

Protocol for ROI analysis using the ENIGMA-DTI template

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The following steps will allow you to extract relevant ROI information from the skeletonized FA images that have been registered and skeletonized according to the ENIGMA-DTI template, and keep track of them in a spreadsheet.

Here we assume that you have a common meta-data spreadsheet with all relevant covariate information for each subject.

- Can be a tab-delimited text file, or a .csv
- Ex) MetaDataSpreadsheetFile.csv :
- The following is an example of a data spreadsheet with all variables of interest. This spreadsheet is something you may already have to keep track of all subject information. It will be used later to extract only information of interest in **Step 6**

subjectID	Age	Diagnosis	Sex	AgeSex	...
UCLA01	23	1	1	23	...
UCLA02	45	1	2	90	...
UCLA03	56	1	1	56	...
UCLA04	27	1	1	27	...
UCLA05	21	1	1	21	...
UCLA06	44	2	2	88	...
UCLA07	35	1	1	35	...
UCLA08	31	1	2	62	...
UCLA09	50	1	1	50	...
UCLA10	29	1	2	58	...

- An example file is provided – ALL_Subject_Info.txt

INSTRUCTIONS

1. Download and install 'R' <http://cran.r-project.org/>
2. Download a copy of the scripts and executables here:
 - http://enigma.ini.usc.edu/wp-content/uploads/2012/06/ROIextraction_info.zipBash shell scripts and compiled versions of the code (**bold**) have been made available to run on Linux -based workstations. Raw code is also provided in the case re-compilation is needed.

The downloaded archive will have the following files:

- **run_ENIGMA_ROI_ALL_script.sh**
- **singleSubjROI_exe**
- singleSubject_FA_ROI.cpp
- **averageSubjectTracts_exe**
- average_subj_tract_info.cpp
- **run_combineSubjectROI_script.sh**

- combine_subject_tables.R
necessary files --
- ENIGMA_look_up_table.txt
- JHU-WhiteMatter-labels-1mm.nii.gz
- mean_FA_skeleton.nii.gz
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- example files --*
- ALL_Subject_Info.txt
- subjectList.csv
- Subject1_FA.nii.gz
- Subject7_FA.nii.gz
- example outputs --*
- Subject1_ROIout.csv
- Subject1_ROIout_avgs.csv
- Subject7_ROIout.csv
- Subject7_ROIout_avgs.csv
- combinedROItable.csv

3. **run_ENIGMA_ROI_ALL_script.sh** provides an example shell script on how to run all the pieces in series.
 - This can be modified to run the first two portions in parallel if desired.
4. The first command - **singleSubjROI_exe** uses the atlas and skeleton to extract ROI values from the JHU-atlas ROIs as well as an average FA value across the entire skeleton
 - It is run with the following inputs
 - ./singleSubjROI_exe look_up_table.txt skeleton.nii.gz JHU-WhiteMatter-labels-1mm.nii.gz OutputfileName Subject_FA_skeleton.nii.gz
 - example -- ./singleSubjROI_exe ENIGMA_look_up_table.txt mean_FA_skeleton.nii.gz JHU-WhiteMatter-labels-1mm.nii.gz Subject1_ROIout Subject1_FA_skel.nii.gz
 - The output will be a .csv file called Subject1_ROIout.csv with all mean FA values of ROIs listed in the first column and the number of voxels each ROI contains in the second column (see **ENIGMA_ROI_part1/Subject1_ROIout.csv** for example output)
5. The second command - **averageSubjectTracts_exe** uses the information from the first output to average relevant (example average of L and R external capsule) regions to get an average value weighted by volumes of the regions.
 - It is run with the following inputs
 - ./averageSubjectTracts_exe inSubjectROIfile.csv outSubjectROIfile_avg.csv

- where the first input is the ROI file obtained from **Step 4** and the second input is the name of the desired output file.
 - The output will be a .csv file called outSubjectROIfile_avg.csv with all mean FA values of the new ROIs listed in the first column and the number of voxels each ROI contains in the second column (see **ENIGMA_ROI_part2/Subject1_ROIout_avg.csv** for example output)
6. The final portion of this analysis is an ‘R’ script **combine_subject_tables.R** that takes into account all ROI files and creates a spreadsheet which can be used for GWAS or other association tests. It matches desired subjects to a meta-data spreadsheet, adds in desired covariates, and combines any or all desired ROIs from the individual subject files into individual columns.
- Input arguments as shown in the bash script are as follows:
 - Table=./ALL_Subject_Info.txt –
 - A meta-data spreadsheet file with all subject information and any and all covariates
 - subjectIDcol=subjectID
 - the header of the column in the meta-data spreadsheet referring to the subject IDs so that they can be matched up accordingly with the ROI files
 - subjectList=./subjectList.csv
 - a two column list of subjects and ROI file paths.
 - this can be created automatically when creating the average ROI .csv files – see **run_ENIGMA_ROI_ALL_script.sh** on how that can be done
 - outTable=./combinedROItable.csv
 - the filename of the desired output file containing all covariates and ROIs of interest
 - Ncov=2
 - The number of covariates to be included from the meta-data spreadsheet
 - At least age and sex are recommended
 - covariates="Age;Sex"
 - the column headers of the covariates of interest
 - these should be separated by a semi-colon ‘;’ and no spaces
 - Nroi="all" #2
 - The number of ROIs to include
 - Can specify “all” in which case all ROIs in the file will be added to the spreadsheet
 - Or can specify only a certain number, for example 2 and write out the 2 ROIs of interest in the next input
 - rois= “all” #"IC;EC"
 - the ROIs to be included from the individual subject files
 - this can be “all” if the above input is “all”

- or if only a select number (ex, 2) ROIs are desired, then the names of the specific ROIs as listed in the first column of the ROI file
 - these ROI names should be separated by a semi-colon ‘;’ and no spaces for example if Nroi=2, rois="IC;EC" to get only information for the internal and external capsules into the output .csv file
- (see **combinedROItable.csv** for example output)

Congrats! Now you should have all of your subjects ROIs in one spreadsheet with only relevant covariates ready for association testing!