MELD Protocol 3 - FreeSurfer Quality Control Version 2

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Abstract

The MELD Project is an international collaboration aiming to create open-access, robust and generalisable tools for FCD detection. To this end, we will train a neural network classifier on MRI features from FCD patients from multiple centres worldwide.

Protocol 3 provides instructions on how to quality control the FreeSurfer reconstructions.

These instructions are based on the freely available protocols on the ENIGMA-epilepsy website http://enigma.ini.usc.edu

We are very grateful to Derrek Hibar, Neda Jahanshad, Roberto Toro, Jerod Rasmussen, Theo van Erp, Esther Walton and Stefan Ehrlich who wrote the orginal ENIGMA protocols and offered them with an unlimited license without warranty!

The main change is correct co-registration of FLAIR to T1 is now checked.

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Guidelines

These instructions are based on the freely available protocols on the ENIGMA-epilepsy website http://enigma.ini.usc.edu/ongoing/enigma-epilepsy/enigma-epilepsy-protocols/

We are very grateful to Derrek Hibar, Neda Jahanshad, Roberto Toro, Jerod Rasmussen, Theo van Erp,

Esther Walton and Stefan Ehrlich who wrote the orginal ENIGMA protocol.

Before start

Ensure that you have logged onto the MELD github (https://github.com/MELDProject/meld) and cloned the repository.

This will contain the essential MELD_QC folder.

You also need to have MATLAB installed.

If you have any questions or run into problems, please feel free to contact the MELD project: (meld.study@gmail.com)

Protocol

Preparing for QC

Step 1.

Extract and Organize Cortical Measures (FreeSurfer)

Open the script for extracting SurfaceArea and Thickness Values: ***extract.sh*** located in the MELD_QC folder.

We want to get the Surface Area and Thickness of each ROI for each subject from FreeSurfer.

The extract.sh script will extract and organize each of the values for each FreeSurfer ROI. The script assumes that your FreeSurfer output are organized as specified in protocol's 1 and 2.

Preparing for QC

Step 2.

Extract and Organize Cortical Measures (FreeSurfer)

Save the **extract.sh** script in the Parent Folder with your FreeSurfer output (*<path>/meld/output/)*.

Preparing for QC

Step 3.

Open the **extract.sh** and ensure that the for loop (line 6) selects the subject folder naming scheme used in this study (i.e. MELD):

*for subj_id in $(ls -d **MELD**_*); do*
#may need to change this so that it selects subjects with FS output

Save the extract.sh script after editing.

Remember to change the permissions to allow you to run the script:

```
chmod u+x extract.sh
```

Preparing for QC

Step 4.

On the command line, you can run the script directly by running:

```
sh <path>/meld/output/extract.sh
```

The result of this step will be two comma-separated (CSV) files that can be opened in your favorite spreadsheet application (i.e. Excel). The first row is a header describing the extracted regions and names for each column. Each row after the first gives the cortical thickness (or surface area)

measures for each subject found in your FreeSurfer directory.

Preparing for QC

Step 5.

Note 1: After running the extract.sh script, open both of the CSV files (CorticalMeasuresENIGMA_ThickAvg.csv and CorticalMeasuresENIGMA_SurfAvg.csv) and make sure that only subjects are listed in the rows of the file. Sometimes if there are other folders in your parent directory those folders can sometimes become included in your final files, if that happens just delete those from your CSV files and save.

Preparing for QC

Step 6.

Note 2: When you edit the files in Excel, be sure to keep them in CSV format when you save!

Preparing for QC - Outlier Detection

Step 7.

This is a simple R script that will identify subjects with cortical thickness and surface area values that deviate from the rest of your subjects.

This step requires that you have R installed and that you have the outlier detection script found called outliers.R which is located in the MELD_QC folder.

Change directories to the location of your **CorticalMeasuresENIGMA_ThickAvg.csv and CorticalMeasuresENIGMA_SurfAvg.csv** generated in Step 1 of the protocols.

cd <path>/meld/output/

Preparing for QC - Outlier Detection

Step 8.

Save the outliers.R script that directory (<path>/meld/output)

Preparing for QC - Outlier Detection

Step 9.

Run:

```
R --no-save --slave < outliers.R > outliers.log
```

This will generate a log file that will tell you which subjects are outliers and for which structures they are outliers for.

Quality Checking Cortical Measures (FreeSurfer)

Step 10.

There are three major steps for quality checking the cortical surface segmentations outputted from FreeSurfer:

1. **Internal Surface Method:** This method uses a Matlab function to plot cortical surface segmentations directly on a subject's scan and collates snapshots from internal slices of the brain into a webpage for easy checking.
2. **External Surface Method:** This is loosely based on the QATools for FreeSurfer. This creates a webpage with external views of the segmentations from different angles**.**
3. **Check FLAIR co-registration (if applicable)**

The following steps will take you through these 3 methods

Quality Checking - The Internal Surface Method

Step 11.

Start Matlab: /usr/local/matlab/bin/matlab

Quality Checking - The Internal Surface Method

Step 12.

Folder containing scripts required is: <path>/meld/MELD_QC/ENIGMA_QC

Add the folder containing all of the required scripts to Matlab's path.

Select: File -> Set Path -> Add Folder -> {OK} -> {Save} -> {Close}

Quality Checking - The Internal Surface Method

Step 13.

In the Matlab console window change directories to the folder with all of your FreeSurfer subject folders.

cd <path>/meld/output/

Quality Checking - The Internal Surface Method

Step 14.

Make a directory to store all of the QC output.

mkdir QC

Quality Checking - The Internal Surface Method

Step 15.

The script we want to run is called **func_make_corticalpngs_ENIGMA_QC.m** with the following parameters:

func_make_corticalpngs_ENIGMA_QC(output_QC_directory, subject_name, select_MRI_image, select_Segmented_image)

We want to set ‘subject_name’ such that ‘subject_name’ + ‘select_Segmented_image’ will form the full name of the segmentation label files (e.g. subj1/mri/aparc+aseg.mgz) and similarly for ‘select_MRI_image’ we want it to give the full name of the registered MRI scan outputted by FreeSurfer (e.g. subj1/mri/orig.mgz).

In the Matlab command window we can do:

```
QC_output_directory='<path>/meld/output/QC/';
FS_directory='<path>/meld/output/';
a=dir(char(strcat(FS_directory,'/MELD_*')));%Choose this so that it
selects %only your subject folders that contain FS output

for x = 1:size(a,1)
    [c,b,d]=fileparts(a(x,1).name); %b becomes the subject_name
    try
        func_make_corticalpngs_ENIGMA_QC(QC_output_directory, b,
[FS_directory, '/', b, '/mri/orig.mgz'], [FS_directory,'/', b,
'/mri/aparc+aseg.mgz']);
    end
    display(['Done with subject: ', b, ': ', num2str(x-2), ' of ',
num2str(size(a,1)-2)]);
end
```

The func_make_corticalpngs_ENIGMA_QC script should take approximately 7 seconds/subject and will output a series of *.png image files separated by individual subject folders.

NB: if you run into problems with this Matlab loop try removing the last "/" in the QC_output_directory variable. So, QC_output_directory='/meld/output/QC/'; would become QC_output_directory='/meld/output/QC';

Quality Checking - The Internal Surface Method

Step 16.

Making the QC Webpage:

To create a webpage for easy viewing of the QC output you just generated in Matlab. Go to the directory where you stored the script ***make_ENIGMA_QC_webpage.sh*** and make sure it is executable:

```
chmod 777 make_ENIGMA_QC_webpage.sh
```

Quality Checking - The Internal Surface Method

Step 17.

Now to run the script, just give the script the full path to the directory where you stored the Matlab QC output files:

```
./make_ENIGMA_QC_webpage.sh <path>/meld/output/QC/
```

NB: If you have trouble running this script, it's possible that you need to fix the line endings in the script before running. You can do this by running this command: sed -i -e 's/\r$//' make_ENIGMA_QC_webpage.sh

This script will create a webpage called **ENIGMA_Cortical_QC.html** in the same folder as your QC output.

Quality Checking - The Internal Surface Method

Step 18.

To open the webpage in a browser in a Linux environment you can probably just type:

```
firefox /meld/output/QC/ENIGMA_Cortical_QC.html
```

In a mac environment you can type

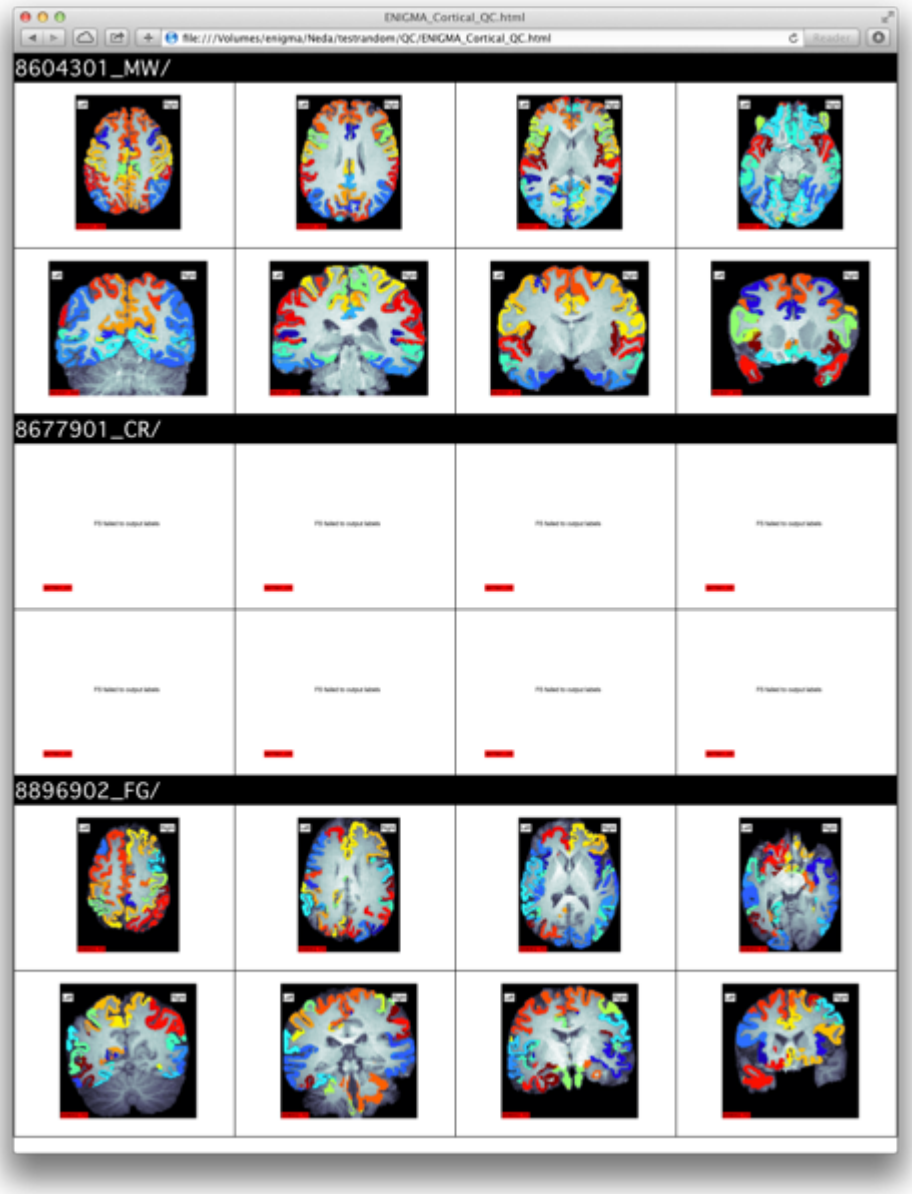
```
open /meld/output/QC/ENIGMA_Cortical_QC.html
```

Note: if you want to check the segmentation on another computer, you can just copy over the whole

/meld/output/QC/ output folder to your computer and open the webpage from there.

Scroll through each set of images. Note that you can click on a subject's files to see a larger version.

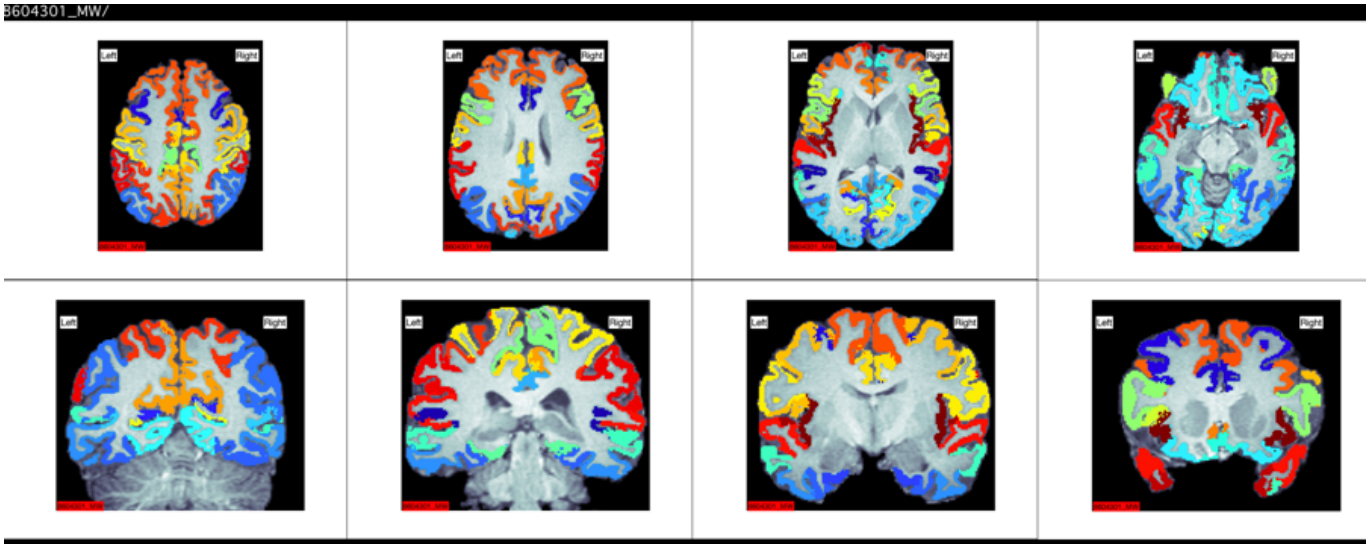
NOTE: you can use the legend.jpg file found in the ENIGMA_QC/ folder as a colored coded reference of each FreeSurfer ROI (split by left/right).



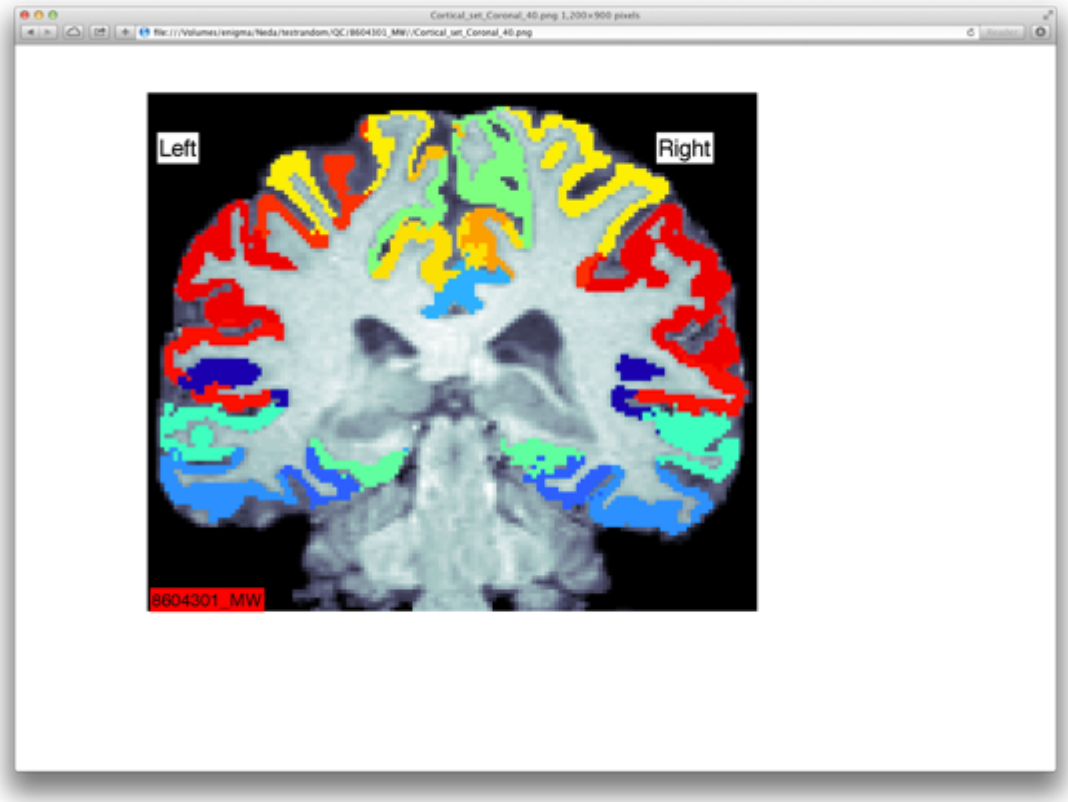
Quality Checking - The Internal Surface Method

Step 19.

Here is an example of a good segmentation:



Here is a close up of a good segmentation:

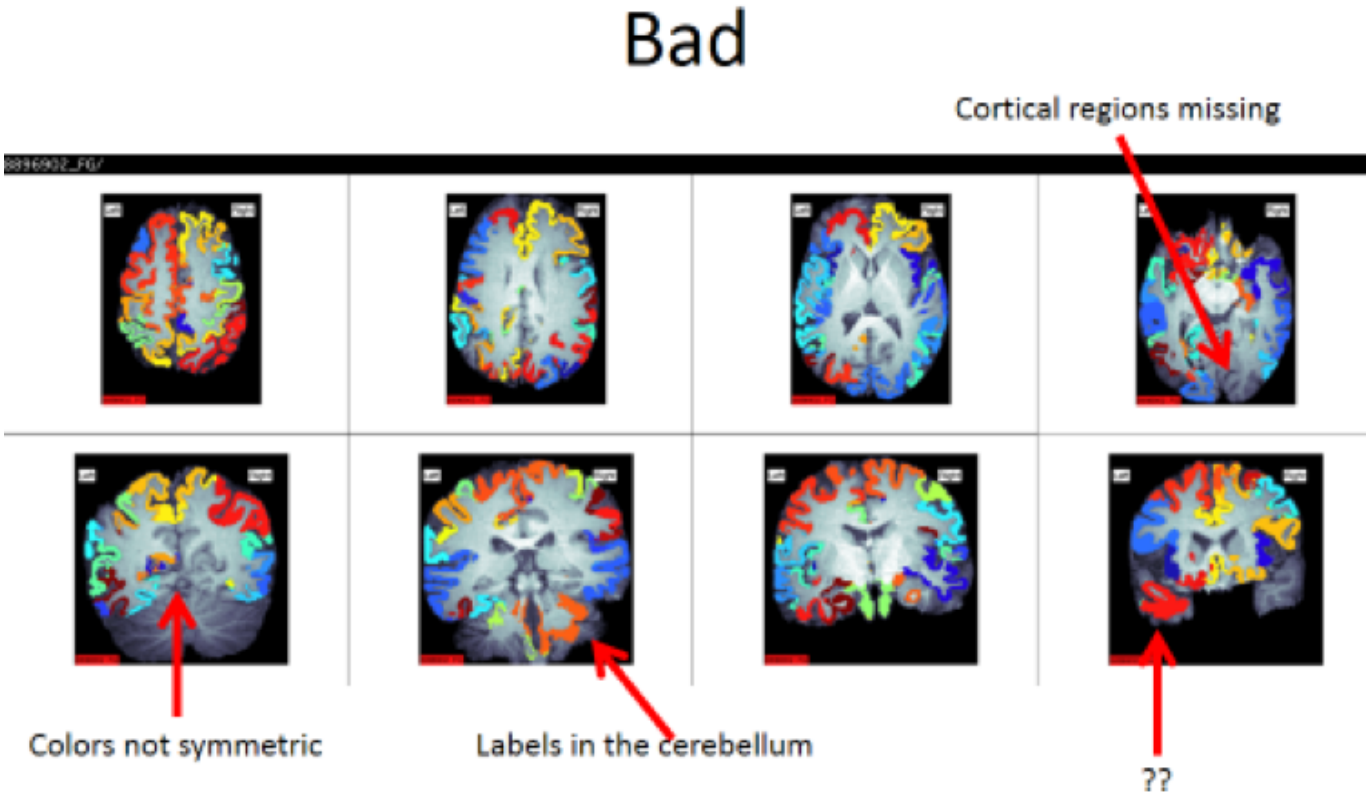


- It is largely symmetrical (colours are symmetrical)
- The grey matter is mostly segmented.
- No skull or cerebellum is wrongly segmented.

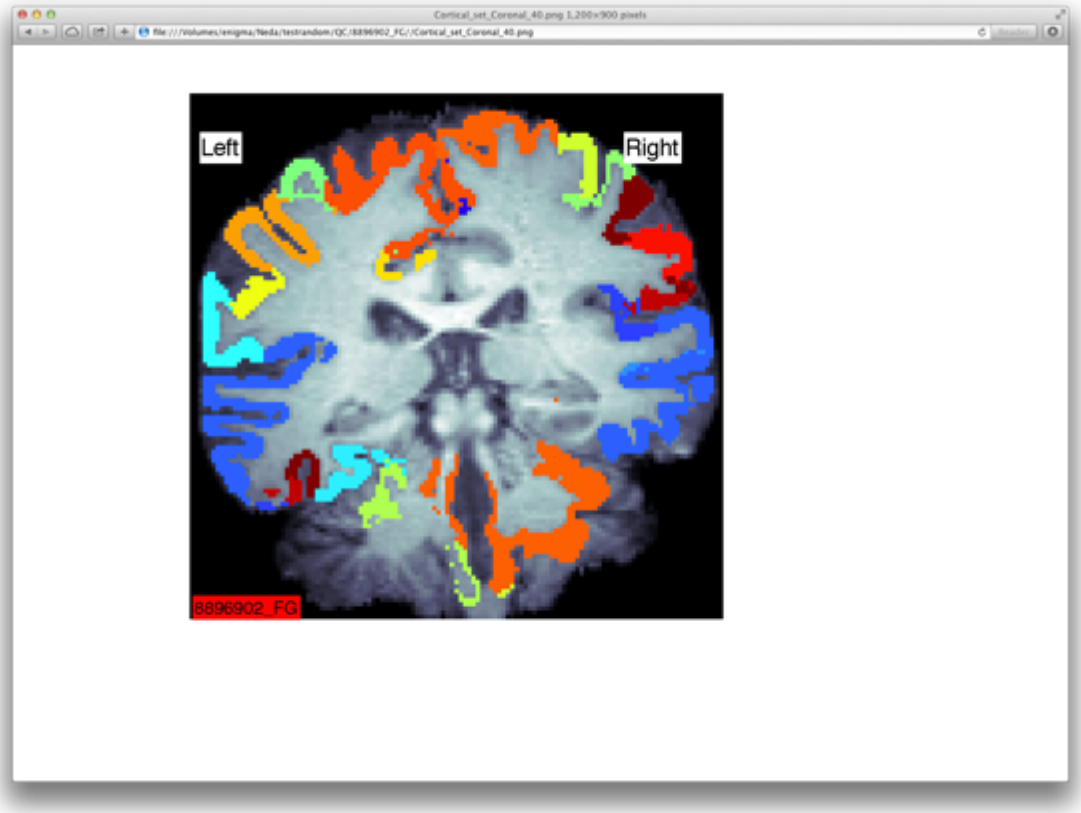
Quality Checking - The Internal Surface Method

Step 20.

Here is an example of a BAD segmentation:



Here is a close-up of a poor segmentation:

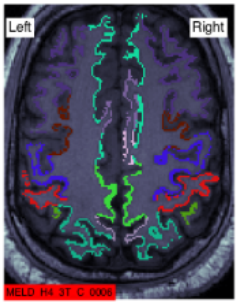


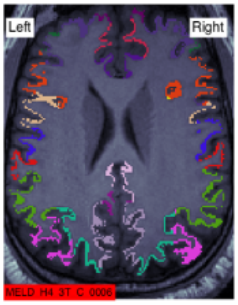
- The colours are not symmetrical
- Part of the cerebellum is segmented
- Part of the grey matter is NOT segmented

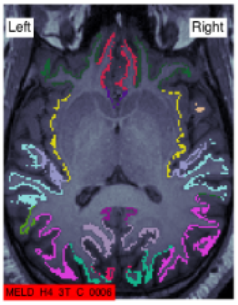
Quality Checking - The Internal Surface Method

Step 21.

Here are more examples of BAD segmentations:

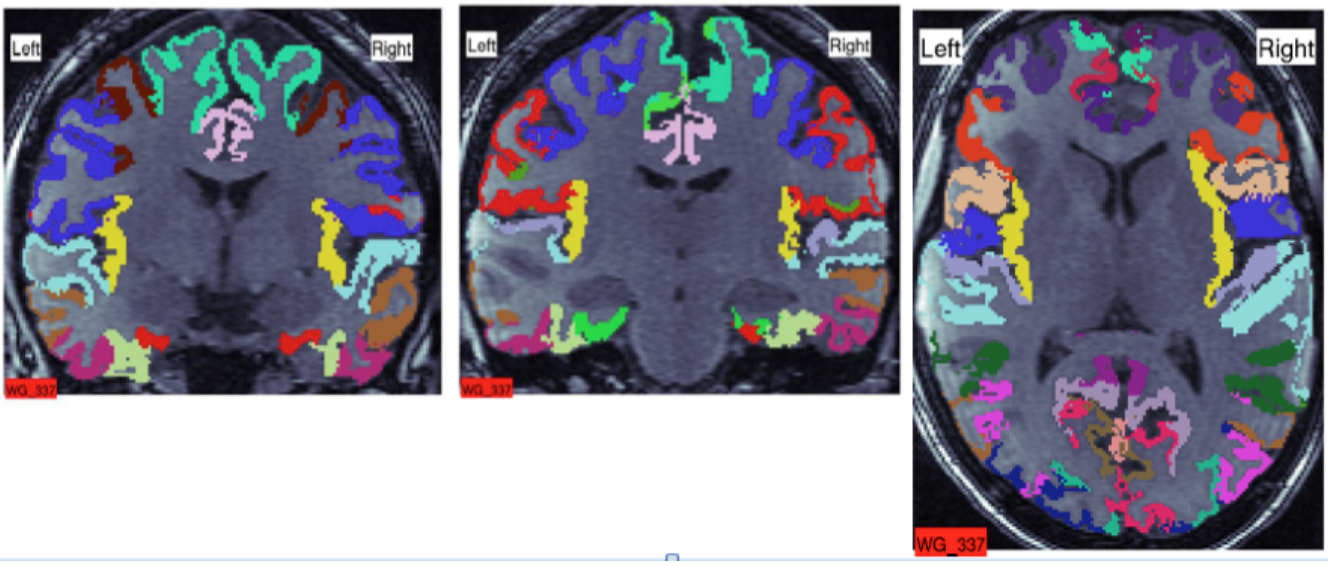






- Although the labels are largely symmetrical, most of the grey matter has not been segmented. The cortex throughout is far too thin.

In this example axial and coronal images show that the outer surface is not correctly delineated due to unsharpness in the raw images, maybe related to motion:



Quality Checking - The Internal Surface Method

Step 22.

Open the data file MELD_[site code]_participants.csv

Rate the quality of the segmentation:

If good, code as 1 in the QC column

If borderline, code as 2 in the QC column

If poor, either attempt to rerun the FreeSurfer segmentation (Supplementary protocol - Rerun FreeSurfer on an individual subject) OR code as 3 in the QC column.

Make sure to save the file back in CSV format!

Please note: in borderline cases, the labels are generally not as symmetrical but there are no very large errors. E.g. no cerebellum is falsely segmented, no global segmentation errors.

We understand that this is quite subjective but this is to give us an overall idea of the data quality. Please do not hesitate to send us an image if you are unsure how to classify the patient / would like more guidance.

Quality Checking - The External Surface Method

Step 23.

Use the script fsqc.sh:

NB: FreeSurfer and its utilities need to be in your path or this script will not run properly. You can type tksurfer on the command line to make sure it is available.

Before you want to work with FreeSurfer, you must make sure three things have happened:

1. The variableFREESURFER_HOME is set (so your computer knows where FreeSurfer is installed):

setenv FREESURFER_HOME <freesurfer_installation_directory>/freesurfer

2. The FreeSurfer set up script must be sourced (so FreeSurfer knows the location of everything it needs):

source $FREESURFER_HOME/SetUpFreeSurfer.csh

3. FreeSurfer has been pointed to a directory of subjects to work on:

setenv SUBJECTS_DIR <path>/meld/output

Quality Checking - The External Surface Method

Step 24.

The script **fsqc.sh** will create a webpage with lateral and medial snapshots of pial surface reconstructions colored with cortical labels. Clicking on the images will display a larger version.

To run the script, first source FreeSurfer's environment variable $SUBJECTS_DIR to point to your subjects directory. For example:

bash
export SUBJECTS_DIR=/meld/output

Quality Checking - The External Surface Method

Step 25.

Next create a directory to contain the snapshots (.tif image files), here we will call it fsqcdir/. Change the working directory to fsqcdir and run the **fsqc.sh** script from there:

mkdir <path>/meld/output/QC/fsqcdir
cd fsqcdir
source <path>/meld/MELD_QC/fsqc.sh

This script will call `tksurfer` in a loop for each subject and output a series of 4 images (.tif) files for each subject. It will also create a website called index.html so that you can easily view the images. You can open the index.html file in any browser, just make sure all of the .tif files are in the same folder if you decide to move the index.html file to a different location (like a local computer).

Quality Checking - The External Surface Method

Step 26.

If you are in a Linux environment you should be able to just type on the command line:

#while in the output fsqcdir output folder

firefox index.html

In a mac environment you can type

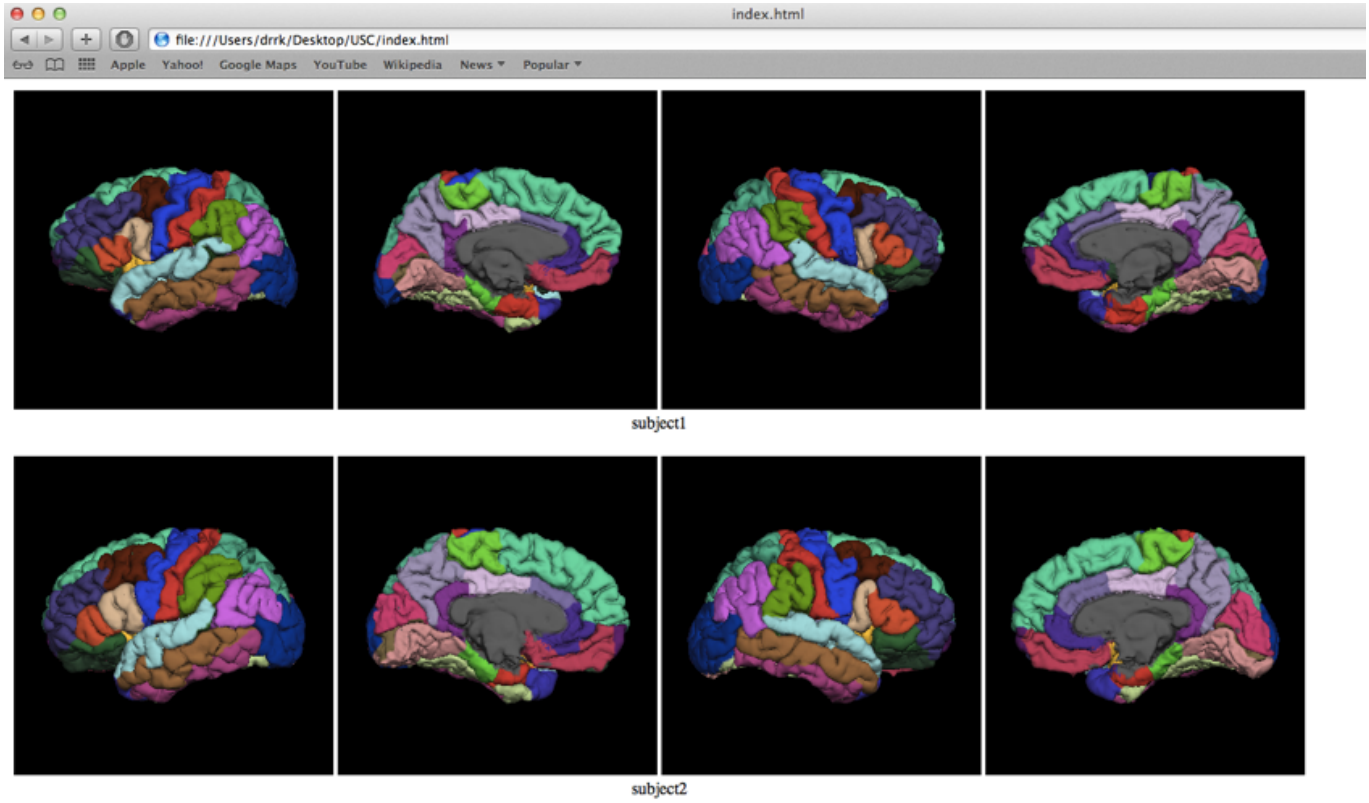
open /meld/output/QC/fsqcdir/index.html

Please note that .tiff is not supported by all browsers (e.g. Google Chrome). It is supported by Safari and Firefox. You may need to install one of these browsers on your linux / mac.

Quality Checking - The External Surface Method

Step 27.

These are examples of good external surfaces:



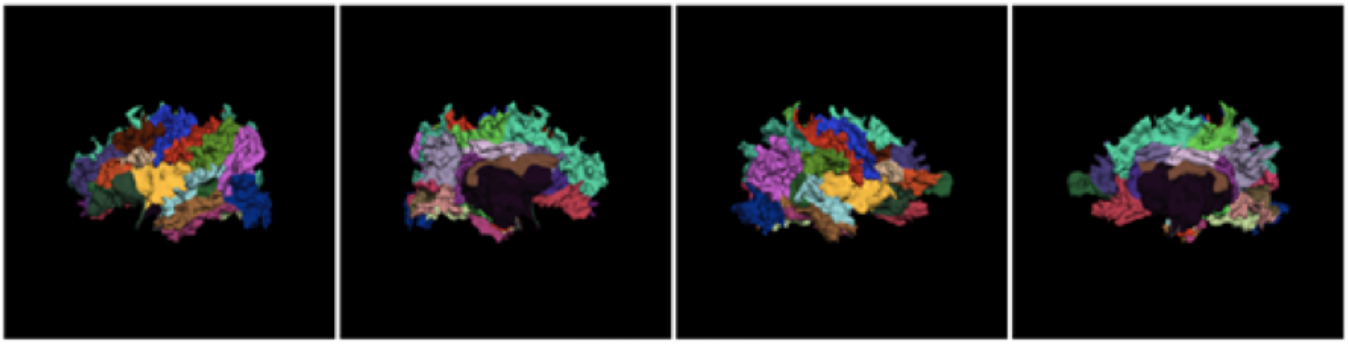
Make sure to:

- Check that all lobes are present, especially the ventral part of the temporal lobe
- Check that labels positions are not different from the general case

Quality Checking - The External Surface Method

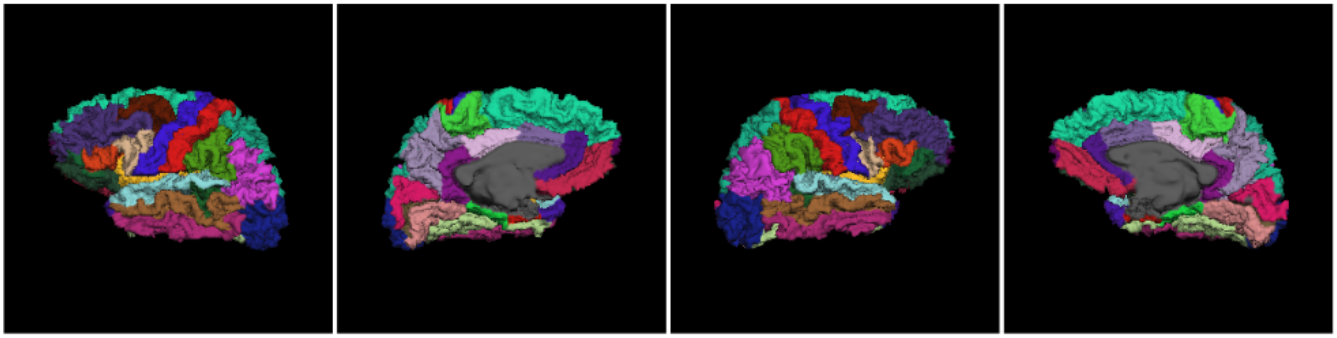
Step 28.

This is an example of a very poor external surface:



000010400245s012a1001

This is another example of a poor external surface:



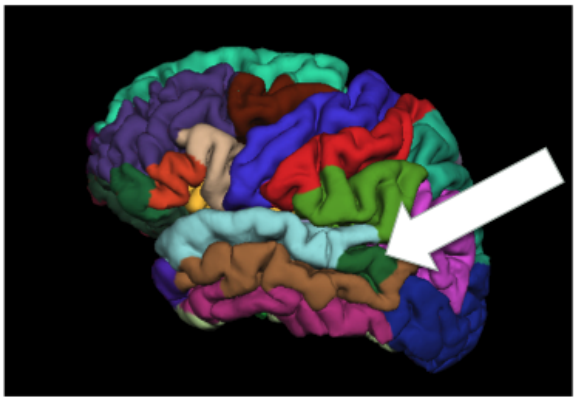
MELD_H4_3T_C_0006

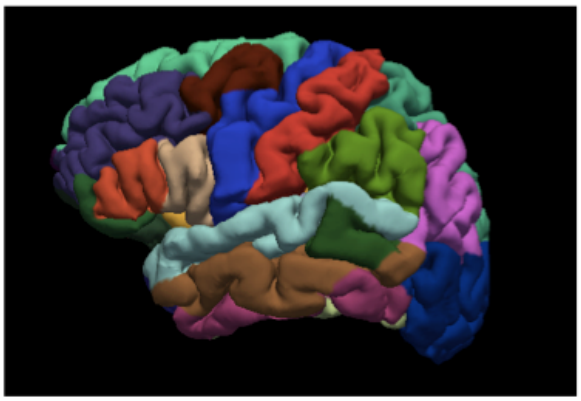
Quality Checking - The External Surface Method

Step 29.

Borderline cases: These are examples of problems that we would code as borderline in the QC column

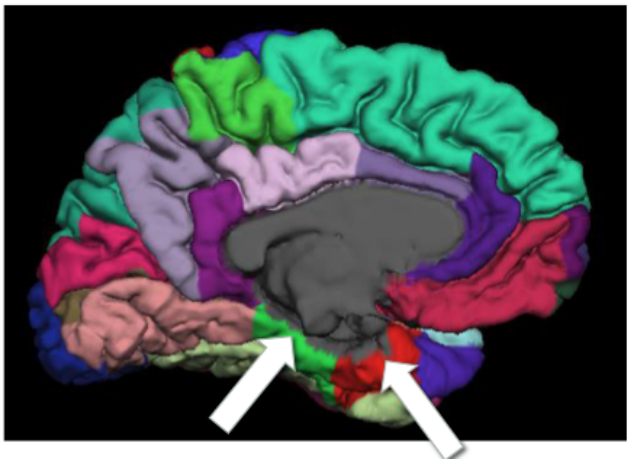
Banks of superior temporal sulcus

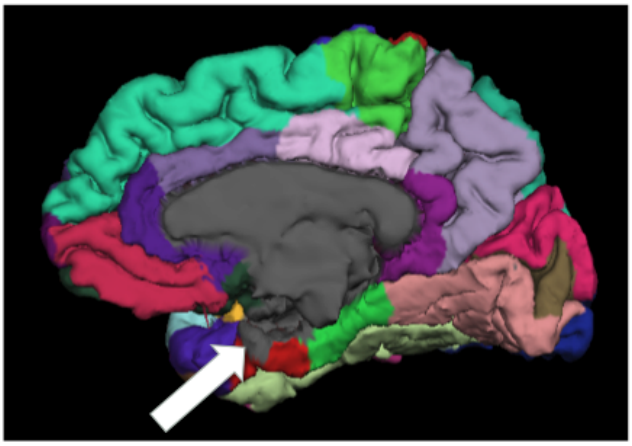




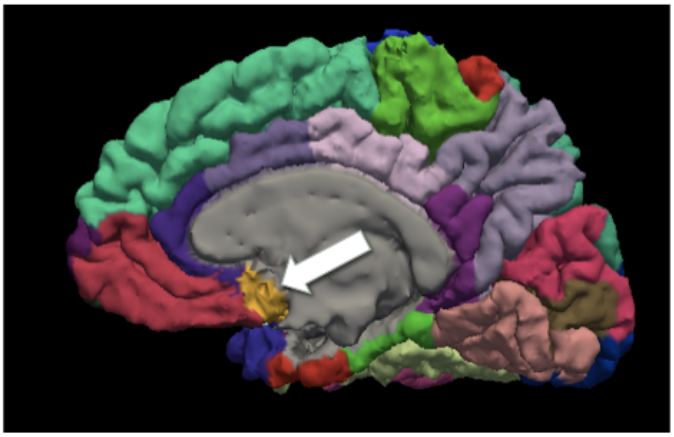
Part of the grey matter of the gyri does seem not to be allocated to parahippocampal

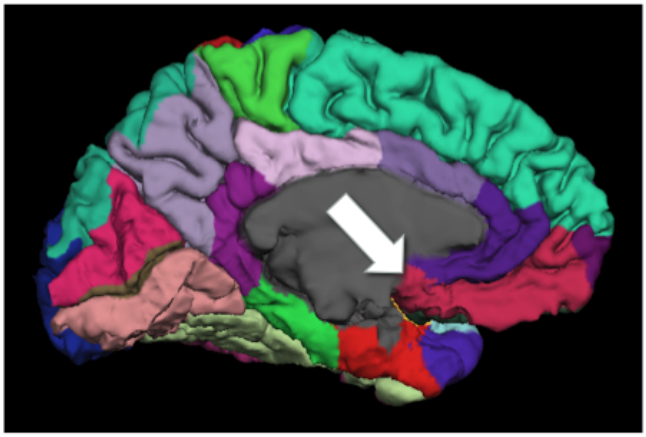
(light green) or entorhinal cortex (red) (but probably to ventricles instead)





Subgenual ACC coded as insula (yellow)





Quality Checking - The External Surface Method

Step 30.

Open the data file MELD_[site code]_participants.csv

Check that the quality of the external surface is the same as the internal surface. If the quality is poorer, recode:

If good, code as 1 in the QC column

If borderline, code as 2 in the QC column

If poor, either attempt to rerun the FreeSurfer segmentation (protocol 2) OR code as 3 in the QC column.

Make sure to save the file back in CSV format!

Please note: unlike in previous ENIGMA-epilepsy studies, DO NOT REMOVE not well segmented regions removed from the CorticaMeasuresENIGMA_SurfAvg.csv or CorticaMeasuresENIGMA_ThickAvg.csv

Quality Checking - FLAIR coregistration

Step 31.

NB: FreeSurfer and its utilities need to be in your path or this script will not run properly. You can type tkmedit on the command line to make sure it is available.

The script **FLAIR_coreg.sh** will create a webpage with coronal, sagittal and axial snapshots of T1 and FLAIR images with the pial and grey-white matter boundary surfaces superimposed. Clicking on the images will display a larger version.

To run the script, first source FreeSurfer's environment variable $SUBJECTS_DIR to point to your subjects directory. For example:

```
bash
export SUBJECTS_DIR=/meld/output
```

Quality Checking - FLAIR coregistration

Step 32.

Next create a directory to contain the snapshots (.tif image files), here we will call it FLAIR_coreg/. Change the working directory to FLAIR_coreg and run the **FLAIR_coreg.sh** script from there:

```
mkdir meld/output/QC/FLAIR_coreg
cd FLAIR_coreg
source <path>/meld/MELD_QC/FLAIR_coreg.sh
```

This script will call `tkmedit` in a loop for each subject and output a series of 4 images (.tif) files for each subject. It will also create a website called index.html so that you can easily view the images. You can open the index.html file in any browser, just make sure all of the .tif files are in the same folder if you decide to move the index.html file to a different location (like a local computer).

Quality Checking - FLAIR coregistration

Step 33.

If you are in a Linux environment you should be able to just type on the command line:

#while in the output fsqcdir output folder

firefox index.html

In a mac environment you can type

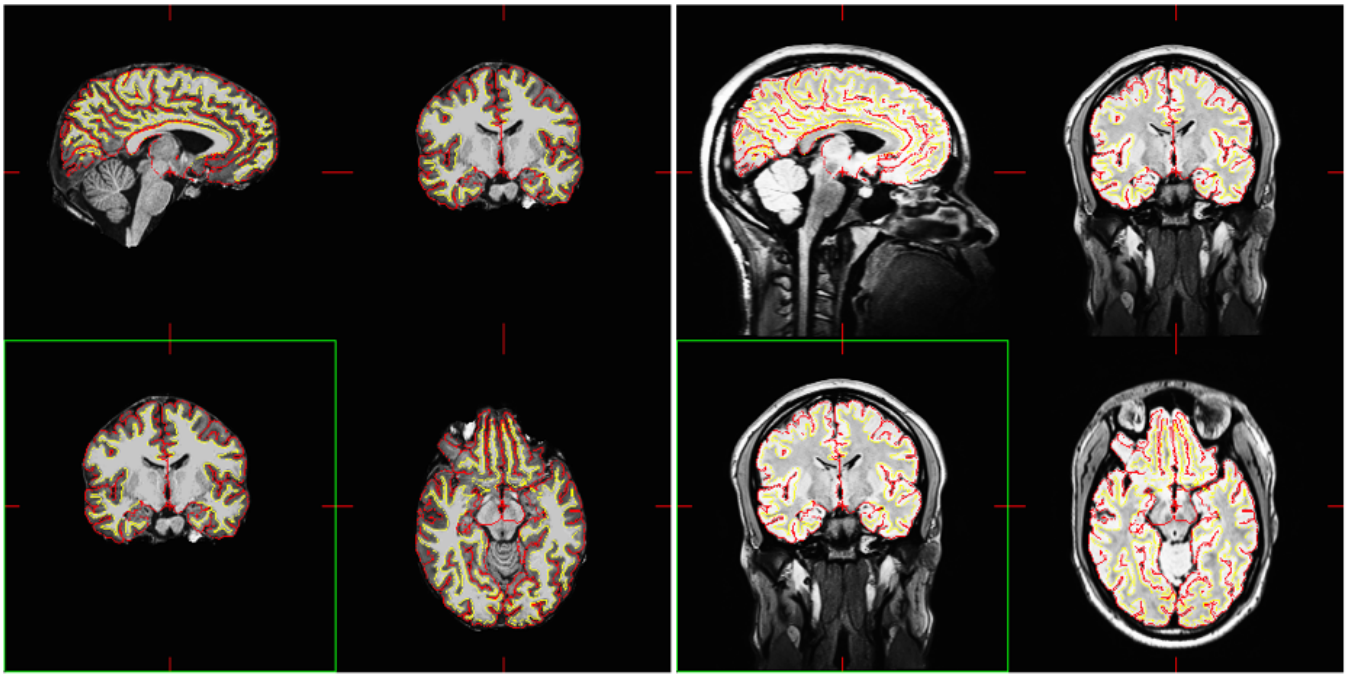
open /meld/output/QC/FLAIR_coreg/index.html

Please note that .tiff is not supported by all browsers. It is supported by Safari and Firefox.

Quality Checking - FLAIR coregistration

Step 34.

This is an example of a correctly co-registered FLAIR scan:



MELD_Htest_3T_C_0001

If the FLAIR is not correctly co-registered you will need to re-run the FreeSurfer reconstruction but change some parameters to ensure that the FLAIR is correctly co-registered this time. **Please contact us via SLACK for more details on the parameters to change (this will depend on**

what your co-registration problem is).

Quality Checking - Summary

Step 35.

Please ensure that you have :

- **checked the quality of any subjects you have rerun**
- **ensured that the FLAIR is correctly co-registered in any participants with FLAIR data**
- **coded the QC in MELD_[site code]_participants.csv for all participants**

Now you can proceed to Protocol 4!

Warnings

PLEASE DO NOT SHARE ANY IDENTIFIABLE DATA

Data sharing only occurs at the level of anonymised demographics information and anonymised data matrices. These are in a template space that cannot be traced back to an individual.