

PROTOCOL

COLLECTION OF MRI DATA



DOWNLOAD
WRAPPER SCRIPTS



PREPARE MRI DATA



RUN WRAPPER
SCRIPTS



WELCOME TO THE
ENIGMA-CNV
WORKING GROUP

BECAUSE EVERY COPY NUMBER VARIANT COUNTS!

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Welcome!

Thank you for your interest in the ENIGMA-CNV working group.

We are grateful for your time invested and hope the data collection will go smoothly.

Overview:

This step-by-step protocol is meant to ease the transition from raw MR data to preprocessed FreeSurfer data and extract measures of:

1. Subcortical and cortical structures including quality measures [derived from the recon-all-function] [mandatory].
2. Segmented brainstem/hypothalamus/hippocampus volumes [optional but highly recommended]
3. Local gyrification index [optional but highly recommended]

Please do not hesitate to reach out for help (enigmacnvhelpdesk@gmail.com) if you experience any issues.

If you already have preprocessed the data using the recon-all function (i.e., called in 1_runfreesurfer.sh/1_runfreesurfer_loop.sh), please go directly to part 4 of the imaging protocol.

Imaging Protocol

1. Organize your data

Imaging data needed:

- Nifti files (*.ni) from raw data (for conversion of raw imaging data unprocessed after extracting files from the scanner, please consult [Appendix 1](#)).

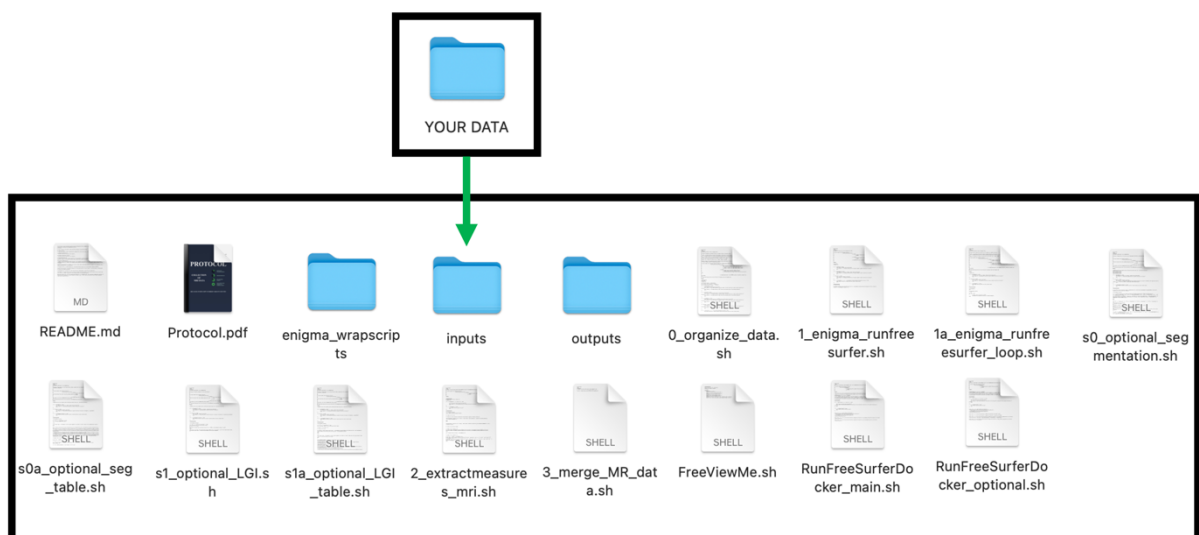
Software needed:

- FreeSurfer (see [Appendix 2](#) for guidance for download),
- R (<https://www.r-project.org>)
- MATLAB (also downloadable through the MATLAB runtime in FreeSurfer, <https://surfer.nmr.mgh.harvard.edu/fswiki/MatlabRuntime>, see [Appendix 3](#) for guidance)

Scripts needed:

- Download the scripts from <https://github.com/ENIGMA-git/ENIGMA-CNV/tree/main/sMRI> containing all necessary scripts. This is a combination of ENIGMA-CNV specific scripts and the freely available ENIGMA scripts found at <https://github.com/npnl/ENIGMA-Wrapper-Scripts> and <http://enigma.ini.usc.edu/protocols/imaging-protocols/>

1. Place the scripts in your folder with the participant data <YOUR DATA>.
2. Please also change the folder name from <YOUR DATA> to <inputs> , so that the folder resembles the image at the bottom:



3. Place the raw data with your .nii files in the “inputs” folder using the following structure: subjXX to subjXX. If you already have the raw .nii files in a folder, you can simply rename the folder to “inputs”.



4. Alternatively, run the 0_organize_data.sh script, which will organize the structure of the data according to the assumed structure in the following scripts.

Usage is:

```
./0_organize_data.sh <subject data header> <directory of  
current data> <path to parent project directory>
```

Note:

- the scripts are sensitive to the naming structure (i.e., naming the folder with the capital letter S instead of s, or leaving the j out in subj). If they are not labeled correctly, the scripts will not run properly.
- The wrapper scripts assume that the .nii files for each subject are labeled the same as the parent folder. I.e., the subj01 folder should include a subj01.nii.gz-file, the subj02 folder should include a subj02.nii.gz-file, etc...
- Feel free to adapt the 1a_enigma_runfreesurfer.sh, 2_extractmeasures_mri.sh, s0_optional_segmentation.sh, s1_optional_LGI.sh, and s1a_optional_LGItable.sh scripts (e.g., change the <subj*> to <your labeling>) if you have a ton of data and/or your own labeling system for each participant, and you do not want to change your labeling.

2. Running the Wrapper scripts

Set the correct FreeSurfer directory in the terminal:

```
export FREESURFER_HOME=/Applications/freesurfer/  
source ${FREESURFER_HOME}/SetUpFreeSurfer.sh  
export PATH=${PATH}:${FREESURFER_HOME}/bin
```

Note the <FREESURFER_HOME> directory may be different on your computer – please change accordingly.

The enigma wrapper scripts allow you to:

i) run on a single subject:

```
./1_enigma_runfreesurfer.sh <subject number> <path to parent  
project directory>
```

```
Example: sh 1_enigma_runfreesurfer.sh subj01  
/Users/runeboen/Documents/ENIGMA-CNV
```

or

ii) loop through several subjects:

```
./1a_enigma_runfreesurfer_loop.sh <path to parent project  
directory>
```

```
Example: sh 1a_enigma_runfreesurfer_loop.sh  
/Users/runeboen/Documents/ENIGMA-CNV
```

We recommend running on a single subject and checking for correct outputs before looping through all subjects. If you ran your code with troubleshooting, you need to clean the outputs folder every time in order your code to work.

Running FreeSurfer on each participant will take time (6-12 h per subject!).

3. Speeding up processing time with parallel

To speed up processing time, parallel can be used.

1. Download here:
<http://www.gnu.org/software/parallel/>
2. You can find an introduction here:
<https://blog.cogneurostats.com/2013/06/10/parallelizing-freesurfer/>

Parallel takes advantage of the number of cores available on your computer (e.g., with 8 cores, you may be able to run 8 simultaneous processes, which will significantly decrease the time to process all of your data!)

You can check your number of cores in the terminal:
`sysctl -n hw.ncpu`

4. Optional analysis

These steps are simple to run on already preprocessed data and only require FreeSurfer version 6.0 or later in addition to download of MATLAB runtime/or MATLAB for the segmentation step. However, for the local gyrification index MATLAB is needed. For more information, please see Appendix 4.

Download MATLAB runtime by typing
`fs_install_mcr R2012b [FreeSurfer v.6]`
`fs_install_mcr R2014b [FreeSurfer v.7]`

i) For hypothalamic subunits, hippocampal segmentation and/or brain stem segmentation, run:

```
sh ./s0_optional_segmentation.sh <parent directory of project> --BrainStem --HippocampusAmygdala --ThalamicNuclei --Hypothalamus
```

Note: For segmentation of the hypothalamus, the newest version of Freesurfer may need to be installed (i.e., Freesurfer v7.2).

ii) For creating a summary table, run the s0a_optional_SEGtable.sh

iii) For local gyrification index, run:

```
sh ./s1_optional_LGI.sh <parent directory of project> --LocalGyrificationIndex
```

iv) For creating a summary table, run the LocalGyrificationIndex.sh file:

```
sh ./s1a_optional_LGItable.sh <parent directory of project> --CreateTable
```

5. Merging files

Now you are ready to extract the relevant data from the reconstruction. Please run the 2_extractmeasures_mri.sh – this will create several .csv files in the outputs folder:

```
./2_extractmeasures_mri.sh <parent directory of project>
```

All the .csv files will need to be merged into a full .csv file. The last step assumes you have installed R as well (if you have not, please do so before running the final shell script below).

Now, run the

```
./3_merge_MR_data.sh
```

This will create the final file called ENIGMA_CNV_ImagingFile.csv in the parent directory.

6. Quality Control

Please visually inspect the ENIGMA_CNV_ImagingFile.csv-file to check for irregularities or other things that might be of concern. All MR images should be visually inspected for artifacts and incidental findings before preprocessing the data.

The outputs of the processed data should undergo the quality control (QC) assessment provided by the ENIGMA consortium (ENIGMA Cortical Quality Control Protocol 2.0; <https://enigma.ini.usc.edu/protocols/imaging-protocols/>)

These scripts must be run for proper quality control of your data. If you have not done this yet, please do so now.

In addition, the following procedure can be done to visually inspect the images using the Freeview tool in FreeSurfer. This could be desirable for any subjects that are marked as outliers during the QC:

For visual inspection of the data, the Freeview tool can be used to visualize the boundaries that are derived from the pial surface files generated after finishing the protocol. Please open Freeview, e.g by manually typing “freeview” in the terminal. Alternatively, use the additional shell script that loads this automatically, named FreeViewMe.sh.

If you want to do this yourself, you can start by loading the following:

For the subcortical segmentation:

1. Load the orig.mgz image.
2. Load the aparc+aseg.mgz file.
3. You may want to change the opacity of the aseg. File to see how well the segmentation did, and slide through the slices to ensure that they look reasonable.

For the surface reconstruction:

1. Load the lh.pial, rh.pial, lh.white and rh.white files.
2. Change the color of the pial and white files to ease visualization (e.g., yellow for lh/rh.pial and red for lh/rh.white). Scroll through the slices to ensure that it captures the boundaries between white and grey matter.

For the FreeViewMe.sh script:

```
sh FreeViewMe.sh <ENIGMA output folder> <subject>
```

Here, you could unload the volume files if you want to look at the grey/white matter boundaries, or unload the surface files to see the parcellation/segmentation. You could also set the opacity to your preferences for visualization of the parcellated/segmented regions of interest (including the segmentation from the optional analyses).

For more information about the Freeview:

<https://surfer.nmr.mgh.harvard.edu/fswiki/FreeviewGuide>

7. Scanner site information

Finally, please fill in “ScannerInfoSheet.csv” with information about the MRscanner and the FreeSurfer version used.

Columns needed:

ScannerSite = name of the scanner site. E.g., NORMENT_Oslo_T3

Sequence = the MR sequence that was used to obtain the mages. E.g., 3D T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE)

FieldStrength = the field strength of the MR scanner measured in Tesla.

E.g., 3T GE HDx

AcquisitionDirection = which direction the MR images were obtained. E.g., Coronal

NumberOfSlices = number of slices of the MR images. E.g., 180

SliceGap = the gap between the slices in mm. E.g., 0mm

VoxelSize_m3= size of the voxels from the MR image in 3D. E.g., 1.25x1.25x1.2

TI = Inversion Time. E.g., 500ms

TE = Echo Time. E.g., 4ms

TR = Repetition Time. E.g., 500ms

FlipAngle = E.g. 8 degrees

Citation = Citation to work that previously used the current data if relevant.

Segmentation = **FreeSurfer** version used. E.g., FreeSurfer (5.1.0)

Segmentation, Optional = **FreeSurfer** version used for the optional analyses. E.g., FreeSurfer (7.2.0)

Note1: The details of the MR scan are usually stored in the .json files that come with the nifti files. From the terminal, you can type ‘cat xxxx.json’. This will provide you with the information you need above.

Note2: FreeSurfer version can be checked manually through the FreeSurfer parent directory, or just check your version by typing <recon-all -version> in the command line.

That’s it! You have now completed the required steps for the collection of the MRI data!

Troubleshooting

Permission errors

If you receive a ‘permission denied error’ when trying to copy files to the FreeSurfer-folder, please try to permit all users to the folder by typing:

```
sudo chmod a+rwX <Freesurfer directory>
```

This will permit all users (a) to read (r), write (w), and execute (x).

If you receive a similar error when trying to run the shell scripts you can try to allow executables by typing:

```
chmod +x <your sh file>
```

Matlab error

You get an error message running the optional analysis stating that Matlab exited, e.g:

ERROR: Matlab is required to run mris_compute_lgi!

Solution:

Your Matlab path may not have been set, so try this to change the path to where your Matlab folder is placed:

```
export PATH=/Applications/MATLAB_R2021a.app/bin/:$PATH
```

Note – the example shows the path to my matlab folder

[/Applications/MATLAB_R2021a.app/bin/], please change it your path!

You could also add the matlab folder to the matlab path:

```
export MATLABPATH=$FREESURFER_HOME/matlab
```

Appendix 1: Conversion of DICOM/PAR/REC to NIFTI files

Only necessary if you do not already have NIFTI-files.

If you have scanned participants yourself, you may have noticed that the scanner outputs are not so user-friendly yet. These outputs are often in DICOM formats (or PAR/REC), and we will convert these files into the more user-friendly NIFTI format (.nii). There are several ways of doing this. You can use your personal favorite or:

- dcm2nii (<https://www.nitrc.org/plugins/mwiki/index.php/dcm2nii:MainPage>)
(also included in the MRICroGL tool for viewing dicom and nifti files – a handy tool for easy visualization/quick visual quality control for MR images
(<https://www.nitrc.org/projects/mricrogl/>))
or
- FreeSurfer (<https://surfer.nmr.mgh.harvard.edu/fswiki/FsFastUnpackData> or
https://surfer.nmr.mgh.harvard.edu/fswiki/mri_convert)

You can also find a list of different dcm to nii tools in the nitrc page for the dcm2nii as shown above.

Note: If you have multiple MR sequences in the dicom to nii conversion, you will get folders with nifti files that correspond to each MR sequence (e.g., T1, diffusion-weighted images...). In this case, we will only use the T1.nii files.

Appendix 2: Downloading FreeSurfer

1. Go to <https://surfer.nmr.mgh.harvard.edu/fswiki/rel7downloads>
2. Choose the appropriate operating system (ie, either Linux, Mac, Ubuntu, or Windows)
- note that for Windows it is necessary to run the Freeview using the Windows subsystem for Linux.

For Mac:

1. Install the pkg file for Mac and follow the instructions. You will also need to download XQuartz (<https://surfer.nmr.mgh.harvard.edu/fswiki/MacOsInstall>)
2. If you receive an error message stating that .pkg file cannot be opened as it is from an unidentified developer – you can find the solution at the bottom of this page: https://surfer.nmr.mgh.harvard.edu/fswiki/FS7_mac

IMPORTANT! Although you may have downloaded Freesurfer, you still need to get a license key and place it in the Freesurfer folder. Please go to <https://surfer.nmr.mgh.harvard.edu/registration.html> and fill in the information. You will then receive an email with a license.txt file that you will need to move to the Freesurfer folder (this may be in the “Applications/freesurfer” or “usr/local/freesurfer” directory).

Congratulations – you have successfully downloaded FreeSurfer!

Appendix 3: Installing Matlab

For users without a Matlab license:

For a few of the included scripts, Matlab is required (which costs money). A free alternative ‘plugin’ is available through the free Matlab R2012b (FreeSurfer 6) or Matlab R2014b runtime (FreeSurfer 7). This can be easily downloaded in the terminal:

```
fs_install_mcr R2012b [for FreeSurfer 6] or  
fs_install_mcr R2014b [for FreeSurfer 7]
```

For users with a Matlab license:

From the FreeSurfer web page (<https://surfer.nmr.mgh.harvard.edu/fswiki/MatlabRuntime>):

If you already have Matlab installed...

If you already have Matlab 2012b/2014b installed on your system, you do not need to install the runtime. Keep in mind that the Matlab version must match the FreeSurfer requirement defined above. All you need to do is create a symbolic link named MCRv80/MCRv84 in your FreeSurfer directory that points to the main matlab install directory. The Matlab root directory can be easily obtained by executing the Matlab command matlabroot. It typically looks like /Applications/MATLAB_R2014b.app (OSX) and /usr/local/matlab/8.4 (Linux). For example, if you already have matlab 2014b, just run the following to make it visible to FreeSurfer:

Appendix 4: Help for optional analyses

I want to run the optional analyses! What to do?

First, make sure that you have Freesurfer v6 or later, the optional analyses will not run with earlier versions.

If you are using an earlier version, you can update your version to the most recent stable release of Freesurfer or use the dockorized version of Freesurfer.

Download:

<https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall>

Release notes:

<https://surfer.nmr.mgh.harvard.edu/fswiki/ReleaseNotes>

You will also need to download MATLAB runtime as described here:

<https://surfer.nmr.mgh.harvard.edu/fswiki/MatlabRuntime>

If you have MATLAB R2012b (for Freeesurfer v6 users) or R2014b (for Freeesurfer v7 users) already installed, you may simply add a link from Matlab application folder to your FreeSurfer directory:

```
cd <FreeSurferRootDirectory> && ln <MatlabRootDirectory> MCRv84
```

Some of the segmentation scripts from previous versions of FreeSurfer may contain bugs, see the latest updates for the segmentations to ensure that you have included the correct scripts:

Brainstem:

<https://surfer.nmr.mgh.harvard.edu/fswiki/BrainstemSubstructures>

Hippocampus and amygdala:

<https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala>

Hypothalamic subunits:

<https://surfer.nmr.mgh.harvard.edu/fswiki/HypothalamicSubunits>

Thalamic nuclei:

<https://freesurfer.net/fswiki/ThalamicNuclei>

and for the local gyrification index (MATLAB is needed!):

<https://surfer.nmr.mgh.harvard.edu/fswiki/LGI>

Now, you are ready to run the optional scripts as described in the main protocol!

Appendix 5. Running the scripts through Docker

We also strongly encourage researchers to run the scripts through Docker if possible. Here, you may download Docker (<https://www.docker.com>) and Freesurfer docker image (<https://hub.docker.com/r/freesurfer/freesurfer>). All the scripts have been tested with dockerized Freesurfer 7.2 version. With the exception of s1_optional_LGI.sh (which will require Matlab).

If you have organized your data (see step 1) and installed Docker and the Freesurfer docker image, you should be able to run the scripts through docker. You could do so by interacting with docker yourself, or you could run the following scripts that will run the main (i.e., 1a_enigma_runfreesurfer_loop.sh and 2_extractmeasures_mri.sh) and optional analyses (i.e., downloads matlab runtime and runs the s0_optional_segmentation.sh script)

```
./RunFreeSurferDocker_main.sh <path to Freesurfer license.txt file>  
<path to parent project directory>
```

Example: sh RunFreeSurferDocker_main.sh
/Applications/Freesurfer/7.2.0/ Users/runeboen/Documents/ENIGMA-CNV

Note that you need to include the path to the freesurfer license text file for the Freesurfer docker image to run. The .txt file can be provided here:
<https://surfer.nmr.mgh.harvard.edu/fswiki/License>

Appendix 6: Overview of shell scripts and their purposes

0_organize_data.sh

= creates and labels folders containing the imaging files (the folder names will be important for the other scripts).

1_enigma_runfreesurfer.sh

= runs the recon-all function on one subject.

1a_enigma_runfreesurfer_loop.sh

= runs the recon-all function on all subjects.

2_extractmeasures_mri.sh

= creates a CSV file that includes measures of area thickness, thickness standard deviation, volume, mean curvature Gaussian curvature, fold index curvature index, subcortical volumes, and Euler number for all subjects.

s0_optional_segmentation.sh

= segments the brainstem/hypothalamus/thalamic nuclei/amygdala/hippocampus (Requires MATLAB runtime or MATLAB).

s0a_optional_seg_table.sh = creates a .csv file for each region containing measures of volume of the segmented areas/subunits/subfields for all participants

s1_optional_LGI.sh = creates measures of a local gyrification index for all participants (Requires MATLAB).

s1a_optional_LGI_table.sh = extracts measures of local gyrification for each region of interest-based on the Desikan-Killiany atlas (Requires MATLAB).

3_merge_MR_data.sh = merges all of the files that are created above to one CSV file (requires R).

FreeViewMe.sh = automatically loads the volumes orig.mgz and aparc+aseg.mgz files and the surface files for white and pial surface for manual inspection of the reconstruction for a specified subject.

RunFreeSurferDocker_main.sh = Calls the dockerized FreeSurfer v7.2.0 package. Will run the 1a_enigma_runfreesurfer_loop.sh and 2_extractmeasures_mri.sh scripts through the dockerized FreeSurfer version.

RunFreeSurferDocker_optional.sh = Calls the dockerized FreeSurfer v7.2.0 package. Will run the optional segmentation analyses as these may not be available in older FreeSurfer versions (e.g., < v6) or does not work due to mac version (problems reported with macOS Big Sur).

The 1_enigma_runfreesurfer.sh (one subject) and 1a_enigma_runfreesurfer_loop.sh (loops through all subjects) scripts will run the recon-all function with the -all argument.