Voxel misassignments and their consequences in DTI skeleton-based group analyses

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Purpose: Diffusion tensor imaging (DTI) is a powerful tool to measure subtle changes in white matter integrity. Tract-based spatial statistics (TBSS) [1] is a widely-used software package to identify differences in DTI-parameters between different subject groups. Given its impact in neuroscience (more than 750 citations, www.webofknowledge.com), it is important to fully understand the potential limitations of this approach. Previous studies have investigated rotational invariance [2], independence of registration approach [3], and anatomical concordance [4] of the method. To what extent TBSS is truly *tract-based* or may misassign voxels from one tract to another, has not been evaluated yet. Here we evaluated this and focussed on two major adjacent fiber tracts in the brain, the cingulum bundle (CB) and the corpus callosum (CC), and quantified false classifications of the TBSS skeletonization and projection algorithm in assigning tract voxels to either one of these two tracts. We further demonstrate that this false classification can have substantial consequences on the statistical inference.

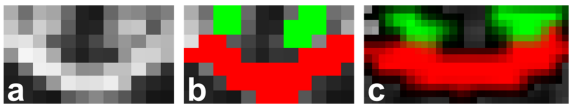

Fig. 1 Follow up of cingulum bundle (CB) and corpus callosum (CC) through the TBSS-procedure. **a** initial FA-map, **b** initial FA-map with segmentated CB (green) and CC (red). The segmentation based on the corresponding tensor-map. **c** FA-map and segmentation after registration.

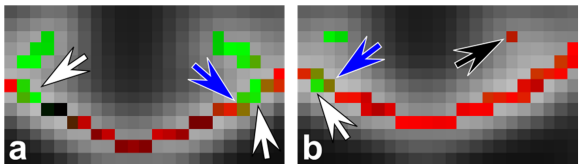

Fig. 2 Misassignment of adjacent white matter tracts in TBSS. **a+b** CB-voxels are assigned to the CC-skeleton (white arrows) and vice versa (black arrow). Blue arrows indicated voxels whose FA-values are a mixture of CB and CC FA-values.

Methods: Imaging: DTI was performed at 1.5T (Symphony, Siemens) for 15 Alzheimer's disease patients and 15 healthy controls using a twice refocused EPI-DTI. Parameters: TR/TE 4700/78 ms, FOV 240 mm, in-plane resolution of 2.5 mm, 50 2. 5 mm thick axial slices, 6 gradient directions (b=1000 s/mm²) and a b=0 image and 10 repetitions.
TBSS analysis: All images were corrected for motion and eddy currents (FSL, FLIRT), while compensating the gradient directions. Images were masked (FSL, BET). The full TBSS pipeline was applied using recommended parameters. A permutation test with n=500 was applied to compare the two groups, with p=0.05 as the threshold for significance.
Evaluation of misassignment: Voxels that clearly belonged to the CB or the CC where segmented in all subjects in native space (**Fig. 1a**) on basis of the main diffusion direction (**Fig. 1b**). The binary maps of CB and CC were transformed and projected to the skeleton using the conventional TBSS pipeline (**Fig. 1c**). This enabled us to reconstruct the true origin of CB and CC skeleton voxels after projection.
Evaluation of potential consequences: The different sources of error in the assignment problem are multifold and unknown. Theoretically, a "perfect" alignment of the different subjects would strongly improve the assignment task. We used a registration approach that incorporates both FA and full tensor information to potentially improve the alignment of the images (DTI-TK, [5]). We applied the same pipeline for these datasets and, using our gold standard segmentations, compared the amount of misassigned voxels. We then compared conventional TBSS statistics with the statistical maps retrieved using the improved alignment.

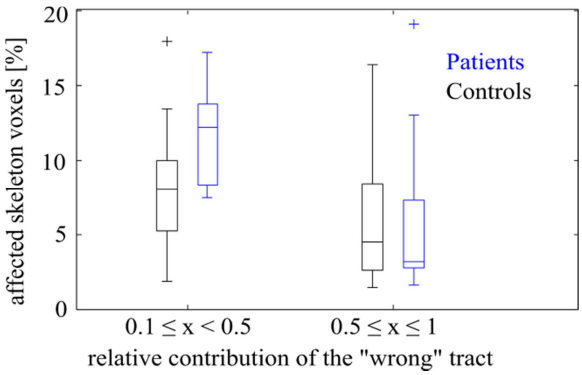

Fig. 3 Fraction of voxels per subject that had a relative contribution x of the "wrong" tract to the FA-values of the skeleton. Patients and controls differed significantly (p=0.02) for 0.1<x< 0.5.

Results: **Figs. 2a/b** show exemplary coronal views of the CB and the CC in two different subjects, as well as the corresponding projected voxels on the FA skeleton in green and red respectively. It is clearly visible that CB voxels are assigned to the CC skeleton (**a**) and vice versa (**b**). The black voxels at the skeleton could not be assigned as originating from the segmentation of either the CB or the CC. The contribution of one tract to the other is not binary, even on a voxel-basis, since the registration and interpolation steps introduce a blurring to the binary segmentations. The blue arrows in **Fig. 2a/b**, for example, point to yellow voxels, where the original colors green and red are mixed. **Fig. 3** shows group-wise boxplots of the subject-specific histogram bins over the relative contribution x of the one white matter tract to the other. On average, when counting voxels with x≥0.1, CC and CB voxels influence each other in 16% of the skeleton voxels that were analyzed. This effect strongly varied from subject to subject but interestingly, also from subject group to subject group (p=0.02 for 0.1 ≤ x < 0.5). The overall number of wrongly assigned voxels (x≥0.1) in patients was about 25 % higher than in controls.
Fig. 4a shows a section of the statistical map obtained using conventional TBSS, with highly significant group differences in both, the CB (top structure), and the CC (bottom structure). **Fig. 4b** was obtained from the data registrated using the full tensor information. Here, we found a significantly lower amount of misassigned voxels ($p<10^{-6}$; total number of misassigned voxels 2%; no significant difference between patients and controls). The group differences in the CB completely disappeared. The group differences in the CC became spatially more homogeneous.

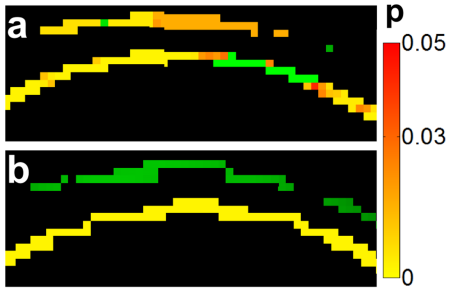

Fig. 4 Sagittal view of TBSS statistical results. The highly significant differences in the CB in **a** (conventional TBSS) completely disappear in **b** (registration on the full tensor information). Upper tract: CB, lower tract: CC.

Discussion: Our findings demonstrate that the TBSS skeletonization and projection algorithm could not distinguish between two major adjacent white matter tracts in a substantial amount of voxels on the skeleton. More specifically, CB voxels where mistaken as CC voxels and vice versa. This is an inherent limitation of the purely FA-based TBSS procedure and it has to be expected that this effect occurs whenever white matter tracts are in close proximity to each other. This strongly influences the statistical inference as illustrated in **Fig. 4** and thus must be borne in mind when interpreting TBSS results.

References: [1] Smith *et al.* 2006, NeuroImage **[2]** Edden *et al.* 2011, J Neurosci Meth **[4]** Keihaninejad *et al.* 2012, PLOS ONE **[3]** Zalesky *et al.* 2011, MRI **[5]** Zhang *et al.* 2005, MICCAI