T1 and DWI data processing pipeline

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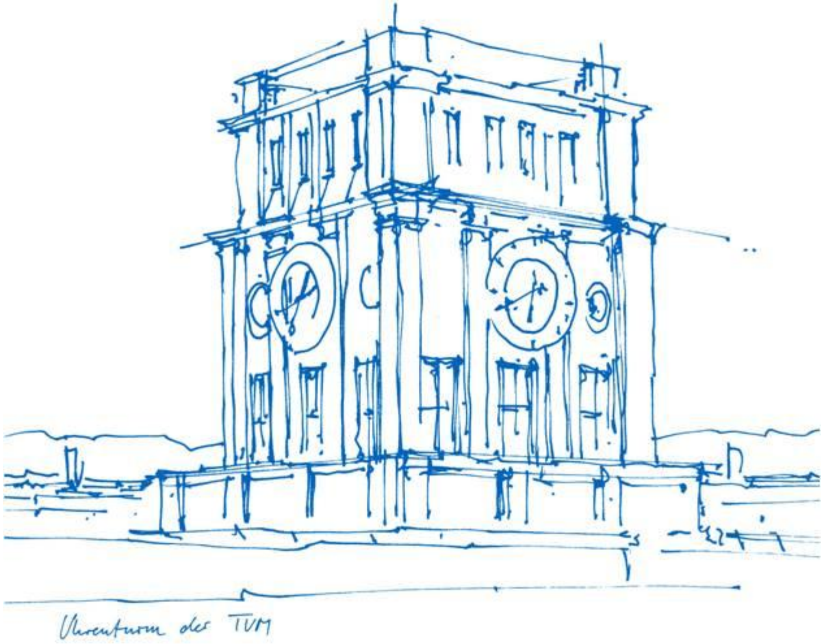


Content

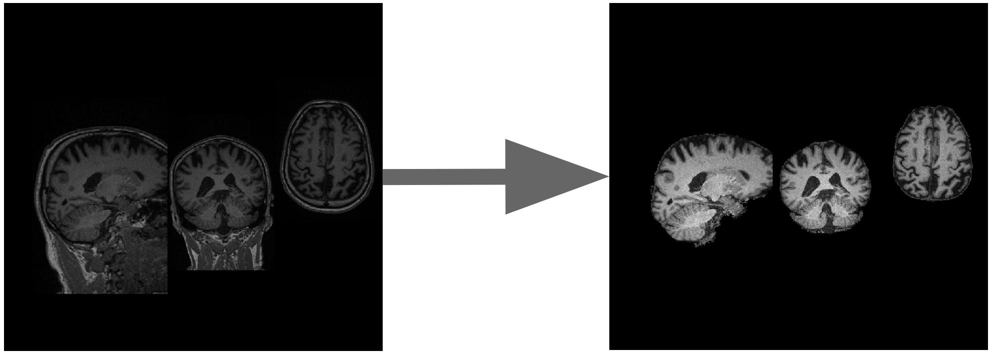
- T1 Processing
  - Extraction
  - Segmentation
  - Reconstruction
  - Registration
  - Atlas

- DWI Processing
  - Diffusion
    - Without Field Map
    - With Field Map
  - Tractography

- GUI/Code comparison

T1 Processing: Extraction

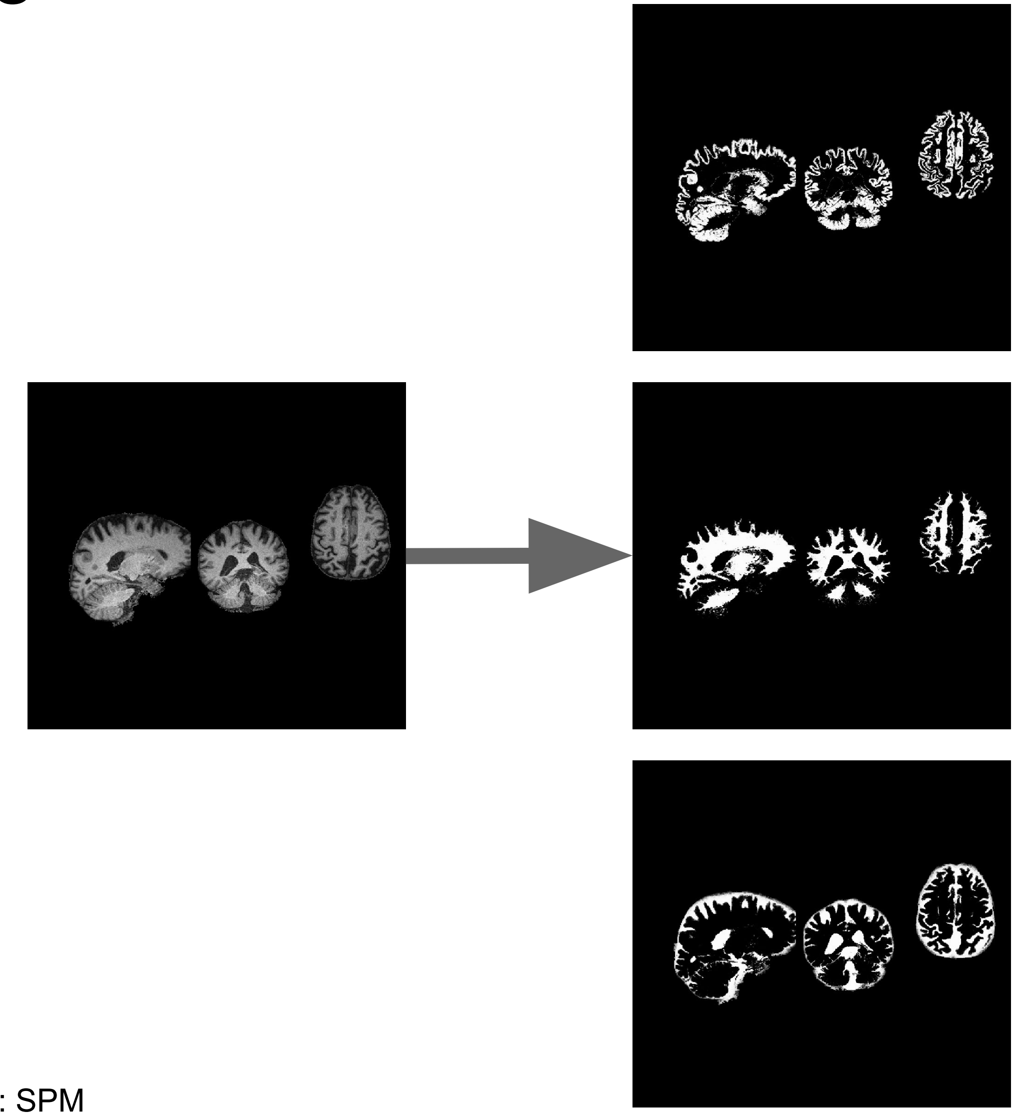
- Input :
  - MRI T1 images
- Output:
  - Brain Region



Data: EiMa 54, First Session, T1; Methodology: SPM

T1 Processing: Segmentation

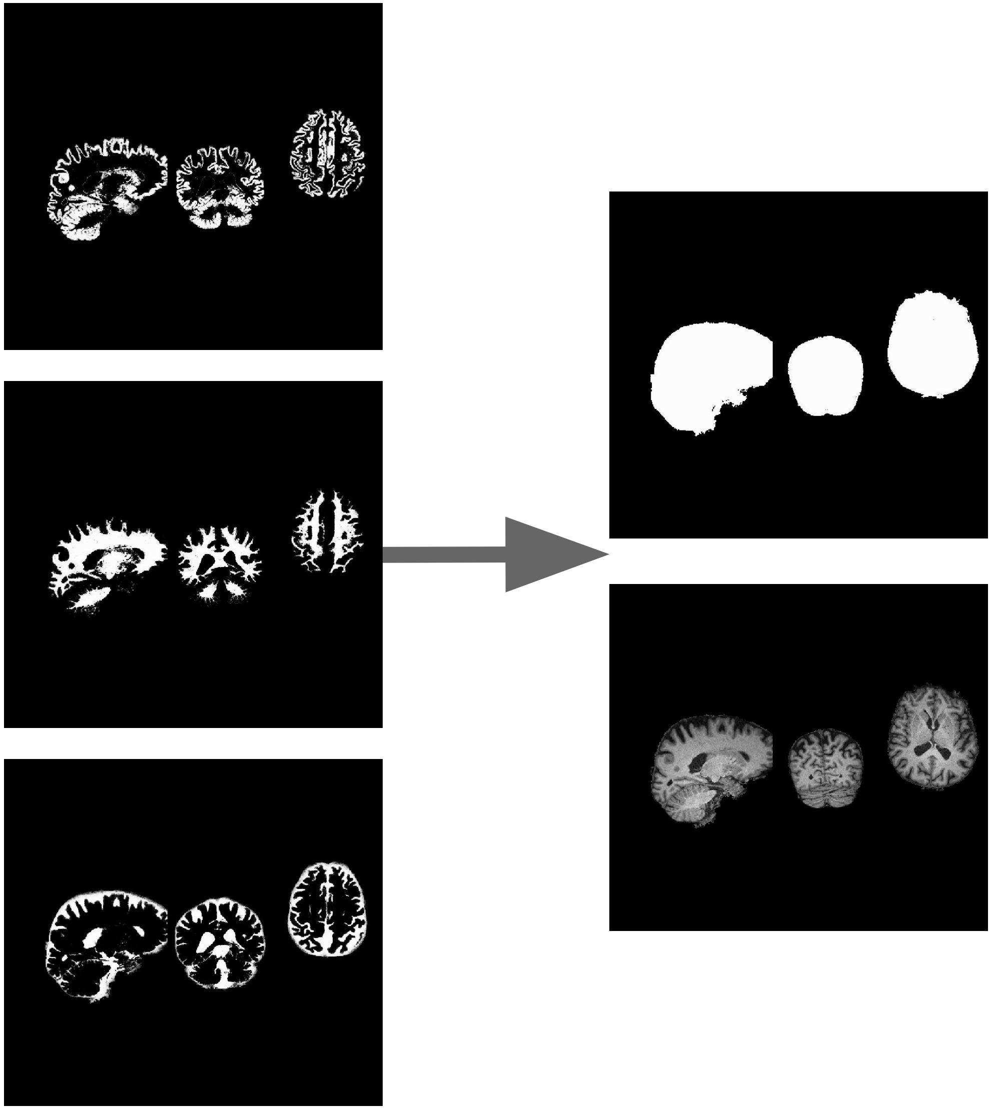
- Input :
    - Extracted Brain from T1
- Output:
    - Grey Matter
    - White matter
    - Cerebrospinal Fluid
    - Bone
    - Soft tissue
    - Others



Data: EiMa 54, First Session, T1; Methodology: SPM

T1 Processing: Reconstruction

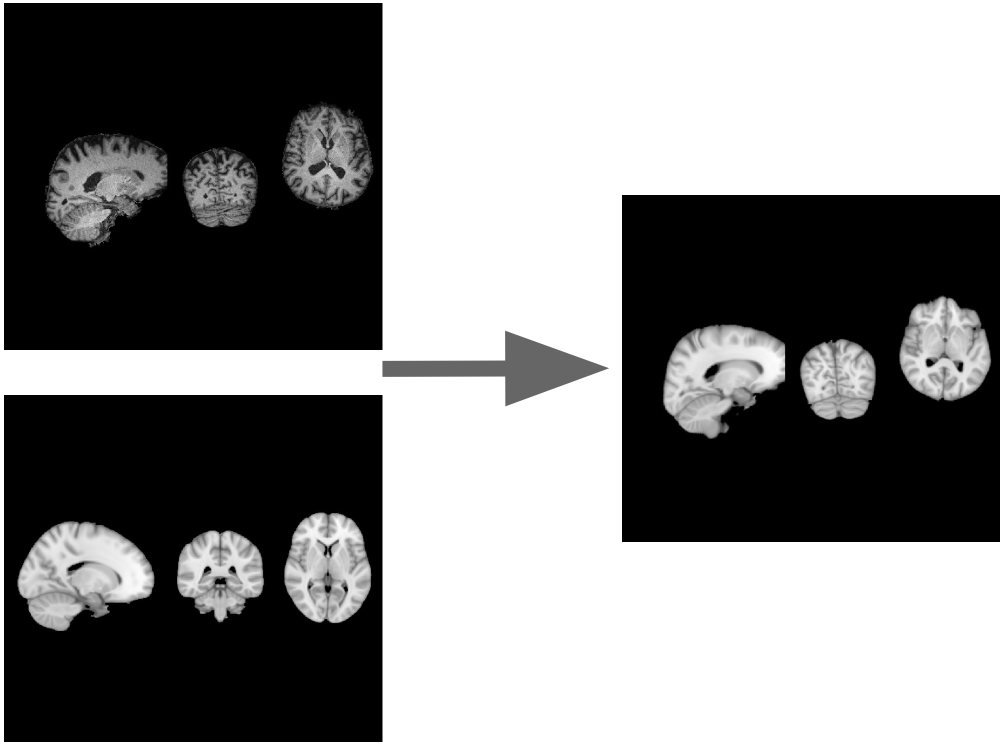
- Input :
    - Grey Matter
    - White matter
    - Cerebrospinal Fluid
- Output:
    - Reconstructed brain
    - Brain Mask



Data: EiMa 54, First Session, T1; Methodology: SPM

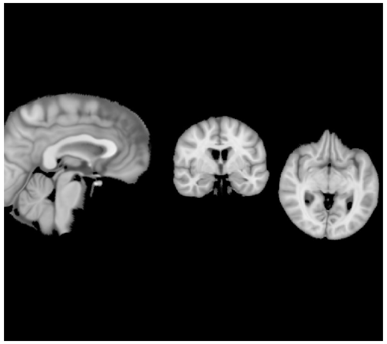
T1 Processing: Registration - MNI152

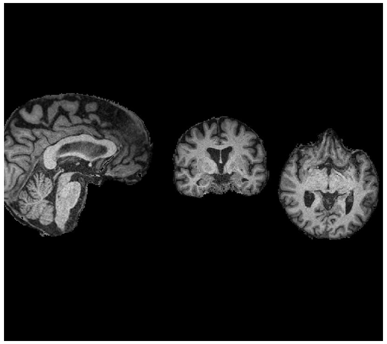
- Input :
  - MNI152_T1_1mm
  - Reference: Reconstructed brain
- Output:
  - Registered image
  - Registration matrix

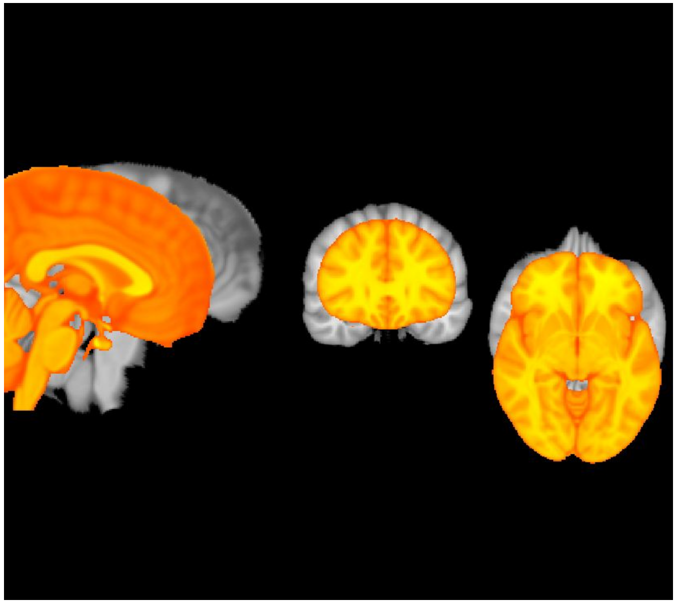


Data: EiMa 54, First Session, T1; Methodology: Antregistration

T1 Processing: Registration - MNI152







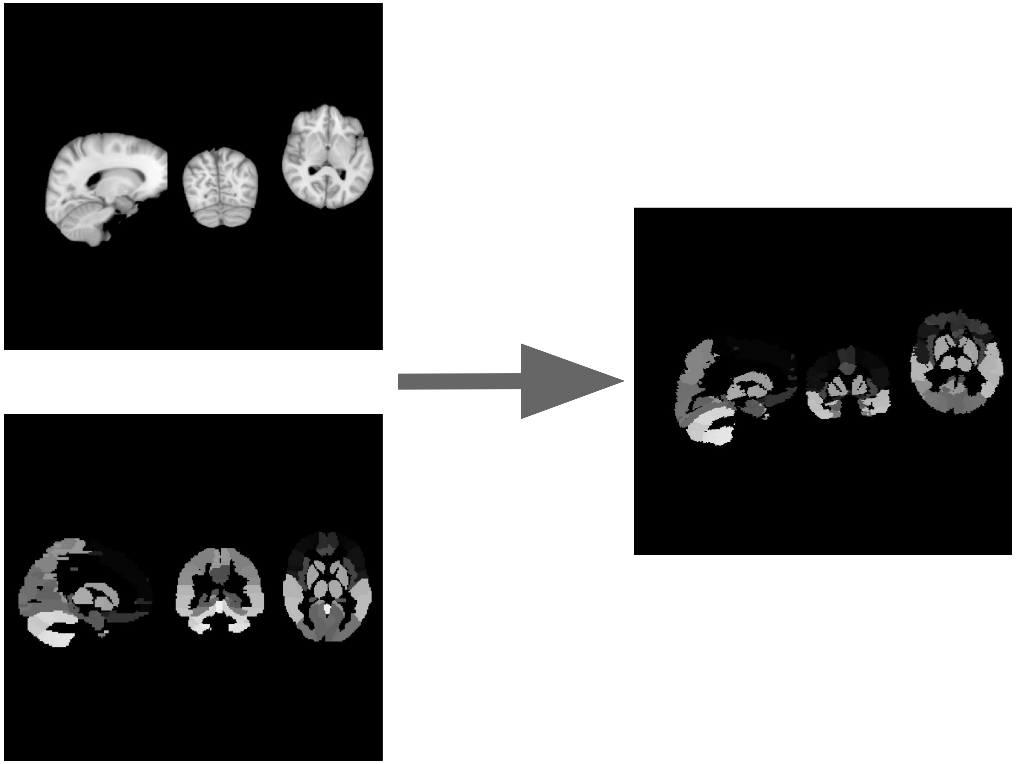
Comparison

- Top right:
  - Reconstructed brain from T1
- Top left:
  - MNI152_T1_1mm_brain after registration
- Bottom: MNI152_T1_1mm_brain
  - Yellow: before registration
  - Grey: after registration

Data: EiMa 54, First Session, T1; Methodology: Antregistration

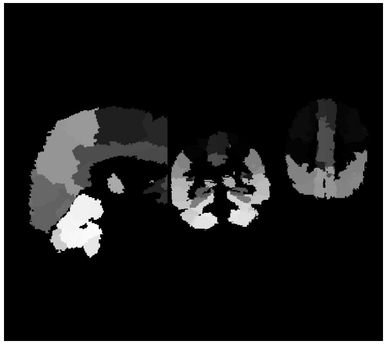
T1 Processing: Atlas - AAL2

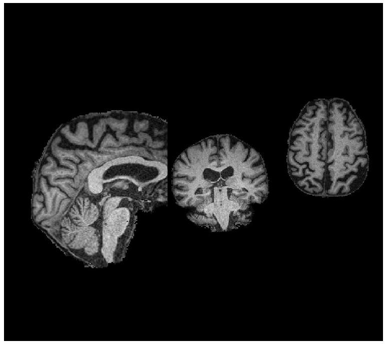
- Input :
    - rAAL2_1mm
    - Reference: Reconstructed brain
- Output:
    - Atlas brain region
    - Transform matrix



Data: EiMa 54, First Session, T1; Methodology: Anttrasformation

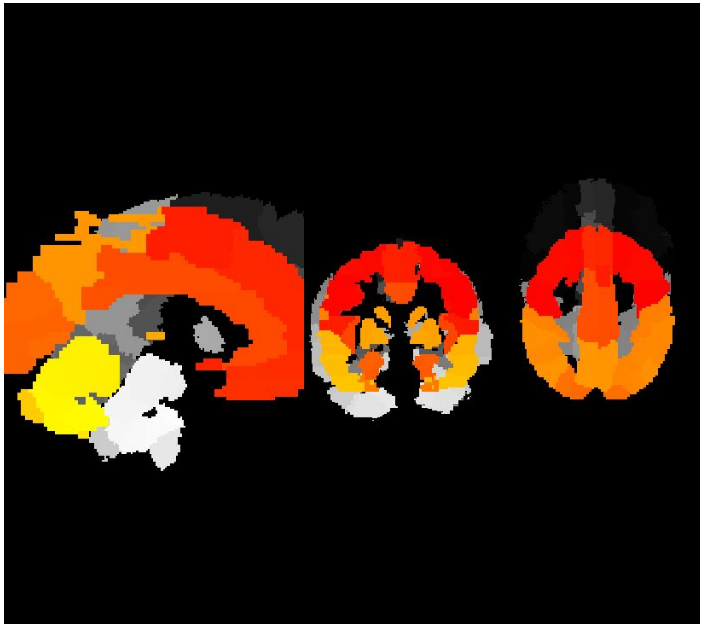
T1 Processing: Atlas - AAL2





Comparison

- Top right:
    - Reconstructed brain from T1
- Top Left:
    - rAAL2_1mm afer atlas
- Bottom:
  rAAL2_1mm
    - Yellow: before registration
    - Grey: after registration

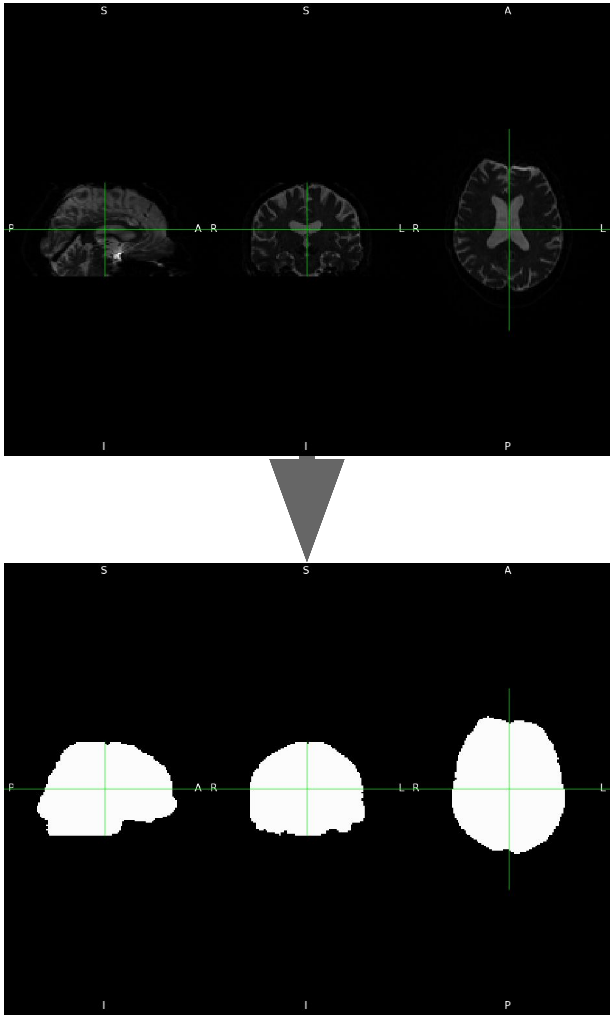


Data: EiMa 54, First Session, T1; Methodology: Anttrasformation

DWI Processing: Extraction

Extraction **without** Field Map

- Input :
  - DWI images
- Output:
  - Brain Mask



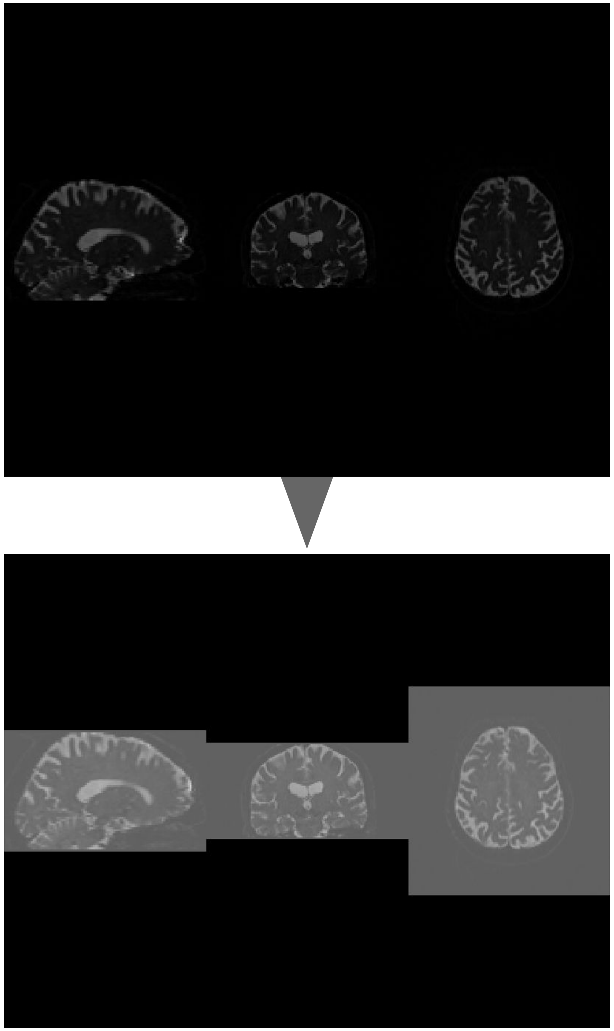
Data: EiMa 54, Second Session, DWI; Methodology: FSL: BET

DWI Processing: Extraction

Extraction **with** Field Map:

Step 1 - Distortion Correction:

- Why?
  - Eddy current



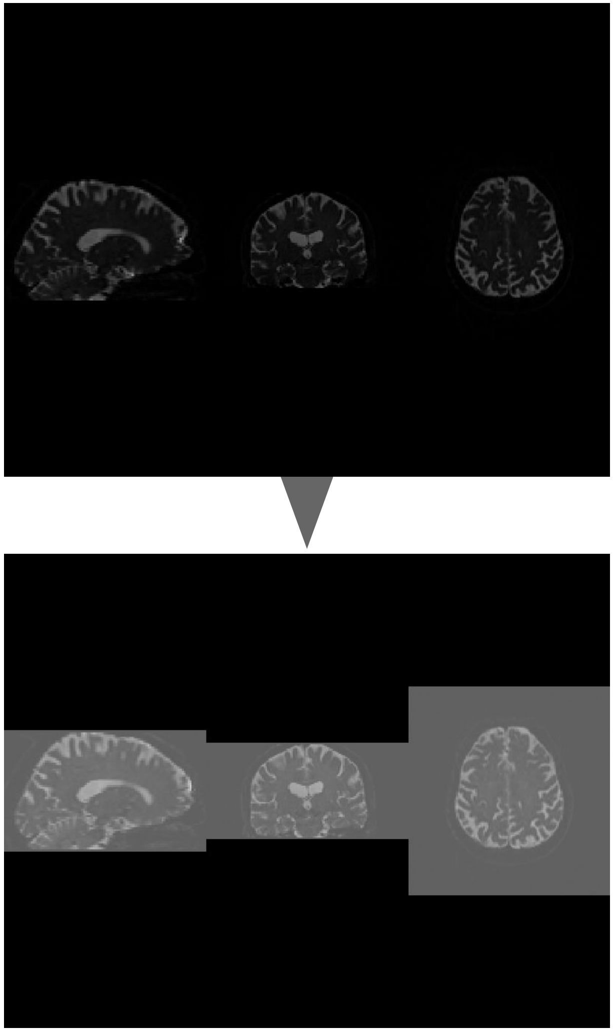
Data: EiMa 54, First Session, DWI; Methodology: FSL: Eddy

DWI Processing: Extraction

Extraction **with** Field Map:

Step 1 - Distortion Correction:

- Input :
  - DWI images
- Output:
  - Corrected images



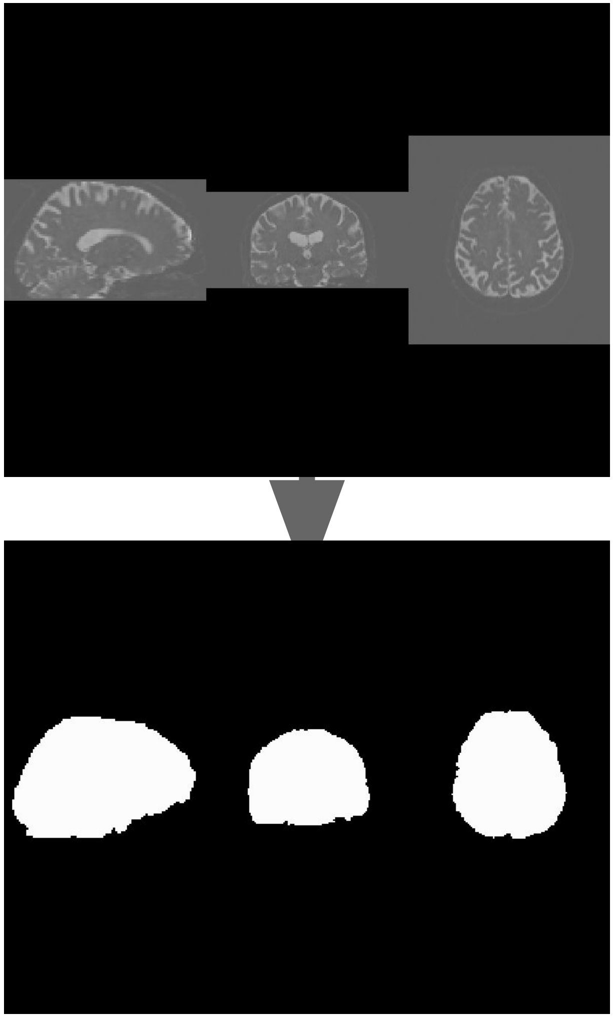
Data: EiMa 54, First Session, DWI; Methodology: FSL: Eddy

DWI Processing: Extraction

Extraction **with** Field Map:

Step 2 - Extraction after Eddy:

- Input :
  - Corrected images
- Output:
  - Brain Mask

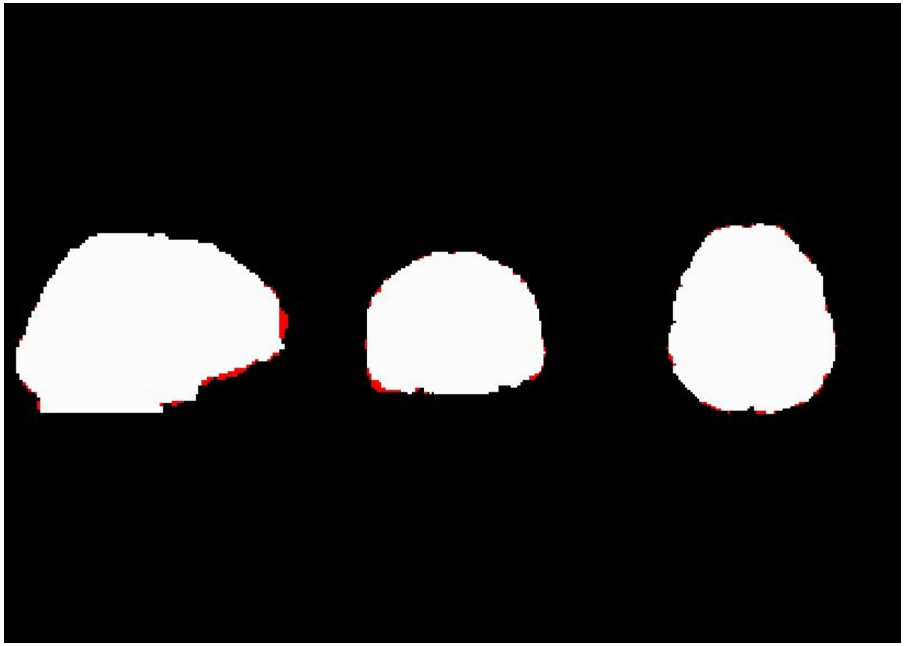


Data: EiMa 54, First Session, DWI; Methodology: FSL: Eddy

DWI Processing: Extraction

Comparison

- Background Red Mask:
  - With Field Map
- Foreground White Mask:
  - Without Field Map



Data: EiMa 54, First/Second Session, DWI; Methodology: FSL: Eddy

DWI Processing: Tractography

Step 1 - bedpostx: It creates all the files necessary for running probabilistic tractography

- Input:
  - Brain Mask
  - B Value
  - B Vector
- Method
  - Bayesian Estimation
  - Markov Chain
  - Monte Carlo sampling
- Output:
  - distributions on diffusion parameters at each voxel.

DWI Processing: Tractography

Step 1 - bedpostx: It creates all the files necessary for running probabilistic tractography

- Bayesian Estimation

$$\pi(\theta \mid x) \propto f(x \mid \theta) \times \pi(\theta)$$

posterior ∝ likelihood × prior

The probability of the white fiber orientation is related to the likely hood of the data multiply the biology prior

DWI Processing: Tractography

Step 1 - bedpostx: It creates all the files necessary for running probabilistic tractography

- Markov Chain

$$P(Xt+1 = x|X0, . . . , Xt) = P(Xt+1 = x|Xt)$$

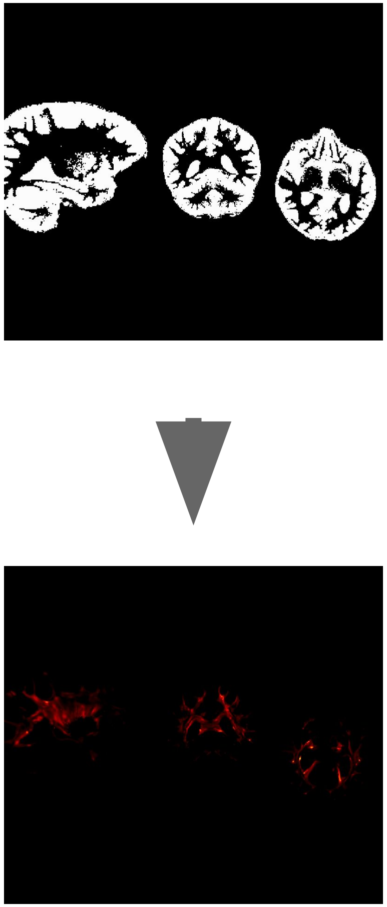
future evolution is conditionally independent of the past given the present

The future white fiber orientation is only related to the current state

DWI Processing: Tractography

Step 2 - PROBTRACKX: Calculate the similarity of voxels nearby to get the connectivity

- Input (Our case):
  - Grey matter Mask
  - CSF Mask
- Output:
  - Connectivity of the white matter

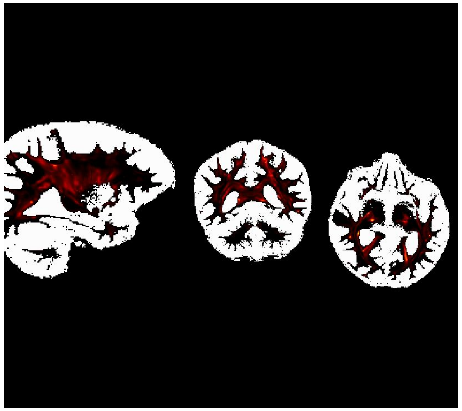


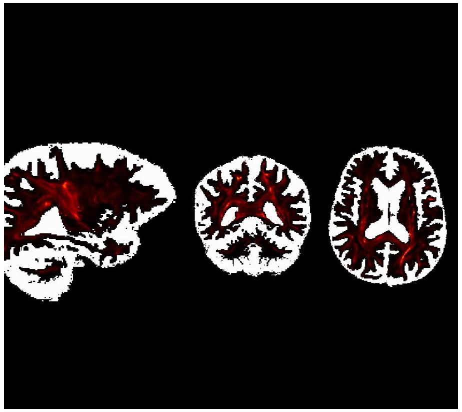
Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

DWI Processing: Tractography

Comparison

- Up:
  - Session 1
    With Field Map
- Output:
  - Session 2
    Without Field Map



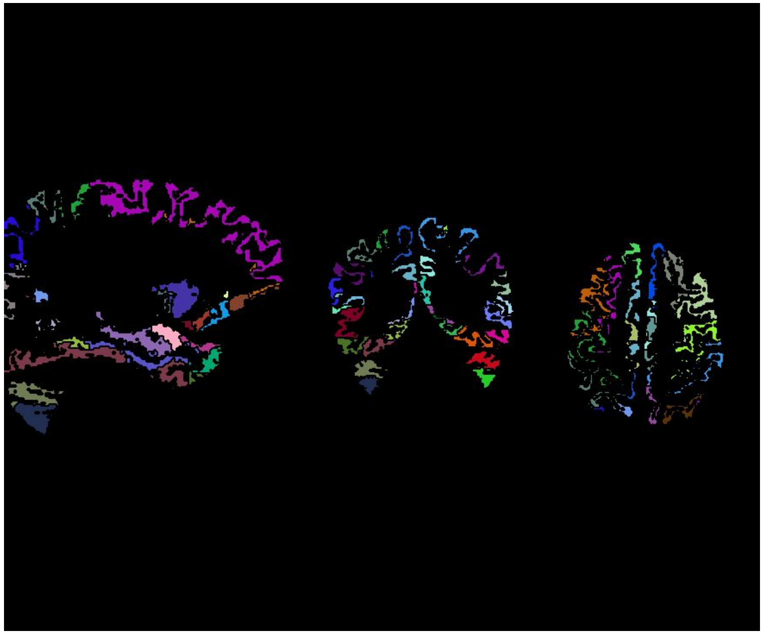


Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

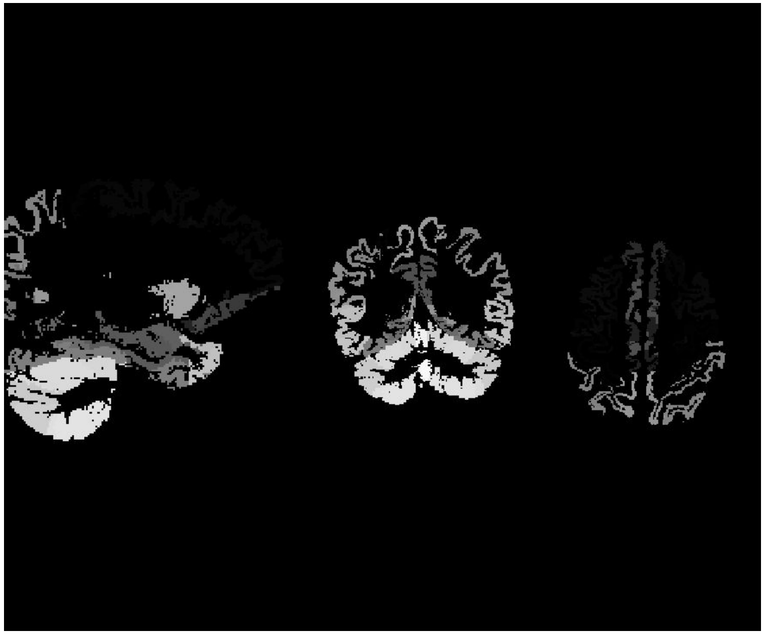
Thanks

T1 Processing: Atlas - AAL2

Comparison



Labeled image

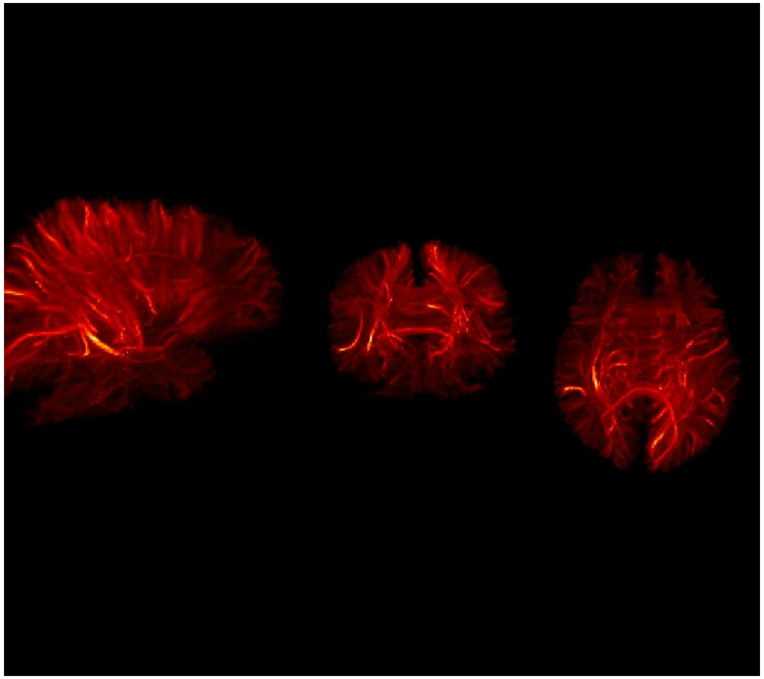


Volume image

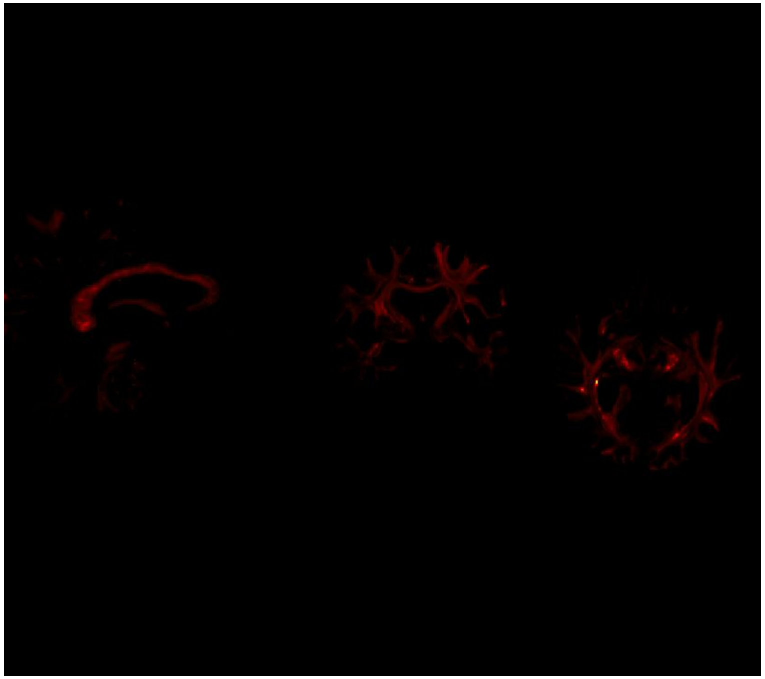
Data: EiMa 54, First Session, T1; Methodology: Anttrasformation

T1 Processing: Tractography

Comparison



Intensity image



Volume image

Data: EiMa 54, First Session, T1; Methodology: Anttrasformation

DWI Processing: BEDPOSTX

BEDPOSTX:

Bayesian Estimation of Diffusion Parameters using **Markov Chain Monte Carlo sampling**

X stands for modelling **Crossing Fibres**

It creates all the files necessary for running probabilistic tractography

DWI Processing: BEDPOSTX

BEDPOSTX:

- Input:
  - Brain volume
  - B-Value, B-Vector, Field Map

- Method
  - Bayesian Estimation
  - Markov Chain Monte Carlo sampling

- Output:
  - **Fibres orientation at each voxel**

DWI Processing: BEDPOSTX

Bayesian Estimation of Diffusion Parameters using **Markov Chain Monte Carlo sampling**

- Bayesian Estimation

$$\pi(\theta\,|x) \propto f(x|\theta) \times \pi(\theta)$$

posterior ∝ likelihood × prior

The white matter fiber orientation $\pi(\theta\,|x)$ is related to the **statistics of the brain volume data** $f(x|\theta)$ multiply the **biology prior knowledge** $\pi(\theta)$

DWI Processing: BEDPOSTX

Bayesian Estimation of Diffusion Parameters using **Markov Chain Monte Carlo sampling**

- Markov Chain

$$P(Xt+1 = x|X0, . . . , Xt) = P(Xt+1 = x|Xt)$$

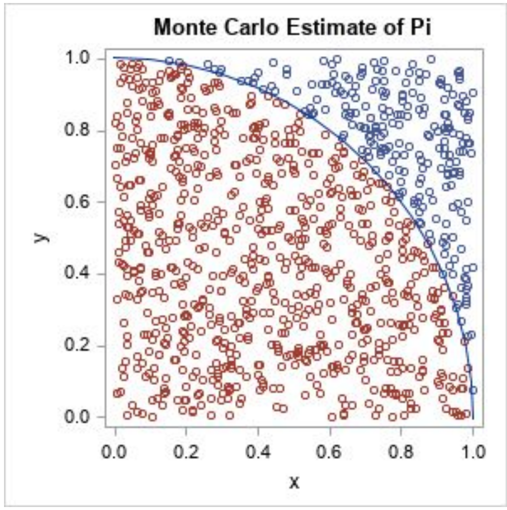
future state is only dependent on the current state

The white matter fiber orientation in next step $P(Xt+1 = x|X0, . . . , Xt)$ is only related to **the current white matter fiber orientation** $P(Xt+1 = x|Xt)$

DWI Processing: BEDPOSTX

Bayesian Estimation of Diffusion Parameters using **Markov Chain Monte Carlo sampling**

- **Monte Carlo sampling**



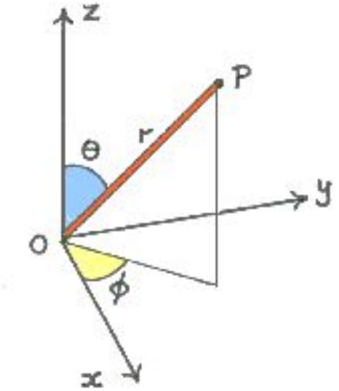
MC utilizes random sampling to approximate numerical results.

DWI Processing: BEDPOSTX

Output: Fibres orientation at each voxel

<i> indicates the i-th fibre

- merged_th<i>samples - theta
- merged_ph<i>samples - phi

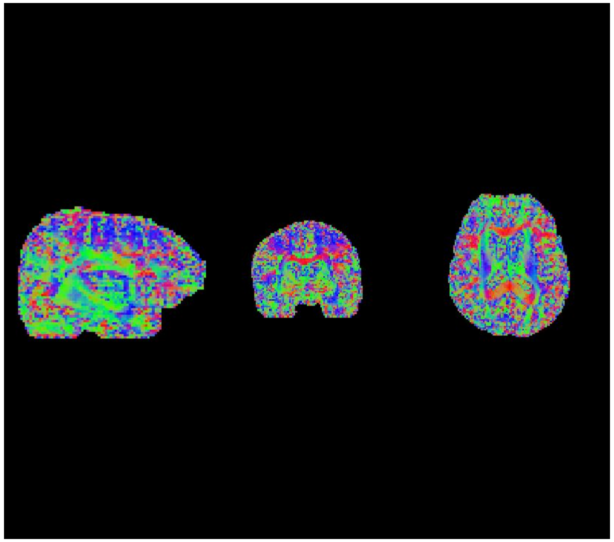


DWI Processing: BEDPOSTX

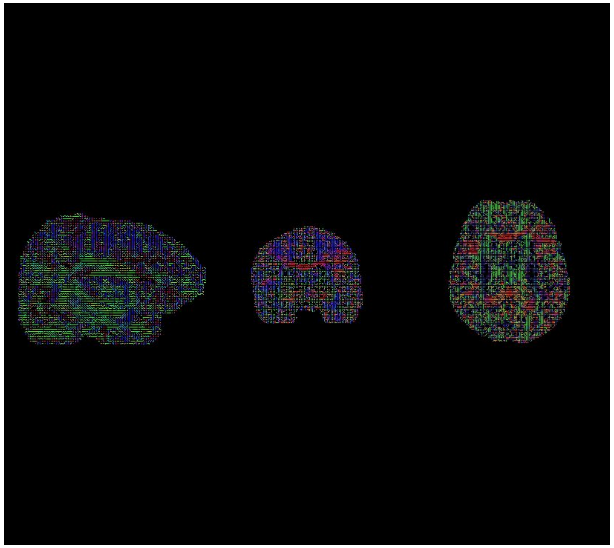
Output: Fibres orientation at each voxel

color indicates the direction

- X
- Y
- Z



color dot image (RBG)

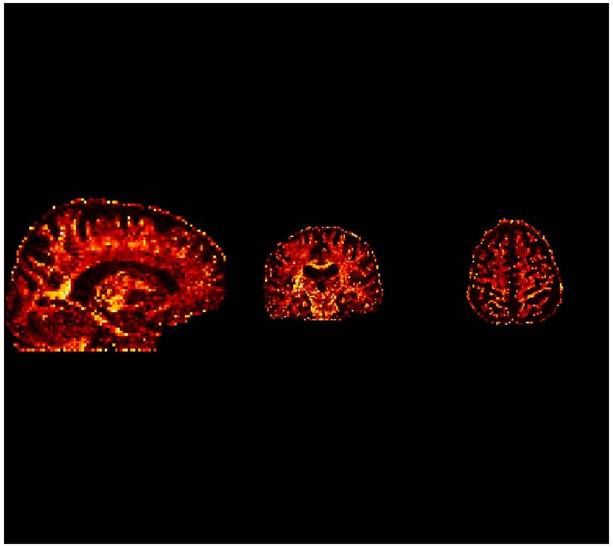


streamline image (RBG)

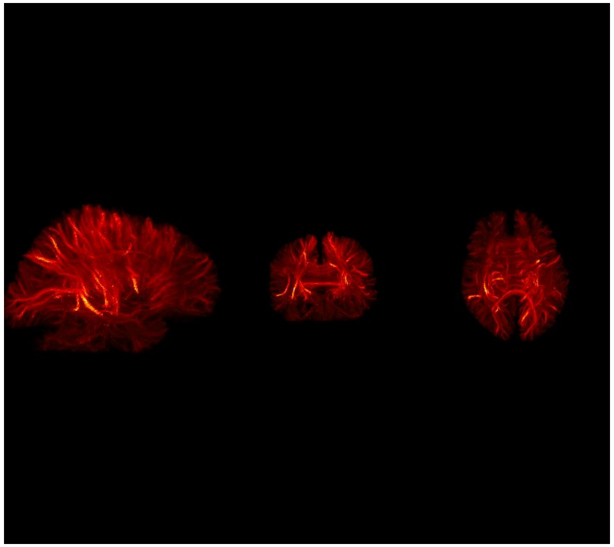
DWI Processing: BEDPOSTX

Output: Fibres orientation at each voxel

- merged_f<i>samples - anisotropic volume fraction



anisotropic fraction



fiber tractography

DWI Processing: PROBTRACKX

PROBTRACKX requires these files from bedpostX directory

- merged_th<i>samples
- merged_ph<i>samples
- merged_f<i>samples
- nodif_brain_mask

PROBTRACKX works in sequence

- drawing an orientation from the bedpostX distributions
- taking a step in this direction
- checking for any termination criteria

DWI Processing: PROBTRACKX

PROBTRACKX still needs these files

- Seed region: defines where the streamlines originate
- Termination masks: stops the streamlines
- Waypoint/exclusion masks: filters out those streamlines not relevant

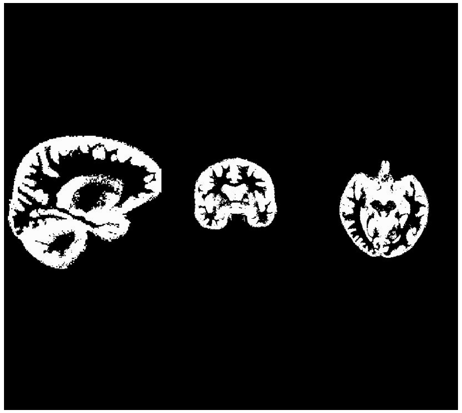
DWI Processing: PROBTRACKX

PROBTRACKX output:

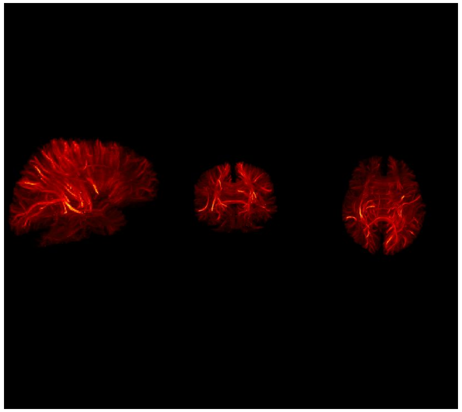
- probtrackx.log: record of the command that was run
- fdt.log: record of FDT GUI
- waytotal: text file containing total number of tracts
- **fdt_paths: Streamline density map**

DWI Processing: PROBTRACKX

PROBTRACKX calculates the similarity of streamline nearby to get the connectivity



- Mask (Our case):
  - Grey matter Mask
  - CSF Mask

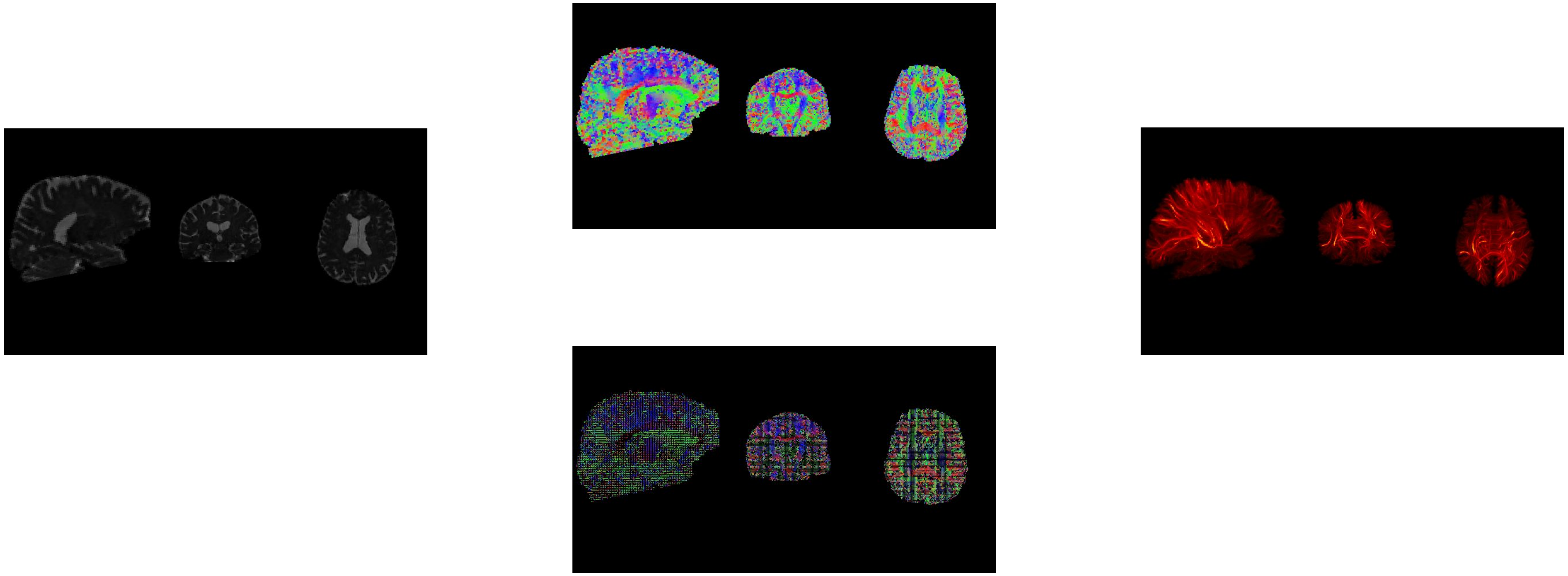


- Output:
  - Connectivity of the white matter

Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

DWI Processing: PROBTRACKX

PROBTRACKX calculates the similarity of streamline nearby to get the connectivity



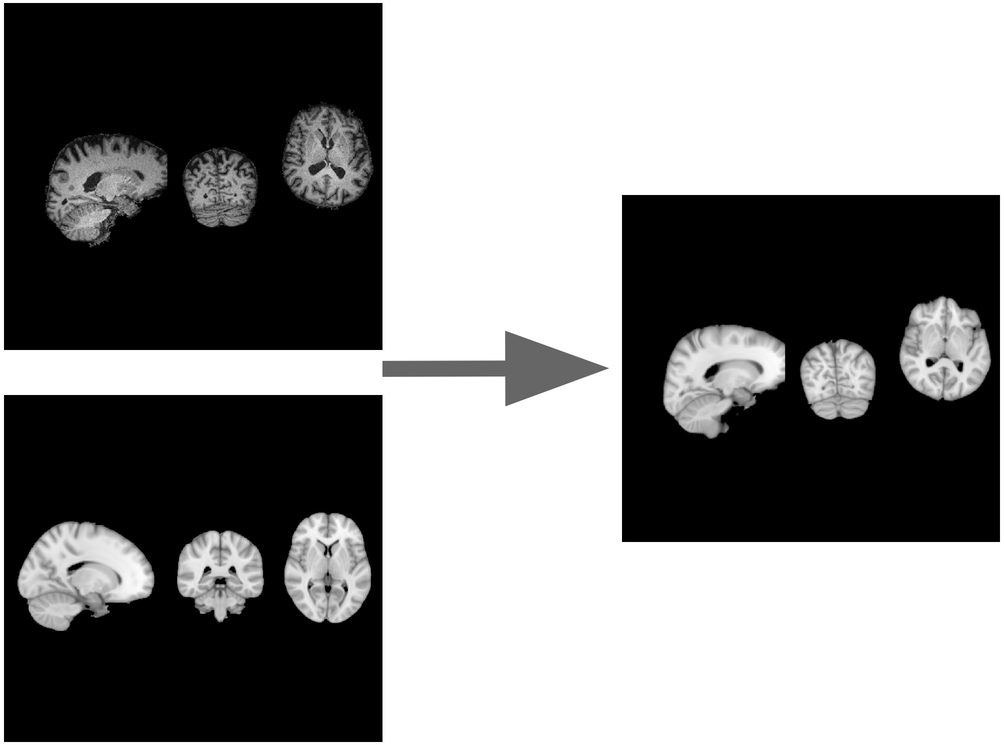
DWI brain volume → Voxel orientation → Tracking

Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

GUI & Code analysis

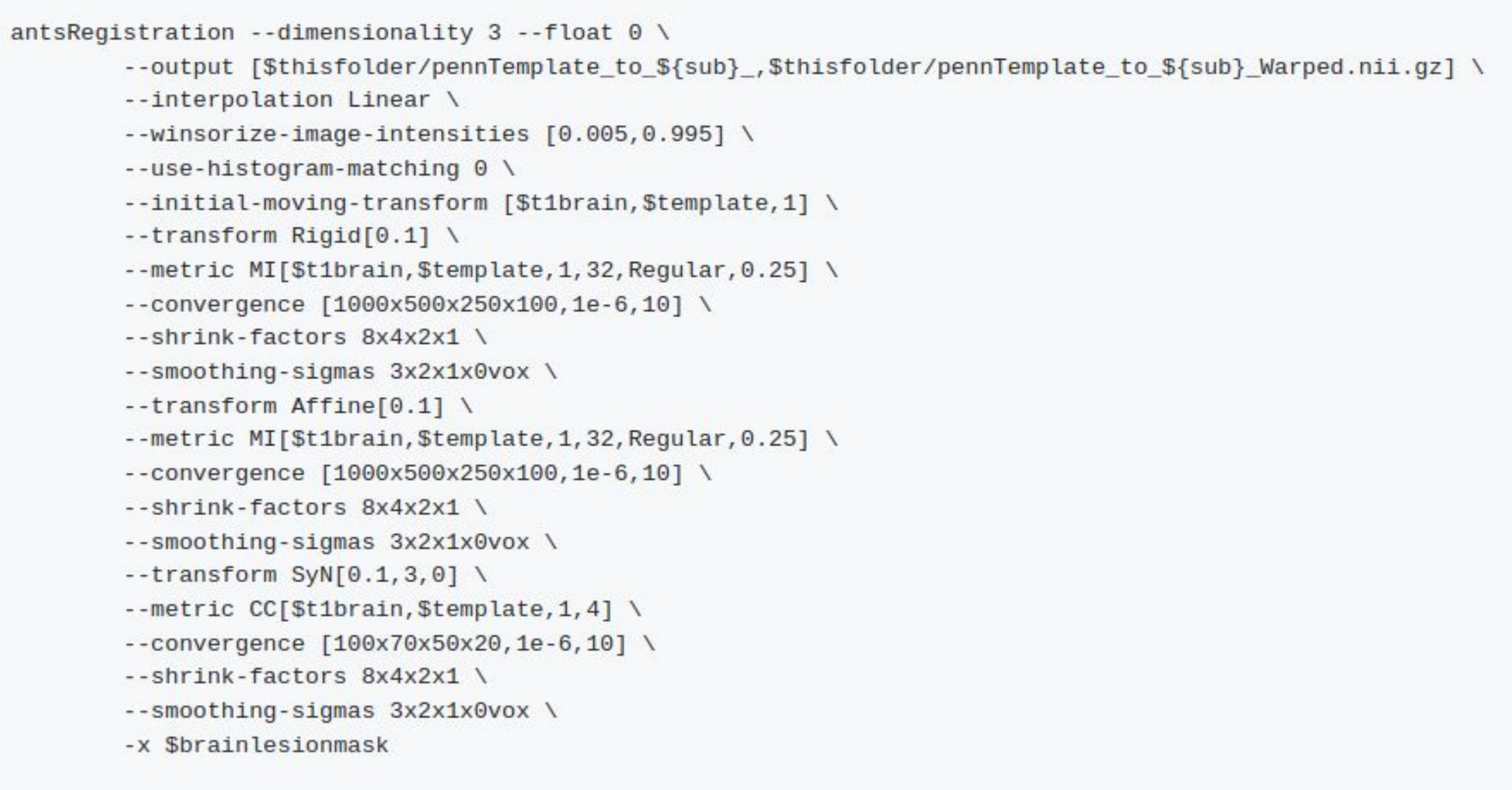
Analysis: ANTS and antsRegistration

- Input :
  - MNI152_T1_1mm
  - Reference: Reconstructed brain
- Output:
  - Registered image
  - Registration matrix



Data: EiMa 54, First Session, T1; Methodology: Antregistration

Analysis: ANTS and antsRegistration



```
antsRegistration --dimensionality 3 --float 0 \
        --output [$thisfolder/pennTemplate_to_${sub}_,$thisfolder/pennTemplate_to_${sub}_Warped.nii.gz] \
        --interpolation Linear \
        --winsorize-image-intensities [0.005,0.995] \
        --use-histogram-matching 0 \
        --initial-moving-transform [$t1brain,$template,1] \
        --transform Rigid[0.1] \
        --metric MI[$t1brain,$template,1,32,Regular,0.25] \
        --convergence [1000x500x250x100,1e-6,10] \
        --shrink-factors 8x4x2x1 \
        --smoothing-sigmas 3x2x1x0vox \
        --transform Affine[0.1] \
        --metric MI[$t1brain,$template,1,32,Regular,0.25] \
        --convergence [1000x500x250x100,1e-6,10] \
        --shrink-factors 8x4x2x1 \
        --smoothing-sigmas 3x2x1x0vox \
        --transform SyN[0.1,3,0] \
        --metric CC[$t1brain,$template,1,4] \
        --convergence [100x70x50x20,1e-6,10] \
        --shrink-factors 8x4x2x1 \
        --smoothing-sigmas 3x2x1x0vox \
        -x $brainlesionmask
```

Data: EiMa 54, First Session, T1; Methodology: Antregistration

Analysis: ANTS and antsRegistration

```
--winsorize-image-intensities [0.005,0.995] \
```

Winsorization replace outliers with the minimum and maximum

```
--use-histogram-matching 0 \
```

Transform the input intensities such that the histogram of the fixed and moving image are matched as closely as possible

Analysis: ANTS and antsRegistration

```
--initial-moving-transform [$t1brain,$template,1] \
```

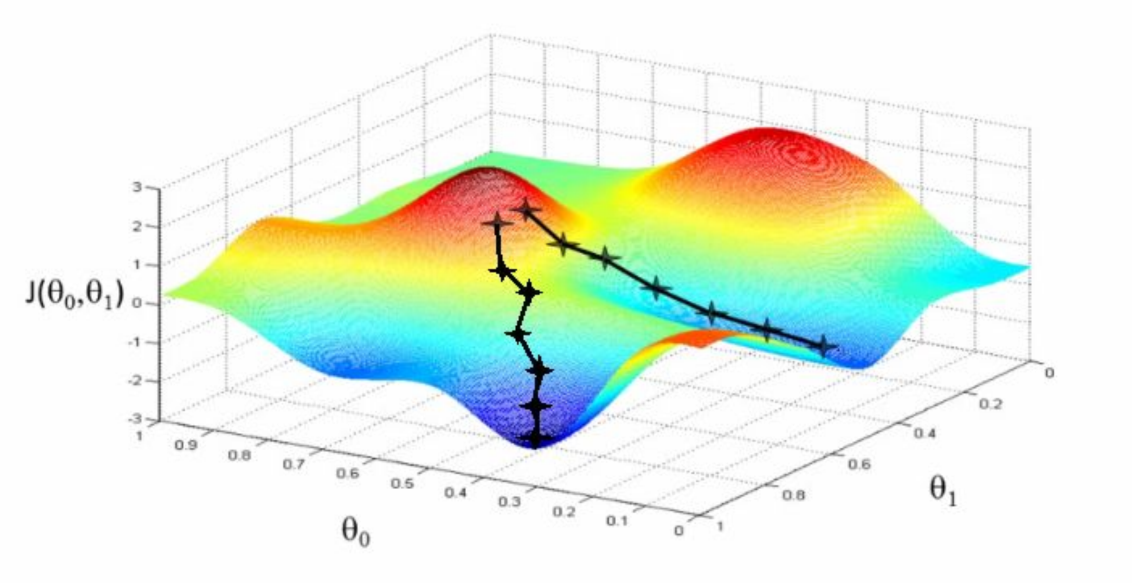
Roughly align the images before registration

Data: EiMa 54, First Session, T1; Methodology: Antregistration

Analysis: ANTS and antsRegistration

```
--transform Rigid[0.1] \
```

Transformation function of registration, most easy one is Rigid, it could also use affin, SyN or the other. The parameter here is the gradient.



Data: EiMa 54, First Session, T1; Methodology: Antregistration

Analysis: ANTS and antsRegistration

```
--metric MI[$t1brain,$template,1,32,Regular,0.25] \
```

Similarity metrics to describe the quality of the registration for example,

MI: Mutual information- check the histogram, good for multi modality

CC: Cross correlation - check the neighbour similarity, good for unimodality

Data: EiMa 54, First Session, T1; Methodology: Antregistration

Analysis: ANTS and antsRegistration

```
--convergence [1000x500x250x100,1e-6,10] \
```

When Similarity is smaller than the threshold, the registration will stop

```
--shrink-factors 8x4x2x1 \
```

The registration is in different resolution levels

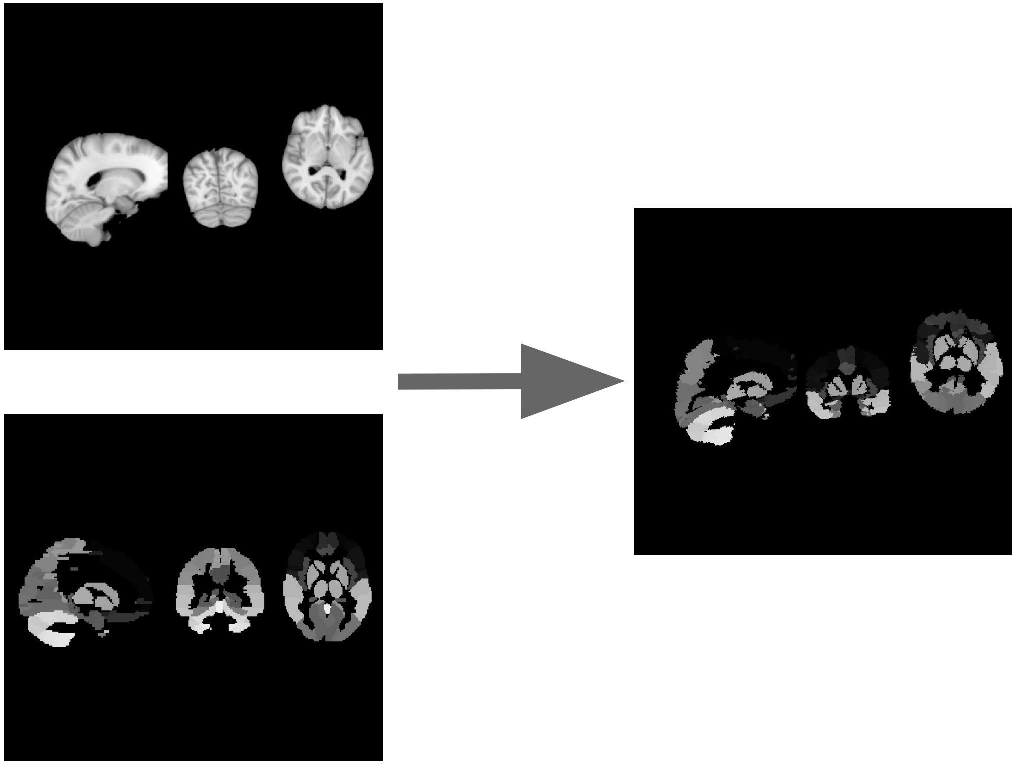
```
--smoothing-sigmas 3x2x1x0vox \
```

Smoothing after the resolution

Data: EiMa 54, First Session, T1; Methodology: Antregistration

Analysis: ANTS and antsApplyTransforms

- Input :
  - rAAL2_1mm
  - Reference: Reconstructed brain
- Output:
  - Atlas brain region
  - Transform matrix



Data: EiMa 54, First Session, T1; Methodology: Anttrasformation

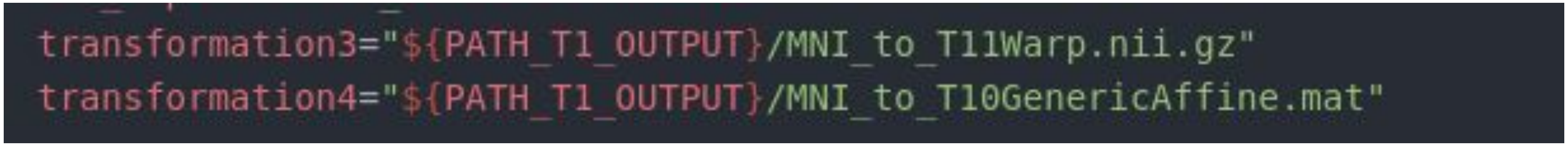
Analysis: ANTS and antsApplyTransforms

```
-d, --dimensionality 2/3/4
-i, --input inputFileName
-r, --reference-image imageFileName
-o, --output warpedOutputFileName

-n, --interpolation Linear, NearestNeighbor
MultiLabel, Gaussian BSpline, CosineWindowedSinc,
WelchWindowedSinc, HammingWindowedSinc,
LanczosWindowedSinc, GenericLabel
```

Data: EiMa 54, First Session, T1; Methodology: Antregistration

Analysis: ANTS and antsApplyTransforms

`-t, --transform transformFileName`



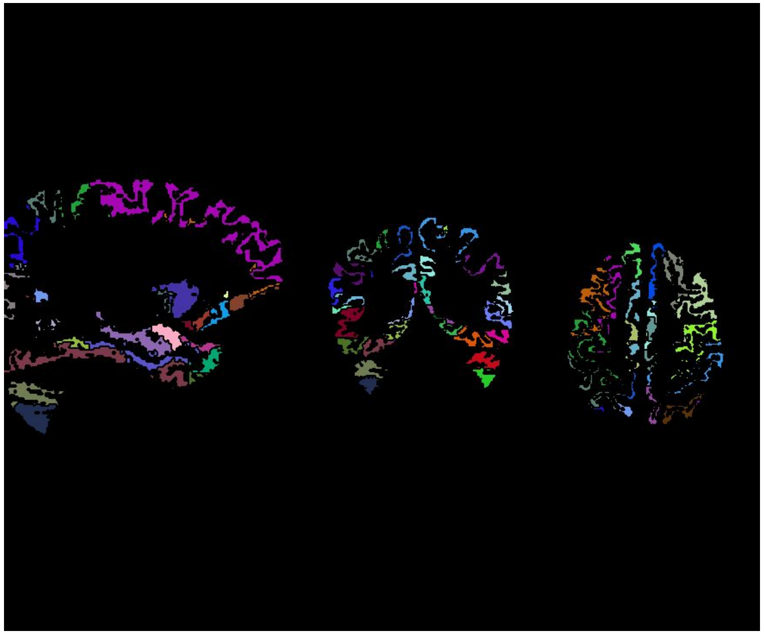
```
transformation3="${PATH_T1_OUTPUT}/MNI_to_T11Warp.nii.gz"
transformation4="${PATH_T1_OUTPUT}/MNI_to_T10GenericAffine.mat"
```

In our case: MNI_to_T11Warp.nii.gz and then MNI_to_T10GenericAffine.mat. Because we would like to use the same transformation from registration, so that the standard brain region could be registered.

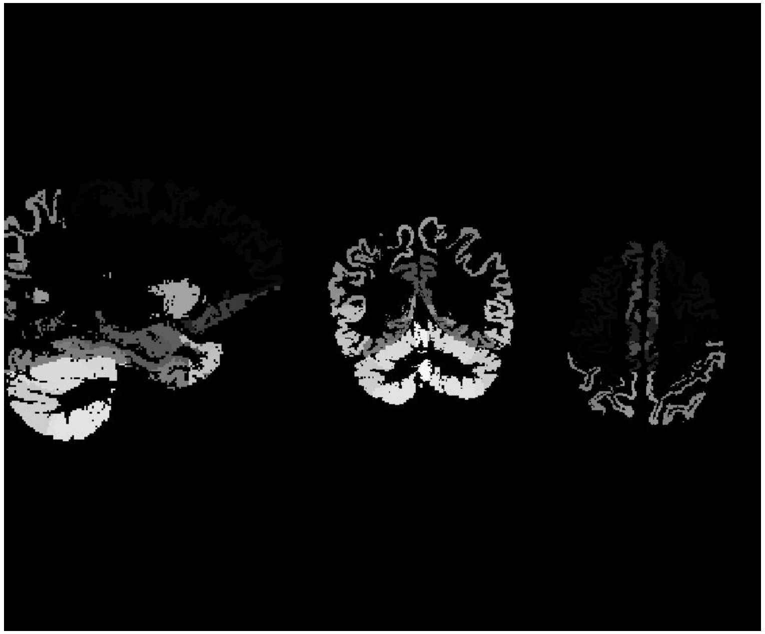
Data: EiMa 54, First Session, T1; Methodology: Antregistration

T1 Processing: Atlas - AAL2

Comparison



Labeled image

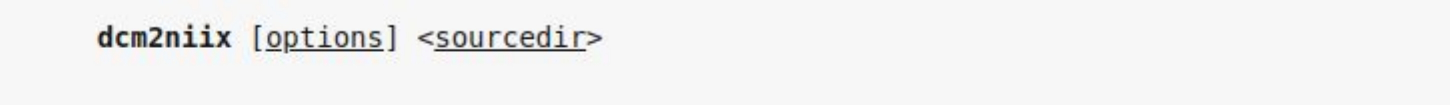


Volume image

Data: EiMa 54, First Session, T1; Methodology: Anttrasformation

Analysis: DWI Processing

1. scanner-specific pre-processing (e.g., conversion from DICOM to NIFTI using for example dcm2niix)



```
dcm2niix [options] <sourcedir>
```

2. Susceptibility-induced distortion correction (fieldmap estimation) using **topup**

   Or **eddy** - Distortion correction using eddy.

3. **bet** - Brain extraction using BET on the distortion-corrected b0 (output of topup)

microstructural analysis or **tractography**

Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

Analysis: DWI Processing

Microstructural analysis

1. dtifit - Fitting of diffusion tensors on eddy-corrected data

2. TBSS - Comparison of the fractional anisotropy maps (or others) between subjects

Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

Analysis: DWI Processing

tractography

1. **bedpostx - Fitting of the probabilistic diffusion model on corrected data**
2. Registration to structural reference image and/or standard space
3. **probtrackx - Probabilistic tractography run on the output of bedpostx**

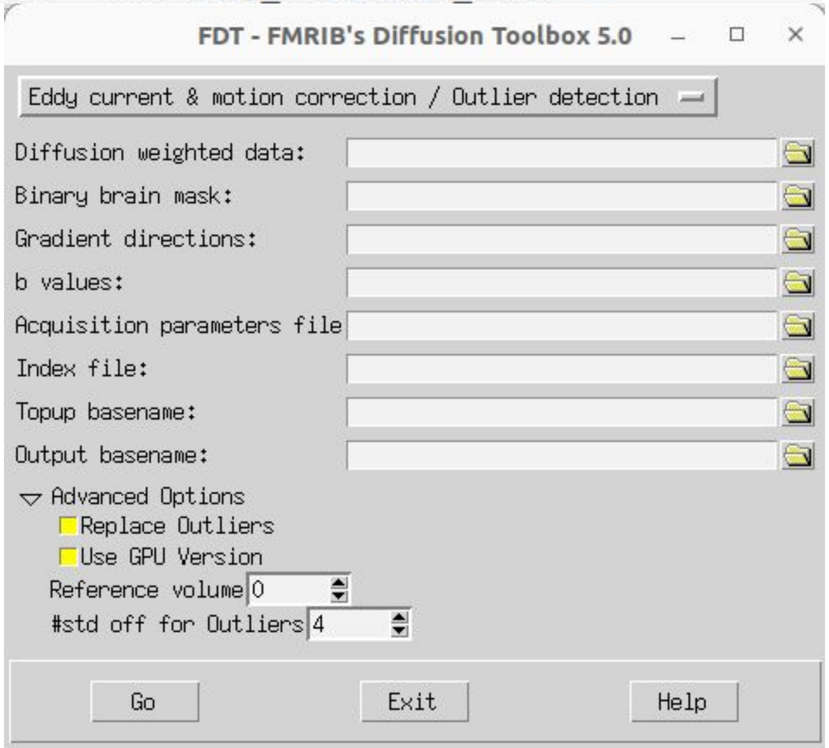
Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

Analysis: DWI Processing

eddy - Distortion correction

```
eddy --imain=data --mask=my_hifi_b0_brain_mask --acqp=acqparams.txt --index=index.txt --
bvecs=bvecs --bvals=bvals --topup=my_topup_results --out=eddy_corrected_data
```

https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/eddy/UsersGuide


FDT - FMRIB's Diffusion Toolbox 5.0

Eddy current & motion correction / Outlier detection

Diffusion weighted data:

Binary brain mask:

Gradient directions:

b values:

Acquisition parameters file

Index file:

Topup basename:

Output basename:

Advanced Options

□ Replace Outliers

□ Use GPU Version

Reference volume 0

#std off for Outliers 4

Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

Analysis: DWI Processing

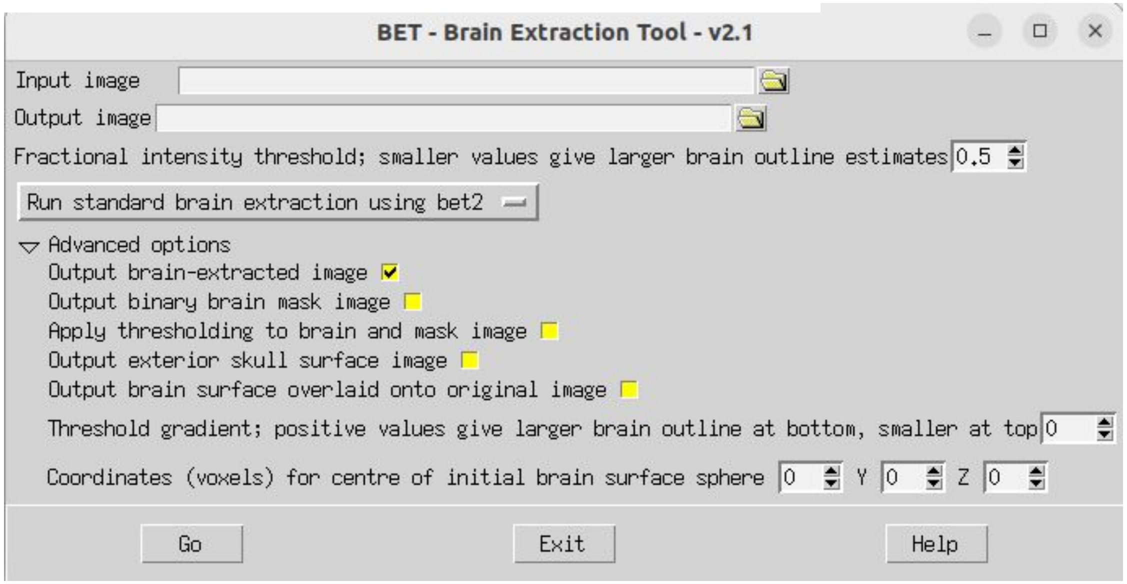
bet - Brain Extraction

```
bet <input> <output> [options]
```

`-f <f>` fractional intensity threshold (0->1); default=0.5; smaller values give larger brain outline estimates

`-m` generate binary brain mask

https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET/UserGuide



BET - Brain Extraction Tool - v2.1

Input image

Output image

Fractional intensity threshold; smaller values give larger brain outline estimates 0.5

Run standard brain extraction using bet2

Advanced options

Output brain-extracted image ☑

Output binary brain mask image □

Apply thresholding to brain and mask image □

Output exterior skull surface image □

Output brain surface overlaid onto original image □

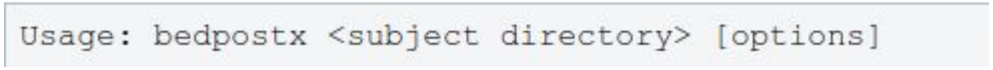
Threshold gradient; positive values give larger brain outline at bottom, smaller at top 0

Coordinates (voxels) for centre of initial brain surface sphere 0 Y 0 Z 0

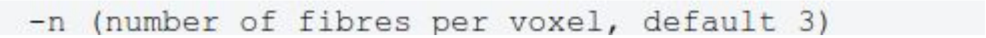
Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

Analysis: DWI Processing

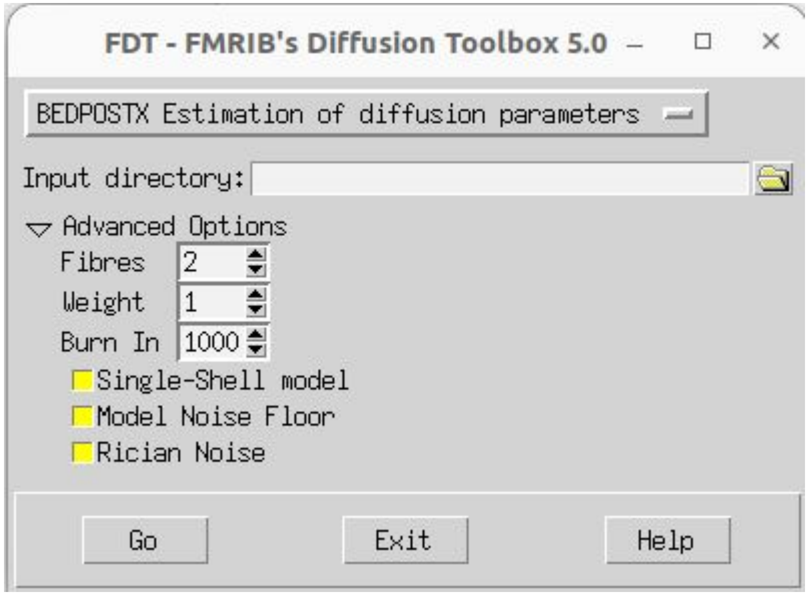
BEDPOSTX - Tool for estimating fibre orientations
Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques.



```
Usage: bedpostx <subject directory> [options]
```



```
 -n (number of fibres per voxel, default 3)
```



FDT - FMRIB's Diffusion Toolbox 5.0

BEDPOSTX Estimation of diffusion parameters

Input directory:

Advanced Options

Fibres: 2

Weight: 1

Burn In: 1000

□ Single-Shell model

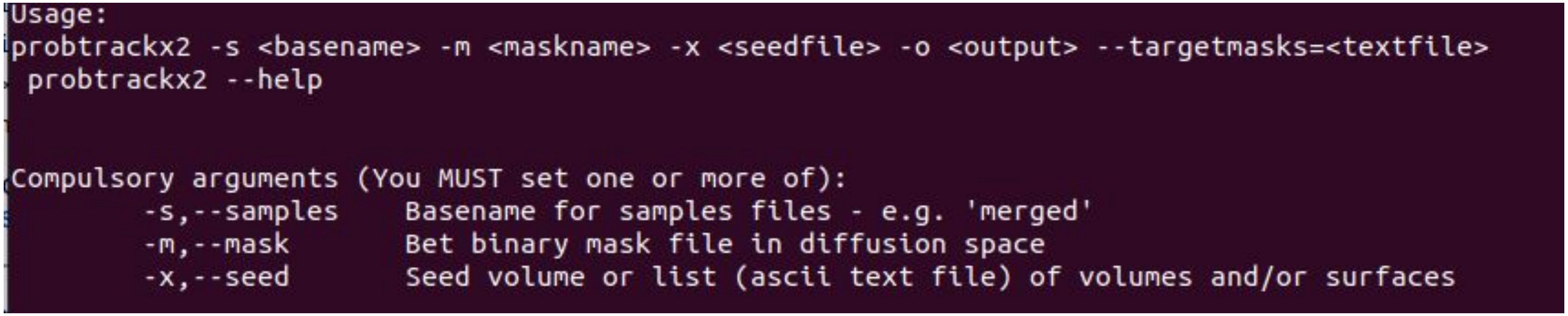
□ Model Noise Floor

□ Rician Noise

https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT/UserGuide

Analysis: DWI Processing

PROBTRACKX - performing probabilistic tractography

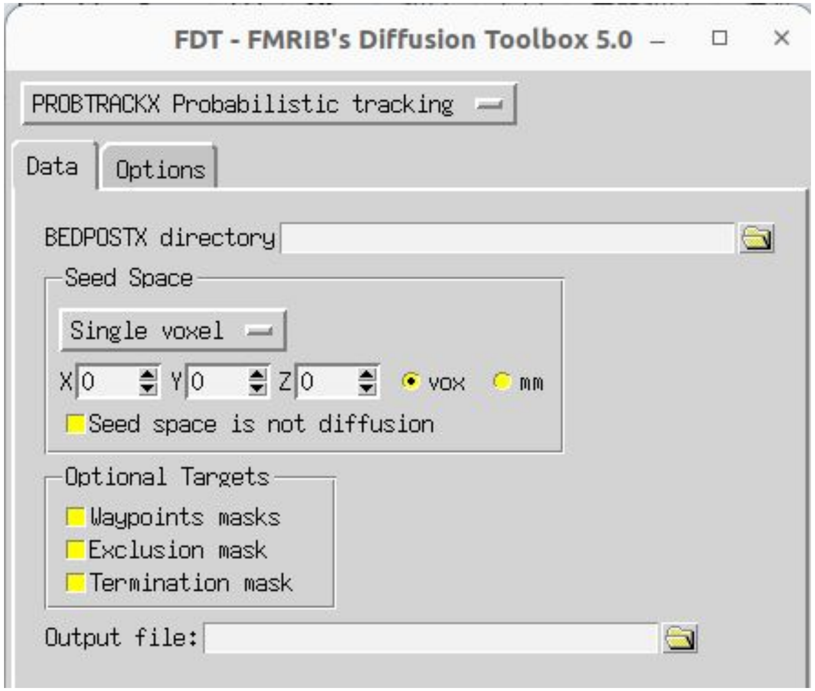


```
Usage:
probtrackx2 -s <basename> -m <maskname> -x <seedfile> -o <output> --targetmasks=<textfile>
 probtrackx2 --help


Compulsory arguments (You MUST set one or more of):
        -s,--samples    Basename for samples files - e.g. 'merged'
        -m,--mask       Bet binary mask file in diffusion space
        -x,--seed       Seed volume or list (ascii text file) of volumes and/or surfaces
```

Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

Analysis: DWI Processing

PROBTRACKX - performing probabilistic tractography



FDT - FMRIB's Diffusion Toolbox 5.0

PROBTRACKX Probabilistic tracking

BEDPOSTX directory

Seed Space

Single voxel

X 0 Y 0 Z 0 ● vox ○ mm

□ Seed space is not diffusion

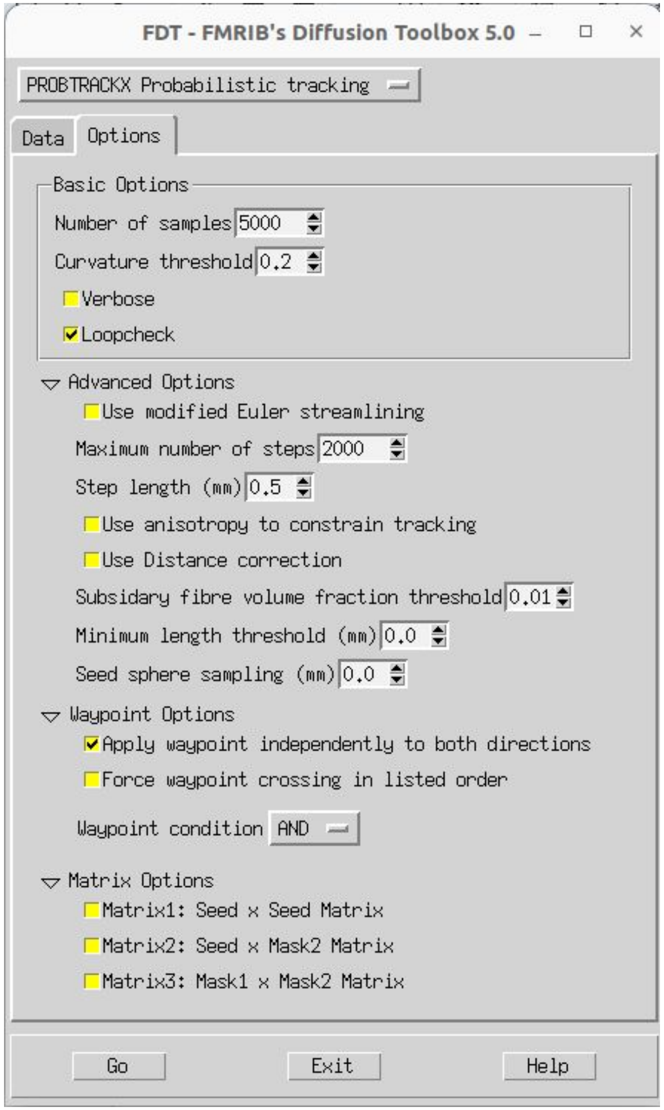
Optional Targets

□ Waypoints masks

□ Exclusion mask

□ Termination mask

Output file:



FDT - FMRIB's Diffusion Toolbox 5.0

PROBTRACKX Probabilistic tracking

Basic Options

Number of samples 5000

Curvature threshold 0.2

□ Verbose

☑ Loopcheck

Advanced Options

□ Use modified Euler streamlining

Maximum number of steps 2000

Step length (mm) 0.5

□ Use anisotropy to constrain tracking

□ Use Distance correction

Subsidary fibre volume fraction threshold 0.01

Minimum length threshold (mm) 0.0

Seed sphere sampling (mm) 0.0

Waypoint Options

☑ Apply waypoint independently to both directions

□ Force waypoint crossing in listed order

Waypoint condition AND

Matrix Options

□ Matrix1: Seed x Seed Matrix

□ Matrix2: Seed x Mask2 Matrix

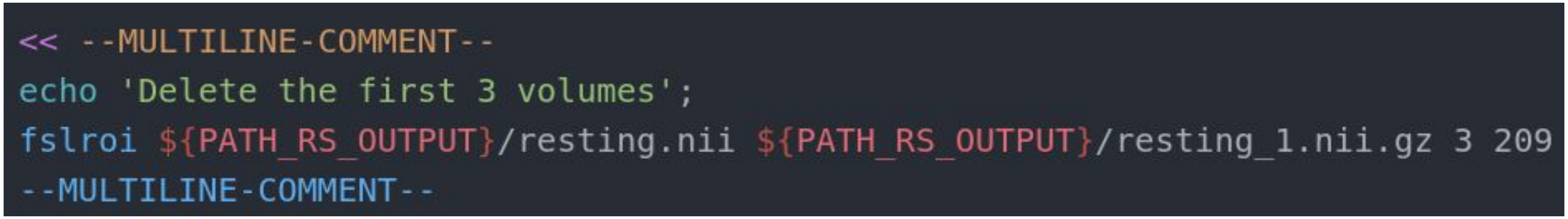
□ Matrix3: Mask1 x Mask2 Matrix

Data: EiMa 54, First Session, DWI; Methodology: FSL:PROBTRACKX

Resting State Processing

Resting State Processing

Select the region of interest

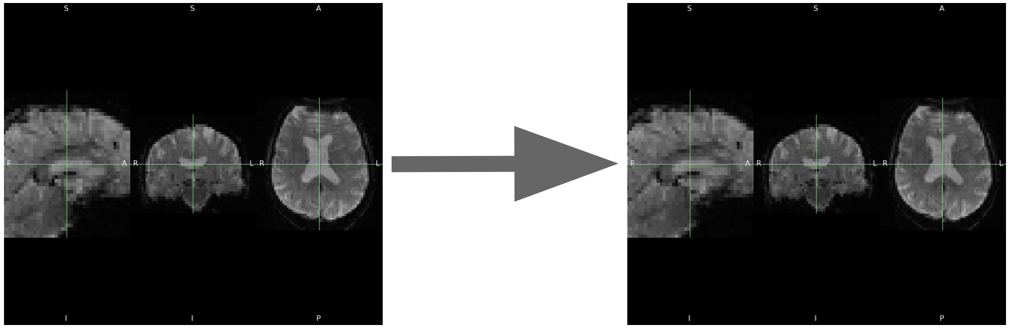


```
<< --MULTILINE-COMMENT--
echo 'Delete the first 3 volumes';
fslroi ${PATH_RS_OUTPUT}/resting.nii ${PATH_RS_OUTPUT}/resting_1.nii.gz 3 209
--MULTILINE-COMMENT--
```

- Input :
  - resting.nii

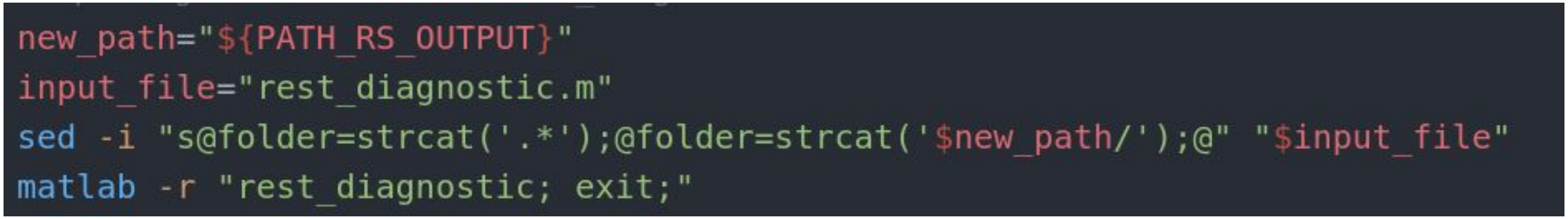
  212 - 3 = 209

- Output:
  - resting_1.nii



Resting State Processing

Diagnostic with tsdiffana, run rest_diagnostic.m in matlab



```
new_path="${PATH_RS_OUTPUT}"
input_file="rest_diagnostic.m"
sed -i "s@folder=strcat('.*');@folder=strcat('$new_path/');@" "$input_file"
matlab -r "rest_diagnostic; exit;"
```

- Input :
    - resting.nii
- Output:
    - The diagnose

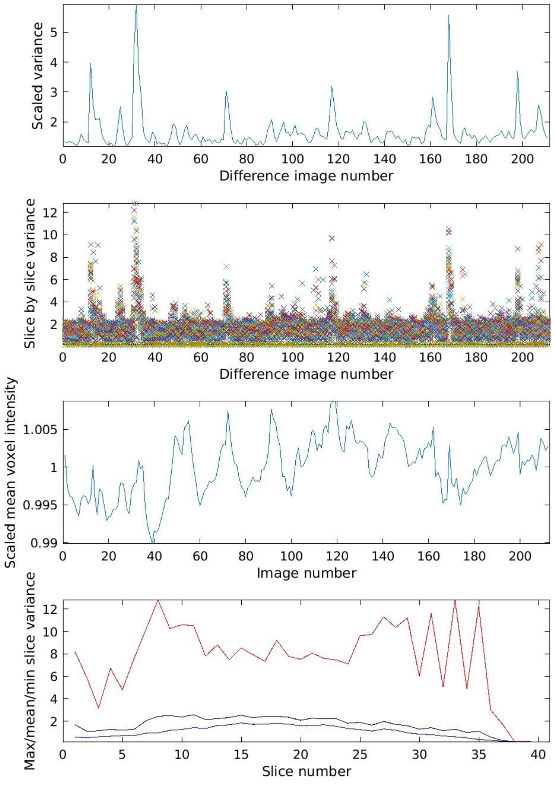
Data: EiMa 54, First Session, RS

Resting State Processing

Diagnostic with tsdiffana, run rest_diagnostic.m in matlab

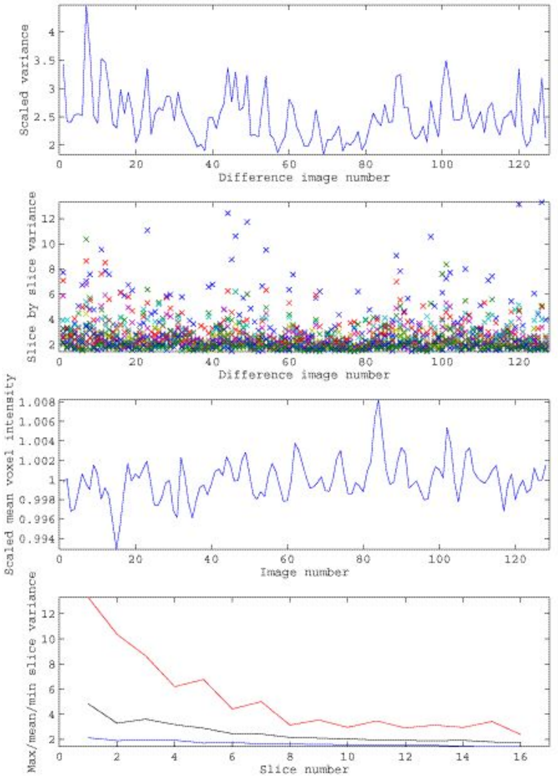
- Input :
  - resting.nii
- Output:
  - The diagnose

Output



First image:
./output/sub-EiMa54/ses-1/func/resting.nii

Normal

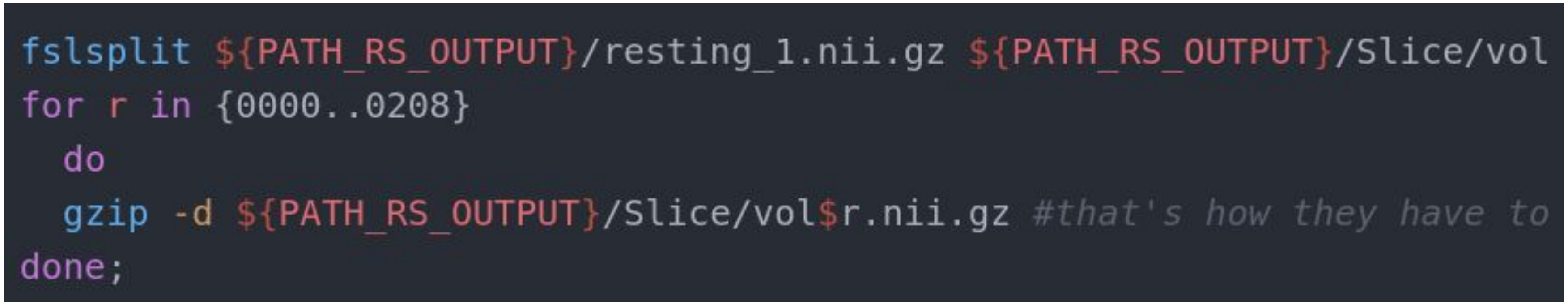


First image:
/cbu/imagers/data/matthew.brett/ER/010160/6/010160-m00 13 EPI 0011.img

Data: EiMa 54, First Session, RS

Resting State Processing

Split into volume, useful for the next step



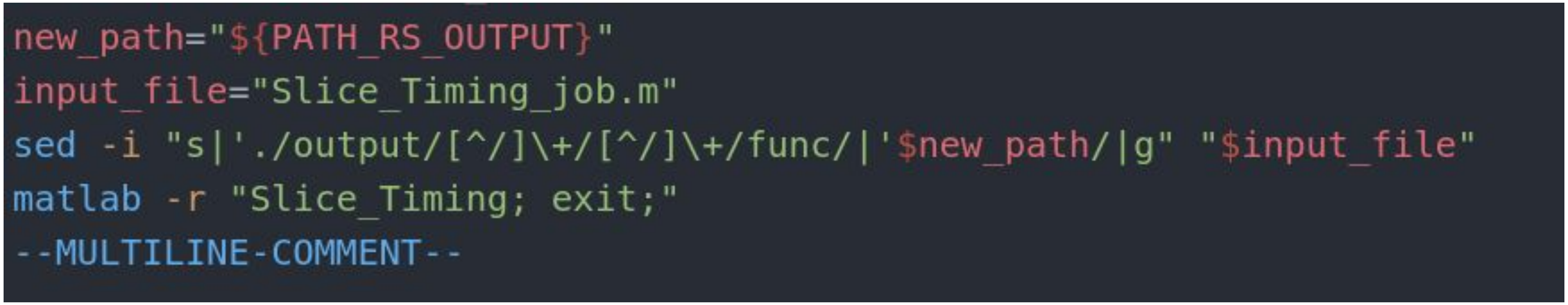
```
fslsplit ${PATH_RS_OUTPUT}/resting_1.nii.gz ${PATH_RS_OUTPUT}/Slice/vol
for r in {0000..0208}
  do
  gzip -d ${PATH_RS_OUTPUT}/Slice/vol$r.nii.gz #that's how they have to
done;
```

- Input :
  - ○ The modified volume(4D)
- Output:
  - ○ Volume of the image (3D volume according to time sequence)

Data: EiMa 54, First Session, RS

Resting State Processing

Volume time correction



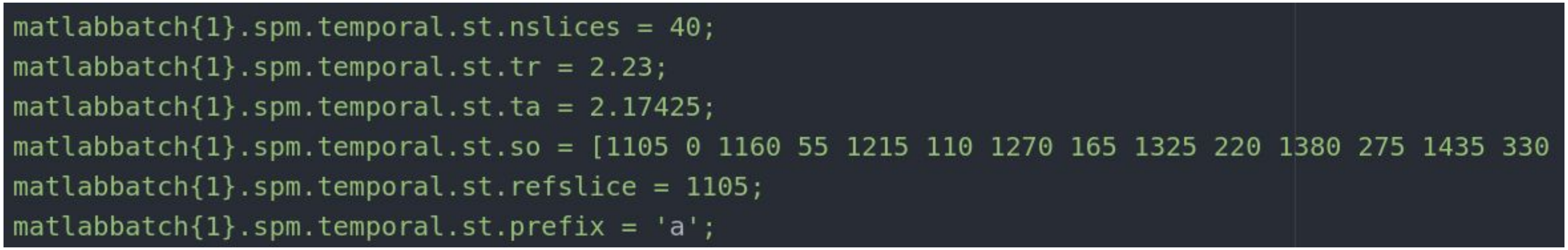
```
new_path="${PATH_RS_OUTPUT}"
input_file="Slice_Timing_job.m"
sed -i "s|'./output/[^/]\+/[^/]\+/func/|'$new_path/|g" "$input_file"
matlab -r "Slice_Timing; exit;"
--MULTILINE-COMMENT--
```

- Input :
    - Volume of the image
- Output:
    - resliced volume

Data: EiMa 54, First Session, RS

Resting State Processing

Slice time correction



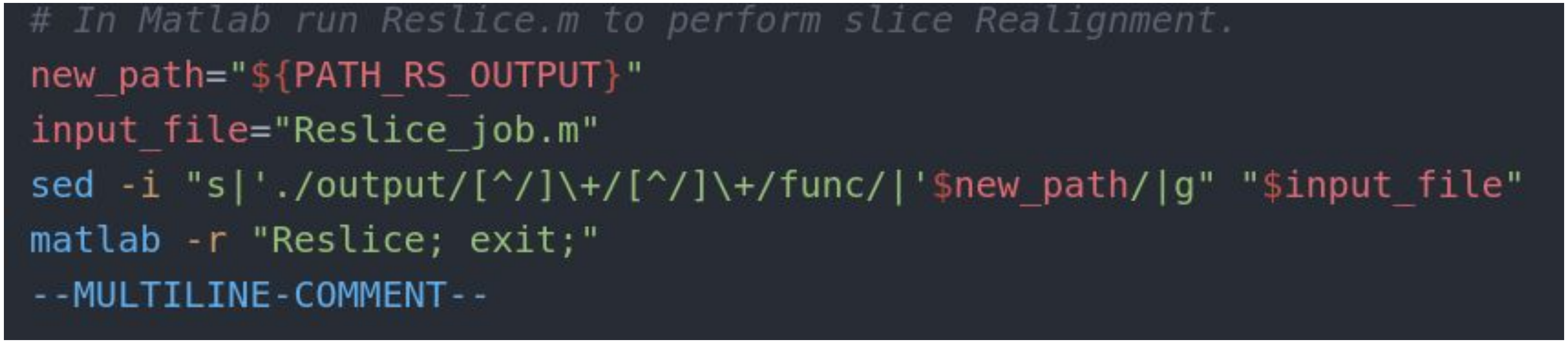
```
matlabbatch{1}.spm.temporal.st.nslices = 40;
matlabbatch{1}.spm.temporal.st.tr = 2.23;
matlabbatch{1}.spm.temporal.st.ta = 2.17425;
matlabbatch{1}.spm.temporal.st.so = [1105 0 1160 55 1215 110 1270 165 1325 220 1380 275 1435 330
matlabbatch{1}.spm.temporal.st.refslice = 1105;
matlabbatch{1}.spm.temporal.st.prefix = 'a';
```

- Input :
    - slice of the image
- Output:
    - re sliced volume

Data: EiMa 54, First Session, RS

Resting State Processing

Slice time realign

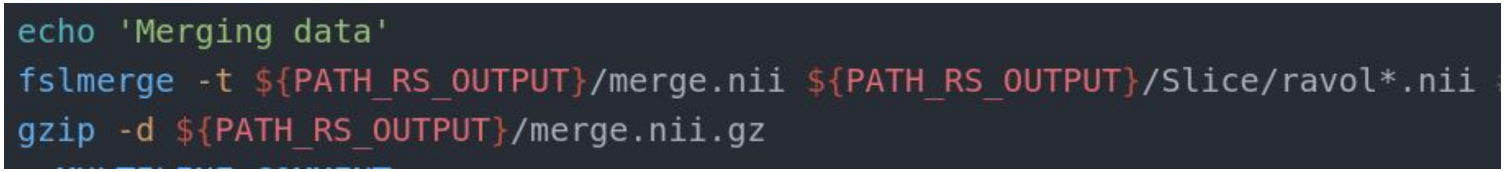


```
# In Matlab run Reslice.m to perform slice Realignment.
new_path="${PATH_RS_OUTPUT}"
input_file="Reslice_job.m"
sed -i "s|'./output/[^/]\+/[^/]\+/func/|'$new_path/|g" "$input_file"
matlab -r "Reslice; exit;"
--MULTILINE-COMMENT--
```

- Input :
    - slice of the image
- Output:
    - resliced volume

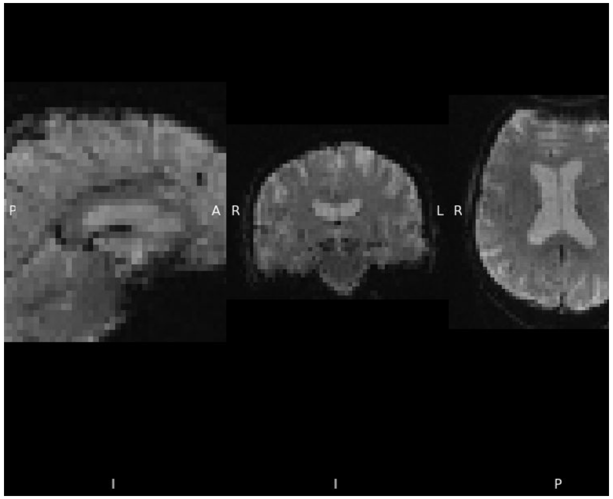
Resting State Processing

Merge the slice



```
echo 'Merging data'
fslmerge -t ${PATH_RS_OUTPUT}/merge.nii ${PATH_RS_OUTPUT}/Slice/ravol*.nii
gzip -d ${PATH_RS_OUTPUT}/merge.nii.gz
```

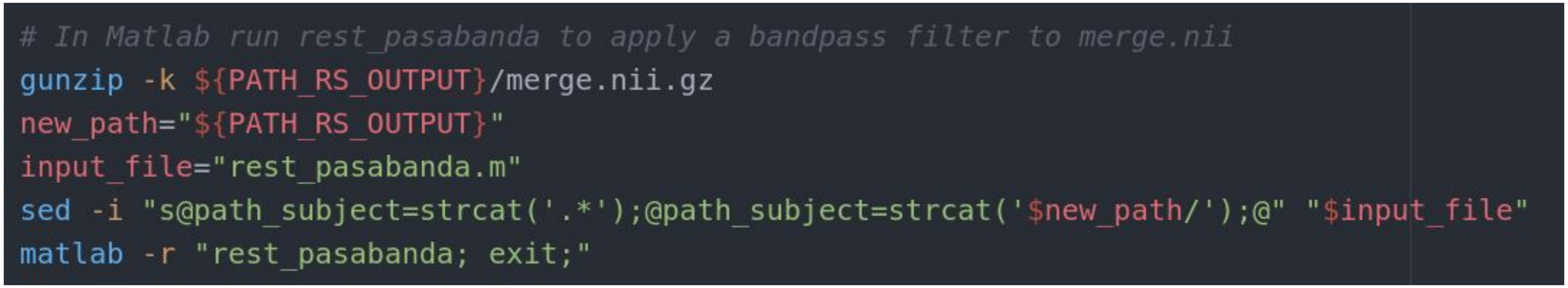
- Input :
  - slice of the image (3D)
- Output:
  - merged one (4D)



Data: EiMa 54, First Session, RS

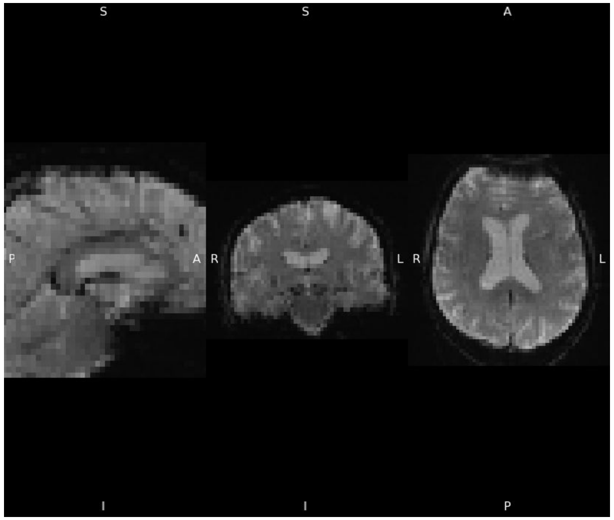
Resting State Processing

Apply a band pass filter



```
# In Matlab run rest_pasabanda to apply a bandpass filter to merge.nii
gunzip -k ${PATH_RS_OUTPUT}/merge.nii.gz
new_path="${PATH_RS_OUTPUT}"
input_file="rest_pasabanda.m"
sed -i "s@path_subject=strcat('.*');@path_subject=strcat('$new_path/');@" "$input_file"
matlab -r "rest_pasabanda; exit;"
```

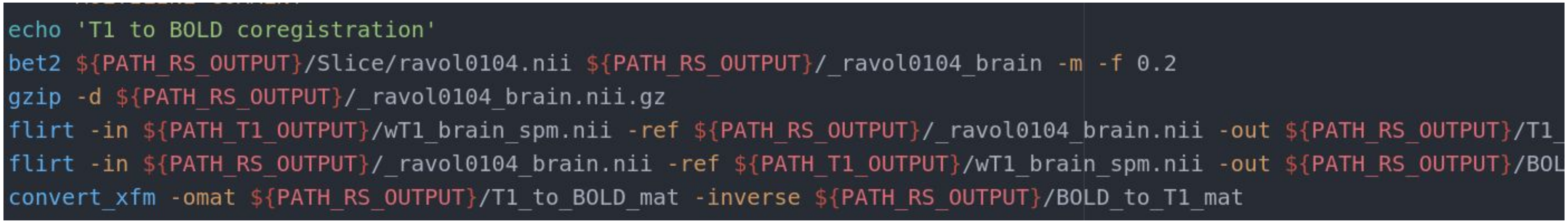
- Input :
    - Merged image
- Output:
    - filtered one



Data: EiMa 54, First Session, RS

Resting State Processing

T1 and BOLD registration



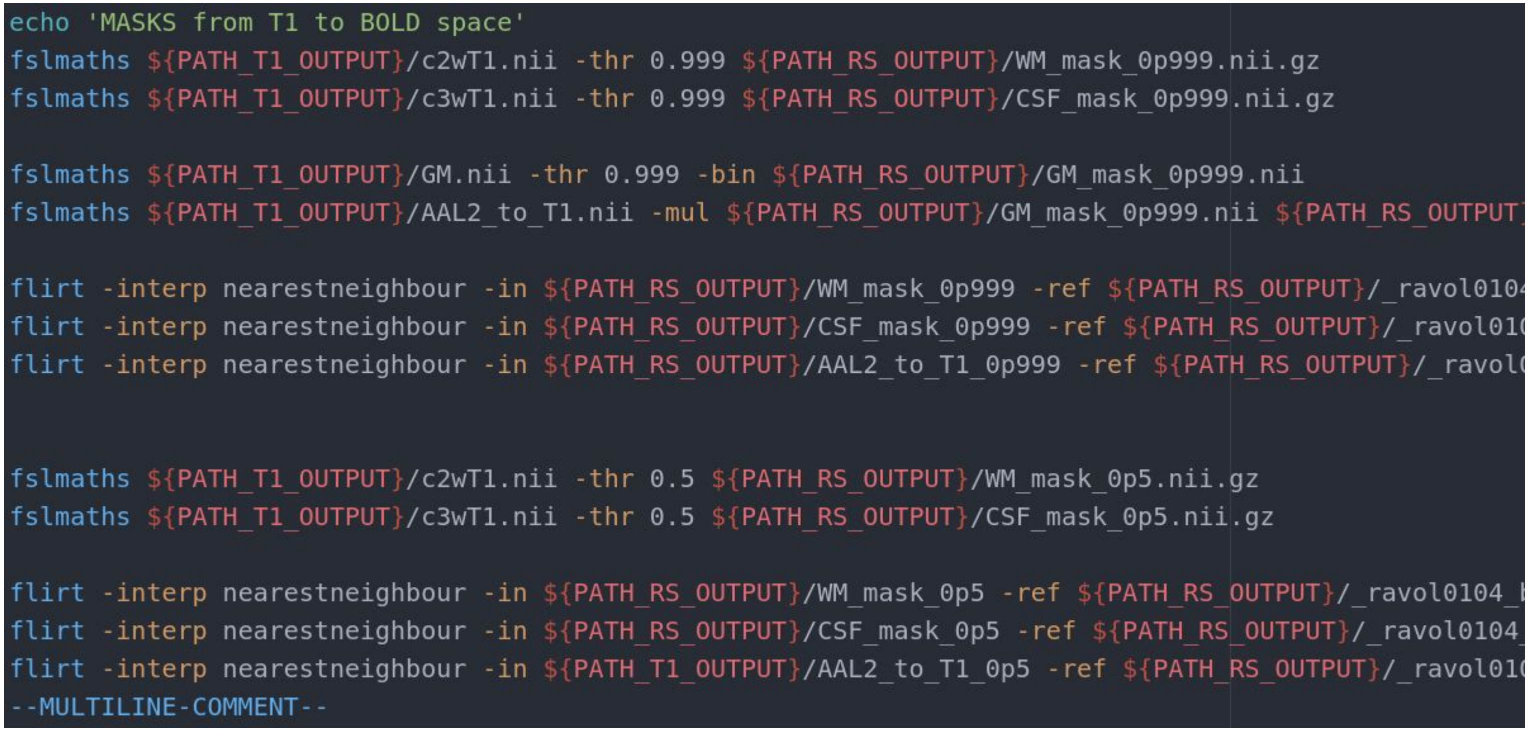
```
echo 'T1 to BOLD coregistration'
bet2 ${PATH_RS_OUTPUT}/Slice/ravol0104.nii ${PATH_RS_OUTPUT}/_ravol0104_brain -m -f 0.2
gzip -d ${PATH_RS_OUTPUT}/_ravol0104_brain.nii.gz
flirt -in ${PATH_T1_OUTPUT}/wT1_brain_spm.nii -ref ${PATH_RS_OUTPUT}/_ravol0104_brain.nii -out ${PATH_RS_OUTPUT}/T1_
flirt -in ${PATH_RS_OUTPUT}/_ravol0104_brain.nii -ref ${PATH_T1_OUTPUT}/wT1_brain_spm.nii -out ${PATH_RS_OUTPUT}/BOL
convert_xfm -omat ${PATH_RS_OUTPUT}/T1_to_BOLD_mat -inverse ${PATH_RS_OUTPUT}/BOLD_to_T1_mat
```

- Input :
  - T1 and Bold images
- Output:
  - registered images and transformation matrices

Resting State Processing

T1 to BOLD masks

Use AAL2 replacing Schaefer



```
echo 'MASKS from T1 to BOLD space'
fslmaths ${PATH_T1_OUTPUT}/c2wT1.nii -thr 0.999 ${PATH_RS_OUTPUT}/WM_mask_0p999.nii.gz
fslmaths ${PATH_T1_OUTPUT}/c3wT1.nii -thr 0.999 ${PATH_RS_OUTPUT}/CSF_mask_0p999.nii.gz

fslmaths ${PATH_T1_OUTPUT}/GM.nii -thr 0.999 -bin ${PATH_RS_OUTPUT}/GM_mask_0p999.nii
fslmaths ${PATH_T1_OUTPUT}/AAL2_to_T1.nii -mul ${PATH_RS_OUTPUT}/GM_mask_0p999.nii ${PATH_RS_OUTPUT

flirt -interp nearestneighbour -in ${PATH_RS_OUTPUT}/WM_mask_0p999 -ref ${PATH_RS_OUTPUT}/_ravol0104
flirt -interp nearestneighbour -in ${PATH_RS_OUTPUT}/CSF_mask_0p999 -ref ${PATH_RS_OUTPUT}/_ravol010
flirt -interp nearestneighbour -in ${PATH_RS_OUTPUT}/AAL2_to_T1_0p999 -ref ${PATH_RS_OUTPUT}/_ravol0


fslmaths ${PATH_T1_OUTPUT}/c2wT1.nii -thr 0.5 ${PATH_RS_OUTPUT}/WM_mask_0p5.nii.gz
fslmaths ${PATH_T1_OUTPUT}/c3wT1.nii -thr 0.5 ${PATH_RS_OUTPUT}/CSF_mask_0p5.nii.gz

flirt -interp nearestneighbour -in ${PATH_RS_OUTPUT}/WM_mask_0p5 -ref ${PATH_RS_OUTPUT}/_ravol0104_
flirt -interp nearestneighbour -in ${PATH_RS_OUTPUT}/CSF_mask_0p5 -ref ${PATH_RS_OUTPUT}/_ravol0104_
flirt -interp nearestneighbour -in ${PATH_T1_OUTPUT}/AAL2_to_T1_0p5 -ref ${PATH_RS_OUTPUT}/_ravol010
--MULTILINE-COMMENT--
```

2 options: threshold use 0.5 or 0.999

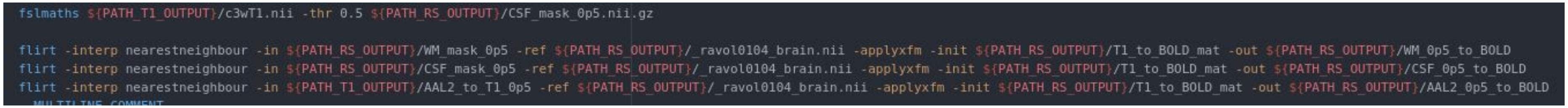
0.5 works better

Data: EiMa 54, First Session, RS

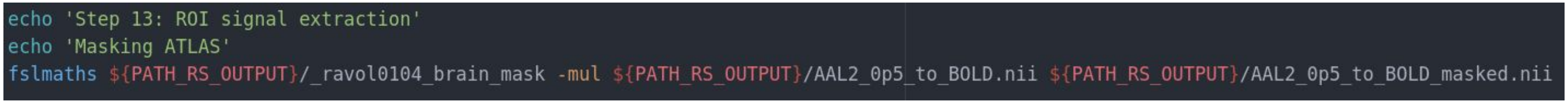
Resting State Processing

In resting state processing: No need to use AAL2_0p5_to_BOLD_masked.nii

We can use AAL2_0p5_to_BOLD.nii



```
fslmaths ${PATH_T1_OUTPUT}/c3wT1.nii -thr 0.5 ${PATH_RS_OUTPUT}/CSF_mask_0p5.nii.gz

flirt -interp nearestneighbour -in ${PATH_RS_OUTPUT}/WM_mask_0p5 -ref ${PATH_RS_OUTPUT}/_ravol0104_brain.nii -applyxfm -init ${PATH_RS_OUTPUT}/T1_to_BOLD_mat -out ${PATH_RS_OUTPUT}/WM_0p5_to_BOLD
flirt -interp nearestneighbour -in ${PATH_RS_OUTPUT}/CSF_mask_0p5 -ref ${PATH_RS_OUTPUT}/_ravol0104_brain.nii -applyxfm -init ${PATH_RS_OUTPUT}/T1_to_BOLD_mat -out ${PATH_RS_OUTPUT}/CSF_0p5_to_BOLD
flirt -interp nearestneighbour -in ${PATH_T1_OUTPUT}/AAL2_to_T1_0p5 -ref ${PATH_RS_OUTPUT}/_ravol0104_brain.nii -applyxfm -init ${PATH_RS_OUTPUT}/T1_to_BOLD_mat -out ${PATH_RS_OUTPUT}/AAL2_0p5_to_BOLD
  MULTILINE COMMENT
```

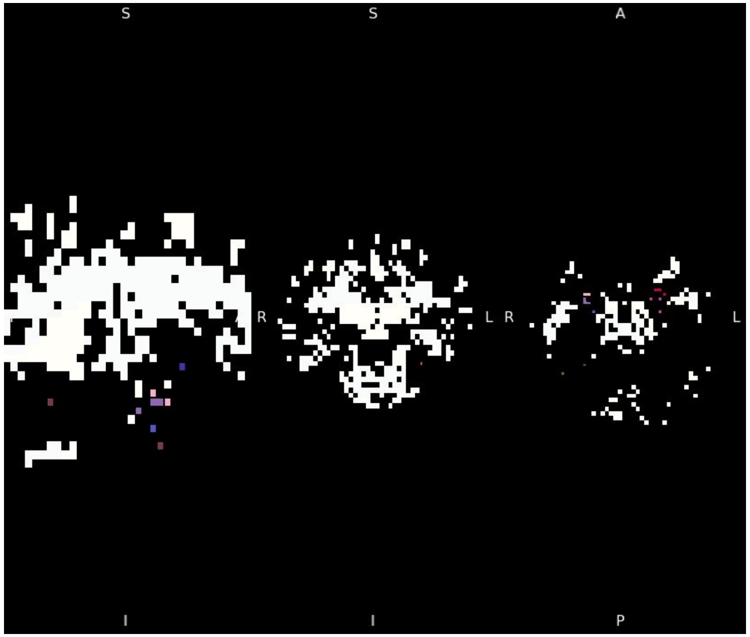


```
echo 'Step 13: ROI signal extraction'
echo 'Masking ATLAS'
fslmaths ${PATH_RS_OUTPUT}/_ravol0104_brain_mask -mul ${PATH_RS_OUTPUT}/AAL2_0p5_to_BOLD.nii ${PATH_RS_OUTPUT}/AAL2_0p5_to_BOLD_masked.nii
```

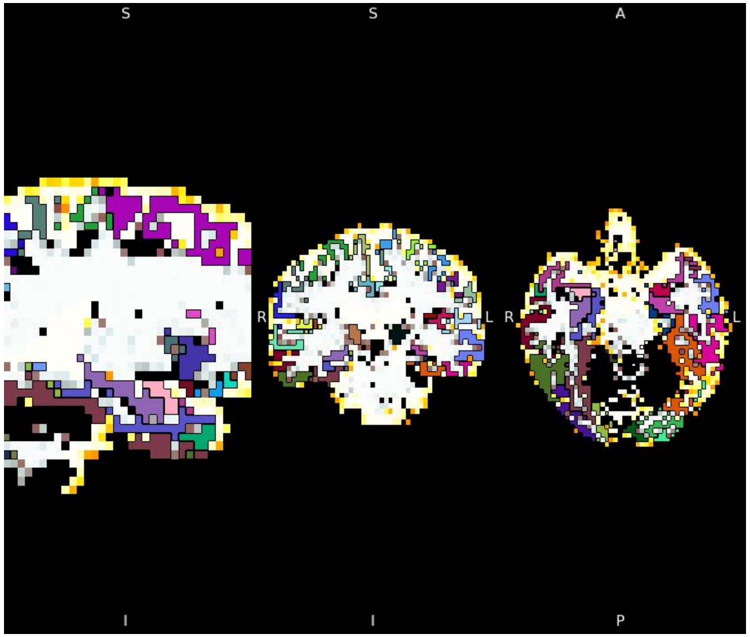
Resting State Processing

T1 to BOLD masks

Use AAL2 replacing Schaefer



0.999

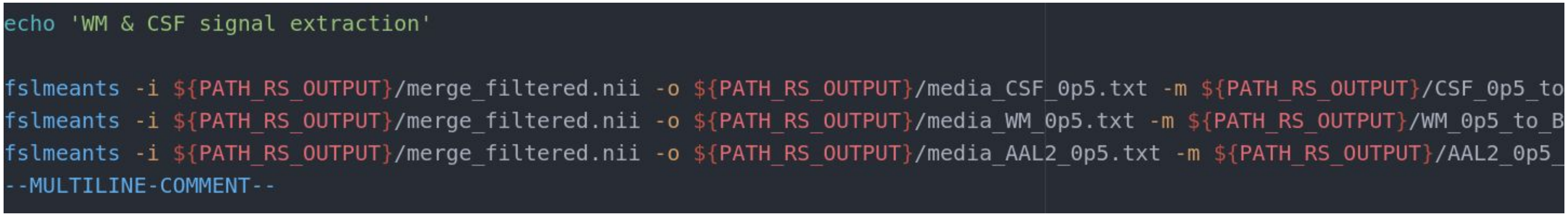


0.5

Data: EiMa 54, First Session, RS

Resting State Processing

extract the average intensity of white matter and grey matter



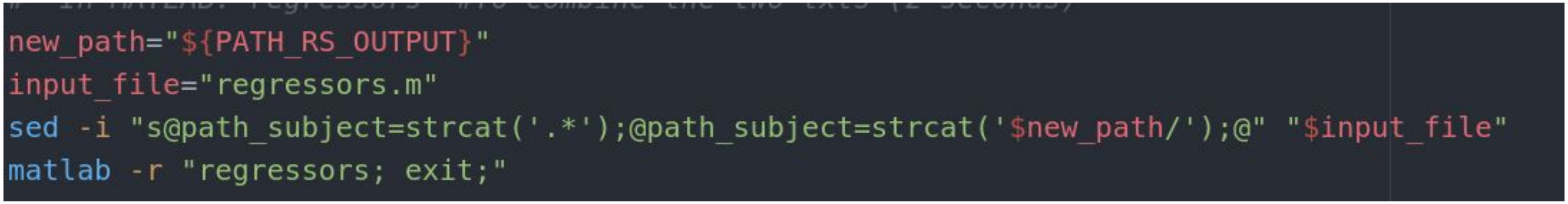
```
echo 'WM & CSF signal extraction'

fslmeants -i ${PATH_RS_OUTPUT}/merge_filtered.nii -o ${PATH_RS_OUTPUT}/media_CSF_0p5.txt -m ${PATH_RS_OUTPUT}/CSF_0p5_to
fslmeants -i ${PATH_RS_OUTPUT}/merge_filtered.nii -o ${PATH_RS_OUTPUT}/media_WM_0p5.txt -m ${PATH_RS_OUTPUT}/WM_0p5_to_B
fslmeants -i ${PATH_RS_OUTPUT}/merge_filtered.nii -o ${PATH_RS_OUTPUT}/media_AAL2_0p5.txt -m ${PATH_RS_OUTPUT}/AAL2_0p5_
--MULTILINE-COMMENT--
```

- Input :
  - CSF and WM mask/Merged files
- Output:
  - text file recording the intensity of CSF and WM

Data: EiMa 54, First Session, RS

Resting State Processing

In matlab combine the signal of CSF and WM



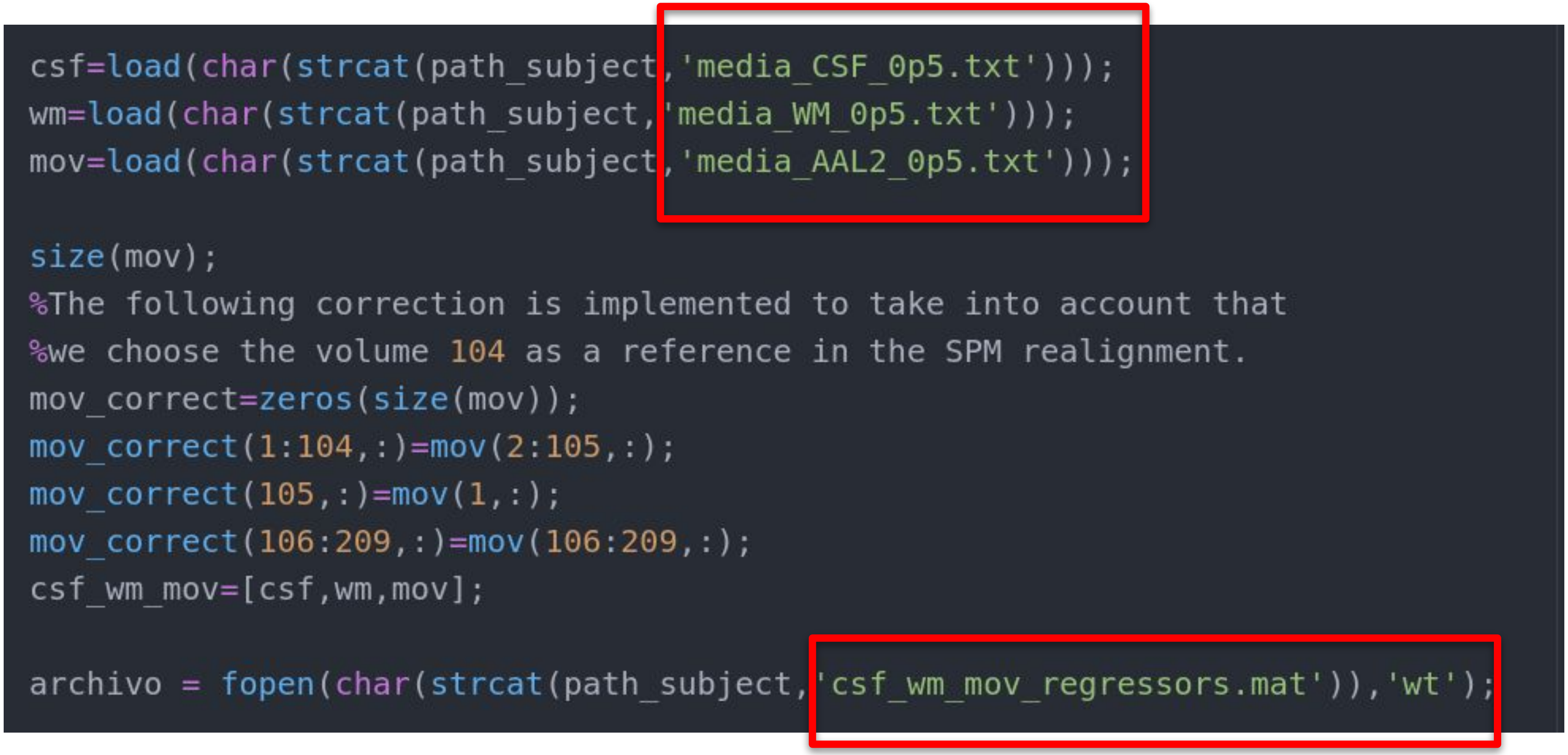
```
#  In MATLAB: regressors  #To combine the two txts (2 seconds)
new_path="${PATH_RS_OUTPUT}"
input_file="regressors.m"
sed -i "s@path_subject=strcat('.*');@path_subject=strcat('$new_path/');@" "$input_file"
matlab -r "regressors; exit;"
```

- Input :
  - CSF and WM ans AAL2
- Output:
  - Regressed CSF WM and AAL2

Data: EiMa 54, First Session, RS

Resting State Processing

In matlab combine the signal of CSF and WM

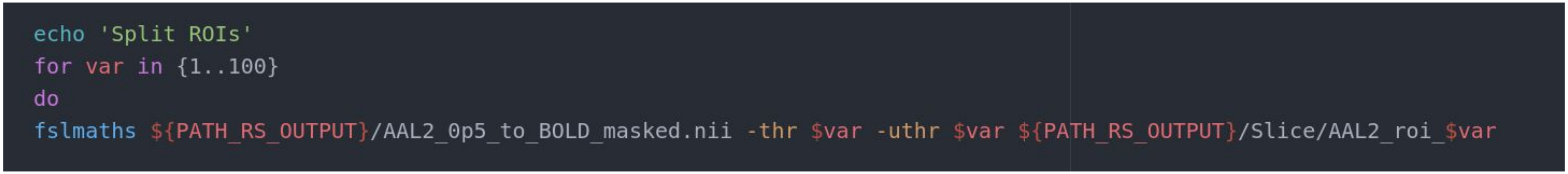


```
csf=load(char(strcat(path_subject,'media_CSF_0p5.txt')));
wm=load(char(strcat(path_subject,'media_WM_0p5.txt')));
mov=load(char(strcat(path_subject,'media_AAL2_0p5.txt')));

size(mov);
%The following correction is implemented to take into account that
%we choose the volume 104 as a reference in the SPM realignment.
mov_correct=zeros(size(mov));
mov_correct(1:104,:)=mov(2:105,:);
mov_correct(105,:)=mov(1,:);
mov_correct(106:209,:)=mov(106:209,:);
csf_wm_mov=[csf,wm,mov];

archivo = fopen(char(strcat(path_subject,'csf_wm_mov_regressors.mat')),'wt');
```

Data: EiMa 54, First Session, RS

Resting State Processing

Split the images into slice



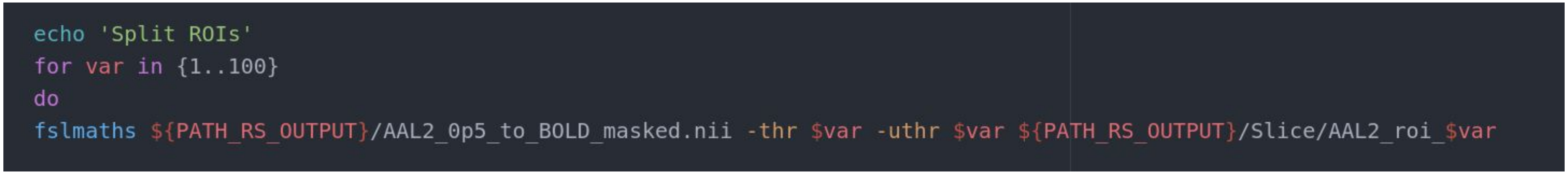
```
echo 'Split ROIs'
for var in {1..100}
do
fslmaths ${PATH_RS_OUTPUT}/AAL2_0p5_to_BOLD_masked.nii -thr $var -uthr $var ${PATH_RS_OUTPUT}/Slice/AAL2_roi_$var
```

- Input :
  - standard brain
- Output:
  - standard brain region

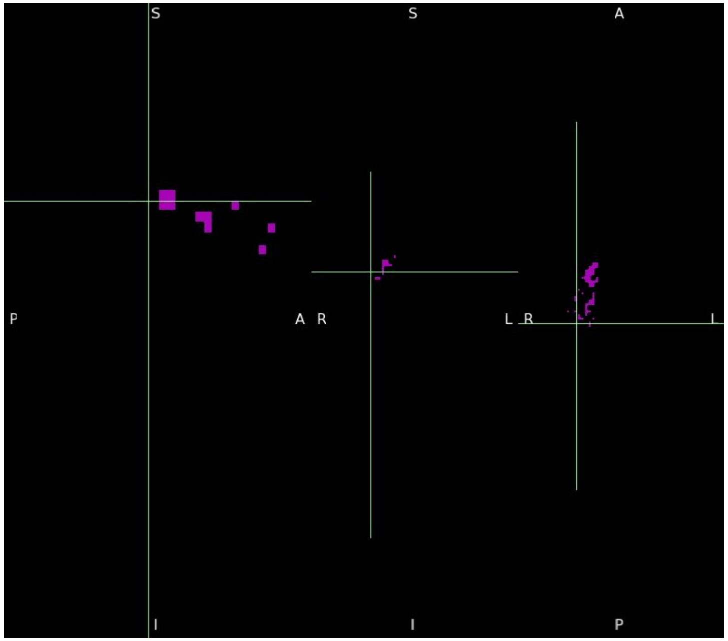
Data: EiMa 54, First Session, RS

Resting State Processing

Split the images into slice

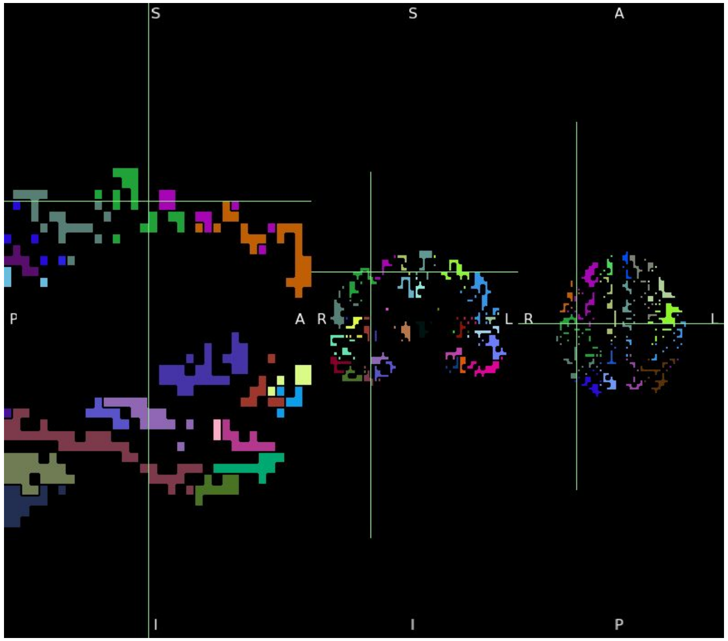


```
echo 'Split ROIs'
for var in {1..100}
do
fslmaths ${PATH_RS_OUTPUT}/AAL2_0p5_to_BOLD_masked.nii -thr $var -uthr $var ${PATH_RS_OUTPUT}/Slice/AAL2_roi_$var
```



Left:
Region 4

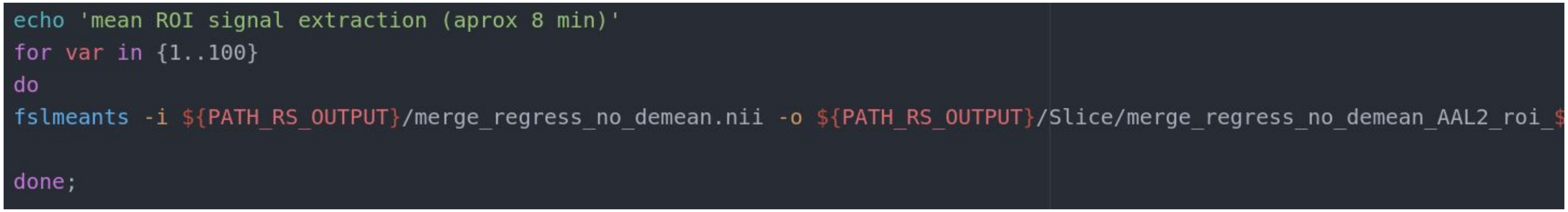
Right:
All AAL2



Data: EiMa 54, First Session, RS

Resting State Processing

Extract the region of interest for each volume

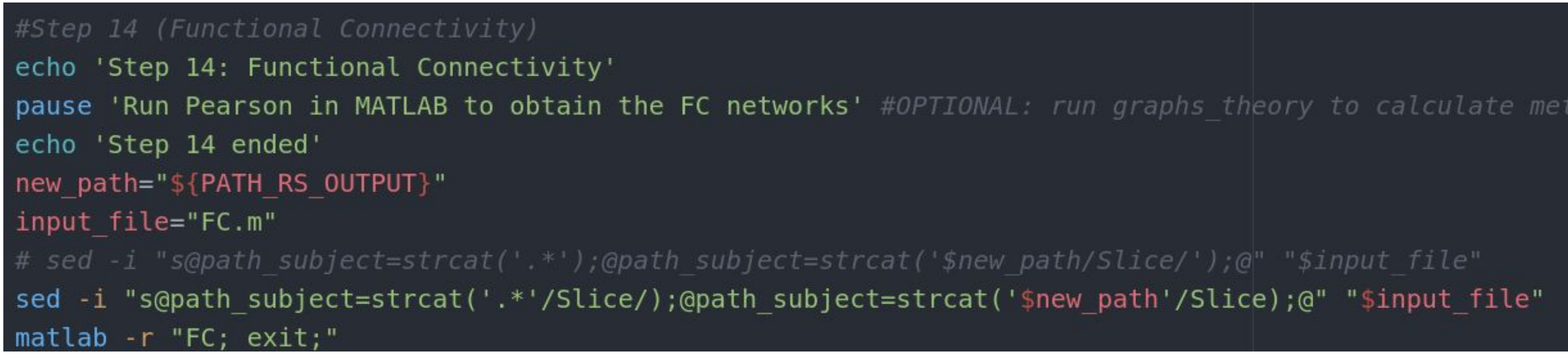


```
echo 'mean ROI signal extraction (aprox 8 min)'
for var in {1..100}
do
fslmeants -i ${PATH_RS_OUTPUT}/merge_regress_no_demean.nii -o ${PATH_RS_OUTPUT}/Slice/merge_regress_no_demean_AAL2_roi_$

done;
```

- Input :
  - standard brain slice
- Output:
  - region of interest

Data: EiMa 54, First Session, RS

Resting State Processing



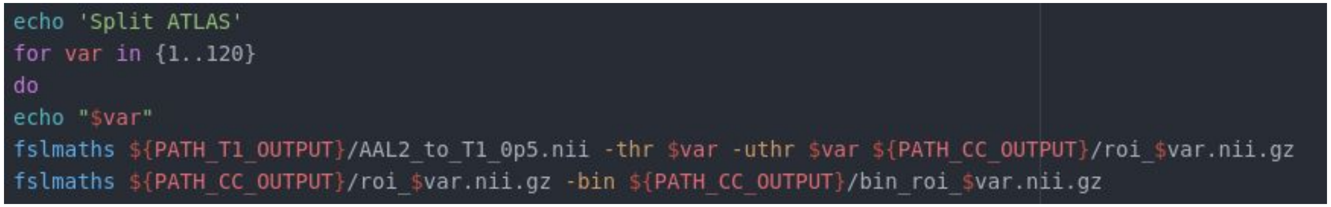
```
#Step 14 (Functional Connectivity)
echo 'Step 14: Functional Connectivity'
pause 'Run Pearson in MATLAB to obtain the FC networks' #OPTIONAL: run graphs_theory to calculate me
echo 'Step 14 ended'
new_path="${PATH_RS_OUTPUT}"
input_file="FC.m"
# sed -i "s@path_subject=strcat('.*');@path_subject=strcat('$new_path/Slice/');@" "$input_file"
sed -i "s@path_subject=strcat('.*'/Slice/);@path_subject=strcat('$new_path'/Slice);@" "$input_file"
matlab -r "FC; exit;"
```

- Input :
  - standard brain slice
- Output:
  - Parameter for the further steps

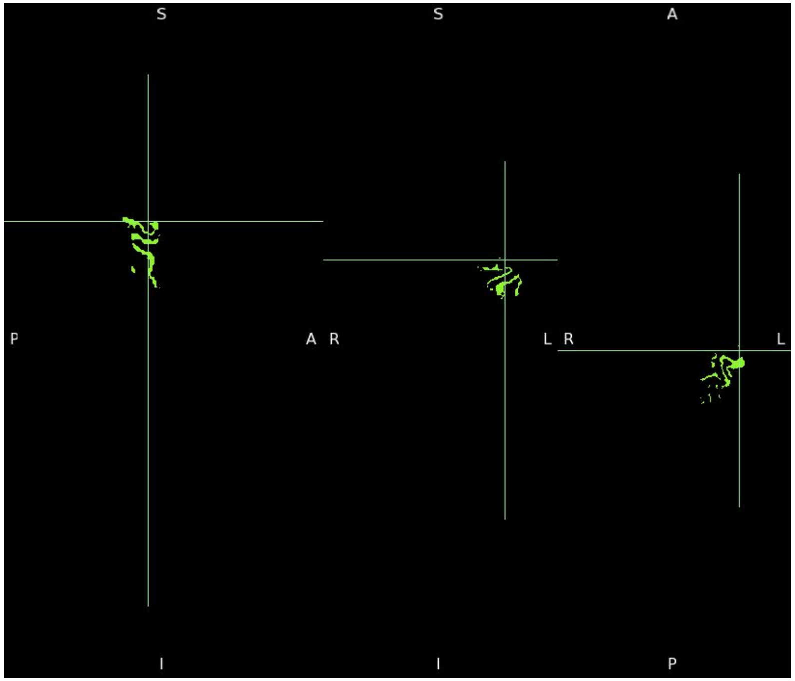
Connectivity Processing

Resting State Processing

Split atlas / Create label

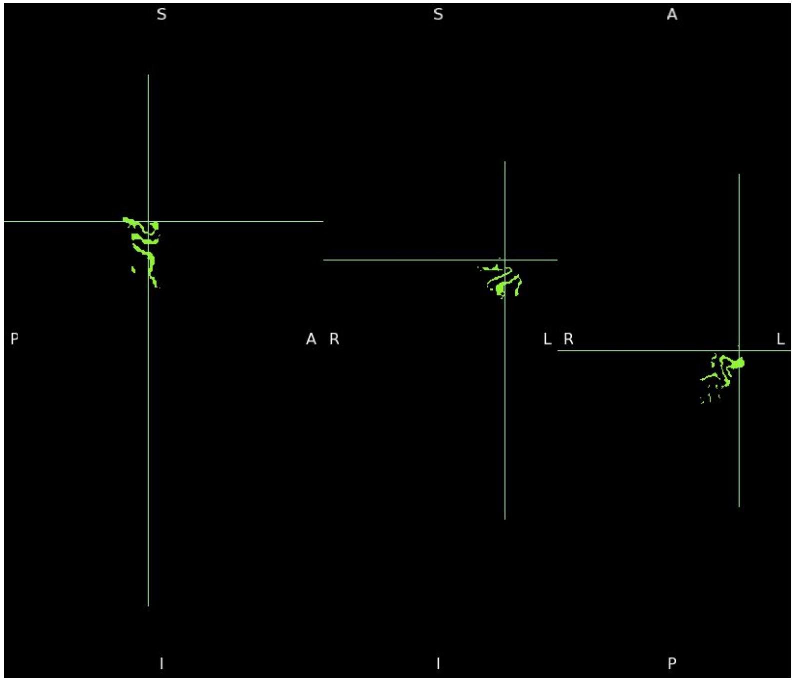


```
echo 'Split ATLAS'
for var in {1..120}
do
echo "$var"
fslmaths ${PATH_T1_OUTPUT}/AAL2_to_T1_0p5.nii -thr $var -uthr $var ${PATH_CC_OUTPUT}/roi_$var.nii.gz
fslmaths ${PATH_CC_OUTPUT}/roi_$var.nii.gz -bin ${PATH_CC_OUTPUT}/bin_roi_$var.nii.gz
```



Left:
Region 1
ROI

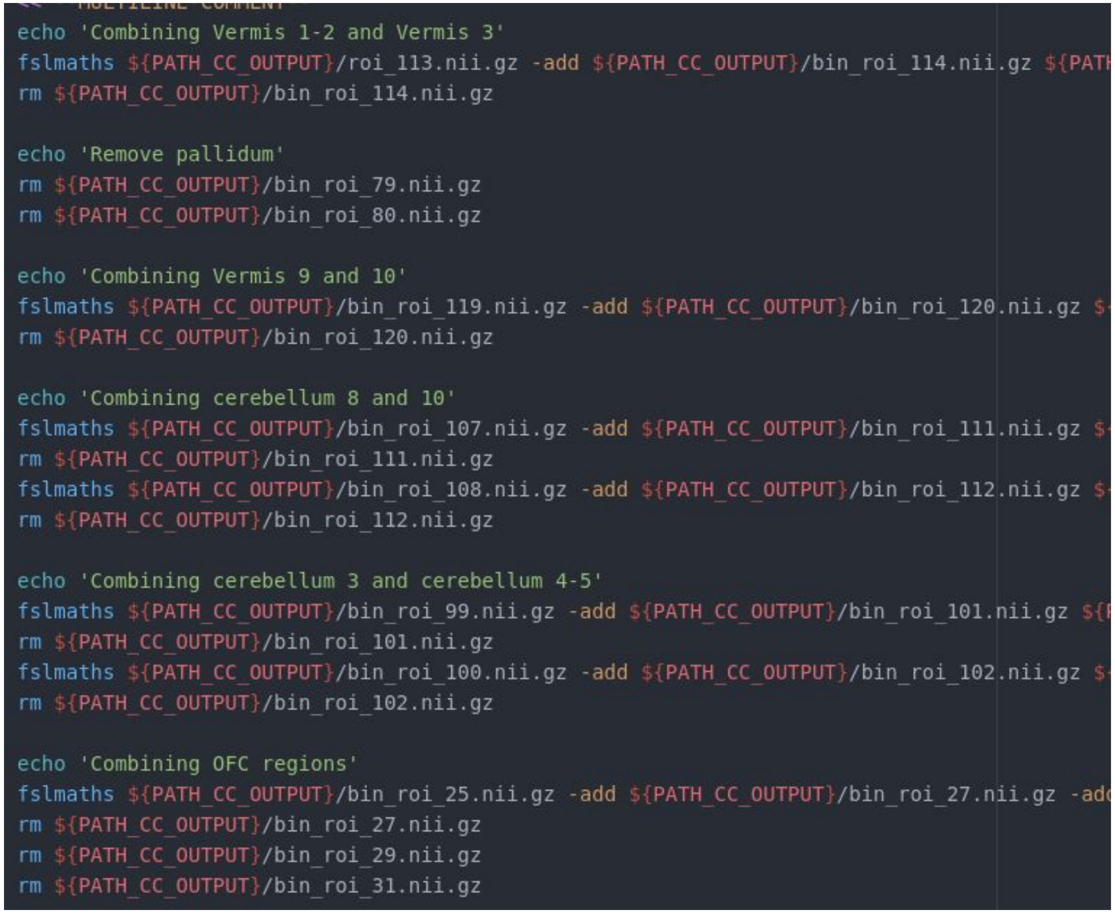
Right:
region1
ROI label



Data: EiMa 54, First Session, RS

Resting State Processing

Merge brain region

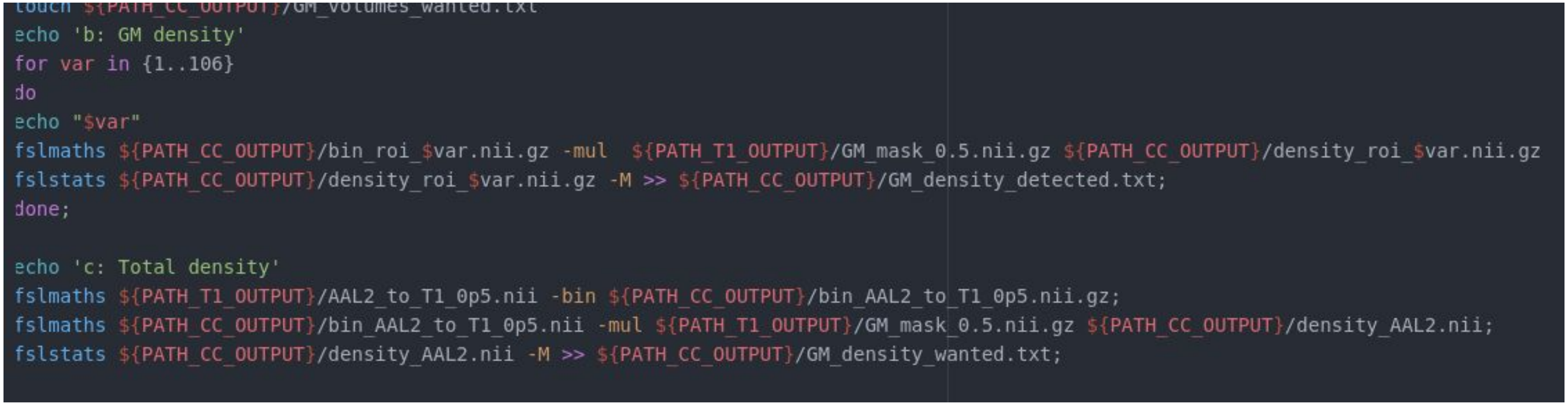


```
<<  MULTILINE COMMENT
echo 'Combining Vermis 1-2 and Vermis 3'
fslmaths ${PATH_CC_OUTPUT}/roi_113.nii.gz -add ${PATH_CC_OUTPUT}/bin_roi_114.nii.gz ${PATH
rm ${PATH_CC_OUTPUT}/bin_roi_114.nii.gz

echo 'Remove pallidum'
rm ${PATH_CC_OUTPUT}/bin_roi_79.nii.gz
rm ${PATH_CC_OUTPUT}/bin_roi_80.nii.gz

echo 'Combining Vermis 9 and 10'
fslmaths ${PATH_CC_OUTPUT}/bin_roi_119.nii.gz -add ${PATH_CC_OUTPUT}/bin_roi_120.nii.gz $
rm ${PATH_CC_OUTPUT}/bin_roi_120.nii.gz

echo 'Combining cerebellum 8 and 10'
fslmaths ${PATH_CC_OUTPUT}/bin_roi_107.nii.gz -add ${PATH_CC_OUTPUT}/bin_roi_111.nii.gz $
rm ${PATH_CC_OUTPUT}/bin_roi_111.nii.gz
fslmaths ${PATH_CC_OUTPUT}/bin_roi_108.nii.gz -add ${PATH_CC_OUTPUT}/bin_roi_112.nii.gz $
rm ${PATH_CC_OUTPUT}/bin_roi_112.nii.gz

echo 'Combining cerebellum 3 and cerebellum 4-5'
fslmaths ${PATH_CC_OUTPUT}/bin_roi_99.nii.gz -add ${PATH_CC_OUTPUT}/bin_roi_101.nii.gz ${
rm ${PATH_CC_OUTPUT}/bin_roi_101.nii.gz
fslmaths ${PATH_CC_OUTPUT}/bin_roi_100.nii.gz -add ${PATH_CC_OUTPUT}/bin_roi_102.nii.gz $
rm ${PATH_CC_OUTPUT}/bin_roi_102.nii.gz

echo 'Combining OFC regions'
fslmaths ${PATH_CC_OUTPUT}/bin_roi_25.nii.gz -add ${PATH_CC_OUTPUT}/bin_roi_27.nii.gz -add
rm ${PATH_CC_OUTPUT}/bin_roi_27.nii.gz
rm ${PATH_CC_OUTPUT}/bin_roi_29.nii.gz
rm ${PATH_CC_OUTPUT}/bin_roi_31.nii.gz
```

Data: EiMa 54, First Session, RS

Resting State Processing

Calculate the density



```
touch ${PATH_CC_OUTPUT}/GM_volumes_wanted.txt
echo 'b: GM density'
for var in {1..106}
do
echo "$var"
fslmaths ${PATH_CC_OUTPUT}/bin_roi_$var.nii.gz -mul  ${PATH_T1_OUTPUT}/GM_mask_0.5.nii.gz ${PATH_CC_OUTPUT}/density_roi_$var.nii.gz
fslstats ${PATH_CC_OUTPUT}/density_roi_$var.nii.gz -M >> ${PATH_CC_OUTPUT}/GM_density_detected.txt;
done;

echo 'c: Total density'
fslmaths ${PATH_T1_OUTPUT}/AAL2_to_T1_0p5.nii -bin ${PATH_CC_OUTPUT}/bin_AAL2_to_T1_0p5.nii.gz;
fslmaths ${PATH_CC_OUTPUT}/bin_AAL2_to_T1_0p5.nii -mul ${PATH_T1_OUTPUT}/GM_mask_0.5.nii.gz ${PATH_CC_OUTPUT}/density_AAL2.nii;
fslstats ${PATH_CC_OUTPUT}/density_AAL2.nii -M >> ${PATH_CC_OUTPUT}/GM_density_wanted.txt;
```

Potential Problem: The GM density is of different region of interest.
But the Total density is all the standard brain AAL2.

Solution: Split the AAL2 into ROI at first, Otherwise the regression part can not work