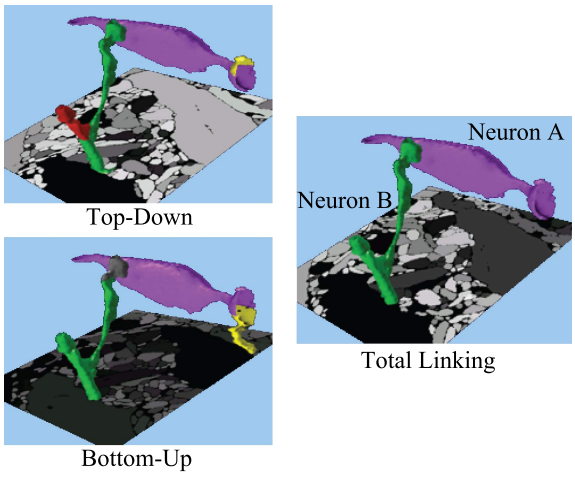

Fig. 5. The Top-Down, Bottom-Up and total linking method. Resolve the linking and segmentation error in the whole stack.

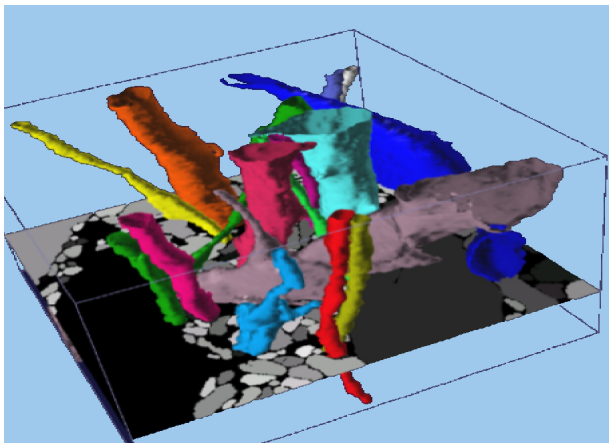

Fig. 6. 3D reconstructions of some neurons in the volume.

neuron A in Bottom-Up order gets an error in linking the consecutive segments and is incorrectly linked two adjacent neurons. With the top-down information in the proposed method, the errors of segmenting two different neurons and the linking of the same neuron are solved.

C. Evaluation

The performance evaluation is done by the 3D topology-based segmentation metric Adaptive Rand error:

$$Adapted\ Rand\ error \triangleq 1 - maximal\ FScore \quad (3)$$

$$FScore = 2 \times \frac{Precision \times Recal}{Precision + Recal} \quad (4)$$

Precision is the proportion of true-positives and all the positives of test outcome and the Recall value is the probability that the ground-truth labels for the pixels are correctly estimated. Figure 6 shows the 3D reconstruction of some neurons in the volume and Table I shows the participant groups and their evaluation values. The human rand error is the error between the segmentation error of two experts who manually segmented and linked neurons. Our performance

TABLE I

EVALUATION VALUES FOR HUMAN AND OTHER PARTICIPANTS.

Name	Rand Error
Human	0.0599
SCI (Utah)	0.1248
FlyEM (Janelia Farm)(2 Groups)	0.1250
Ours (NUS)	*0.1664*
Rhoana (Harvard)	0.1725
SPLab (Czeck)	0.4664

is ranked third but so close to top ones. In order to reach to the accuracy of the human, the segmentation stage plays an important role. Since, a small error in segmentation leads to a large error in linking stage as well. One of the segmentation problems is occurred due to the thin neurons in each image where the membrane pixels are so close together in a tube like shape of neurons in each slice.

Extracting more representative features, which could help the classifier provide better probability map can be considered as future work. Moreover, graphical modelling approaches would be supportive to provide the segmentation with minimum error.

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