Introduction
Recent studies of contrast in white and gray matter (WM/GM) of the central nervous system (CNS) have provided evidence for theories predicting phase (and frequency shift) dependence on tissue architecture, in the cellular and subcellular components of tissue [2-4]. Combining the well-defined (anisotropic) architecture of the rat spinal cord dorsal column (DC) transection model of axotomy, we study areas of neuronal damage consistent with expected pathology of Wallerian and retrograde degeneration [5,6]. 3D frequency shift mapping of spinal cords bi-directional to the injury were generated from 3D multi-gradient echo (MGE) phase, and used to investigate progressive degeneration over several weeks.

Methods
Transection of the dorsal column was performed on anesthetized healthy Sprague-Dawley rats at the cervical (C6) level. Injured cords were excised at 3-weeks (n = 7) and 8-weeks (n = 8) post injury, and compared with healthy controls (n = 6). 3D MGE images were acquired on 20mm-long sections of ex-vivo paraformaldehyde-perfused cord (35μm × 35μm × 1000μm, TE = 4.2 + 3.7ms, Echoes = 6, TR = 35ms, FA = 20°, NA = 20, 1mm-thick slices). Phase images were unwrapped, homodyne filtered, and converted to frequency maps [1]. Region of interest (ROI) analysis of the fasciculus gracilis (FG, ascending) and corticospinal (CST, descending) tracts were evaluated in 1mm MRI slices around the injury site, for each time-point. Frequency maps of the first 4 echoes were averaged to increase overall SNR.

Results and Discussion
Significant damage to the DC at the injury site (0 mm) and immediately adjacent slices (± 1mm) due to mechanical injury as well as local effects (potential injury) are not Wallerian or retrograde, and thus not evaluated (Fig. 1, red and yellow highlights). Slices further away, up to ± 8 mm, are evaluated (Fig. 1). Frequency shift values of healthy FG and CST WM in Fig. 2 (black lines) are consistent in each tract across several animals and along the spinal cord, providing a pre-injury (control) baseline.

Wallerian degeneration occurs relatively quickly after axonal injury, starting with disintegration of most axons (3-4 days post injury) followed by myelin debris formation and removal over several weeks. This is reflected in the consistent frequency shifts measured along the path of Wallerian degeneration in the FG (1x10^-6 ppm decrease, +2mm to +8mm) and CST (1x10^-6 ppm increase, -2mm to -8mm) at 3-weeks post injury (Fig. 2 A, B, darker line, ‘Wallerian’). At 8-weeks, frequency shifts in both the FG and CST are significantly decreased compared to control and 3-week post injury values (Fig. 2 A, B, lighter line, ‘Wallerian’).

Retrograde degeneration is a relatively slower process compared to Wallerian degeneration, characterized by shrinking of axons (‘axonal die-back’), leaving some myelin debris in its wake. Frequency shifts measured along retrograde degeneration paths in the FG (-2mm to +8mm) and CST (+2mm to -8mm) are similar to baseline values at up to 3 mm from the injury site, then show increased frequency (around 2x10^-6 ppm) consistently for the remaining length, which persists at 8-weeks post injury (Fig. 2 A, B, ‘Retrograde’).

Both types degeneration are mediated by inflammatory agents (microglia, astrocytes, and macrophages) that have been known to proliferate at different rates in each tract [7]. The CST microstructure has a smaller inter-axonal distance compared with the FG, and may restrict diffusion of these agents. In turn, degeneration of myelin and its subsequent removal may occur at different rates in the FG compared to CST in the Wallerian degeneration case, leading to the observed difference in frequency shifts at 3-weeks post injury (decreases for FG, increases for CST). Due to the relatively slow progression of retrograde degeneration, proliferation of inflammatory agents is (most likely) consistent in both tracts, producing similar frequency shift patterns (Fig. 2 A, B, ‘Retrograde’).

Conclusion
Our results, (especially at ± 5 mm from the injury site), agree with previous studies that correlated MR of spinal cord injury with histology for myelin and axons [5, 6]. While a clear pattern is seen for frequency shifts in the retrograde degeneration of the FG and CST, the pattern is less distinct in the two tracts for Wallerian degeneration. However, these results provide characterization of both types of degeneration that are distinct and differentiable using frequency shift mapping. Furthermore, this study suggests that 3D GRE imaging of longer spinal cord segments can be adapted for in-vivo imaging in future studies to provide more significant insights into spinal cord injury.

References

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