

# Voxelwise Analysis of FA Data: Session and Subject Variability, Gaussianity and Dependence on Acquisition

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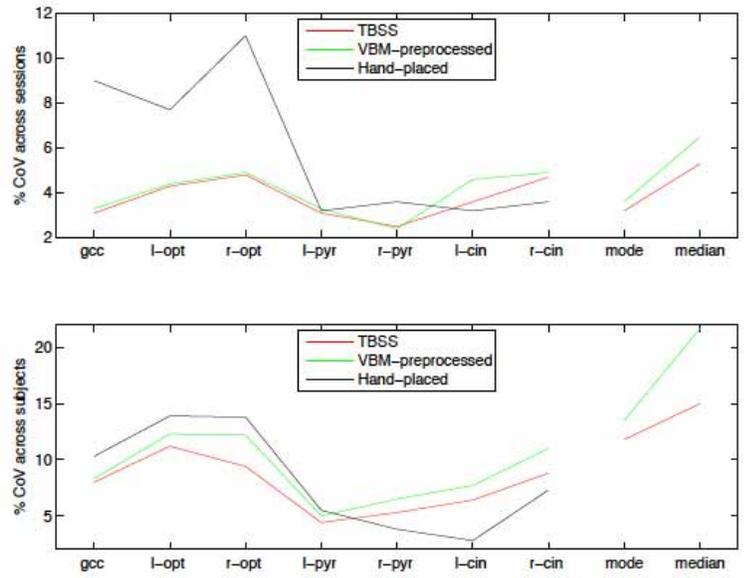
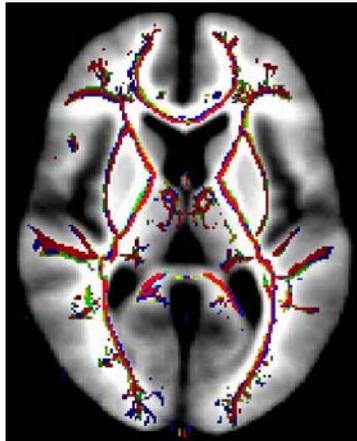
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**INTRODUCTION** Many imaging studies are starting to use FA images in voxelwise (“VBM-style”) statistical analyses, in order to localise brain changes related to development, degeneration and disease. We have recently proposed (ISMRM06 & NeuroImage, in submission) an approach to voxelwise analysis of FA data that attempts to solve major problems relating to alignment issues and spatial smoothing extent. We refer to our new approach as Tract-Based Spatial Statistics (TBSS). TBSS pre-aligns all subjects’ FA data to a common FA image, creates a mean-FA-based white-matter skeleton image, and then projects each subject’s FA image onto this skeleton, before carrying out cross-subject voxelwise statistical analysis. TBSS aims to improve the sensitivity, objectivity and interpretability of analysis of multi-subject diffusion imaging studies. Here we investigate: a) the cross-subject Gaussianity of FA data after pure nonlinear registration compared with TBSS preprocessing; b) the cross-session and cross-subject variability in FA when pre-processed by pure nonlinear registration, TBSS and by hand; and c) the dependence of cross-subject FA variability on different DTI acquisitions - 6 vs. 60 directions and standard imaging vs. generalized autocalibrating partially parallel acquisitions (GRAPPA).

**OVERVIEW OF TBSS** 1) Identify a common registration target and align all subjects’ FA images to this target using nonlinear registration. At this stage, perfect alignment is not expected or required. 2) Create the mean of all aligned FA images and apply “thinning”, to create a skeletonised mean FA image. Threshold to suppress areas of low mean FA and/or high inter-subject variability. 3) Project each subject’s FA image onto the skeleton, by filling the skeleton with FA values from the nearest relevant tract centre. This is achieved, for each skeleton voxel, by searching perpendicular to the local skeleton structure for the maximum value in the subject’s FA image. 4) Carry out voxelwise statistics across subjects on the skeletonised FA data. TBSS is implemented as part of FSL [www.fmr.ox.ac.uk]; the nonlinear registration used is IRTK [www.doc.ic.ac.uk/~dr/software].

**TESTING FOR GAUSSIANTITY** It is of interest to test whether projecting data onto the FA skeleton improves the Gaussianity of the cross-subject distribution of FA. In [1] it was shown that there were a large number of voxels whose cross-subject distribution was significantly non-Gaussian. We tested two datasets, 36 controls and 33 schizophrenics, using the Lilliefors test to find voxels where the cross-subject distribution was significantly non-Gaussian. The threshold was 0.05: we expect to find 5% of voxels failing the test in purely Gaussian data. We ran the test on each dataset in three ways. Firstly, we tested all voxels after the initial nonlinear registration (and before skeletonisation); this is similar therefore to the VBM-based investigation reported in [1]. Secondly, we masked this aligned data with the mean FA skeleton, and investigated just these voxels - i.e., looking at skeleton voxels, but before projecting the aligned data onto the skeleton. Finally, we tested the skeletonised data after full TBSS preprocessing, i.e. after projection onto the skeleton. The percentage of voxels found to be non-Gaussian in the controls were (resp. for the three tests): 17.8, 7.0, 6.6. In the schizophrenics the % were: 19.2, 8.1, 7.5. Thus it is clear that with the “VBM-style” analysis, we find a large number of voxels with a non-Gaussian distribution (4x more than predicted by chance, in agreement with [1] for unsmoothed VBM-preprocessed data). Interestingly, the spatial distribution of these tends to be away from the tract centres, as judged visually, and as shown by the great reduction in the percentages in the second tests, where the aligned data is only tested at the skeleton voxels. For the fully TBSS-processed data, the test failure rate is reduced still further, to rates not far above the 5% expected by chance, as one would hope if the variability simply reflects subject variability in perfectly corresponding locations.

**REPEATABILITY TESTS** Next, we investigated the repeatability of FA, both across sessions and across subjects. Data: 8 healthy subjects, each scanned on 3 occasions, 1.5T Siemens Sonata, 60 directions, 3 repeats, 2x2x2mm<sup>3</sup>. We estimated % coefficient of variation (100 x std.dev. / mean) across sessions or subjects at each skeleton voxel. We first measured CoV at 7 voxels placed in the centre of various white matter tracts on the mean FA image; the genu of the corpus callosum, left/right optic radiation, left/right pyramidal tract, and left/right superior cingulum bundle. As well as estimating CoV for the TBSS-preprocessed data at these points, we also found CoV for data before the skeletonisation, after just the nonlinear registration, which we therefore refer to as being “VBM-preprocessed” (though no spatial smoothing was applied). Thirdly, we estimated CoV by carefully choosing the relevant voxels of interest by hand on each original FA image separately. Ideally, this hand placing has the advantage of adapting to tract localisation changes across subjects, but potentially suffers from subjectivity/user-error. In the easiest to define, thickest tracts, hand definition of the voxel in this way should give a close to optimal CoV. We also obtained global summary statistics (median and mode) across the whole brain for CoV in the TBSS and VBM-preprocessed cases. VBM-preprocessed results are only reported for voxels where the mean FA across all subjects and sessions is > 0.2, to avoid bias through inclusion of potentially high CoV values in low mean FA voxels. Likewise, the TBSS skeleton was thresholded at



0.2. The graphs show the inter-session and inter-subject variability. Cross-session variability with TBSS is generally lower than VBM preprocessing and generally considerably lower than with hand-placing. Cross-subject variability with TBSS preprocessing is consistently lower than with VBM preprocessing and lower than hand-placing in 4 out of seven points of interest. The results suggest that TBSS is successful in aligning equivalent structures across sessions/subjects and that it improves alignment further than pure nonlinear registration has achieved here. With TBSS the inter-session CoV is generally between 3% and 5% (mode 3%), and the inter-subject CoV is generally between 5% and 15% (mode 12%). These figures should prove useful when carrying out power calculations for planned DTI studies.

**DEPENDENCE ON ACQUISITION** Data: 18 normals x 4 acquisitions at 1.5T: 6 and 60 diffusion encoding directions (4 repeats in 4.2 min vs. 1 in 9.2 min), both without parallel imaging and with GRAPPA (acc. factor 2, 72 ref. lines), phased array headcoil. Averaging repeats increases SNR; parallel imaging lowers distortions at the expense of SNR and introducing some structured artifacts. Nonlinear registration based on B0 images with no diffusion weighting, (sometimes used in VBM preprocessing), is somewhat sensitive to the latter. We applied TBSS and estimated voxelwise cross-subject CoV. The table shows median (across voxels) CoV. In general, the FA skeletons (shown in different colours in the figure) were very similar across all 4 acquisitions, and the FA variability is not strongly dependent on acquisition.

	Directions	6 (4 repeats)	60
No parallel imaging		15.0%	15.5%
GRAPPA		15.9%	17.4%

[1] Jones et al., NeuroImage 26:546-554, 2005.