## Reconstruction of 3-D Fabric Structure and Fiber Nets in Skeletal Muscle via In Vivo DTI

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## Introduction

Microscopic examination of skeletal muscle has revealed a complex architecture with the muscle fibers tethered transversely by crisscrossing perimysium fibers [1] and this provides the structural basis for the transmission of passive stress perpendicular to the myofiber fiber direction and of the active stress along it [2]. On the other hand, the established asymmetry of the diffusion tensor in the transverse direction (unequal secondary and tertiary eigenvalues) hints of the presence of gross muscle asymmetry in that direction [3]. DTI MRI has been employed successfully to reconstruct the fiber tracts in isolated human calf [4] and thigh muscles [5], and to elucidate additional structure, such as "fabric" in axial slices of the human calf [6] and "sheets" in the whole myocardium [7]. By considering multiple slices and reconstructing the primary and secondary eigenspaces, we pursue here the reconstruction of the muscle fabric inspired by the putative bidirectional force transmission in isolated skeletal muscles of the calf and thigh.

## **Methods and Results**

<u>Image Acquisition</u>: The subjects were placed supine with feet first into the scanner, and with legs relaxed and placed parallel to the magnetic field. Diffusion tensor imaging (DTI) data was collected on a 3T full-body Siemens Trio scanner (Siemens Medical Systems, Erlangen, Germany) using a combination of an eight-channel spine coil and a flexible body matrix surface coil. Diffusion-weighted images were acquired using a single-shot twice-refocused spin-echo EPI sequence with the following parameters: TR/TE = 3000/71 ms, FOV = 25x25 cm<sup>2</sup>, slice thickness = 10 mm, matrix = 76x76, and N<sub>ex</sub> = 10. Diffusion weighted gradients were applied along 30 non-collinear directions with a nominal b-value of 550 s/mm<sup>2</sup>. Water excitation was performed using a spatial-spectral RF pulse and seven axial slices were acquired near the midsection of the left thigh and calf.

<u>*Tractography*</u>: Fiber tracking of the primary and secondary eigenvectors was performed on the vastus medialis of each subject with *TrackVis* software [8] using the interpolated streamline algorithm with 0.5 mm step size [9]. One random seed per voxel was used and the stop criterion was an orientation change between points >20 degrees for both the primary and secondary tracts. Tracts with lengths less than 20mm were removed and a B-Spline filter was used to smooth the tracts. Examples of superimposed primary and secondary tracts in two muscles of the calf and thigh are given in Figures 1 and 2. The network forms a fabric which is consistent with the orientation of the myofibers relative to the aponeuroses.

<u>Data Processing</u>: The tracts for both the primary and secondary eigenvectors were exported to MATLAB® (The Mathworks, Inc., Natick, MA) to be further analyzed. Nets were constructed through an algorithm that first identifies nodes of crossing primary and secondary tracts, and the net is grown through the addition of nodes that share common tracts. The algorithm then finds defect-free rectangular nets and counts their nodes (i.e. a 4x6 net has 24 nodes). Figure 3 shows an example of how the nets are identified and counted.

## **Discussion and Conclusion**

Each tract in Figures 1 and 2 is colored according to anatomical orientation; blue is superior/inferior, red is left/right, and green is posterior/anterior. While the primary tracts represent the direction of muscle fibers, the secondary tracts should not be confused with a physical fiber. The myofibers and interfiber matrix adapt in response to muscle strain both in the axial and transverse direction, and this adaptation gives rise to structures that restrict diffusion preferentially. The secondary tracts establish a fabric organization of the muscle, whereby the interfiber matrix acts as a transverse yarn (weft) woven through the myofibers (warp). Counting the nets and/or their nodes provides a quantitative method of characterizing the organization of the secondary structure. Such metrics can potentially be linked to muscle quality, since it is plausible to expect that higher quality muscle has a more ordered fabric in order to deliver better bidirectional force transmission. To test the robustness of the fabric, the statistical variation of the above two metrics obtained by repeating the net reconstruction ten times for three subjects differing in fitness level is given in Table 1. Although the mean values vary significantly between the subjects, the standard deviation (which expresses the repeatability of the fabric reconstruction) remains relatively small. In conclusion, evidence as been given here about the existence of a skeletal muscle fabric by constructing it and formulating topological metrics to quantify its degree of organization (muscle quality).

**References:** [1] Passerieux, E, et al., *J Struct Biol*, 2006. 154:206-216; [2] Purslow, PP, *Comp Biochem Physiol*, 2002. A133:947-966; [3] Karampinos, DC, et al., *Ann Biomed Eng*, 2009. 37:2532-46; [4] Heemskerk, AM, et al., *Magn Reson Med*, 2009. 61:467-472; [5] Kermarrec, E, et al., *Am J Roentgenol*. 2010. 195:352-356; [6] Chen, D, et al., *ISMRM*, 2010. no. 3242; [7] Tseng WY et al., J Magn Reson Imag 17:31-42, 2003; [8] Wang, R, et al., *Proc. ISMRM*, 2007. no. 3720; [9] Conturo, T, et al., *P Natl Acad Sci Usa*, 1999. 96: p. 10422-27.



**Figures:** (1) Tracking of primary and secondary eigenvectors of gastrocnemius medialis and lateralis. (2) Tracking of primary and secondary eigenvectors in vastus lateralis. (3) Crossing of primary and secondary tracts in the gastrocnemius lateralis and an example showing 20-node (closed circles) and 8-node (open circles) nets.

	Subject 1 (mean ± stdev)	Subject 2 (mean ± stdev)	Subject 3 (mean ± stdev)
Net Count	116.7 ± 6.8	140.1 ± 10.3	$65.3\pm6.9$
Mean Net Size	$10.6\pm0.6$	$10.9\pm0.7$	$8.2\pm0.2$