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Segmentation of nerve fibers using multi-level gradient watershed and fuzzy systems

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ABSTRACT

Objective: This paper presents an algorithm based on multi-level watershed segmentation combined with three fuzzy systems to segment a large number of myelinated nerve fibers in microscope images. The method can estimate various geometrical parameters of myelinated nerve fibers in peripheral nerves. It is expected to be a promising tool for the quantitative assessment of myelinated nerve fibers in related research.

Materials and methods: A novel multi-level watershed scheme iteratively detects pre-candidate nerve fibers. At each immersion level, watershed segmentation extracts the initial axon locations and obtains meaningful myelinated nerve fiber features. Thereafter, according to *a priori* characteristics of the myelinated nerve fibers, fuzzy rules reject unlikely pre-candidates and collect a set of candidates. Initial candidate boundaries are then refined by a fuzzy active contour model, which flexibly deforms contours according to the observed features of each nerve fiber. A final scan with a different set of fuzzy rules based on the *a priori* properties of the myelinated nerve fibers removes false detections. A particle swarm optimization method is employed to efficiently train the large number of parameters in the proposed fuzzy systems.

Results: The proposed method can automatically segment the transverse cross-sections of nerve fibers obtained from optical microscope images. Although the microscope image is usually noisy with weak or variable levels of contrast, the proposed system can handle images with a large number of myelinated nerve fibers and achieve a high fiber detection ratio. As compared to manual segmentation by experts, the proposed system achieved an average accuracy of 91% across different data sets.

Conclusion: We developed an image segmentation system that automatically handles myelinated nerve fibers in microscope images. Experimental results showed the efficacy of this system and its superiority to other nerve fiber segmentation approaches. Moreover, the proposed method can be extended to other applications of automatic segmentation of microscopic images.

1. Introduction

Clinical and research evaluations of regeneration and repair of nerve fibers require the detection and extraction of nerve fiber features that have been imaged using various modalities. A quantitative cross-sectional analysis of highly clustered myelinated nerve fibers is important to related research. When this task is performed by manual segmentation, it is labor-intensive and time-consuming because thousands of nerve fibers appear in each image. Therefore, an image segmentation system with the ability to segment a large number of myelinated nerve fibers in a short time is desired for use by neuropathologists and neuroscientists. In this study, we focus on developing a rule-based method for the segmentation of cross-sectional myelinated nerve fibers in microscope images. The segmented results could be used in researches such as crushed nerve experiments and studies.

Contemporary cell segmentation tools are generally based on standard methods such as thresholding, Hough transform, morphological operations, active contour model (ACM), and watershed transform approach. Using the thresholding method, Weyn et al. [1] presented a system that can segment a large number of cells in a microscope image, but the segmentation results depend greatly on the threshold value. In addition, this method may require human intervention at each segmentation step. In watershed segmentation [2], the immersion level of the watershed is a key factor. If the contrast between the cells and the background is significant, cells can be found at a high immersion level. Conversely, if the contrast is weak or unclear, cells may be distinguishable only at a

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low immersion level. Thus, traditional watershed transformations using only one immersion level may fail to extract all the cells. Vanhamel et al. proposed a hierarchical watershed segmentation method that applies a gradient watershed to scale-space diffusion filtered images for color segmentation [3]. However, the diffusion results deviate significantly from the true boundary. The ACM is a deformable shape model that offers a powerful approach to image segmentation because it can incorporate continuity and curvature together with *a priori* knowledge, *e.g.*, the shape of objects. However, the conventional ACM using only one set of empirical weights cannot handle complicated cell cross-sections, which usually have fuzzy boundaries and are located close to each another.

Some cell segmentation methods combine multiple stages to provide more robust segmentation results. Mouroutis et al. [4] determined the possible locations of cell nuclei using the compact Hough transform and then used a likelihood function to optimize the cell boundaries. Fok et al. [5] first obtained the inner boundaries of nerve fibers using an elliptical Hough transform and then used the ACM to refine the outer boundaries of the nerve fibers. Finally, they used a false positive (FP) detection system to improve the detection performance. Garrido and Perez [6] developed an automatic cell segmentation approach that uses the elliptical Hough transform to obtain cell locations and then refined the results using Grenander's deformable template model [7]. A number of other studies related to cell segmentation have also been reported [8–12]. Although the abovementioned studies have provided promising results with the given images, various issues remain unsolved. These issues include semi-automatic segmentation [1], inflexible ACM weights [5], and a small number of extracted cells [4–6,11]. In our preliminary study [12], a simple method was proposed to check the feasibility of a fuzzy system in detecting nerve fiber boundaries. The method uses two fuzzy systems with empirically determined parameters. Thus, this segmentation method is more sensitive to image conditions and detects relatively few nerve fibers. The patterns of peripheral nerves are relatively heterogeneous. Our present algorithm does not attempt to deal with all possible cases of neuropathological analysis for peripheral nerves, but simplifies the problem by focusing on slides of myelinated nerve fibers.

To address the abovementioned issues, this paper proposes a new method that uses a multi-level gradient watershed scheme in combination with three fuzzy systems (MWFS) for automatic segmentation of nerve fibers. The three fuzzy systems handle the phases of detection, refinement, and confirmation. In the multi-level gradient watershed scheme, pre-candidate nerve fibers are extracted at each level and are then examined by the first fuzzy rule system for certain nerve fiber conditions. If the pre-candidate nerve fibers satisfy these conditions, they become candidates and are added to a specific buffer. During the refinement phase, the boundaries of the candidates are refined by the second fuzzy system, which is a rule-based ACM. Finally, a third fuzzy rule system based on *a priori* cellular characteristics is used to confirm whether each refined candidate is a FP or a true nerve fiber.

The proposed method offers several advantages over the conventional methods. In most cross-sectional nerve fiber images, the gray level variations in areas of myelin sheath are quite large and the contrast between the myelin sheath areas and background is not uniform. In the detection phase, the multi-level gradient watershed method can extract a higher percentage of nerve fibers under these image conditions than the traditional watershed segmentation method using a fixed watershed level. Subsequently, the MWFS algorithm performs refinement and confirmation. In each phase, information on the location and other properties of the nerve fibers is embedded in the corresponding fuzzy system, making nerve fiber segmentation more efficient and robust. The particle swarm optimization (PSO) method [13] is used to obtain the large number of parameters required by the proposed fuzzy systems. Experimental results show that the proposed automatic segmentation approaches the ground truth, which corresponds to manual segmentation by experts.

2. Materials and related technology

2.1. Data acquisition

A set of cross-sectional images of nerve fibers was acquired from the National Cheng Kung University Hospital in Tainan, Taiwan. Five

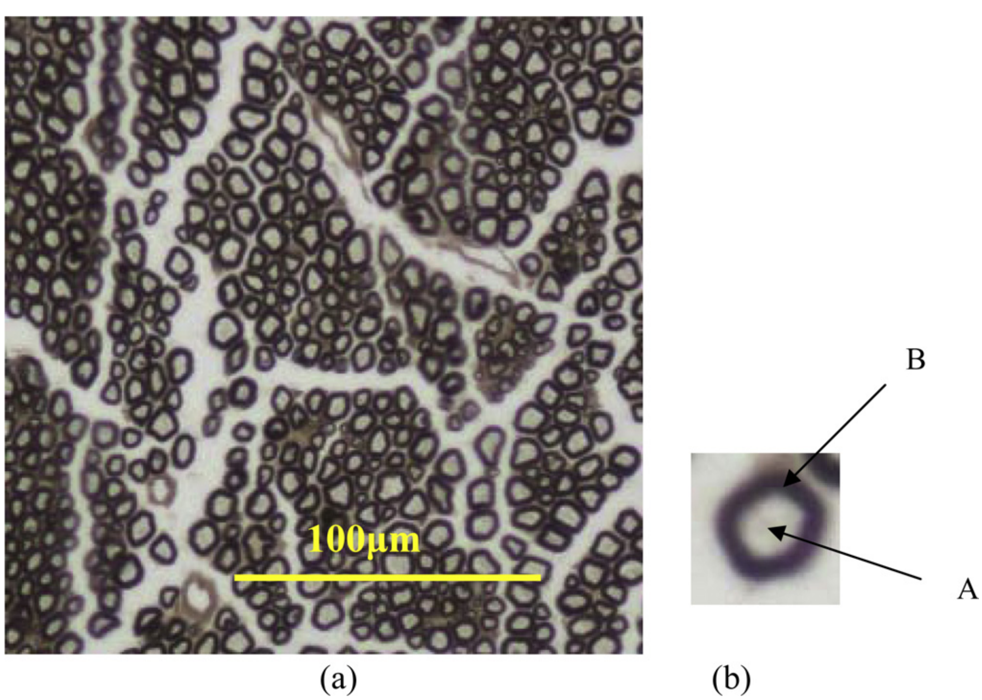

Fig. 1. Microscopic image: (a) typical nerve-fiber cross-section, 300 × 300 pixels; (b) enlarged single nerve fiber, with arrow A pointing to the axon and arrow B pointing to the myelin sheath (MS).