Diffusion Tensor Imaging of Fresh and Formalin Fixed Porcine Hearts: A Comparison Study of Fiber Tracts

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Targeted Audience: Biomedical engineers, researchers and biomechanical engineers.

Background: In biological tissues diffusion of water molecules is anisotropic in nature since they are restricted by the tissue structure. Diffusion tensor imaging (DTI) quantifies the amount of anisotropic diffusion exhibited by water molecules within a tissue [1-2]. Processing DTI images facilitate non-invasive characterization and visualization of fiber architecture and fiber trajectories within the region of interest [3]. The purpose of this study is to qualitatively analyze the effect on fiber trajectories of pig hearts pre and post formaldehyde fixation based on its fractional anisotropic (FA) values.

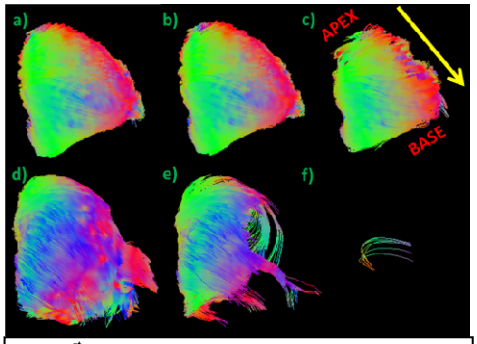

Fig. 1: 1st Row: Fibers tracked across the entire LVM of the fresh heart. **2nd Row:** Fibers tracked across the entire LVM of the formalin fixed heart. Tracking parameters, **1st Column:** FA [0.1, 1]; **2nd Column:** FA [0.2, 1]; **3rd Column:** FA [0.3, 1]; The yellow arrow shows the direction from apex to base of the myocardium.

Methods: Ex-vivo DTI was performed on two porcine hearts before and after formalin fixation on a 3T MRI scanner (Tim Trio, Siemens Healthcare, Erlangen, Germany). After the animals were euthanized, their hearts were dissected and stored in ice for 5 hours. A balloon was inserted into the left ventricle (LV) to inflate the hearts. A diffusion-weighted (DW) single shot spin-echo (SE) echo planar imaging (EPI) sequence was used to acquire multi-slice short axis views of the heart covering the entire LV. Post scanning, the hearts were fixed using 10% neutral buffered formalin solution and stored at room temperature for 10 days and re-scanned using the same imaging parameters. Imaging parameters included: diffusion encoding directions=256; TE=90ms; TR=7000, 8000ms; slice thickness=2mm; matrix=128x128; FOV=256x256mm^2; b-values=0,1000s/mm^2; slices=42, 45 (depending on the size of the heart); acquisition voxel=2x2x2mm^3. All diffusion tensor images were masked to obtain the LV myocardium and eddy current induced artifacts were corrected using FSL (FMRIB, Software Library). Explore DTI (ISI, The Netherlands) was used to obtain a tensor map and then track the fibers across the entire volume of the LV myocardium (LVM), using a deterministic algorithm [4]. For fiber tracking, following tracking constraints were used: maximum turning angle: 45 degrees; fiber length range: 50mm to 500mm. The lower limit of the FA value was varied in both the hearts to evaluate the effect of formalin fixation on fiber tracking associated with diffusivity. Three ranges of FA value were considered, [0.1, 1], [0.2, 1] and [0.3, 1]. Next we defined a small region of interest (ROI) on both the hearts and tracked fibers passing through the defined ROI.

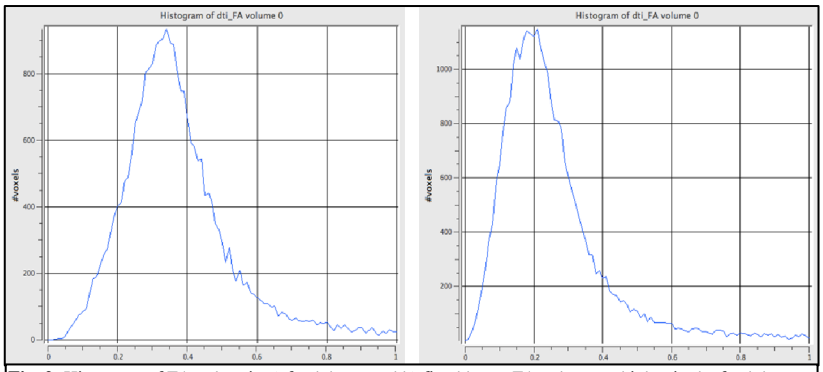

Fig. 2: Histogram of FA values in a) fresh heart and b) fixed heart. FA values are higher in the fresh heart.

Results: Figure 1 compares the tracts across the entire LVM for a fresh (Fig 1 a, b, c) and formalin fixed (Fig 1 d, e, f) heart. Each column displays fiber tracts for a fixed FA range (1st column [0.1, 1], 2nd column [0.2, 1] and third column [0.3, 1]). As the lower limit of FA increased, less number of fibers were tracked in the fixed heart when compared to the fresh heart. Furthermore, only a fraction of fibers were tracked with FA range [0.3, 1] in the fixed heart [Fig. 1. f]. Figure 2 displays the histogram of FA values in a fixed and fresh heart. It is evident from the plot that the FA values in a fresh heart are higher compared to a fixed heart. Figure 3 shows fiber tracts in both the fresh and fixed heart through a defined ROI. In Fig 3(a, b) we noticed that in FA range [0.2, 1] the tracts in the fresh heart are smoother than the ones in the fixed heart.

Discussion: Our study demonstrated that formalin fixation made the diffusion pattern of the cardiac muscle fibers more isotropic. A one-to-one mapping of the fiber tracts between the fresh and formalin fixed heart (Fig 1) was not feasible because they were orientated differently inside the scanner. Instead, this study showed that the volume of fiber tracts changed with formalin fixation, in spite of keeping the tracking parameters exactly the same for pre and post fixation. With respect to the amount of fibers tracked through a defined ROI (Fig 3) we can observe that the fibers tracked in the fresh heart with FA values [0.2, 1] is close to the amount of fibers tracked in the formalin fixed heart with FA values [0.1, 1].

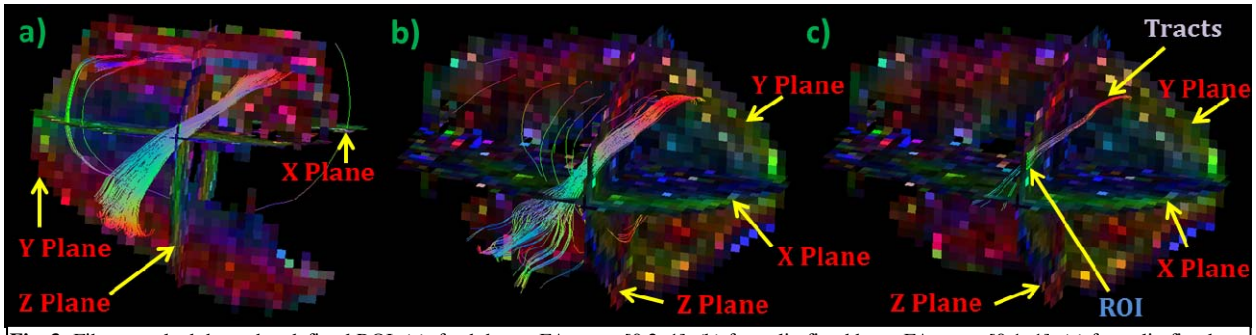

Fig. 3: Fibers tracked through a defined ROI. (a) fresh heart, FA range [0.2, 1], (b) formalin fixed heart FA range [0.1, 1] (c) formalin fixed

References:

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