# Hyper-acute evaluation of spared white matter in mouse model contusion SCI using in vivo DTI

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## Introduction

The extent of spared white matter is critical for functional recovery after contusion spinal cord injury (SCI). The SCI diagnosis in both human patients and animal models may benefit from the accurate assessment of the extent of the spared white matter. Unfortunately, the current gold standard histology can not be applied non-invasively. There is currently no accurate methodology for the noninvasive determination of spared white matter content, Herein, we present the use of in vivo DTI for the acute and non-invasive method for the determination of spared white matter content in mouse model of SCI. Using mouse model contusion SCI, the acquired *in vivo* DTI maps, relative anisotropy (RA), axial (λ||) and radial (λ⊥) diffusivity, and mean diffusivity (Tr(D), provided acute evaluation of spared white matter content with histological validation.

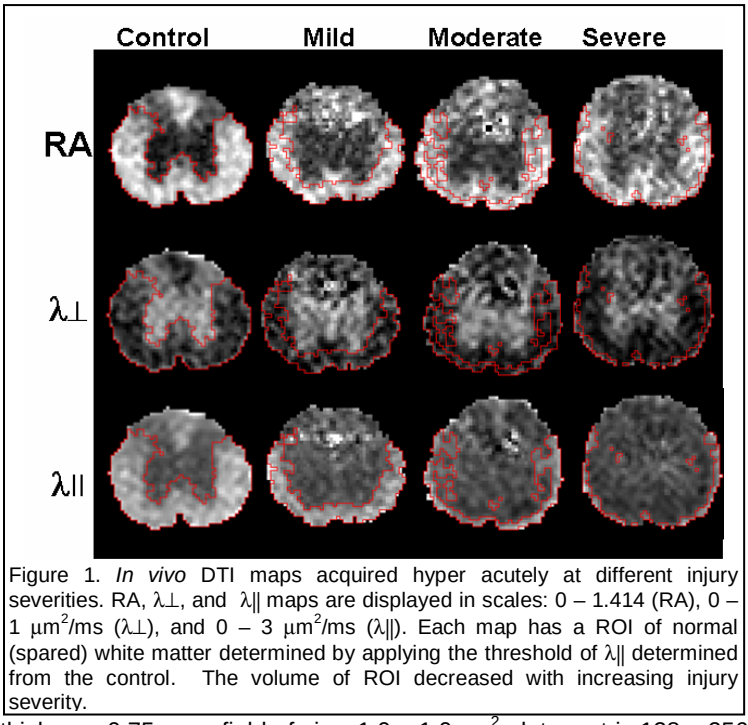


Figure 1. *In vivo* DTI maps acquired hyper acutely at different injury severities. RA, λ⊥, and λ|| maps are displayed in scales: 0 – 1.414 (RA), 0 – 1 μm$^2$/ms (λ⊥), and 0 – 3 μm$^2$/ms (λ||). Each map has a ROI of normal (spared) white matter determined by applying the threshold of λ|| determined from the control. The volume of ROI decreased with increasing injury severity.

## Methods

Twelve 10-week-old C57BL/6 female mice underwent contusion spinal cord injury at the T9 vertebral level. The injury severity was introduced by changing impacting distance at 0, 0.6, 0.7, and 0.9 mm for control, mild, moderate, and severe injury (n = 3 for each group). *In vivo* DTI observation was performed immediately on a 4.7 T magnet using the previously described set up (1). A spin-echo diffusion-weighted sequence was modified to acquire images with respiratory gating. All images were obtained with acquisition parameters of TR 1.2 sec (gated acquisition), TE 38 msec, Δ 18 msec, δ 7 msec, slice thickness 0.75 mm, field-of-view 1.0 × 1.0 cm$^2$, data matrix 128 × 256 (zero filled to 256 × 256), total data acquisition time ~ 2.5 hrs, (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), and (1,0,-1), and b = 0 and 0.758 ms/μm$^2$. Image resolution was 78 × 39 × 750 μm$^3$. Perfusion fixation was performed immediately after DTI observation and tissue was harvested. Silver stain and immunohistochemical staining for neurofilament and myelin basic protein were performed on 3-μm thick tissues to evaluate axon and myelin integrity of the white matter.

## Results and Discussion

As previously reported (2), the diffusion anisotropy of white matter is largely preserved due to the decreased axial and radial diffusivities for all contusion injured cords resulting in no visible difference seen in the injured white matter (Fig. 1). However, the injured and normal appearing white matter tracts are clearly distinguished in λ|| maps. The normal appearing (spared) white matter was quantitatively segmented by applying a threshold calculated using the control white matter λ|| (Fig. 1). The delineated ROI correlated with silver (morphology of myelinated axon) and SMI-31 (phosphorylated neurofilament positive) staining results (Fig. 2). These results suggest that in vivo DTI derived λ|| can non-invasively assess spared white matter content in hyper-acute phase of SCI as expected from previous study (3).

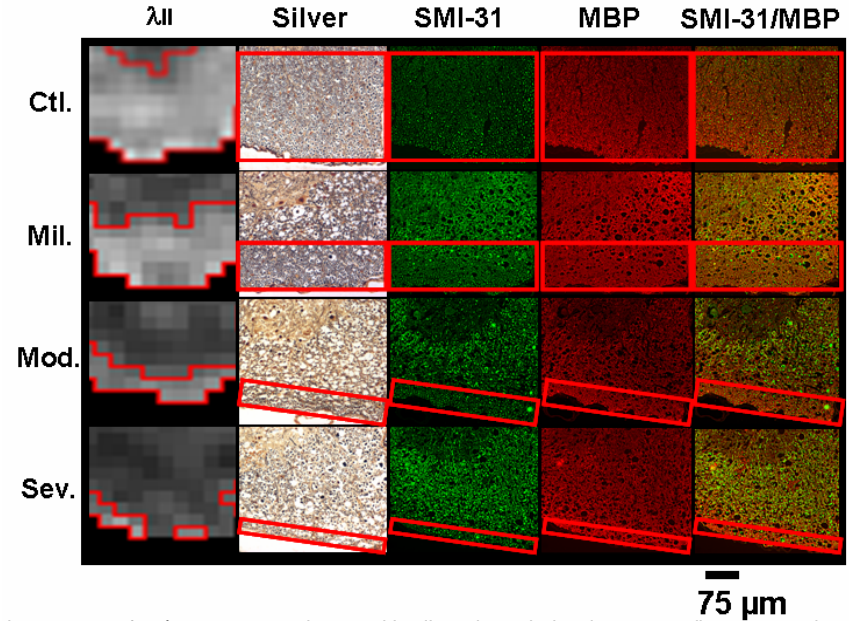


Figure 2. *In vivo* λ|| maps are shown with silver (morphology), SMI-31 (intact axons) and MBP (intact myelin) stain results. Both silver and SMI-31 showed decreased volume of normal appearing (spared) white matter as injury severity increases. For all injury severity, no apparent loss in MBP staining intensity is seen, a consistent result expected from the normal λ⊥.

**References:** 1. Kim *et al.,* Neurobiol. Dis., 2006. 2. Loy *et al.,*J. Neurotrauma, 2007. 3. Kim *et al.*, Magn. Reson. Med., 2007.
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