Skeleton Thickness Biases Statistical Power in Skeleton-Based Analyses of Diffusion MRI Data

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INTRODUCTION: Diffusion tensor imaging is a powerful tool for probing tissue microstructural organization and orientation. A key feature is that it naturally provides *rotationally invariant* information[1]. To this end, significant effort has focused on optimizing DTI acquisitions to ensure that the data are *statistically rotationally invariant* [2,3], so that the orientation of a fibre population within the brain has minimal impact on the quality of quantitative results and statistical inferences. Against this backdrop, we report on an observation that will be of interest to those developing skeletonization-based statistical methods for group comparisons of DTI data (such as TBSS[4]). Without due care and attention to their implementation, we show that such methods can *reintroduce* a rotational dependence. Specifically, we demonstrate that the power to detect group differences can depend on the orientation of the head in the scanner or on the orientation of a fibre within the head. While the root cause and solution to this problem is trivial, the effect on statistical inference is not – and should be set in the context that skeletonization-based methods are clearly becoming increasingly popular (e.g., TBSS alone has 144 citations on ISI Web of Knowledge in 2006-2009).

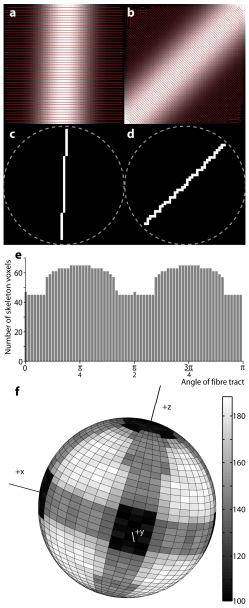

Figure 1: Skeletonization

Overview of Skeletonization Based Methods:

1. Anisotropy data (usually fractional anisotropy, FA) undergo a non-linear co-registration and spatial interpolation;
2. The mean FA image (averaged across the co-registered FA images) is searched in a tract-perpendicular direction to locate a one-voxel thick skeleton of local FA maxima;
3. Each point in the skeleton is populated by a vector of values from the individual FA images;
4. Statistical tests are then applied on the skeletonised data (e.g. [5])

METHODS: We illustrate the problem using both phantom and real data.

Phantom FA Data To illustrate the rotational variation inherent in skeletonization, a 2D Gaussian fibre was simulated in MATLAB (The Mathworks, Natick, MA) for 72 discrete orientations ranging from $0 \le \theta < \pi$ (e.g. Fig 1a,b). Each of these fibres was then skeletonised in 2D, searching for the maximum along quantised tract-perpendicular directions (Fig 1c, d). Similarly, a 3D FA sheet was simulated for all combinations of 43 angles of $0 \le \theta \le \pi$ and 43 angles of $-\pi \le \phi \le \pi$. Each individual dataset was then multiplied by a spherical mask and subjected to a 3D skeletonization.

Experimental Data: DTI data were acquired from twenty healthy subjects (15m/ 5f; 33 +/-6.2 yrs), on a GE 3T HDx system, using a peripherally gated twice-refocussed EPI sequence with 6 b=0 images and 60 isotropically distributed gradients[16]. TE 87 ms/ TR ~20s; isotropic resolution 2.4mm; matrix 96x96; 60 slices; ASSET factor = 2;

Manipulation of Experimental Data: Nonlinear regression was used to fit a single tensor to the DW-data in each voxel. Using FLIRT, all FA maps were rotated either by $+\pi/8$ or $-\pi/8$ in the axial plane, to produce 2 complete datasets (n = 20 in each), rotated by $\pi/4$ relative to each other, and with similar smoothing. These data sets were then skeletonised using TBSS[4] (Fig 2a). An FA offset of +0.25 was applied to an ROI placed in the right internal capsule (IC) and optic radiation (OR) in the first ten subjects of each data set. Each of the two 'new' datasets was then statistically analysed to determine group differences between the first ten and second ten subjects by randomisation testing with threshold-free cluster enhancement (TFCE). If the statistical analysis were rotationally invariant, we would expect the results to be almost identical for the two datasets.

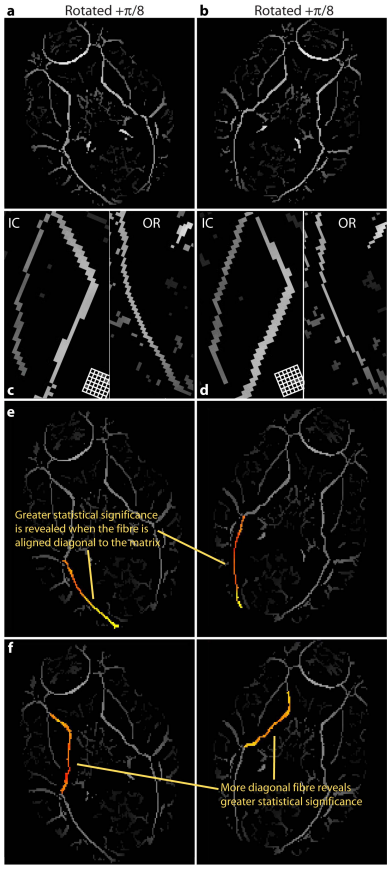

Figure 2: TBSS analysis

RESULTS: *Simulated FA Data:* Due to the geometric interaction of skeletonization processes with the imaging matrix, the number of voxels on a skeleton will clearly vary as a function of orientation for both 2D and 3D simulated fibres. In 2D, there is a $\sim\sqrt{2}$ increase in skeleton thickness for fibres diagonal to the imaging matrix over those parallel (Fig 1e). Fig 1f shows the number of voxels in the 3D skeleton for each fibre orientation, plotted on the surface of a sphere. There is a $\sim\sqrt{2}$ increase in skeleton thickness for fibres diagonal to the imaging matrix, and $\sim\sqrt{3}$ increase in skeleton thickness for fibres doubly diagonal, over those parallel.

Manipulated Data Fig 2c-2f show the important consequence of this seemingly innocuous geometrical property - the statistical significance of the FA offset, determined by the skeletonization-statistical testing pipeline, is dependent on the orientation of the tract. For example, the significance of the offset in the OR is higher (the tracts appear more yellow) when it runs diagonally than when running vertically.

DISCUSSION: Skeletonization of a tract, reducing it to a 'one-voxel thick' sheet, is unavoidably rotationally variant. For example, Fig 1c, d and Fig 2c, d show that the skeleton consists of more voxels in the diagonal case. This rotational variance is amplified when statistical analysis methods, such as TFCE, which recruit statistical support from adjacent voxels, are applied. Diagonally oriented fibres have more neighbours on the skeleton, from which support can be recruited and changes are therefore more likely to be judged as significant. Thus, orientation influences skeletal thickness, and thickness biases statistical sensitivity. While some developers have already proposed software patches in response to our observations (e.g. TBSS has recently been modified), it is important to highlight the on-going effort to ensure the rigour of DTI analysis tools - especially considering the large number of studies previously affected.

References: 1. Basser PJ *et al.* Biophys J 1994; 66:259-; 2. Batchelor PG *et al.* Magn Reson Med 2003; 49:1143-; 3. Jones DK. Magn Reson Med 2004; 51:807-; 4. Smith SM et al. NeuroImage 2006; 31:1487-; 5. Smith SM and Nichols TE. NeuroImage 2009; 44:83-;