Feature Measures for the Segmentation of Neuronal Membrane using a Machine Learning Algorithm

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ABSTRACT

In this paper, we present a Support Vector Machine (SVM) based pixel classifier for a semi-automated segmentation algorithm to detect neuronal membrane structures in stacks of electron microscopy images of brain tissue samples. This algorithm uses high-dimensional feature spaces extracted from center-surrounded patches, and some distinct edge sensitive features for each pixel in the image, and a training dataset for the segmentation of neuronal membrane structures and background. Some threshold conditions are later applied to remove small regions, which are below a certain threshold criteria, and morphological operations, such as the filling of the detected objects, are done to get compactness in the objects. The performance of the segmentation method is calculated on the unseen data by using three distinct error measures: *pixel error*, *wrapping error*, and *rand error,* and also a pixel by pixel accuracy measure with their respective ground-truth. The trained SVM classifier achieves the best precision level in these three distinct errors at 0.23, 0.016 and 0.15, respectively; while the best accuracy using pixel by pixel measure reaches 77% on the given dataset. The results presented here are one step further towards exploring possible ways to solve these hard problems, such as segmentation in medical image analysis. In the future, we plan to extend it as a 3D segmentation approach for 3D datasets to not only retain the topological structures in the dataset but also for the ease of further analysis.

Keywords: Neuronal membrane segmentation, machine learning, image morphology, feature selection.

1. INTRODUCTION

With the recent advancements in the automated collection of large image datasets of nano-scale Electron Microscopy (EM) images of brain tissue [1, 2], have resulted in interest in the neuroscience community to develop computational algorithms for an automated analysis system in order to understand the structures and connectivity more accurately and automatically [3]. The development of such a system is important as it would help neuroscientists better understand the maps of the partial or the complete brain [4]. To develop such a system, the first step is to find connectomes in the tissue in order to understand the complete connectivity in the brain map. To find the connectomes, first synapses should be identified along with the axons and dendrites or wires must be traced through the images [5].

Segmentation usually is the first and the most challenging phase in automated analysis. In the given datasets, there are two types of boundaries in the images; boundaries between neurites and boundaries of the intracellular organelles. The most challenging part in this problem is how to deal with both types of boundaries while retaining the topological structure of the brain maps [5]. Previously, several methods have been proposed for similar data to track and segment axons, such as, machine learning technique [5], hierarchical classification [6], graph cuts [7], and semi-automated level-set segmentation and active contour methods [8, 9]. However, active contour models and level-set methods are computationally expensive and require a starting point in each image and do not perform well where boundaries are noisy and have artifacts [10]. We have addressed one such problem in our previous work described in [10, 11] for delineating the boundaries of endothelial cell cytoplasm, but the problem in the data in this research is manifoldly more difficult. In this data, we have to account for the topological neuronal structures as well as the boundaries of neurons along with the small elements in the images, such as mitochondria. Sometimes, the boundaries are either missing or badly affected due to noise and/or other artifacts. To address these problems, we modified the algorithm in [11] and introduced some edge-sensitive features, such as Gradients of 3 by 3 patches for each pixel, obtained from each image in the given data in the existing features vector. Therefore, in this research we have taken an approach based on machine learning technique. In this paper, we present a semi-automated segmentation technique using an SVM based machine learning algorithm on a high dimensional feature space generated from patch-based multiple-features and a set of small training examples to build an algorithm in order to detect neuronal structures in EM images of brain tissue. In this approach, we have chosen a training image, which contains both types of boundaries and trained with a Support Vector

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Machine (SVM) as a classifier. The SVM has been used in many applications in the past and has consistently outperformed many algorithms in classification of the data samples [12]; that is why we opted to work with SVM as a classifier. In short, SVMs create an n-dimensional features space for each object and background, and then define a hyper-plane that best separates samples from the two classes [13]. This hyper-plane then determines the classes of unknown samples in the test feature space relative to the position of each test sample in two different classes, ‘‘membrane’’ and ‘‘non-membrane’’. The rest of the paper is organized as follows: Section 2 presents data gathering methods. Section 3 presents a segmentation method. The results of the segmentation algorithm are presented in Section 4. Finally, Section 5 presents some concluding remarks and future work.

2. IMAGE DATASETS

Given two datasets containing a stack of 30 sections of the Drosophila acquired through a serial section Transmission Electron Microscopy (ssTEM) [14], one of which has manually traced binary groundtruth. The corresponding boundaries as labels are provided with “0” for the objects and “1” for the rest of background pixels [5]. In this paper, we have taken just one section of the training data along with its manually labeled groundtruth for the training of our machine learning classifier, as shown in the Figure 1(a), to reduce the computational complexities and increase the efficiency in the learning process.

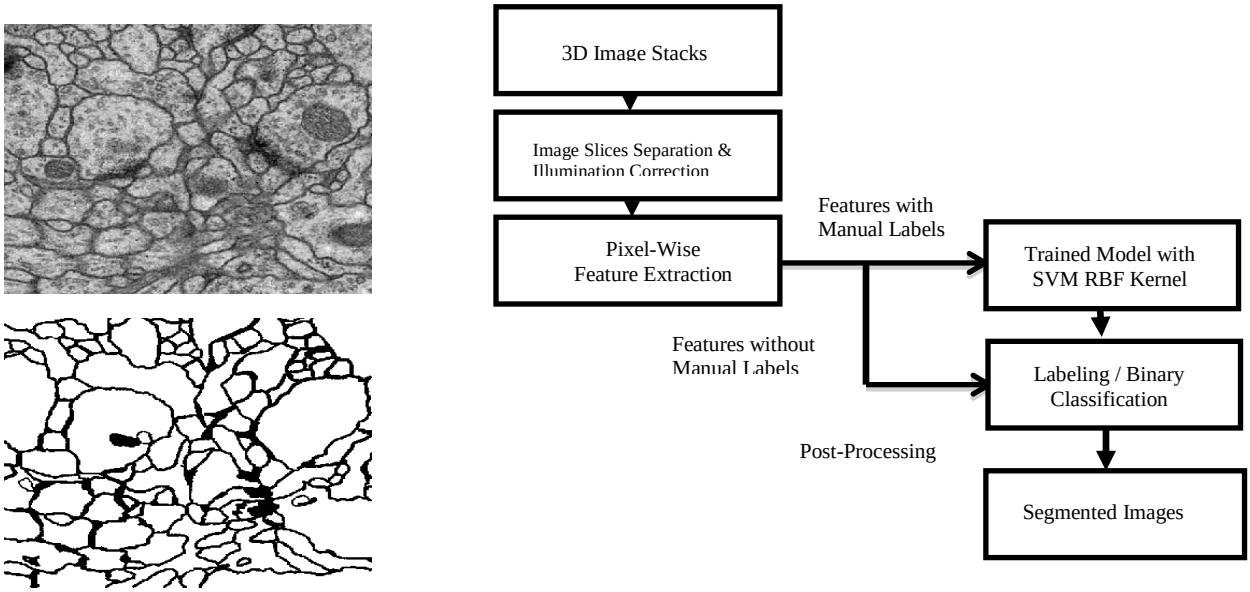

Fig.1.(a) Training image and its respective manually labeled groundtruth. (b) Flow Chart of the Segmentation Algorithm.

3. SEGMENTATION METHOD

For the segmentation, first features are extracted and then these features are normalized to a range between [-1, +1]. For the classification purposes, these features are extracted along with the respective groundtruth data to create a binary classifier that is then applied to the rest of the image feature spaces generated from the test images to get the binary results. In the next step, some post-processing operations are applied, such as: morphological and shape thresholds are set up on these binary images to produce improved and clean binary images. The flow chart of the segmentation algorithm is given in Figure 1(b). The features extracted from these images are explained in the next section.

3.1. Feature Selection

A set of 24 distinct features, taken from 3 by 3 neighborhood patches of pixel intensities, of the image and some edge-sensitive feature patches, is extracted to describe feature information for a candidate pixel; as described in Equation (1).

$$\vec{\mathbf{FV}} = \left[\mathbf{I}_{3x3}, \mathrm{M}, \mathrm{R}, \mathrm{E}, \mathrm{m}_{2,3,4}, \mathrm{G}_{3x3}\right] \quad \textbf{(1)}$$

Where I_{3x3} is the intensity and M, R, m and G are median, range, energy, 2^{nd}, 3^{rd} and 4^{th} order spatial moments and gradient of the image patch respectively. These 24 features are taken from n by n neighboring pixels from the candidate pixel where n is the number of pixels, here n =3, in the neighborhood of the candidate pixel of the image. Gradients can be utilized to increase the visibility of edges and other details present in an image and are widely used in artificial vision and/or biological vision systems [15]. Some of these features are already discussed in detail elsewhere [11, 16].

4. EXPERIMENTAL RESULTS

All components of the algorithms are implemented in MATLAB and using the LIBSVM package [17], unless otherwise stated. The results obtained from two sets of 512 x 512 x 30 pixel images are promising and show a clear detection of neuronal structures and some small elements such as mitochondria. In this method, we have chosen only one image of 512 x 512 pixels as shown in Figure 1, along with manually labeled groundtruth for the training to obtain the classifier that was then applied on the rest of the test image datasets, with unknown groundtruth, of the ISBI 2012 EM Segmentation Challenge to get binary results [14, 18]. An SVM-RBF based classifier with 10-fold cross-validation is obtained in a set of 512 x 512 training samples extracted from the original training image with its manually labeled ground-truth. The best training machine was obtained by setting different ranges of, gamma (width of the kernel), cost and weight parameters in the SVM-RBF kernel, $K(x; xi) = \exp(-\gamma \| x-xi \|2)$, which is then opted to perform the classification on the test datasets. We trained the SVM classifier on a set of 512 x 512 training samples and then applied this classifier without further modification to given test datasets of 30 images of size 512 x 512 pixels to get binary classification. The binary images of test image are presented in the Figures 2. After finding the binary images using SVM, we then applied morphological operation for filling of the detected objects. Then, some thresholds; such as: minimum and maximum areas and shape indices, defined as: 4π x (Area/Perimeter2), of the detected objects, are applied to get the compactness of the objects and remove small elements and noise detected through SVM classification. This step also removes some important elements as well along with the noise from the binary images, which can be avoided by introducing some more edge-sensitive and data relevant features in the classification or in the setting up of the thresholds; which are yet to be explored.

4.1. Segmentation Performance Measures

The performance of the segmentation method is calculated on the test data by using three distinct error measures: pixel error, warping error and rand error as explained in [5] with their respective ground-truth. These measures are defined as: pixel error (PE), the number of image pixels on which machine and human boundary labeling disagree, random error (RE), based on Rand index [5], which is an error measure of the frequency with which the two segmentations disagree over whether a pair of pixels belongs to the same or different objects, and wrapping error (WE) metric which tolerates disagreements over boundary locations while penalizing topological disagreements and it can be used as a cost function for supervised learning in boundary detection, as explained in [5]. In short, the PE is a convenient cost function for supervised learning, but is too sensitive to boundary locations. RE measures the similarities between two data groups and evaluates whether the grouping of pixels into separate objects is correct, but small differences in the location of object boundaries penalize and make RE slightly higher; while merging and splitting of objects tend to increase RE by a large amount [5]. To overcome these shortcomings, the same group introduced wrapping error for the digital topology of boundary detection and possesses the properties such as: it tolerates minor differences in boundary locations, penalizes topological disagreements and serves as a convenient cost function in supervised learning [5]. The trained SVM classifier achieves the best precision level in these three distinct errors at 0.23, 0.016 and 0.15 respectively on the given dataset [14, 18]. In another performance analysis of our method on one dataset of 30 sections whose manually labeled images are available, we calculated errors using pixel by pixel matching. By keeping one section as a training sample and the remaining 29 sections for classification and verification purposes, we found the accuracies of the segmentation results given in Table 1. We then generated Receiver Operating Characteristics (ROC) curves to measure sensitivity and specificity of these images, presented in the Table 1 (b). The range of accuracies using pixel by pixel measure reaches from ~71 % to ~77 % on the segmented results. The original along with their segmented images are presented in Figure 2.

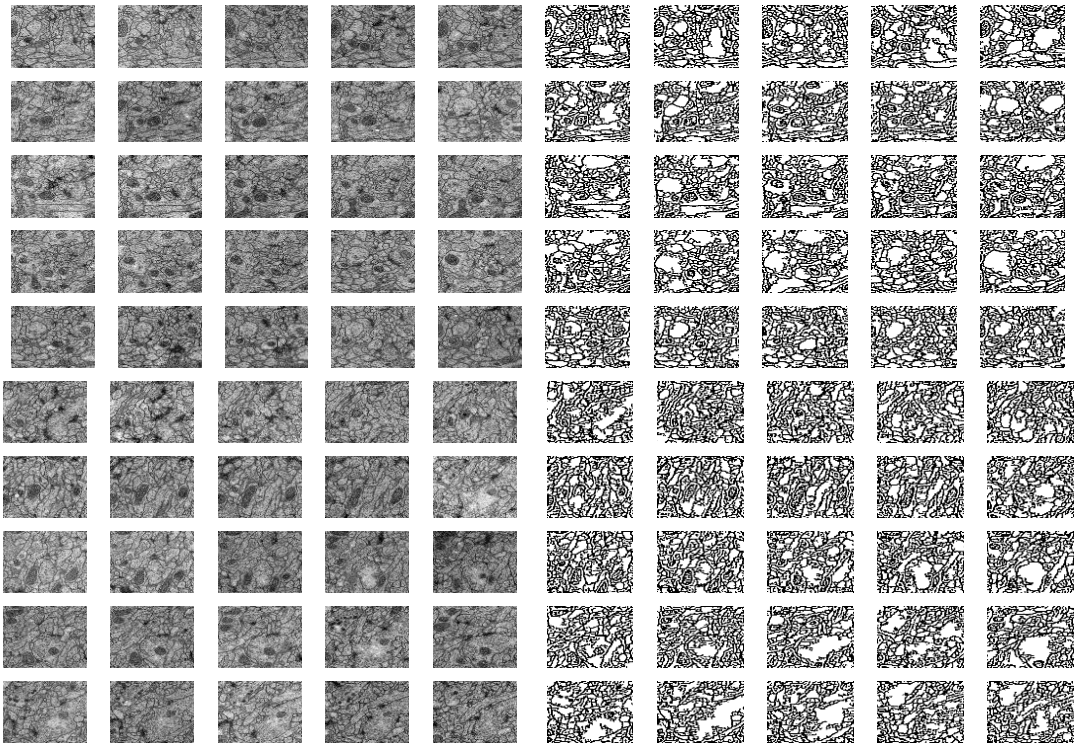

Fig. 2. Segmentation results on two datasets of stack of sizes 512 x 512 x 30 pixels. (a) Left panel: Original 30 images of each datasets. (b) Right panel: Binary images obtained using SVM Classifier and post-processing operations.

Image #	Accuracy (%)	Image #	Accuracy (%)
2	75.6	**17**	71.4
3	74.2	**18**	72.7
4	77. 4	**19**	74.2
5	76.1	**20**	76.5
6	72.4	**21**	78.5
7	75.1	**22**	75.7
8	73.1	**23**	72.3
9	74.0	**24**	76.7
10	71.8	**25**	75.0
11	75.9	**26**	75.8
12	74.6	**27**	73.7
13	75.4	**28**	75.6
14	76.7	**29**	74.3
15	72.5	**30**	75.6
16	72.1		

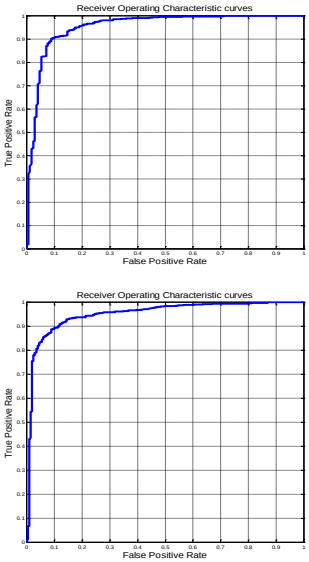

Table 1. Table presents the image accuracies and TP, FP, TN and FN, respectively, on a set of 29 images. Note: The first image in the set of 30 sections was used in the SVM classifier training process. The graphs on the right of the table represent two sample ROC curves of the segmentation specificity and sensitivity.

5. CONCLUSIONS

In this work, we develop a method for segmentation using a SVM based machine-learning technique with a 24-dimensional patch-based feature vector for neuronal structures for EM images in brain tissue samples. The method first finds a set of distinct features representing each pixel and then binary classifies them into, 0 and 1. Later, different shape

and morphological measures are applied on the binary images to remove unwanted regions from the binary results and to produce clean segmented images. We showed the method presented worked well on many images in this difficult task on the given dataset. The results produced using the algorithm presented here are an effort towards exploring possible ways to solve hard problems, like segmentation, in medical image analysis. Future work in this research includes the extension of current technique to a 3D segmentation approach for the 3D datasets and the search of additional features in the machine learning for detecting all neuronal structures simultaneously, and to explore other machine learning techniques, and how to avoid removing the objects of interest in the thresholds criteria in the last step of the algorithm.

REFERENCES

[1] K. Hayworth, N. Kasthuri, R. Schalek, and J. Lichtman; "Automating the collection of ultrathin serial sections for large volume TEM reconstructions," *Microscopy and Microanalysis*, 2006.

[2] W. Denk and H. Horstmann; "Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure," *PLoS Biol*, vol. 2, no. 11, 329, 2004.

[3] E. Jurrus, T. Tasdizen, P. Koshevoy, P. T. Fletcher, M. Hardy, C. Chien, W. Denk, and R. Whitaker; "Axon tracking in serial block-free scanning electron microscopy" , *MIAAB*, 114–119, 2006.

[4] Cardona, S. Saalfeld, S. Preibisch, B. Schmid, A. Cheng, J. Pulokas, P. Tomancak and V. Hartenstein; "An Integrated Micro and Macroarchitectural Analysis of the Drosophila Brain by Computer-Assisted Serial Section Electron Microscopy", *PLoS Biol* , vol. 8, no. 10, 2010.

[5] V. Jain, B. Bollmann, M. Richardson, D. R. Berger, M. N. Helmstaedter, K. L. Briggman, W. Denk, J. B. Bowden, J. M. Mendenhall, W. C. Abraham, K. M. Harris, N. Kasthuri, K. J. Hayworth, R. Schalek, J. C. Tapia, J. W. Lichtman and H. S.Seung; "Boundary Learning by Optimization with Topological Constraints," *IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*, 2010, 2488-2495, 2010.

[6] A. U. Koethe, M. Helmstaedter, W. Denk, and F. Hamprecht, "Segmentation of SBFSEM volume data of neural tissue by hierarchical classification," in *DAGM*. Springer, 142–152, 2008.

[7] H. Yang and Y. Choe; "Cell tracking and segmentation in electron microscopy images using graph cuts," *IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, 2009, 306-309, 2009.

[8] J. H. Macke, N. Maack, R. Gupta, W. Denk, B. Sch¨olkopf and A. Borst; "Contour-propagation algorithms for semi-automated reconstruction of neural processes*," Journal of Neuroscience Methods*, vol. 167, no. 2, 349-357, 2008.

[9] A. Vazquez-Reina, E. Miller, and H. Pfister; "Multiphase geometric couplings for the segmentation of neural processes," *CVPR*, 2009.

[10] S. Iftikhar and A. A. Bharath; "Cell boundary analysis using radial search for dual staining techniques", *Proc. SPIE 7259, 72593M*, 2009.

[11] S. Iftikhar, A. R. Bond, A. I. Wagan, P. D. Weinberg and A. A. Bharath; "Segmentation of endothelial cell boundaries of rabbit aortic images using a machine learning approach," *Int. J. of Biomed Imaging*, vol. 1, 270247, 2011.

[12] Y. Y. El-Naqa, M. N. Wernick, N. P. Galatsanos and R. M. Nishikawa; "A support vector machine approach for detection of microcalcifications," IEEE Transactions on, Medical Imaging, vol. 21, no. 12, 1552-1563, 2002.

[13] C. Cortes and V. Vapnik; "Support-vector networks," *Machine Learning*, vol. 20, no. 3, 273-297, 1995.

[14] The challenge website: "http://fiji.sc/wiki/index.php/Segmentation_of_neuronal _structures_in_EM_stacks_challenge__ISBI_2012".

[15] E. H. Marr and "Theory of Edge Detection". *Proceedings of the Royal Society of London. Series B, Biological Sciences.* Vol. 207, no. 1167, 187-217, 1980.

[16] S. Cao, S. Iftikhar, and A. A. Bharath. "Patch-based Feature maps for pixel-level image segmentation." *Proceedings of the 20th European , Signal Processing Conference (EUSIPCO)*, 2263,2267, 27-31, 2012.

[17] C. C. Chang, C. J. Lin, Libsvm: a library for Support Vector Machines. 2011, http://www.csie.ntu.edu.tw/~cjlin/libsvm.

[18] S. Iftikhar and A. Godil. "The Detection of Neuronal Structures using a Patch-based Multi-features and Support Vector Machines Learning Algorithm". *Proc. of ISBI 2012 EM Segmentation Challenge*.