High Resolution Imaging of Human Nerves with MicroCT

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THE intraneural topography of peripheral nerves is critical in the development of neural interfaces. Peripheral nerves consist of multiple fascicles within a loose collagen matrix. An early seminal study suggested that fascicles have a plexiform organization [5], but others have suggested fascicles are cable like [1]. Studies that characterize nerve cuff stimulation via computer simulations assume fascicles are cable like and extrude 2D histological cross sections [4] to create 3D reconstructions. A cable like organization of fascicles indicates that axons are spatially contained within fascicles. Nerve cuff electrodes can selectively activate axons within a fascicle in peripheral nerves [6,7]. MRI techniques have been proposed to study the intraneural topography of upper extremity nerves [2], but they do not reliably distinguish all fascicles. To optimize implant location and cuff dimensions, a new method must be developed to resolve fascicular organization. We have validated microCT as a viable imaging technique to trace fascicles in human cadaveric peripheral nerves.

The median nerve from a cadaveric arm was dissected and extracted. The dissected nerve was then preserved in 10% formalin. The nerve was stained with 0.3% Phosphotungstic Acid [3] and stored in 100% ethanol to be microCT imaged. The nerve was CT imaged at a 20 μm resolution (μCT) using a 40-80 kVp xray source, 0.5 mA max current, and 0.05 degree rotational precision (ImageIQ, Cleveland, OH). A 3D reconstruction was created for a 2 cm nerve section. After imaging the nerve, histological cross sections were collected to serve as a gold standard. The nerve section was immersed in sucrose gradients of 10%, 20%, and 30%. The sample was then block embedded in O.C.T. (Optimum Cutting Temperature) compound for cryostat sectioning. The frozen blocks were sliced with a thickness of 20 μm, stained with methylene blue, and imaged with a Zeiss AxioImager microscope. MicroCT images were validated by comparing them to the corresponding histological cross sections every 0.2 mm along a 0.5 cm section. Both the cryostat and microCT images were analyzed using custom MATLAB (The Mathwork, Inc. Natwick, MA) software. The fascicles in the images were traced and the number of fascicles were recorded for each image.

There were 20.9±1.5 (20-24) and 20.4±1.9 (19-25) fascicles across all cross sections using microCT and standard histological techniques, respectively. The mean difference of fascicle count between the microCT and histological cross sections was .9±.77 fascicles. There is a 95% confidence that the error in fascicle count is less than 2.16 fascicles, or approximately 10% error in fascicle count between the two methods. This value is acceptable considering the high fascicle count in upper extremity nerves. There was a higher false positive rate of fascicle detection in the microCT images due to the decreased contrast inherent to the microCT images.

MicroCT is an attractive novel, method to map the intraneural nerve anatomy at a high resolution. MicroCT enables 3D dimensional reconstruction of the nerve while keeping the tissue sample intact. This imaging modality can be used as a tool to develop neural interfacing technology for use in human peripheral nerves.

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