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Multi-scale segmentation of neurons based on one-class classification

Paul Hernandez-Herrera [a,*], Manos Papadakis [a,b], Ioannis A. Kakadiaris [a]

[a] Computational Biomedicine Lab, Department of Computer Science, University of Houston, TX 77204, USA
[b] Department of Mathematics, University of Houston, TX 77204-3008, USA

HIGHLIGHTS

- A multi-scale framework to segment the neurons.
- A mathematically rigorous general approach for the normalization of the response of a multi-scale ensemble of linear filters.
- A multi-scale framework to compute the Laplacian of the 3D image stack and an approach to compute as many decision functions as the number of scales (one for each scale) used for segmentation.
- A mathematical justification for using different low-pass filters to compute the Laplacian and the Hessian matrix.
- An extensive experimental evaluation of the performance of our approach on a number of datasets, including all of the DIADEM competition.

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ABSTRACT

Background: High resolution multiphoton and confocal microscopy has allowed the acquisition of large amounts of data to be analyzed by neuroscientists. However, manual processing of these images has become infeasible. Thus, there is a need to create automatic methods for the morphological reconstruction of 3D neuronal image stacks.
New method: An algorithm to extract the 3D morphology from a neuron is presented. The main contribution of the paper is the segmentation of the neuron from the background. Our segmentation method is based on one-class classification where the 3D image stack is analyzed at different scales. First, a multi-scale approach is proposed to compute the Laplacian of the 3D image stack. The Laplacian is used to select a training set consisting of background points. A decision function is learned for each scale from the training set that allows determining how similar an unlabeled point is to the points in the background class. Foreground points (dendrites and axons) are assigned as those points that are rejected as background. Finally, the morphological reconstruction of the neuron is extracted by applying a state-of-the-art centerline tracing algorithm on the segmentation.
Results: Quantitative and qualitative results on several datasets demonstrate the ability of our algorithm to accurately and robustly segment and trace neurons.
Comparison with existing method(s): Our method was compared to state-of-the-art neuron tracing algorithms.
Conclusions: Our approach allows segmentation of thin and low contrast dendrites that are usually difficult to segment. Compared to our previous approach, this algorithm is more accurate and much faster.

1. Introduction

Neurons are the main part of the nervous system. They allow processing and transmission of information. Thus, to understand the neuronal process at the cellular level, it is necessary to develop mathematical models allowing simulation of the neuronal function. In another direction, new research (Chowdhury et al., 2014) suggests that anorexia nervosa affects the morphology structure of the neuron, such as the dendritic length and dendritic branches. Hence, it is necessary to trace the neuron from a 3D image stack to extract the morphology representation of the neuron. The first step of this process is the segmentation of the neuron from the background. Recent developments in confocal and multiphoton microscopy allow the acquisition of large volumes of neuronal images. Manual processing of these images is infeasible, as it would require an excessive amount of manual effort and would be likely to suffer from human errors. All of these reasons establish the need for the development of methods for the automatic segmentation of

* Corresponding author.
E-mail address: phernandez7@uh.edu (P. Hernandez-Herrera).

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neurons from the 3D image stack. The main challenges to address when developing a segmentation algorithm are: (i) irregular cross-section (i.e., not semi-elliptical cross-sections such as those of vessels) of dendrites due to structures attached to the dendrites (spines); (ii) variability in the size of the dendrites to be segmented; (iii) thin dendrites can be as small as one voxel radii (depending on the voxel size); (iv) thin dendrites appear as low contrast objects; (v) contrast variation across different datasets due to different acquisition modalities; and (vi) noise.

The remainder of the paper is organized as follows: in Section 2, previous work in the segmentation of neurons is presented. In Section 3, an overview of our algorithm is presented, Section 4 our approach for neuron segmentation is presented with mathematical detail, Section 5 reports results in real datasets, and our conclusions are presented in Section 6.

2. Previous work

Different approaches have been proposed to segment neurons from the background of the 3D image stack, including machine learning (ML) algorithms. The most common approach in ML is supervised learning where the user usually trains a support vector machine (SVM) classifier which allows separation of the training data in a high-dimensional space. The training data usually consist of two classes, positive samples corresponding to the structure of interest (dendrites) and negative samples corresponding to the background. The main difference between the various supervised learning approaches is the selection of the feature vector. Gonzalez et al. (2009) used 3D steerable filters to create rotationally invariant feature vectors that are less sensitive to the irregularities of dendrites. Jimenez et al. (2015) proposed using isotropic low-pass, high-pass and Laplacian filters to compute a set of features that are computationally efficient. Santamaria-Pang et al. (2015) used the eigenvalues of the Hessian matrix as descriptors to learn the local geometry of the dendrites. The main limitation of these approaches is the assumption that training and testing samples follow the same distribution, which may not be true due to the large variety in datasets (different imaging technologies and preparations with various resolutions and labeling methods (Brown et al., 2011)). These methods require re-training when the assumptions are not satisfied and a different model must be created for each dataset. This process is usually difficult since it requires properly selecting the positive and negative samples. In addition, the user needs previous knowledge in ML to set the correct parameters of the classifier. Furthermore, due to the variability in the size of the dendrites, these approaches usually have difficulty segmenting thin dendrites.

The segmentation process can also be implemented by thresholding. Janoos et al. (2009) used a non-linear diffusion filter to de-noise the image. Thus, the neuron was segmented using a global threshold. Chothani et al. (2011) proposed enhancing dendrites using a multi-scale center-surround filter and applying a threshold to the enhanced image to segment neurons. Then, a post-processing step was employed to remove regions with a small number of voxels. Xie et al. (2011) used the triangle method over the histogram of the 3D image stack to compute a global threshold for segmentation. Then, a morphological closing operation was employed to fill artificial holes produced by the segmentation. Xiao and Peng (2013) used the average intensity of the 3D image stack as a threshold value to create an over-segmentation of the dendrites. These approaches assume that the staining is homogeneous, which is usually incorrect. As a consequence, these approaches are not robust to segmentation errors. In addition, it is difficult to know in advance the correct threshold value for segmentation. For a complete review of neuron segmentation algorithms see Meijering (2010) and Donohue and Ascoli (2011).

Recently, our team (Hernandez-Herrera et al., 2014) proposed a semi-automatic method based on one-class classification for the segmentation of neurons. First, a monoscale isotropic Laplacian filter was proposed to detect a training set consisting of points belonging to the background. These points are used to train a single decision function that allows determining how similar an arbitrary point is to the points in the background class. Finally, the unlabeled points rejected as background are labeled as foreground. A limitation of Hernandez-Herrera et al. (2014) is that a-priori knowledge of the likely size of dendrites to be segmented is required to determine the suitable scale of the mono-scale isotropic Laplacian filters used in the training step. In addition, neurons with a high degree of heterogeneity in dendrite diameter are difficult to segment since a single scale will not cover the range of dendrite diameters.

In this paper, we propose a multi-scale approach that eliminates the drawbacks of our previous approach. The core philosophy of our approach is still the one-class classification segmentation approach (Hernandez-Herrera et al., 2014), but the requirement of the user to provide the likely width of the neuron to segment is dropped. More specifically, our contributions in this work are the following:

(i) A multi-scale framework to segment the neurons.
(ii) A mathematically rigorous general approach for the normalization of the response of a multi-scale ensemble of linear filters motivated by the ad-hoc normalization of Gaussian low-pass filters used in Lindeberg (1998).
(iii) A multi-scale framework to compute the Laplacian of the 3D image stack.
(iv) An approach to compute as many decision functions as the number of scales (one for each scale) used for segmentation.
(v) A mathematical justification for using different low-pass filters to compute the Laplacian and the Hessian matrix.
(vi) An extensive experimental evaluation of the performance of our approach on a number of datasets, including all of the DIADEM competition and the BigNeuron dataset.

Compared to Hernandez-Herrera et al. (2014), the method presented here (i) allows detection of neurons with a high degree of heterogeneity in dendrite diameter; (ii) allows a fair comparison of the response to the Laplacian filter at different scales; (iii) allows an automatic selection of samples belonging to the background of the 3D image stack using several scales and allows creation of a partition of the 3D image stack for each scale; and (iv) allows creation of specific decision functions for each scale. Due to these advantages over our previous approach, the Multi-scalE Segmentation Of Neuron (MESON) algorithm is less sensitive to the varying sizes of the dendrites to be segmented.

3. Materials

3.1. Data acquisition and segmentation

The first step of the analysis of neurons is the acquisition of the 3D image stacks. Neurons are imaged using microscopy and the voxels usually have anisotropic size where the $x-y$ dimensions have the same size but the z dimension usually has a different value than the $x-y$. This property creates an elliptical cross-section of the dendrites in the 3D image stack. Hence, models that assume circular cross-section usually fail to accurately detect dendrites. Another property due to the acquisition protocols is that dendrites usually have a decreasing intensity profile where the maximum value is reached at the center of the dendrite and the intensity decreases from the center to the boundary of the dendrite.