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Postmortem diffusion MRI of the entire human spinal cord at microscopic resolution

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ABSTRACT

The human spinal cord is a central nervous system structure that plays an important role in normal motor and sensory function, and can be affected by many debilitating neurologic diseases. Due to its clinical importance, the spinal cord is frequently the subject of imaging research. Common methods for visualizing spinal cord anatomy and pathology include histology and magnetic resonance imaging (MRI), both of which have unique benefits and drawbacks. Postmortem microscopic resolution MRI of fixed specimens, sometimes referred to as magnetic resonance microscopy (MRM), combines many of the benefits inherent to both techniques. However, the elongated shape of the human spinal cord, along with hardware and scan time limitations, have restricted previous microscopic resolution MRI studies (both *in vivo* and *ex vivo*) to small sections of the cord. Here we present the first MRM dataset of the entire postmortem human spinal cord. These data include 50 μm isotropic resolution anatomic image data and 100 μm isotropic resolution diffusion data, made possible by a 280 h long multi-segment acquisition and automated image segment composition. We demonstrate the use of these data for spinal cord lesion detection, automated volumetric gray matter segmentation, and quantitative spinal cord morphometry including estimates of cross sectional dimensions and gray matter fraction throughout the length of the cord.

1. Introduction

The spinal cord is an essential part of the central nervous system that is responsible for transmitting neuronal signals between the brain and body. In addition to its role as the primary conduit for sensory and motor function, the spinal cord is affected by several debilitating human diseases including multiple sclerosis, amyotrophic lateral sclerosis, and various forms of spinal cord injury (Minagar and Rabinstein, 2012). Lesions to the spinal cord can produce a range of symptoms including pain, paresthesias, weakness, and paralysis. Because of its clinical importance, the spinal cord is frequently a target for imaging research (Wheeler-Kingshott et al., 2014). Historically, light microscopy-based histology has been the major imaging modality used for investigating the spinal cord (Fix, 2008; Sengul et al., 2013; Standring, 2016; Watson et al., 2009). One of the most widely known histological atlases of the spinal cord is *Gray's Anatomy* (Standring, 2016) (now in its 41st edition), which has since been digitized to create a white matter atlas of the human spinal cord (Lévy et al., 2015). A more recent landmark histological atlas is the *Atlas of the Spinal Cord of the Rat, Mouse, Marmoset, Rhesus, and Human* (Sengul et al., 2013). These histologic studies offer sub-cellular in-plane resolution and many unique contrasts based on chemical (*e.g.* cresyl violet) and immunohistochemical (*e.g.* acetylcholinesterase) tissue stains.

While histology remains the gold standard for anatomic and pathologic studies of the spinal cord, it is not without limitations. Histological techniques are labor intensive and require destruction of the sample due to the need for tissue sectioning. The resulting tissue slices are frequently affected by distortions from fixation, embedding, and sectioning. Moreover, each piece of tissue can be stained only once, thus limiting what can be visualized with any given specimen. For these reasons, histologic studies are typically limited to a small number of individual slices, often with large intervening gaps, and therefore lack the ability to represent the three-dimensional anatomy and connectivity of the cord. For example, Gray's Anatomy includes only a single slice through the cervical cord (C4), and the Sengul et al. atlas, one of the most comprehensive of its kind, includes only one slice for each of the

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31 vertebral levels. Most clinical pathologic assessments of the spinal cord are similarly sparse in their sampling and can potentially miss focal lesions, which may occur anywhere throughout the length of the cord.

Magnetic resonance imaging (MRI) of the spinal cord can overcome many of the drawbacks inherent to conventional histology, albeit at the cost of lower in-plane spatial resolution. Contiguous 3D images can be acquired along the full cord, and multiple contrasts can be visualized within the same tissue. Notably, several MRI contrasts, including diffusion MRI (dMRI), are quantitative and provide additional information about tissue microstructure that is beyond the actual image resolution. However, techniques for spinal cord MRI have historically been limited; in particular, troublesome factors include the spinal cord's small cross-sectional area, artifacts from adjacent bone-soft tissue interfaces, and physiologic motion of blood and cerebrospinal fluid (Cohen-Adad and Wheeler-Kingshott, 2014). Despite these challenges, there have been many notable *in vivo* MRI studies of the human spinal cord including those utilizing dMRI, though they are limited in slice number and resolution (Farrell et al., 2008; Massire et al., 2016; Rasoanandrianina et al., 2017; Taso et al., 2014).

Postmortem microscopic resolution MRI of fixed specimens, sometimes referred to as magnetic resonance microscopy (MRM), offers an alternative approach that addresses many of the limitations of other spinal cord imaging modalities. Using exogenous contrast agents, long scan times, and specialized equipment, superior resolution and contrast can be achieved along the entire cord while maintaining most of the benefits inherent to MRI. Previous MRM studies of the human spinal cord have demonstrated the incredible three-dimensional anatomic detail that this technique can provide, but they have typically been confined to small sections of the cord due to hardware limitations (Bergers et al., 2002; Bot et al., 2004; Gilmore et al., 2009; Mottershead et al., 2003; Nijeholt et al., 2001). Here, we present the first microscopic resolution dMRI dataset of the entire postmortem human spinal cord, made possible by multi-segment acquisition and automated image composition. These data offer a unique opportunity to explore the three-dimensional anatomy and connectivity of the spinal cord at unprecedented resolution. We further demonstrate the use of these data for both pathologic lesion detection and for automated volumetric gray matter segmentation and morphometric analysis using a recently published deep learning method (Perone et al., 2017).

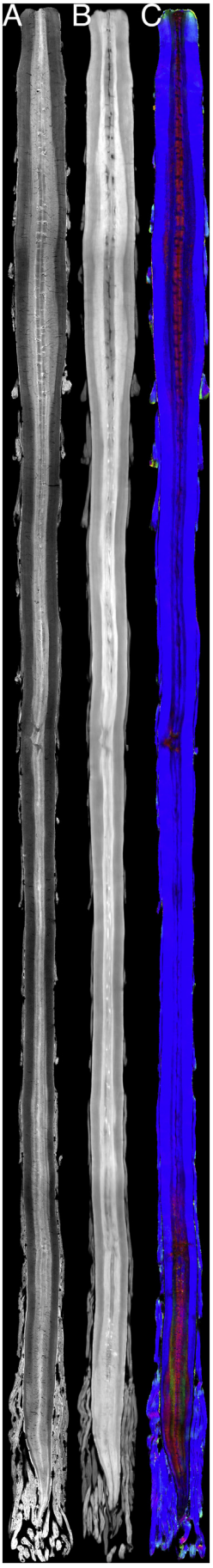
2. Materials and methods

2.1. Specimen procurement

An entire human spinal cord, from pyramidal decussation to cauda equina, was obtained from a deceased adult male in his sixties at the time of death, with no known history of neurologic disease. After death and prior to spinal cord removal, the cadaver was gravity perfused with isotonic saline through the right carotid artery until all visible blood was flushed from the vascular system. The time between death and spinal cord harvest (postmortem interval) was approximately 18 h, and during this time the cadaver was maintained at 4°C. Spinal cord extraction was performed by a trained neurosurgeon using standard surgical instruments and took approximately 1 h to complete. The dissected cord measured approximately 46 cm from pyramidal decussation to filum terminalis.

2.2. Specimen preparation

Immediately after extraction, the dura was opened longitudinally and the spinal cord was sutured to a block of closed-cell polystyrene foam in a fully extended position *via* the attached dural flaps. This was done to ensure that the cord remained straight and suspended during subsequent fixation. The specimen was immersion-fixed in a 10% formalin solution for two weeks at 4 °C and then transferred to a



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