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Automatic identification and quantitative morphometry of unstained spinal nerve using molecular hyperspectral imaging technology

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ABSTRACT

Quantitative observation of nerve fiber sections is often complemented by morphological analysis in both research and clinical condition. However, existing manual or semi-automated methods are tedious and labour intensive, fully automated morphometry methods are complicated as the information of color or gray images captured by traditional microscopy is limited. Moreover, most of the methods are time-consuming as the nerve sections need to be stained with some reagents before observation. To overcome these shortcomings, a molecular hyperspectral imaging system is developed and used to observe the spinal nerve sections. The molecular hyperspectral images contain both the structural and biochemical information of spinal nerve sections which is very useful for automatic identification and quantitative morphological analysis of nerve fibers. This characteristic makes it possible for researchers to observe the unstained spinal nerve and live cells in their native environment. To evaluate the performance of the new method, the molecular hyperspectral images were captured and the improved spectral angle mapper algorithm was proposed and used to segment the myelin contours. Then the morphological parameters such as myelin thickness and myelin area were calculated and evaluated. With these morphological parameters, the three dimension surface view images were drawn to help the investigators observe spinal nerve at different angles. The experiment results show that the hyperspectral based method has the potential to identify the spinal nerve more accurate than the traditional method as the new method contains both the spectral and spatial information of nerve sections.

1. Introduction

In spinal cord injury or some central nervous system (CNS) diseases with compromised fiber architecture and function such as the Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS), visualization of morphological and biochemical events of nerve fibers would be of great value in surgery and the search for therapy leading to neurofunctional recovery or compensation (Dyakin et al., 2010; Mamata et al., 2006; Wilson et al., 2004). Therefore, the identification and quantitative neuroanatomical morphology of nerve fibers is an important description method in investigating the development or pathological abnormalities of CNS. Morphometric analysis of nerve fibers is often used as a complemented method for histopathological examination in both clinical and research settings such as the experiments involving nerve injury and recovery (Nassiri et al., 2010; Oliveira et al., 2001), the examination of regenerative nerve phenomena (Mezin et al., 1994), the topological distribution of nerves (Zhao et al., 2010), the experimental models of hyperalgesia, neuropathic pain and its treatments (Yasuda et al., 2005), etc. Usually, morphometric studies are focused on some morphological parameters of nerve fibers, for instance the myelinated fiber size and number, fiber perimeter, fiber diameter, myelin sheath thickness, etc. (Herrmann et al., 1999; Lauria et al., 2011; Romero et al., 2000; Zhao et al., 2010). These morphological parameters can be achieved manually by experienced neuroscientists or with the aid of semi-automatic or fully automated computer-assisted programs which are capable of automating the process to a varying degree.

Traditionally, manually methods have been used by investigators to measure fiber diameters and estimate fiber size (Donovan, 1967; Mezin et al., 1994; Nehrer-Tairych et al., 2000; Romero et al., 2000). The workload demanded by this kind of approach was greatly dependent on the nerve cross surface and fiber size population. Moreover, this method can introduce unavoidable subjective bias by the profile selection the neuroscientists made. To increase the numbers and speed of measurements, the semi-automatic morphometry methods were proposed (More et al., 2011). Semi-automatic morphometry method refer to the use of a digital tablet or similar to trace around nerve images. This

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method is faster than manual techniques, allowing more sample areas to be analyzed. However, it is still unsuitable for study nerves with a large fiber population. In addition, most of these manually and semi-automatic nerve morphometry methods are extremely tedious, labour intensive and time-consuming. It also makes the investigators fatigue to get the subjective decisions which is the potential sources of errors (Urso-Baiarda and Grobbelaar, 2006).

To overcome these problems, fully automated nerve morphometry methods have been developed and used with the advent of more advanced computer technology. The application of automatic image processing based on images of nerve cross sections has drawn much attention from image processing and neurology communities. Many investigators have presented some automated nerve morphometry algorithms such as edge detection, template matching, active contours, snakes, canny, neural networks, zonal graphs, and region growing to identify the nerve fibers automatically and calculate the morphological parameters quantitatively based on the gray or color images of nerve sections (Jurrus et al., 2010; More et al., 2011; Romero et al., 2000; Usson et al., 1991; Ying-Lun et al., 1996; Zhao et al., 2010).

These studies show that the automated nerve morphometry methods are faster and more objective than the manually and semi-automatic methods. Currently, most of the automated nerve morphometry methods are based on the images captured by light microscopy (Hunter et al., 2007; Weyn et al., 2005), transmission electron microscope (TEM) (Chapman et al., 2012; Jurrus et al., 2010; Zhao et al., 2010), and scanning electron microscope (SEM) (Carreras et al., 2010; Cowin et al., 2011; More et al., 2011). These microscopes can provide high spatial resolution images of nerve cross sections which can be used for nerve fiber identification with pattern recognition algorithms. However, these automatic nerve morphometry methods are associated with some other problems. First, the RGB color or gray images captured by traditional microscope only contained the structural information of nerve fibers, which influenced the accuracy of the automatic morphometry methods and lead to the algorithms complicated. Second, the nerve cross sections need to be stained with some reagents such as toluidine blue before observing with the light microscopy, TEM or SEM (da Silva et al., 2007), which lead to the tissues preparation process extremely labour intensive and time-consuming. In addition, these histological sections contain nerve fiber structures with inhomogeneous staining densities, which lead to smooth variations of the average luminance in some regions of the image (Romero et al., 2000).

The molecular hyperspectral imaging (MHI) technology may offer a solution for these constraints. Hyperspectral imaging is part of a class of techniques commonly referred to spectral imaging or spectral analysis, which is a technology that integrates conventional imaging and spectroscopy to attain both spatial and spectral information from an object (Gowen et al., 2007; Landgrebe, 2002). It was originally developed for remote sensing applications such as agriculture, mineralogy, military, environment, etc. (Adam et al., 2010; Alexander and Goetz, 1981). Hyperspectral imaging can take advantage of the spatial relationships among the different spectra in a neighborhood, allowing more elaborate spectral-spatial models for a more accurate segmentation and classification of the image (Picon et al., 2009). In recent years, this technology has been used for characterization and monitoring of applications in life science field. Researchers have developed various microscopic multispectral or hyperspectral imaging systems for the biochemical analysis of various biological tissues (Bouillard et al., 2010; Leavesley et al., 2012). For example, Afromowitz developed a multispectral imaging system to evaluate burn depth of skin (Afromowitz et al., 1988). This is the early use of spectral imaging technology in biomedicine field. Then Papadakis presented a method for the quantitative assessment of the uptake by histologic samples of stains used in pathology to label tissue features of diagnostic importance based on a spectral microscope system (Papadakis et al., 2003). Afterwards, MacKinnon developed a similar system to analyze the hematoxylin and eosin-stained tissue sections from cervical biopsies (MacKinnon et al., 2007). All these studies have shown that the MHI technology can obtain both images (structural information) and spectra (biochemical information) of biological tissues which has the significant advantages in the area of life science.

In the past ten years, we have developed a pushbroom microscopic hyperspectral imaging system and apply it to the immunohistochemical analysis for the protective effect of erythropoietin (EPO) on diabetic retinal cells quantitatively, feature extraction of leukemic blood cells, etc. (Li et al., 2009a,b; Li et al., 2010). Although this system can capture microscopic hyperspectral images of tissues, it needs a pushbroom procedure in one spatial dimension which makes the system complex and time consuming. In this paper, an acousto-optic tunable filter (AOTF) based MHI system was developed and used for capturing the molecular hyperspectral images of nerve cross sections. AOTF is a rapid wavelength-scanning solid-state device that operates as a tunable optical band pass filter (Jackel et al., 1996). The acoustic wave is generated by radio-frequency signals, which are applied to the crystal via an attached piezoelectric transducer. Unlike in the grating based pushbroom microscopic hyperspectral imaging system, no motion of the system or sample is required to obtain a complete scene of molecular hyperspectral images. This makes the structure of the new system based on AOTF more simple and compact, which is suitable for nerve fibers observation. In addition a new automatic identification and quantitative nerve morphometry method for segmentation, recognition, and measurement of nerve fibers based on the molecular hyperspectral images is also presented. Unlike those existing automated nerve morphometry methods, the proposed method can recognize nerve fibers using their spectral signatures instead of their gray values. This characteristic makes it possible to analysis the no-stained nerve fiber sections directly and automatically. The hyperspectral based automated nerve morphometry algorithm, the implementation of this algorithm, the experimental process, and the performance of the proposed method are illustrated and discussed in the following sections.

2. Materials and methods

2.1. Animal and tissue preparation

The spinal nerve samples used in this study was obtained from female New Zealand White Rabbits (2.5–3.0 kg) to be used for control data in an unrelated experiment. All operations were carried out in department of Orthopaedics, Zhongshan Hospital, China after an ethic committee approval. These rabbits were kept for two weeks with ordinary food and water, until the beginning of the experiment. The rabbits were anatomized in the prone position and the ventral and dorsal roots of spinal nerves were cut off (from 5 to 10 mm length). Then, these nerve roots were placed on the aluminum foil papers filled with OCT (Tissue-Tek optimal Cutting Temperature compound, Sakura USA). The samples were cooled rapidly with sub-liquid nitrogen and placed into refrigerator to freeze with subzero 80 °C for 24 h. After this, the transverse 10 μm thickness sections were cut (subzero 20 °C) with a freezing microtome (Leica CM1800, Germany), collected in a knife-mounted OCT bath and transferred to a clean glass slide.

2.2. Image acquisition

In order to assess the performance of the hyperspectral based approach, both the traditional microscope images and the