# Introduction

This procedure involves the normalization of FA and MD images through a pipeline that minimizes across-session test-retest reproducibility error (Jacobacci et al., 2019).

Normalization of DTI images to MNI152\_T1\_1mm template is performed using ANTs (antsRegistration command) via an intermediate individual FA template also created using ANTs (antsMultivariateTemplateConstructionTool).

For this script to work correctly, DWI images must already be pre-processed and the DTI model fitted. FSL and ANTs need to be previously installed and $FSLDIR and $ANTSPATH variables need to be set. These scripts were tested using FSL version 5.0.9 and ANTs version 2.2.0

# File organization

Files must be organized in the following way:

One subjects directory folder, which will contain one folder per subject.

Each subject folder will contain:

* one folder called FA, with all FA images for that subject (multiple sessions)
* one folder called MD, with all MD images for that subject (multiple sessions)
* one folder called B0, with all B0 images for that subject (multiple sessions)

FA, MD and B0 images need to be in the same space (i.e., coregistered). FA and MD maps are coregistered because they are produced from the DTI model fitting step. However, this is particularly important for the B0 image. If you extract the B0 after eddy correction, the FA and MD images will be aligned to it.

***<Subjects\_directory>***

***|***

***|***

***|\_SUBJECT0001***

***| |***

***| |\_\_\_>FA: 0001\_FA.nii.gz, 0002\_FA.nii.gz, etc. (Multiple sessions per subj.)***

***| |***

***| |\_\_\_>MD: 0001\_MD.nii.gz, 0002\_MD.nii.gz, etc.***

***| |***

***| |\_\_\_>B0: 0001\_B0.nii.gz, 0002\_B0.nii.gz, etc.***

***|***

***|***

***|\_SUBJECT0002***

***... etc***

NOTE: Scripts are written so that images are read using the format specified here: i.e. Subject folder names maintaining the format SUBJECTXXXX and images inside maintaining the format XXXX\_FA.nii.gz, XXXX\_MD.nii.gz, XXXX\_B0.nii.gz

# Script execution

The main script is executed on the terminal via bash with the following input parameters:

***<Subjects\_directory>*** is the full path to the folder where subject folders are stored. Inside each subject folder FA, MD and B0 folders are required. These folders will contain the FA, MD and B0 images, respectively, for all the sessions of that subject.

If your CPU allows for multi-threading you can perform several jobs in parallel. You can check the number of jobs in your CPU by opening the terminal and typing **nproc**

***<n\_jobs>*** is the number of jobs to be used to parallelize template creation (paralellization is automatic)

Example call:

First, make sure you are on the directory where the scripts are located.

For example, if the scripts are located in **/home/mycomputer/path-to-dir/SCRIPTS** then type in terminal the following:

**cd /home/mycomputer/path-to-dir/SCRIPTS**

Once you are placed in the correct directory, set the subjects directory. To do this, type in terminal the following:

**Subjects\_directory=/home/mycomputer/path-to-dir/SUBJECTS\_FOLDER**

**n\_jobs=8**

**bash 0\_MainScript\_DTINormalizationtoMNI152T1ViaIndividualFATemplate.sh $Subjects\_directory $n\_jobs**

NOTE: All scripts must be in executable mode. Type in terminal:

**chmod +x <script-name>.sh**

# Script details

The main script will perform these steps:

| **Step number** | **Script name** | **Description** | **Input parameters** | **Input files** | **Output files** |
| --- | --- | --- | --- | --- | --- |
| 1 | 1\_Erode\_PadImages.sh | This script will erode brilliant voxels on the border of FA images, caused by eddy currents in CSF and apply the same erosion to the corresponding MD and B0 images. It will also pad FA, MD and B0 images with 10 voxels all around. | directory | FA, MD and B0 images  $Subjects\_directory/SUBECT0001/FA/0001\_FA.nii.gz  $Subjects\_directory/SUBECT0001/MD/0001\_MD.nii.gz  $Subjects\_directory/SUBECT0001/B'0/0001\_B0.nii.gz | Eroded and padded FA, MD and B0 images  $Subjects\_directory/SUBECT0001/FA/0001\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/MD/0001\_MD\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/B0/0001\_B0\_ero\_padded.nii.gz |
| 2 | 2\_Create\_Multi-modal\_file.sh | This script will create the txt file required to supply it to ANTs-MultivariateTemplateCreation algorithm for the creation of the intermediate template/s | directory | Eroded and padded FA, MD and/or B0 images  $Subjects\_directory/SUBECT0001/FA/0001\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/MD/0001\_MD\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/B0/0001\_B0\_ero\_padded.nii.gz | One txt file per subject with all the images that will be used for template creation on steps 3 and 4  $Subjects\_directory/SUBECT0001/Multi-modal\_images.txt |
| 3 | 3\_CreateInitialFATemplateANTs\_MVT.sh | This script will create an initial FA template to be used as seed using antsMultivariateTemplateConstruction tool | directory, n\_jobs | All eroded and padded FA images from each subject  $Subjects\_directory/SUBECT0001/FA/0001\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0002\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0003\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0004\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0005\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0006\_FA\_ero\_padded.nii.gz  etc | One initial FA individual template per subject  $Subjects\_directory/SUBECT0001/INIT\_FA\_MVT\_template0.nii.gz  One initial b0 individual template per subject  $Subjects\_directory/SUBECT0001/INIT\_FA\_MVT\_template1.nii.gz |
| 4 | 4\_CreateIndividualFATemplateANTs\_MVT.sh | This script will create an Individual FA template to be used as seed using antsMultivariateTemplateConstruction tool | directory, n\_jobs | All FA images from each subject  $Subjects\_directory/SUBECT0001/FA/0001\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0002\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0003\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0004\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0005\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/FA/0006\_FA\_ero\_padded.nii.gz  etc | One individual FA template per subject  $Subjects\_directory/SUBECT0001/SUBJECT0001\_FA\_template0.nii.gz  One individual b0 template per subject  $Subjects\_directory/SUBECT0001/SUBJECT0001\_FA\_template1.nii.gz |
| 5 | 5\_NormalizeIndividualFATemplates\_toT1\_MNI152\_1mm.sh | This script will perform normalization of FA Templates to MNI152\_T1\_1mm template using ANTs' antsRegistration command | directory, n\_jobs | Individual FA templates from each subject  $Subjects\_directory/SUBECT0001/SUBJECT0001\_FA\_template0.nii.gz | Normalized individual FA template and tranformations  $Subjects\_directory/SUBECT0001/MNI152\_T1\_1mm\_brain\_fixed\_SUBJECT0001\_FA\_template0\_moving\_setting\_is\_forproduction\_SUBJECT0001\_FA\_template0.nii.gz\_warped.nii.gz  $Subjects\_directory/SUBECT0001/MNI152\_T1\_1mm\_brain\_fixed\_SUBJECT0001\_FA\_template0\_moving\_setting\_is\_forproduction\_inv.nii.gz  $Subjects\_directory/SUBECT0001/MNI152\_T1\_1mm\_brain\_fixed\_SUBJECT0001\_FA\_template0\_moving\_setting\_is\_forproduction1InverseWarp.nii.gz  $Subjects\_directory/SUBECT0001/MNI152\_T1\_1mm\_brain\_fixed\_SUBJECT0001\_FA\_template0\_moving\_setting\_is\_forproduction1Warp.nii.gz  $Subjects\_directory/SUBECT0001/MNI152\_T1\_1mm\_brain\_fixed\_SUBJECT0001\_FA\_template0\_moving\_setting\_is\_forproduction0GenericAffine.nii.gz |
| 6 | 6\_ApplyTransf\_FAtoT1\_MNI152\_1mm.sh | This script applies transformations to take FA and MD images to MNI152\_T1\_1mm template | directory | Eroded and padded FA and MD images  $Subjects\_directory/SUBECT0001/FA/0001\_FA\_ero\_padded.nii.gz  $Subjects\_directory/SUBECT0001/MD/0001\_MD\_ero\_padded.nii.gz | FA and MD images warped to MNI152 space  $Subjects\_directory/SUBECT0001/FA/0001\_FA\_warped2MNI152\_T1\_1mm.nii.gz  $Subjects\_directory/SUBECT0001/MD/0001\_MD\_warped2MNI152\_T1\_1mm.nii.gz |
| 7 | 7a\_Check\_normalizationFAandMD\_toMNI152\_T1.sh | This script creates gifs for quality control of the normalization. | directory | FA and MD images warped to MNI152 space  $Subjects\_directory/SUBECT0001/FA/0001\_FA\_warped2MNI152\_T1\_1mm.nii.gz  $Subjects\_directory/SUBECT0001/MD/0001\_MD\_warped2MNI152\_T1\_1mm.nii.gz | Gif images of normalized FA and MD over MNI152 T1 template  $Subjects\_directory/SUBECT0001/0001\_FAoverMNI152\_T1.gif  $Subjects\_directory/SUBECT0001/0001\_MDoverMNI152\_T1.gif |
| 7b\_CheckNormalizationFA\_toMNI152\_T1\_HTML.sh | This script creates an HTML webpage, to be opened in any browser, showing normalization results for FA for quality control. | directory | Gif images of normalized FA and MD over MNI152 T1 template  $Subjects\_directory/SUBECT0001/0001\_FAoverMNI152\_T1.gif  $Subjects\_directory/SUBECT0001/0001\_MDoverMNI152\_T1.gif | It will produce an html web page  $Subjects\_directory/check\_FAoverMNI152.html |
| 7c\_CheckNormalizationMD\_toMNI152\_T1\_HTML.sh | This script creates an HTML webpage, to be opened in any browser, showing normalization results for MD for quality control. | directory | Gif images of normalized FA and MD over MNI152 T1 template  0001\_FAoverMNI152\_T1.gif  0001\_MDoverMNI152\_T1.gif | In the <directory> folder, it will produce an html web page  $Subjects\_directory/check\_MDoverMNI152.html |

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