

High Resolution Isotropic DTI of Human Intervertebral Disc Tissue

A. C. Wright¹, S. Pickup², D. Hornig¹, G. D. O'Connell³, and D. M. Elliott³

¹Laboratory for Structural NMR Imaging, Department of Radiology, University of Pennsylvania, Philadelphia, PA, United States, ²Small Animal Imaging Facility, Department of Radiology, University of Pennsylvania, Philadelphia, PA, United States, ³McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, PA, United States

Introduction

The objective of this work is to measure the micro-architecture of collagen fibers in the annulus fibrosus (AF) of the intervertebral disc (IVD) using diffusion tensor imaging (DTI) methods at high isotropic resolution. Within the AF of the IVD the structural organization of collagen fibers is believed to contribute to the distribution of mechanical forces. However, details of the three-dimensional (3D) structure, changes with degeneration, and reorientation behavior in response to mechanical loading have been difficult to measure. In principle, the non-destructive and non-perturbing nature of MRI is ideal for following changes in fiber microstructure under stress; yet due to low SNR the fibers are not easily resolved (1). Most previous MRI studies of AF microstructure until now have inferred fiber integrity from lower-resolution DTI or relaxation parameter maps (2-4). DTI more directly probes microstructure, as it measures the direction of water diffusion along the collagen fibers. We present here DTI data at 90 μm isotropic resolution, the highest done to date on disc tissue. The collagen fibers in our section from the anterior AF of a human lumbar IVD are directly resolved, supporting our hypothesis that fiber tracts determined via DTI correspond to actual fibers.

Methods

Specimen preparation: Human IVD tissue was acquired by an IRB approved protocol with informed consent. A cylindrical plug of AF tissue approximately 1 cm dia. was excised from the anterior of a human lumbar IVD (L5S1, age 76). The AF tissue section was placed in neutral buffer, then frozen in a vacuum sealed bag to maintain *in situ* hydration. Prior to the MRI experiment, the tissue section was thawed at room temperature for about 4 hrs, and placed in a Delrin specimen holder filled with perfluorinated oil to minimize magnetic susceptibility differences near the specimen boundary. The specimen holder then was mounted in the RF resonator.

Data acquisition: Hardware: DTI data were acquired on a high-field (9.4 T) vertical-bore MRI microimaging system (Varian, Palo Alto, CA), equipped with 45 mm i.d., 100 G/cm tri-axial gradients and a custom-made 22 mm i.d. loop-gap RF resonator for high SNR. **Pulse sequence:** All images were generated with a 3D multi-spin echo imaging pulse sequence employing bipolar diffusion weighting gradients in the first echo time and variable direction and amplitude crusher gradients around each refocusing pulse. Imaging parameters: TR=1s, ETL=6, echo spacing=12 ms, voxel size=90x90x90 μm^3 , 6 diffusion directions, $\delta\Delta = 9/10$ ms, 2 averages, scan time=17 hrs.

Data analysis: Initial data analysis was performed in the IDL programming environment with custom written code. Raw data were transformed into magnitude images and the multiple echoes summed to improve SNR. The apparent diffusion coefficient (ADC), and diffusion tensor eigenvectors and eigenvalues were calculated using the free software DTI-Studio (5). Calculation of fiber angles was performed with IDL scripts and also with the free software ImageJ (NIH). The Fiber Assignment by Continuous Tracking (FACT) algorithm of DTI-Studio was used to estimate the tracts of collagen fibers. Fiber angles obtained from DTI data were compared with those of directly resolved fibers.

Results

A single MRI slice, orthogonal to the spinal axis, shows the lamellae as bands of different intensity (Fig. 1a), but does not resolve individual fibers. However, the principle eigenvector of water diffusion (Fig. 1b and 1c), shows an approximate $\pm 60^\circ$ variation in adjacent MRI data were re-sliced along planes revealing the fibers (spinal axis \sim vertical with the MR image, where color refers to the principle diffusion eigenvector (Fig. 2b). A histogram of angles in this slice shows two groups: $+60^\circ \pm 24^\circ$ and $70^\circ \pm 12^\circ$ (Fig. 2c). Further agreement between the MR images and calculations from the DTI data is supported qualitatively by fiber tracts obtained from the FACT algorithm, shown penetrating the lamellae. The 3D isotropic diffusion-weighted parallel to the lamellae (Fig. 2a), directly

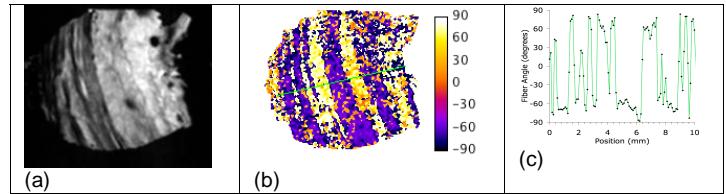


Fig. 1 (a) MRI slice of a human AF section. (b) DTI data from the same slice, showing the angle (color in degrees) of the principle diffusion eigenvector from an axis normal to the image plane. (c) Line profile drawn across the DTI data (green line in (b)).

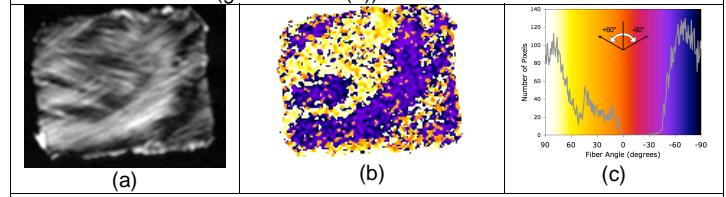


Fig. 2 (a) Reformatted slice from MRI data set, orthogonal to image in Fig. 1a. (b) Same slice from DTI data, showing principle diffusion eigenvector angle from image vertical. (c) Histogram of angles in (b).

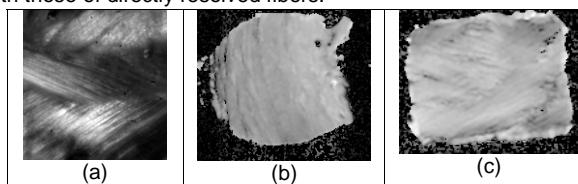


Fig. 4 (a) Optical microscopy image of AF fibers (different specimen). (b, c) Reformatted slices from the 3D ADC map, corresponding to Figs. 1a and 2a.

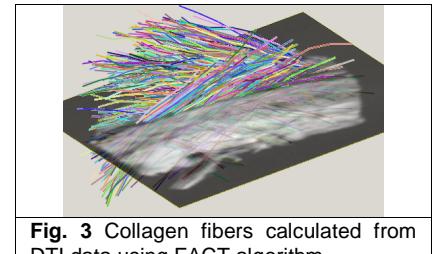


Fig. 3 Collagen fibers calculated from DTI data using FACT algorithm.

parallel to the lamellae (Fig. 2a), directly

vertical (Fig. 2b). The re-sliced DTI data corresponds to the angle from image vertical of the principle diffusion eigenvector (Fig. 2c). Further agreement between the MR images and calculations from the DTI data is supported qualitatively by fiber tracts obtained from the FACT algorithm, shown penetrating the lamellae. The 3D isotropic diffusion-weighted parallel to the lamellae (Fig. 2a), directly

Discussion

These DTI data represent the highest resolution yet reported for AF tissue. By directly resolving the fiber bundles, the data support the hypothesis that the principle eigenvector of the diffusion tensor points along the direction of the collagen fibers. Furthermore, the collagen fiber angles measured here are comparable to optical microscope results in the literature (angle = 45-62°) (6) (see Fig. 4a). The source of fiber contrast is unclear, but possibly due to a combination of local differences in water concentration, relaxation rates, magnetic susceptibility, and diffusion. The latter however seems small when ADC maps are examined (Fig. 4b). Limitations of this DTI technique include a long scan time and the use of an AF section rather than a whole IVD. A next step will be to incorporate tension or compression into the DTI experiment.

Acknowledgements

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