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Using nonlinear diffusion and mean shift to detect and connect cross-sections of axons in 3D optical microscopy images

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ABSTRACT

The morphology of neuronal axons has been actively investigated by researchers to understand functionalities of neuronal networks, for example, in developmental neurology. Today's optical microscope and labeling techniques allow us to obtain high-resolution images about axons in three dimensions (3D), however, it remains challenging to segment and reconstruct the 3D morphology of axons. These include differentiating adjacent axons and detecting the axon branches. In this paper we present a method to track axons in 3D by identifying cross-sections of axons on 2D images and connecting the cross-sections over a series of 2D images to reconstruct the 3D morphology. The method can separate adjacent axons and detect the split and merge of axons. The method consists of three steps, modified nonlinear diffusion to remove noise and enhance edges in 2D, morphological operations to detect edges of the cross-sections of axons in 2D, and mean shift to track the cross-sections of axons in 3D. Performance of the method is demonstrated by processing real data acquired by confocal laser scanning microscopy.

1. Introduction

The morphology of axons in three dimensions plays an important role for researchers to understand axonal functions and connectivity (Feng et al., 2000). Neuroscientists (Kasthuri and Lichtman, 2003) have been using the morphology of motor axons including their structure and branch patterns to study axonal development. For example, three-dimensional image stacks of motor axons can be obtained using confocal laser scanning microscope (Feng et al., 2000). The discovery of the jellyfish green fluorescent protein (GFP) as a vital stain enables researchers to stain individual axons (Chalfie et al., 1994) and because the GFP chromophore is derived entirely from the polypeptide chain, GFP can be used to label living cells with minimal perturbation (Feng et al., 2000). Researchers have been using yellow fluorescent protein (YFP), a spectral variant of GFP, to label axons in vivo (Sanes and Lichtman, 1999) in muscle. The muscle is then sectioned and imaged by confocal microscopy to investigate axonal organization and development.

Challenges in extracting the morphology of axons in 3D include correctly segmenting axons from the background and separating each axon from its neighbors. Methods have been proposed to segment and track 2D and 3D tubular objects similar to axons, including the NeuronJ developed by E. Meijering et al. (2004) to track neurites, a model-based approach to extract microtubules from electron tomography volume (Jiang et al., 2006), and a semi-automatic method based on cost map to segment microtubules from total internal reflection fluorescence microscopy images (Hadjidemetriou et al., 2005). A probabilistic region merging-based technique was proposed by Srinivasan et al. (2007) to extract centerline of axons in 3D. Repulsive force-based active contour has also been developed to track axons (Cai et al., 2006). Saban et al. (2006) developed a three-step method to extract microtubules from time-lapse fluorescence microscopy images. Among the methods that have been proposed to segment and track 3D tubular objects, active contour methods (snakes) (Kass et al., 1987) are very powerful for image segmentation and widely used in object tracking (Zimmer et al., 2002; Ray et al., 2002; Ray and Acton, 2004). These methods can generate good boundaries, however, they require manual intervention to initialize the process and have difficulties to accommodate topological deformation (Zimmer et al., 2002; Ray et al., 2002). Recently, methods based on level set (Sethian, 1996) have been proposed to track tubular objects. While such methods can track topological deformation like merge or split, they have difficulties detecting weak boundaries. Computational cost for level set-based methods, which transform a two-dimensional problem to a three-dimensional one, can be very high, making it difficult to process large-volumes of images (Malladi et al., 1995; Mukherjee et al., 2004; Osher and Fedkiw, 2001). In tracking 3D

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tubular objects, Aylward et al. (1996, 2002) developed a method by approximating the medial axes of blood vessels as intensity ridges and tracking the ridges by estimating the local vessel directions. Quek and Kirbas (2001) described a method based on 3D wave propagation to extract vessels in magnetic resonance angiography. Krissian et al. (2000) developed a method based on a parametric model of a circular cylinder with a Gaussian cross-section to model the vessel radius and segment blood vessels in the brain in magnetic resonance imaging data. Multiscale analysis is also used to model blood vessels of various sizes by finding the optimal distance for computing gradient information at a given scale. By selecting a response function based on the eigenvalues of the Hessian matrix of the image, Sato et al. (1998) proposed a method to combine the three eigenvectors of the Hessian matrix to detect blood vessels. Al-Kofahi et al. (2002) presented a template-based method to detect 3D neurons by applying a set of templates guided by a generalized 3D cylinder model to search for the edges of the neurons. Roysam et al. used a model-based algorithm for tracing the retinal vasculature in an exploratory manner and to estimate parameter settings under minimal description length principle (Can et al., 1999; Tsai et al., 2004).

In this paper, we present a method to segment the cross-sections of axons in 2D images and then track the segmented cross-sections in 3D image by connecting the results among slices. Since the raw data contain noise, we apply nonlinear operations to remove noise and preserve edges for the next step of edge detection. To overcome the problem that axons may be adjacent to each other, which makes them difficult to be separated, we use morphological operations to detect the correct cross-section of each axon in 2D image. In the third step, we connect the segmentation results by applying a mean shift tracking technique across 2D image slices to reconstruct the axons in 3D. Results obtained by using the method on real data show that it can segment and track multiple axons in very difficult cases and correctly identify split and merge of axons.

This paper is organized as follows. In Section 2, we introduce the three steps of our method, edge enhanced nonlinear diffusion, morphology operations, and mean shift tracking, respectively. The motivation of each step is also discussed. Experimental results are used to demonstrate the performance of the method in Section 3. Discussion and ongoing research are presented in Section 4.

2. Method

The procedures we employed are illustrated in Fig. 1, namely, modified nonlinear diffusion for noise removal and edge enhancement, adaptive morphological operations for edge detection, and mean shift for tracking.

2.1. Image acquisition

A small piece of the omohyoid muscle of a transgenic mouse was harvested. The muscle consists of motor axons in a peripheral muscle nerve and the axons express YFP in their cytoplasm under the control of the regulatory element of the ubiquitous nervous system gene Thy-1 (Feng et al., 2000). The tissue sample was mounted flat onto a slide, immersed in a mounting medium, and covered with a cover slip. It was scanned horizontally using a confocal laser scanning microscope from the top to the bottom (the x-direction), and left to right (the y-direction). After scanning in the x and y directions, the microscope focused at the next x–y plane to acquire a 3D image stack (the z-direction). The resolution of the raw 3D image stack in the x–y plane was 0.1 μm per pixel. The z step size is 0.2 μm. After acquiring the 3D image stack, the data were down-sampled in the x, y, z directions by two. Therefore the data have a resolution of 0.2 μm in the x and y directions, and 0.4 μm in the z-direction. The matrix size of the resampled data is $512 \times 512 \times 126$ in the x, y, z direction, respectively.

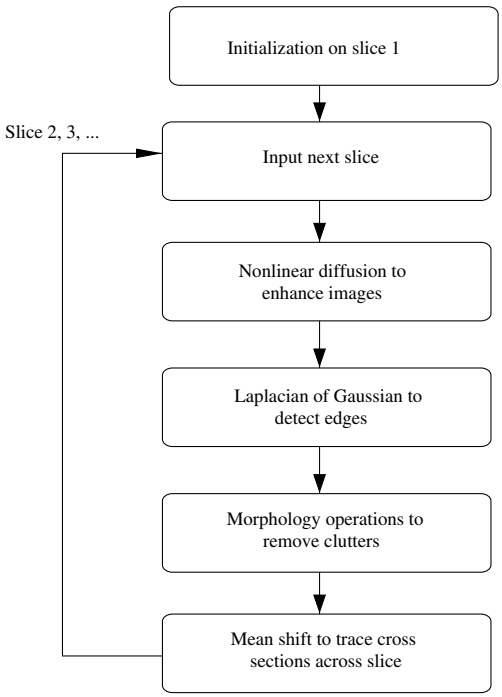

Fig. 1. Flow chart of the proposed method.

2.2. Nonlinear diffusion

In recent years, nonlinear diffusion methods (Perona and Malik, 1990) have proved to be very effective in image processing, especially in biomedical applications (Weickert and Schnorr, 2000; Catte et al., 1992; Weickert, 2001; Niessen et al., 1994; Gilboa et al., 2001; Krim et al., 1999; Lysaker et al., 2003; Dong et al., 2003), since they usually require a preprocessing step to remove noise while preserving fine structures such as edges and boundaries in the images. It has been shown that the partial differential equation (PDE)-based method preserves image transitions better than spatially averaging techniques such as moving average and Gaussian filtering (Weickert, 1999; Moisan et al., 2002). However, PDE-based methods such as the filter proposed by Perona and Malik (1990) have the drawbacks of oversmoothing and creating blocky effects in the results, and thus lose some important fine structures in the images. Rudin–Osher–Fatemi (ROF) (Rudin et al., 1992) developed an approach by minimizing the total variation (TV) norms of images under some given conditions to circumvent oversmoothing. This approach has been used as a regularization method for many other applications where one needs to identify discontinuous functions (Koepfler et al., 1994; Chan et al., 2003; Dong et al., 2003).

We developed a modified nonlinear diffusion method to enhance edges and remove noise simultaneously as a preprocessing step. The motivation is to overcome the oversmoothing and blocky effects of the Perona–Malik equation and achieve better edge enhancements than the TV-norm filters, which cannot increase image contrast due to the design of the diffusion function. Moreover, our method does not create speckles as the second-order derivative method does (You and Kaveh, 2000). The modified nonlinear diffusion method solves the following equation: