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A neurocomputational method for fully automated 3D dendritic spine detection and segmentation of medium-sized spiny neurons

Yong Zhang [a,1], Kun Chen [a,b,1], Matthew Baron [c], Merilee A. Teylan [c], Yong Kim [c], Zhihuan Song [b], Paul Greengard [c], Stephen T.C. Wong [a,*]

[a] The Ting Tsung and Wei Fong Chao Center for Bioinformatics Research and Neurosciences Imaging and The Center for Bioengineering and Informatics, The Methodist Hospital Research Institute, Weill Cornell Medical College, Houston, TX 77030, USA
[b] State Key Laboratory of Industrial Control Technology, Institute of Industrial Process Control, Zhejiang University, Hangzhou 310027, China
[c] Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10065, USA

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ABSTRACT

Acquisition and quantitative analysis of high resolution images of dendritic spines are challenging tasks but are necessary for the study of animal models of neurological and psychiatric diseases. Currently available methods for automated dendritic spine detection are for the most part customized for 2D image slices, not volumetric 3D images. In this work, a fully automated method is proposed to detect and segment dendritic spines from 3D confocal microscopy images of medium-sized spiny neurons (MSNs). MSNs constitute a major neuronal population in striatum, and abnormalities in their function are associated with several neurological and psychiatric diseases. Such automated detection is critical for the development of new 3D neuronal assays which can be used for the screening of drugs and the studies of their therapeutic effects. The proposed method utilizes a generalized gradient vector flow (GGVF) with a new smoothing constraint and then detects feature points near the central regions of dendrites and spines. Then, the central regions are refined and separated based on eigen-analysis and multiple shape measurements. Finally, the spines are segmented in 3D space using the fast marching algorithm, taking the detected central regions of spines as initial points. The proposed method is compared with three popular existing methods for centerline extraction and also with manual results for dendritic spine detection in 3D space. The experimental results and comparisons show that the proposed method is able to automatically and accurately detect, segment, and quantitate dendritic spines in 3D images of MSNs.

Introduction

Dendritic spines are post-synaptic parts of glutamatergic synapses. Dendritic spines undergo activity-dependent structural remodeling, which has been proposed to be involved in learning and memory (De Roo et al., 2008; Yuste and Bonhoeffer, 2001). Spines with larger heads are dynamically more stable, express larger numbers of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate type (AMPA-type) glutamate receptors, and contribute to stronger synaptic connections. By contrast, spines with smaller heads contribute to weaker or silent synaptic connections (Kasai et al., 2003). In addition, synaptogenesis associated with experience-dependent learning and environmental complexity is reflected in changes in the number of spines (De Roo et al., 2008; Leuner et al., 2003; Muller et al., 2002). Structural changes in dendritic spines also accompany long-term synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission (Luscher et al., 2000; Yuste and Bonhoeffer, 2001). LTP-inducing stimuli have been shown to increase the proportion of large spines while LTD-inducing stimuli have been shown to decrease the proportion of large spines and cause retraction of spines (Toni et al., 1999; Zhou et al., 2004). LTP and LTD are thought to be involved in learning and memory (Bliss and Collingridge, 1993; Kandel, 2001).

The striatum is a subcortical region of the cerebrum and is the major input station of the basal ganglia system. Abnormalities in striatal function are associated with neurological and psychiatric diseases (Greengard, 2001), including Parkinsonism, schizophrenia, Attention Deficit Hyperactivity Disorder (ADHD), mental depression, and drug addiction. Compared to other brain regions, the striatum is relatively large and remarkably homogeneous. About 95% of all striatal neurons have a similar morphology and are referred to as medium-sized spiny neurons (MSNs) (Greengard et al., 1999). MSNs receive midbrain dopaminergic input, which serves to modulate excitatory glutamatergic input from the prefrontal cortex (Hyman and Malenka, 2001). The initial site of interaction between dopamine and glutamate is within the dendritic spines of MSNs. Notably, the changes

* Corresponding author.
[1] Co-First Authors.

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of density or morphology of dendritic spines of MSNs in the striatum have been observed in several disease models and are likely associated with neuronal function and pathology (Day et al., 2006; Deutch et al., 2007; Kalivas, 2009; Kim et al., 2009; Lee et al., 2006; Robinson and Kolb, 2004). Dendritic spine morphology of MSNs is highly heterogeneous compared to that observed in other types of neurons, such as pyramidal neurons in the cerebral cortex and hippocampus (Kim et al., 2009; Shen et al., 2009). Acquisition and accurate analysis of high resolution images of dendritic spines are highly challenging but necessary tasks for better understanding of many neurological and psychiatric diseases. Modern fluorescence microscopy techniques, such as confocal scanning microscopy and 2-photon excitation laser scanning microscopy, provide powerful tools to image neurons at relatively high resolution. With these advanced modern imaging techniques, the analysis of dendritic spines, however, remains largely manual. Manually extracting spine measurements is a labor intensive process, suffers from substantial subjective bias, and often yields inaccurate spine extraction.

In order to overcome the aforementioned difficulties, researchers seek a 2D or 3D automatic way to analyze dendrites and spines. Previous works on dendritic spine detection can be roughly divided into two groups: *classification-based methods* (Rodriguez et al., 2008) and *centerline extraction-based methods* (Janoos et al., 2009; Zhang et al., 2007). Classification-based methods separate points into different groups using a certain classifier. For example, Rodriguez et al. (2008) propose an automated 3D spine detection approach using point clustering. In this method, only the distances from the points to the closest point of the surface are used as the clustering criteria, which may cause spurious spines. Centerline extraction-based methods detect all the possible centerlines of certain objects in the image and treat dendritic spines as small protrusions attached to the dendrites. Koh et al. (2002) adopt the thinning method to extract centerlines and applies the grassfire propagation technique to assign each dendritic point a distance to the medial axis of the dendritic structure. Since segmentation is achieved by global thresholding and very limited geometric information is considered for spine detection, this method may detect pseudo spines. Zhou et al. (2009) use a local binary fitting model of level sets to do the spine segmentation, followed by a label-based 3D thinning strategy to do the medial axis extraction and a grassfire approach to do the spine detection. This method requires heavily designed post-processing procedures to remove pseudo medial axes from the 3D thinning method and is limited to processing relatively simple neuron structures. Zhang et al. (2007) proposes a 2D tracing algorithm that uses a curvilinear structure detector for dendrite and spine centerline analysis. This method requires several parameters to be fine-tuned and currently works only on 2D projections of images. Thus, 3D information is lost, and spines orthogonal to the projection plane are missed. Janoos et al. (2009) present a method for dendritic skeleton structure extraction using a curve-skeletons approach based on the medial geodesic function which is defined on the reconstructed isosurfaces. Some existing commercial software tools, such as Imaris,[2] perform semi-automated dendrite and spine analysis and visualization, but they are limited in their scope and capability for fully automated analysis of neuron images with complex neuron structures.

In dendritic spine detection, object centerline extraction plays an important role in analyzing the morphology of dendritic backbones and segmenting dendritic spines. Centerline extraction has been widely used in a variety of application areas. For example, researchers in computer vision utilize object skeletons to specify animation (Bloomenthal, 2002; Siddiqi et al., 2002). In visualization and computer graphics, centerlines are used as a compact representation of complex 3D models (Cornea et al., 2007; Zhou and Toga, 1999). In the medical imaging area, anatomical structure segmentation and modeling of blood vessels and nerve structures involve centerline extraction as a necessary step (Bouix et al., 2005). Centerline extraction has also been used in 3D virtual navigation to utilize symmetric features to generate meaningful paths through a scene or an object (Perchet et al., 2004; Wan et al., 2002). Conventional methods for centerline extraction can be grouped into skeletonization methods and model-based methods. Skeletonization is based on the notion that the geometric medial axis captures the object topology. Lee et al. (1994) propose a 3D topological and geometrical preserving thinning method to detect centerlines. Cornea et al. (2005) generate a repulsive force field (RFF) over a discretization of the 3D object and use topological characteristics of the resulting vector field, such as critical points and critical curves to extract the curve-skeleton. Bouix et al. (2005) utilize an average outward flux measure to distinguish skeletal points from non-skeletal ones and combines this measure with a topology-preserving thinning procedure. In practice, the skeletons generated by these aforementioned methods contain many spurious branches due to noise which causes irregularities on the binarized surface. In addition, many real branches are missed.

Model-based methods apply explicit geometric shape models to extract centerlines. Sato et al. (1998) introduce a multi-scale Gaussian smoothing and Hessian-based technique to model the local line structure for centerline measurements. Frangi et al. (1998) propose a generalized centerline measurement using all eigenvalues simultaneously. Manniesing et al. (2006) improve Frangi et al.'s method by applying a non-linear anisotropic Hessian-based diffusion along the local line directions. The advantages of model-based methods include a natural ability to capture local image features, robustness to noise, and accuracy. However, most of these models on 3D image computing are limited due to increased computation complexity dealing with multi scales and difficulty in scale selection. The recently launched highly automated tool, Neuronstudio (Rodriguez et al., 2008), contains tools aimed at neuron structure tracing and reconstruction. However, it still requires user interaction while the tracing and reconstruction is not optimized for processing MSNs.

In this work, we explore the potential to improve model-based methods and apply it to the application of dendritic spine detection and segmentation in 3D high resolution images of dendritic spines. Fig. 1 shows the image processing pipeline of the proposed method. First, a series of pre-processing methods are applied to the input images to remove noise and improve the image quality. Second, a gradient vector field is calculated and normalized using a Generalized Gradient Vector Flow (GGVF) framework with an enhanced smoothing strategy. Then a series of feature points are detected by tracking along the gradient vectors, and these feature points are used for further eigen-based analysis. Third, the eigen-analysis-based method is applied to the feature points to detect spines. The proposed eigen-analysis method is novel compared to previous methods, as it automates the selection of scales when calculating second-order derivatives of the image, and the scale selection is adaptive and specific for each individual point. Since the derivative calculation and eigen-analysis are applied only to those feature points, and scale selection is improved and automated, spine detection is more accurate and faster compared to previous methods. Furthermore, we propose to use a combination of various shape measurements and spine geometric features to improve the spine detection performance. Finally, a fast marching method is employed to segment the spines from the previously detected spine central regions, and spine segmentation is further improved by a series of post-processing steps, including spine head connection and pseudo spine removal.

The remainder of this paper is organized as follows: The next section is composed of our image acquisition method, the detailed spine detection algorithm and discussion on parameter selection. The third section reports experimental and comparison results, followed by conclusions in the last section.

[2] Imaris: http://www.bitplane.com/