## Potential of Diffusion Tensor Imaging as a Virtual Dissection Tool for Cardiac Muscle Bundles: A Pilot Study

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Introduction: Diffusion-based tractography has the potential to delineate complex cardiac muscle fiber architecture [1]. Although many cardiac diffusion tensor imaging (DTI) studies have been conducted, segmentation of cardiac muscle bundles has been rarely explored. The ultimate goal of such bundle-based segmentation would be to reveal the organization of the cardiac muscle fiber bundle architecture. As an initial step to achieve the goal, we performed a DTI study on a fixed porcine heart specimen. The study was carried out in two phases on the left ventricle (LV) of the fixed specimen. In the first phase, we explored the dependency of tractography on three fiber tracking constraints: fractional anisotropy (FA) threshold, maximum turning angle, and minimum fiber length. In the second phase, we performed segmentation of long muscle bundles (> 50 mm) using deterministic tractography and evaluated the feasibility of the muscle bundle segmentation in the given specimen. Materials and Methods: A porcine heart fixed in 10% neutral buffered formalin and stored at room temperature for 6 months was used for the study. DTI data was acquired using a single shot spin-echo echo planar imaging (EPI) sequence with following parameters: TR/TE = 7000/90 ms, 256 diffusion weighting directions (b = of  $1000 \text{ s/mm}^2$ ) and a minimally diffusion-weighted image (b = 0 s/mm^2),  $128 \times 128$  acquisition matrix, 37 slices with thickness of 2 mm, isotropic voxel of  $2 \times 2 \times 2$  mm<sup>3</sup>. Acquired DTI data underwent eddy-current correction using FSL (FMRIB Software Library, Oxford, UK) to remove EPI related distortions. DTIFIT (included in FSL) was used to generate FA, eigenvalues, eigenvectors, and other diffusion tensor derivatives. The right ventricle was masked out from the images to facilitate evaluation of the LV. Phase 1: Multiple FA ranges with interval of 0.1 was explored to investigate the distribution of FA values across the specimen using ROI editor. Two other tracking constraints (maximum turning angle and minimum fiber length) were also studied to determine their influence on the tractography results. Phase 2: Muscle segmentation was performed using ExploreDTI (ISI, The Netherlands) based on the information from phase 1. First, large and long bundles were selected from the tracking results obtained with seeding FA of 0.2. Minimum fiber length was limited to 50 mm to make this pilot study simple. Next, extraction of the bundle of interest was performed applying the same ROI to the tractography result with seeding FA of 0.1. Phase 2 was repeated for other bundles of interest. Z-component of principal eigenvectors was used for segmenting inner/outer layers (subendo/subepicardium).

**<u>Results:</u>** Most FA values were < 0.5 and were most frequently observed in the range of [0.2, 0.3]. [Figure 1. a - f] Tractography results with seeding FA values of 0.1 and 0.2 showed drastic difference in the number of fibers tracked.[Figure 1. g, h] However, when the minimum fiber length was set to 10 mm, we could observe more shorter fibers being tracked in unfilled areas when compared to the minimum fiber length set to 50 mm.[Figure 1. i] We have also applied a few maximum turning angles to tract the fibers; however, the difference was subtle compared to the result obtained with varying seeding FA value or fiber length. Based on this, seeding FA value of 0.2 was selected to perform initial tractography while other tractography constraints were kept constant (i.e. maximum turning angle = 30 degrees, minimum fiber length = 50 mm). After the initial tractography, to fill untracked area with more muscle fibers, seeding FA value of 0.1 was applied with the same ROIs [Figure 2]. Figure 3 shows segmentation of three long muscle bundles with subendo and subepicardium were segmented. [Figure 3]

**Discussion:** We explored FA distribution, maximum turning angle, and minimum fiber length threshold dependency of tractography in a fixed heart specimen. Among three tracking constraints, seeding FA value and minimum fiber length threshold noticeably affected the tractography results. Maximum turning angle did not significantly affect the muscles in the mid-wall, while more tracked voxels were observed in the subendocardium with increased turning angle. Z-component of the principal eigenvector turned out to be useful to segment inner-/outer-layers, while a long distance tractography underperformed in these areas. Actual FA values might

a. FA[0.01, 0.1] b. FA[0.1, 0.2] c. FA[0.2, 0.3] d. FA[0.3, 0.4] e. FA[0.4, 0.5]

 f. FA[0.1, 0.4]
 g. FA[0.2, 1.0],
 h. FA[0.1, 1.0],
 i. FA[0.1, 1.0],

 For Most voxels
 min. fiber length = 50 mm
 min. fiber length = 50 mm
 min. fiber length = 10 mm



Figure 1. FA distribution and tractography examples by varying tracking constraints



Figure 2. Demonstration of Segmentation Steps.

be different from the fresh specimen or in vivo heart depending on postmortem interval (PMI) and fixation process details. It has been reported that fixation does not change fiber orientation [2]. Hence tractography result will not be affected by the fixation. Similar approach (reported here) can be easily extended to muscles of other anatomy.

References: 1. Sosnovik et al. Circulation. 2012. 2. Holmes et al. MRM. 2000.



Figure 3. Segmented muscle bundles and subepi/subendocardium