# DTI preprocessing, statistical and cluster analysis

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ABSTRACT

This protocol outlines preprocessing, statistical and cluster analyses that were applied in 'Using whole-brain diffusion tensor analysis to evaluate white matter structural correlates of delayed visuospatial memory and one-week motor skill retention in nondemented older adults: A preliminary study' (https://doi.org/10.1371/journal.pone.0274955).

The following software packages are required to follow this protocol: FSL, MRtrix, Advanced Normalization Tools (Github: https://github.com/ANTsX/ANTs). Figures were created via AFNI.

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PROTOCOL CITATION

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GUIDELINES

Double-check your images after each step!

## Preprocessing data

1 Double-check if your native and original images are in FLOAT32 and oriented with the MNI.
-Convert to float-
**fslmaths dwidata.nii.gz dwi.nii.gz -odt float**

2 DWI Denoising:
dwidenoise -datatype float32 -noise level_noise.nii.gz [IN] [OUT_denoised]
**dwidenoise -datatype float32 -noise level_noise.nii.gz dwi.nii.gz dwi_denoised.nii.gz**

3 mrdegibbs [OUT_denoised] [OUT_Gibbs]
**mrdegibbs dwi_denoised.nii.gz dwi_Gibbs.nii.gz**

4 need brain – bet doesn't work as well as dwi2mask
**dwi2mask -fslgrad bvecs.txt bvals.txt dwi_Gibbs.nii.gz temp_brain_mask.nii.gz**

5 Run 'nodif_brain_mask.sh' script [e.g.,**bash nodif_brain_mask.sh dwi_Gibbs.nii.gz 4214_52 temp_brain_mask.nii.gz**] to get temporary nodif brain masks for eddy. Rename both of these nodif files with the prefix 'dwi_Gibbs_' e.g., 'dwi_Gibbs_brain_mask' for eddy. Delete the 'temp_brain' file.

6 Run eddy correction and get rotated vector file
**eddy --imain=dwi_Gibbs.nii.gz --mask=dwi_Gibbs_brain_mask.nii.gz --index=index.txt --acqp=acqparams.txt --bvecs=bvecs.txt --bvals=bvals.txt --fwhm=0 --flm=quadratic --slm=linear --out=eddy_unwarped_images --data_is_shelled**

7 Nodif and Brain extraction on the data.nii.gz
**fslroi data.nii.gznodif_data0 1**



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**dwi2mask -fslgrad eddy_unwarped_images.eddy_rotated_bvecs.txt bvals.txt data.nii.gz temp_brain_mask.nii.gz**

8 Run 'nodif_brain_mask.sh' script [e.g.,**bash nodif_brain_mask.sh data.nii.gz 4214_52 temp_brain_mask.nii.gz**] output is 'nodif_brain_mask.nii.gz' and 'nodif_brain.nii.gz' rename and add 'data' in front, so 'data_nodif_brain'. Delete 'temp_brain' file.

9 Bias Field correction (via ANTs):
**dwibiascorrect ants data.nii.gz data_bias.nii.gz -fslgrad eddy_unwarped_images.eddy_rotated_bvecs.txt bvals.txt -mask data_nodif_brain_mask.nii.gz -bias bias_image.nii.gz -ants.b [100,3]**

10 Upsample DWI images for better coregistration with MNI template
**mrgrid -vox 1.25 data_bias.nii.gz regrid data_bias_HR.nii.gz**

11 Fit tensor model
**dtifit -k data_bias_HR.nii.gz -o DTI_map_w-linear_ -m nodif_brain_mask.nii.gz -r eddy_unwarped_images.eddy_rotated_bvecs.txt -b bvals.txt -w**

12 Optional: create bash script to generate group template
Copy all the nodif_brain.nii.gz images into one folder and change directory to it. Then run:
**bash buildtemplateparallel.sh -d 3 -o template -c 0 -r 1 -n 0 -i 3 *.nii.gz**

***WarpImageMultiTransform 3 [in: FA map in native space] [out: FA map in template space] -R templatetemplate.nii.gz [warp file] [affine file]***

13 At this point all participant FA maps should be in template space. Create a 4D (x,y,z + time) file with all your maps:
Insert all your FA in template space in one folder and run:
**fslmerge -t [output] *.nii.gz**

14 Create then apply mask from all subjects
**fslmaths all_FA.nii.gz -max 0 -Tmin -bin mean_mask -odt char**
**fslmaths all_FA.nii.gz -mas mean_mask all_FA.nii.gz**

15 Create a mean of the FA over-time and create white matter mask (thresholded at FA>0.20):
**fslmaths all_FA -Tmean mean_FA**
**fslmaths mean_FA -thr 0.20 -bin WM_mask**

16 Apply your mask to your smoothed maps:
**fslmaths all_FA_smooth -mas mean_mask all_FA_smooth**



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17 At this point you should have a FA map 'all_FA.nii.gz' for all subjects.

## Linear regression

18 Create linear regression script (that applies model voxel-by-voxel) using the LM.m function in MATLAB by using:
LM (file_ID1, raw_image_subject, mask)

e.g., **LM (covariates.txt, all_FA_smooth.nii, WM_mask.nii)**

19 Resulting files will be the FDR-corrected p-value and tstat maps for each variable.

## Clusterize and apply white matter atlas to identify clusters

20 Example is for first behavioral variable 'score_1'
**3dcalc -ascore_1_FDR.nii-expr 'a*1' -prefixscore_1_FDR_corrected.nii.gz**
**3dcalc -a score_1_tStat.nii -expr 'a*1' -prefix score_1_tStat_corrected.nii.gz**

21 Split scores tStat into positive and negative (this provides you with positive and negative correlations):

**Fslmathsscore_1_tStat_corrected.nii.gz -thr 0 positive_score1.nii.gz**
**Fslmathsscore_1_tStat_corrected.nii.gz -uthr 0 negative_score1.nii.gz**
*For negative images, need to multiply by -1
**Fslmaths negative_score1.nii.gz -mul -1 negative_score1.nii.gz**

22 Get FDR-corrected p-value clusters that are less than 0.01 and that are at least 100 voxels in size
**3dClusterize -nosum -1Dformat -inset score_1_FDR_corrected.nii.gz -idat 0 -ithr 0 -NN 2 -clust_nvox 100 -1sided RIGHT_TAIL 0.99 -pref_map Clust_mask_motor.nii.gz**

**fslmaths Clust_mask_motor.nii.gz -bin Clust_mask_motor.nii.gz**

23 Coregister the template to MNI:

**antsRegistrationSyN.sh -d 3 -m /Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/group_template/templatetemplate.nii.gz -f /usr/local/fsl/data/standard/MNI152_T1_1mm_brain.nii.gz -o /Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/template_to_MNI/templatetemplate_to_MNI -t s**



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24 Check that ROIs are Binarized (use nearest neighbor command when the images are binarized). Behavioral variables are 'score_1' and 'motor'

Transform clusters to MNI:

**antsApplyTransforms -d 3 -i Clust_mask_motor.nii.gz -o Clust_mask_motor_MNI.nii.gz -r/usr/local/fsl/data/standard/MNI152_T1_1mm_brain.nii.gz -t [/Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/template_to_MNI/templatetemplate_to_MNI1Warp.nii.gz,0] -t [/Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/template_to_MNI/templatetemplate_to_MNI0GenericAffine.mat,0] -n NearestNeighbor**

25 Transform scores tstat (positive and negative) to MNI space:

**antsApplyTransforms -d 3 -ipositive_score1.nii.gz-opositive_score1_MNI.nii.gz -r/usr/local/fsl/data/standard/MNI152_T1_1mm_brain.nii.gz -t [/Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/template_to_MNI/templatetemplate_to_MNI1Warp.nii.gz,0] -t [/Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/template_to_MNI/templatetemplate_to_MNI0GenericAffine.mat,0]**

**antsApplyTransforms -d 3 -inegative_score1.nii.gz-onegative_score1_MNI.nii.gz -r/usr/local/fsl/data/standard/MNI152_T1_1mm_brain.nii.gz -t [/Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/template_to_MNI/templatetemplate_to_MNI1Warp.nii.gz,0] -t [/Users/syschaef/Desktop/4214_DTI/MRtrix_preproc/template_to_MNI/templatetemplate_to_MNI0GenericAffine.mat,0]**

26 Mask the tstat scores with significant cluster files:

Positive:
**fslmathspositive_score1_MNI.nii.gz -mas Clust_mask_motor_MNI.nii.gzpositive_score1masked_MNI.nii.gz fslmathspositive_score1masked_MNI.nii.gz -bin Clust_mask_motor_positive_MNI.nii.gz**

Negative:
**fslmathsnegative_score1_MNI.nii.gz -mas Clust_mask_motor_MNI.nii.gznegative_score1masked_MNI.nii.gz fslmathsnegative_score1masked_MNI.nii.gz -bin Clust_mask_motor_negative_MNI.nii.gz**

27 Custom code extracted significant cluster locations from the JHU white matter atlas, but use



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the atlas of your choice and identify clusters by using '**fslmaths**' (see fslwiki for tutorial)



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