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Identification and segmentation of myelinated nerve fibers in a cross-sectional optical microscopic image using a deep learning model

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HIGHLIGHTS

- A new method for fully automated identification and segmentation of human myelinated nerve fibers in a cross-sectional optical-microscopic image was proposed.
- It used a deep learning model of a convolutional neural network.
- The results of accuracy evaluation were promising in comparison with previously reported automated methods.
- By training the model with more sample data, higher performance can be achieved than it is.

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ABSTRACT

Background: The morphometric analysis of myelinated nerve fibers of peripheral nerves in cross-sectional optical microscopic images is valuable. Several automated methods for nerve fiber identification and segmentation have been reported. This paper presents a new method that uses a deep learning model of a convolutional neural network (CNN). We tested it for human sural nerve biopsy images.
Methods: The method comprises four steps: normalization, clustering segmentation, myelinated nerve fiber identification, and clump splitting. A normalized sample image was separated into individual objects with clustering segmentation. Each object was applied to a CNN deep learning model that labeled myelinated nerve fibers as positive and other structures as negative. Only positives proceeded to the next step. For pretraining the model, 70,000 positive and negative data each from 39 samples were used. The accuracy of the proposed algorithm was evaluated using 10 samples that were not part of the training set. A *P*-value of <0.05 was considered statistically significant.
Results: The total true-positive rate (TPR) for the detection of myelinated fibers was 0.982, and the total false-positive rate was 0.016. The defined total area similarity (AS) and area overlap error of segmented myelin sheaths were 0.967 and 0.068, respectively. In all but one sample, there were no significant differences in estimated morphometric parameters obtained from our method and manual segmentation.
Comparison with existing methods: The TPR and AS were higher than those obtained using previous methods.
Conclusions: High-performance automated identification and segmentation of myelinated nerve fibers were achieved using a deep learning model.

1. Introduction

Morphometric analysis of myelinated nerve fibers of peripheral nerves in a *trans*-sectional optical microscopic image is valuable in research and clinical settings. In a clinical setting, the analysis can used to evaluate the severity of damage and to infer the underlying pathophysiology of peripheral neuropathies (Said, 2002). The distribution of the diameters of myelinated nerve fibers is a significant indicator of nerve degeneration; thus, measurements of the density distribution are routinely taken from biopsy images. A healthy nerve exhibits bimodal distribution, with peaks at 3–6 μm (i.e., small fibers) and 9–13 μm (i.e., large fibers) (O'Sullivan and Swallow, 1968). In contrast, some neuropathies have characteristic pathological features in their distribution patterns. For example, in some kinds of familial amyloid polyneuropathies, the number

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of small fibers decreases from the early stage of illness, whereas large fibers are comparatively preserved. They cannot be diagnosed by the evaluation of the density distribution alone, but it can assist with the diagnosis (Koike et al., 2004). Morphometric analysis has been widely performed in research studies to evaluate physiological and morphological changes during aging Ceballos et al. (1999), regeneration Schröder (1972), and responses to outer stresses (Mackinnon et al., 1984). Moreover, it has been used to investigate peripheral nerve changes in animal models of diseases (Oliveira et al., 2013; da Silva et al., 2016).

The manual detection and measurement of myelinated nerve fibers are labor intensive and time consuming because each sample is typically composed of hundreds of nerve fibers. Thus, several semiautomated (Silva da et al., 2007; More et al., 2011; Isaacs et al., 2014) and automated (Campadelli et al., 1999; Romero et al., 2000; Wang et al., 2012; Novas et al., 2013; Novas et al., 2016) methods for nerve fiber identification and segmentation have been proposed to facilitate this process. Here we define a semiautomated method that requires any manual operation, for example, manually setting up parameters or manipulating software. The number (or density) and diameters of myelin sheaths and myelinated nerve axons are commonly measured parameters; thus, the goal of semiautomated and automated methods is to essentially provide the segmentation of each myelin sheath in sample images. Most current automated methods are designed for the (1) identification and segmentation of candidate objects and (2) elimination of false positives (FPs) in accordance with prespecified criteria.

To automate the identification and segmentation processes, clustering algorithms (Campadelli et al., 1999; Wang et al., 2012; Novas et al., 2016), gray level thresholding (Romero et al., 2000), and watershed transform (Wang et al., 2012) have been utilized. Furthermore, active contour models and Hough transform have been used in the field of nerve cell segmentation of a brain specimen (Fok et al., 1996). Although FPs can be eliminated later, accurate segmentation is desirable to generate as few FPs as possible. A recently proposed method (Novas et al., 2016) used competitive learning (Uchiyama and Arbib, 1994), which is a clustering algorithm, to segment candidate areas of myelinated nerve fibers. Clustering on pixel intensities was conducted, and pixels of the darkest cluster were extracted as target areas. Although this method exhibits high-performance segmentation of the laryngeal nerves of rats, the optimal number of clusters is not predetermined and thus needs to be determined for each evaluation. This disadvantage becomes more obvious when the method is applied to human clinical samples because in such samples, the density of nerve fibers can decrease to various degrees, leading to difference in color distributions among samples. Different color distributions require different optimal numbers of clusters (Fig. 1).

As misidentification in the above processes is inevitable, accurate and robust systems for recognizing true positives (TPs; i.e., myelinated nerve fibers) and eliminating FPs are required. Previous methods used rule-based criteria based on myelinated nerve fiber features, which included nerve size (Campadelli et al., 1999; Romero et al., 2000; Novas et al., 2016), ratio of d/D (Campadelli et al., 1999; Romero et al., 2000), circularity (Campadelli et al., 1999; Romero et al., 2000; Wang et al., 2012), and existence of a hole (Campadelli et al., 1999; Romero et al., 2000). Despite these efforts, errors are still made due to variabilities in myelinated nerve fiber shapes and other structures such as unmyelinated nerve fibers, Schwann cells, myelin ovoids, and parts of connective tissues. The highest TP rate (TPR) in previous methods was not more than 96% (Wang et al., 2012). Furthermore, accurate judgment is assumed to be more difficult in human biopsied samples of neuropathies than in animal samples because there can be various types of deformed nerve fibers. Finally, the number of some structures, such as Schwann cells, often increases due to pathological processes (Behse et al., 1975).

Until recently, image recognition has mainly depended on rule-based algorithms, including the above-mentioned methods. Such rules are generated from observed patterns and features. How-

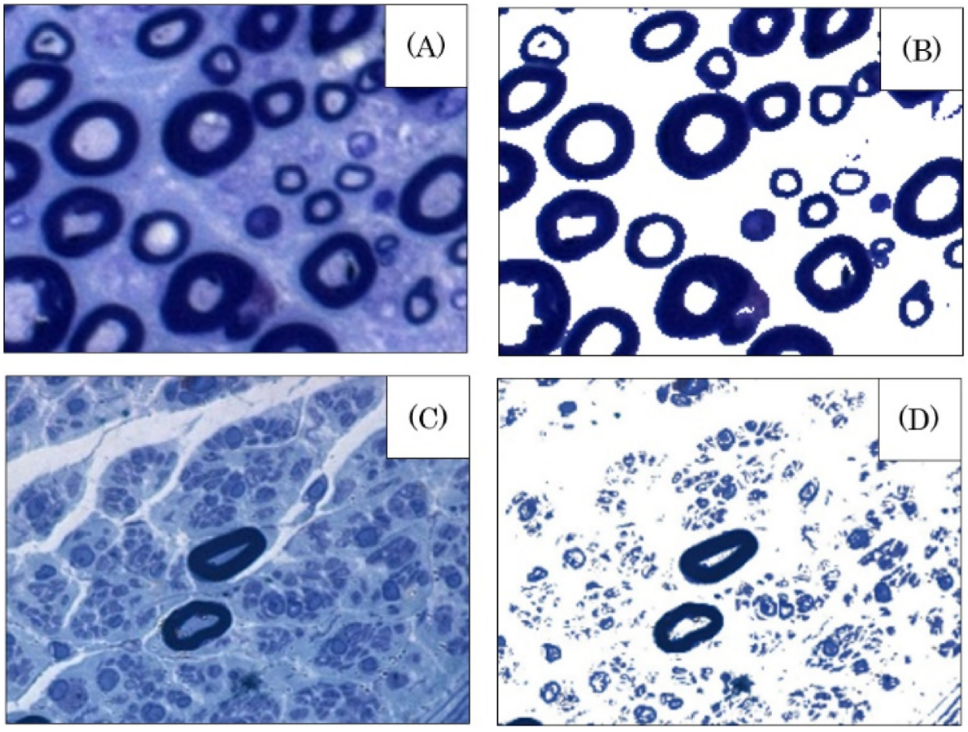

Fig. 1. Segmentation using competitive learning of three clusters: (A) an example of myelinated nerve fibers of normal density; (B) the pixels of the darkest cluster in (A) are extracted. (C) An example of severe neuronal loss, and (D) the pixels of the darkest cluster in (C) are extracted. In a sample with severe neuronal loss, three clusters are inadequate to extract only myelin sheaths due to differences in color distribution.