

# Validation of a clinical protocol for DTI of articular cartilage in whole knee specimen using a new method for one-to-one correlation of DTI with histology

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**Introduction:** Diffusion tensor imaging (DTI) of the articular cartilage has demonstrated high accuracy to detect (95%) and classify (75%) early cartilage damage as assessed by histology in ex vivo experiments at high field MRI (17.6 T) with high spatial resolution and high signal to noise ratios (SNR).<sup>1</sup> Recently, in vivo DTI of the articular cartilage has demonstrated high accuracy (90%) for the early diagnosis of osteoarthritis (OA).<sup>2</sup> In vivo DTI of articular cartilage is now performed at 7 T with low resolution (0.6 mm in plane) and lower SNR. Therefore, DTI data acquired with a clinical scanner needs further histologic validation in conditions mimicking the in vivo acquisition. The aim of this work is to introduce a new method for one to one correlation of DTI with histology on whole knee specimens measured with the same conditions of in vivo acquisition.

**Methods:** The right knee of a 50 year-old female donor was imaged in a 7-T scanner (Siemens Healthcare) with a dedicated birdcage transmit, 28-channel receive knee coil (QED). MRI protocol included a 3D fat-saturated T1-weighted gradient-echo (GRE)-sequence for cartilage morphology and segmentation (TE/TR = 6.12/38 ms, matrix = 654x512, flip angle = 20°, in-plane resolution = 0.25x0.25 mm<sup>2</sup>, slice thickness = 1 mm, bandwidth = 500 Hz/Pixel, slices = 96 (sagittal)/54 (axial), parallel imaging acceleration factor=3, acquisition time = 10:40 min (sagittal)/5:41 min (axial)) and a line-scan diffusion tensor imaging (LSDTI) sequence with same parameters as used in vivo (TE/TR/TR<sub>eff</sub> = 46/180/2890 ms, matrix = 256x128, rotation angle ( $\alpha$ ) = 20°, resolution=0.6x0.6 mm<sup>2</sup>, slice thickness = 2 mm, *b*-values = 0, 450 s/mm<sup>2</sup>, 6 directions, bandwidth = 500 Hz/Pixel, slices = 25 (sagittal)/10 (axial), acquisition time = 1:50 h).<sup>2</sup>

After MRI, the knee specimens were prepared for histology. The patella, tibia and femur were extracted from the knee specimen. Cartilage plates were divided into 42 blocks (Fig. 1). The border lines between the blocks were drawn onto the cartilage surface and photographed for subsequent identification of the blocks on a 3D model reconstructed from MRI images. Each block underwent histology with safranin-O for assessment of the proteoglycan distribution and with picrosirius red to depict the architecture of the collagen matrix with polarized light microscopy (PLM). Safranin-O slices were scanned with a Miramax-Midi microscope (Carl-Zeiss) and assembled together for comparison with the MRI slices. The degree of cartilage damage was assessed on the safranin-O slides using the OARSI score (range from 0=healthy to 6=bone remodeling) by two readers in consensus.<sup>3</sup> Picrosirius red slices were scanned with the Metripol microscope for PLM and assembled together for comparison with MRI.<sup>4</sup>

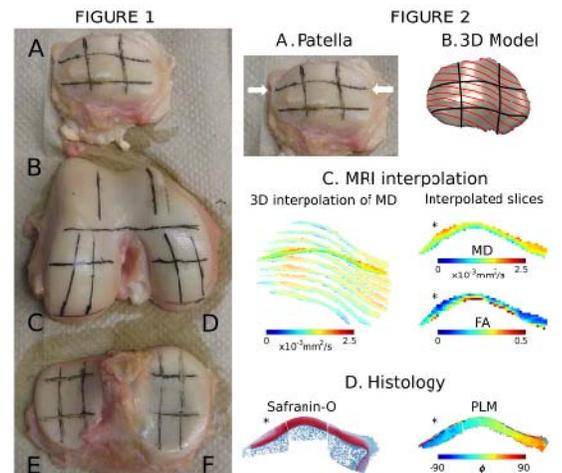
For correlation of MRI with histology, the segmentation of the T1-w GRE images was used to generate a 3D model of each cartilage plate using the Poisson Surface Reconstruction algorithm (Fig. 2B).<sup>5</sup> On the 3D model, the positions of the MRI slices will be known because GRE and LSDTI images were acquired with the same image orientation. Border lines were traced over the articular surface of the 3D model to provide the same block division as that of the articular cartilage (Fig. 2A, B). Trilinear interpolation of the parameter maps to the position of the border lines allowed a one-to-one comparison of MRI parameter maps and histology (Fig. 2C, D). A block mean MD and FA were calculated. One-way ANOVA tests were performed to test the difference in diffusion parameters between cartilage plates, between the diffusion parameters in the deep and superficial regions of the blocks and between OARSI groups. A trend of diffusion parameters with the OARSI score was assessed with the Cuzick test.

**Results:** Figure 3 shows examples of the MD and FA parameter maps. There were no differences either in MD ( $P=0.53$ ) or in FA ( $P=0.66$ ) among the cartilage plates (mean MD:  $(1.06 \pm 0.18) \times 10^{-3}$  mm<sup>2</sup>/s; mean FA:  $(0.23 \pm 0.06)$ ), but a significant ( $P < 0.00005$ ) difference between the superficial and deep portions of the blocks both in MD (superficial:  $(1.15 \pm 0.19) \times 10^{-3}$  mm<sup>2</sup>/s; deep:  $(0.95 \pm 0.20) \times 10^{-3}$  mm<sup>2</sup>/s) and FA (superficial:  $(0.19 \pm 0.06)$ ; deep:  $(0.27 \pm 0.08)$ ). Histology (OARSI) scoring of blocks demonstrated a low grade of cartilage ( $0.55 \pm 0.88$ ) damage in almost every cartilage plate: Patella (n=3 OARSI 0, n=3 OARSI 1; n=2 OARSI 2 and n=1 OARSI=3), femoral trochlea (n=1 OARSI 0, n=2 OARSI 1), medial femoral condyle (n=5 OARSI 0; n=1 OARSI 1), lateral femoral condyle (n=4 OARSI 0, n=1 OARSI 1, n=1 OARSI 4), tibia medial (n=5 OARSI 0; n=4 OARSI 1) and tibia lateral (n=8 OARSI 0; n=1 OARSI 1). There was no statistical difference between the samples pooled according their OARSI scores in MD ( $P=0.16$ ) or FA ( $P=0.94$ ). However, there was a significant ( $P < 0.05$ ) trend of increasing MD with the OARSI score (Fig. 3). Figure 2C shows how in the patella of the correlation between regions with increased MD and/or reduced FA with areas showing abnormality in safranin-O and/or PLM (Fig. 2).

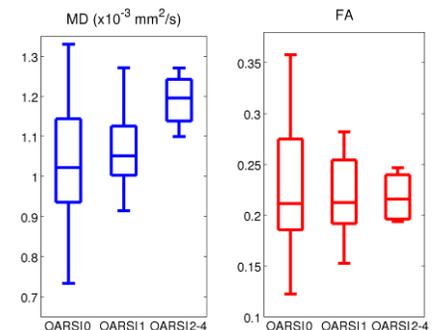
**Discussion:** The use of a whole knee specimen is essential to provide accurate validation of DTI in equal conditions as the in vivo acquisition. Validation with whole knee specimens accounts for the presence of partial volume effects with surrounding tissue, uses the same experimental set-up (coil, coil loading...) as in vivo with the same pulse sequences. Careful 3D reconstruction and orientation of the cartilage plate is critical to guarantee an accurate correlation with histology. Our data indicate potential of DTI to detect damaged cartilage with OARSI grade 2 or higher. However, in spite that only 4 samples had OARSI  $\geq 2$  we could see a significant trend of increased MD with OARSI.

**Conclusion:** We introduced a method for one-to-one correlation of MRI data with histology using whole knee specimens and showed the potential of DTI to detect early cartilage damage (OARSI 2).

**References:** [1] Raya et al. Radiology (in press). [2] Raya et al. Radiology 2012;262:550. [3] Pritzker KP, et al. Osteoarthritis Cartilage. 2006;14:13. [4] Kaminsky W, et al. Chem Soc Rev. 2004;33:514. [5] Kazhdan M et al. Eurographics Symposium on Geometry Processing 2006; Sardinia, Italy.



**Figure 1:** Block definition on all cartilage plates: patella (A), the femoral trochlea (B), the lateral (C) and medial (D) femoral condyles, and lateral (E) and medial (F) tibia. **Figure 2:** Correlation of DTI with histology. (A) Patellar cartilage with arrows indicating the position of histology. (B) 3D model with the position of the DTI images (red lines) and the border lines (black lines). (C) Interpolation of the MD maps to the position of histology (D) Safranin-O and PLM histology sections. Asterisk (\*) indicates an area of PG loss (OARSI = 2) and altered collagen structure.



**Figure 3:** Box plots showing the change of MD and FA with the OARSI score.