ENIGMA Cerebellum VBM Protocols
Version 2.0

These protocols have been developed by the ENIGMA-Ataxia working group for voxel based morphometry of the cerebellar grey (and white) matter using optimised normalisation of the cerebellum to SUIT space (Diedrichsen et al, 2006, *NeuroImage*; www.diedrichsenlab.org/imaging/suit.htm) using T1-weighted (and optionally, T2-weighted) anatomical MRI images.

These methods are intended for use in normative samples and in cohorts with cerebellar atrophy. It is anticipated that they would be appropriate to most clinical populations, barring those with gross artefacts or large lesions. Images must be visually inspected to exclude such effects, preferably by a qualified radiologist or experienced radiographer.

These protocols are freely available for use by the neuroimaging community. If you use this procedure in your work, please acknowledge the ENIGMA-Ataxia working group, and provide appropriate attribution & citation of the constituent software, including SPM12, the SUIT toolbox, and the CERES pipeline.

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Version Control

Version 2.0 October, 2018 Ian Harding, Sid Chopra
- New naming convention of the files in the "input" directory to allow for different data types (e.g., T1 and T2) from the same subject.
- Addition of a preprocessing step to reset the starting position of the images; Should mitigate most segmentation failures.
- 3rd party cerebellar isolation. The provided scripts use the CERES pipeline (volbrain.upv.es), but options to omit this step or use other isolation approaches are available
- Option to include a T2 or FLAIR image increase automation
- Packaging of scripts into a GUI to streamline functionality

Version 1.0 September 2017 Ian Harding, Carlos Hernandez-Castillo
Processing pipelines for SUIT normalization and quality assurance, and ICV estimation

Software & Setup

1. MATLAB (has been tested on version 2016a and 2018a)

2. SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/)

3. ENIGMA-SUIT Toolbox (suit32e.zip)
   (https://drive.google.com/open?id=1Rn1huVfJo-tPsVDLu9KCmdV4HCJt4Y4l)
   - The SUIT toolbox provided in this link MUST be used – there are some ENIGMA-specific modifications and add-ons. **DO NOT USE OTHER VERSIONS OF SUIT**
   - Unzip and place the 'suit' folder in the 'spm12/toolbox/' directory.

4. ENIGMA-Ataxia Scripts (ENIGMA_Ataxia.zip)
   (https://drive.google.com/open?id=1Rn1huVfJo-tPsVDLu9KCmdV4HCJt4Y4l)
   - Unzip and place the 'ENIGMA_Ataxia' folder (with "ENIGMA_suit" and "shared" subfolders) in the 'spm12/toolbox/' directory

Path Setup: Ensure the SPM12 folder and all subfolders are in your MATLAB path. To check or edit path, type: "pathtool", click "Add with Subfolders", and select your spm12 folder.

Directory Structure and Data Set-Up:

Create a directory or folder called "enigma", with "input" and "suit" subdirectories.

The "input" directory will contain your unprocessed data and will be used as the starting point in all ENIGMA analyses (you will only need to create it once).

In your "input" folder, place the scans of all your participants in "nii.gz" format, labelled as:

Controls:	cont_01_t1.nii.gz	cont_02_t1.nii.gz	…
FRDA:	frda_01_t1.nii.gz	frda_02_t1.nii.gz	…
SCA3:	sca3_01_t1.nii.gz	sca3_02_t1.nii.gz	…
etc.			

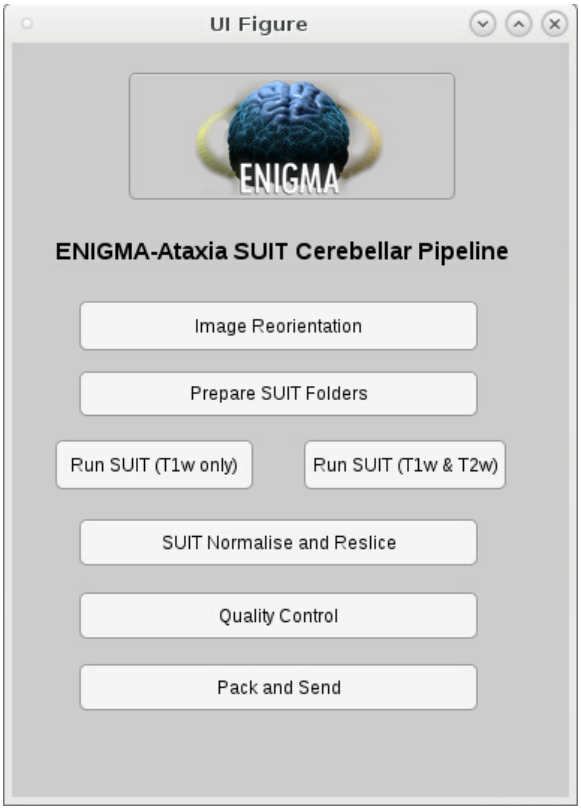
If T2-weighted or FLAIR volumes with similar spatial resolution to the T1 are available, please also include them in the "input" folder as <subj_##>_t2.nii.gz (e.g., cont_01_t2.nii.gz). Including a T2/FLAIR will optimise processing in most cases, but is not essential.

Ensure subject names are all the same length: *4chars_2nums_t#.nii.gz*

VERSION UPDATE: This naming convention differs slightly from Version 1.0 of the pipeline ("_t1" has been added). Use this MATLAB code to rename files from <name>.nii to <name>_t1.nii if necessary:

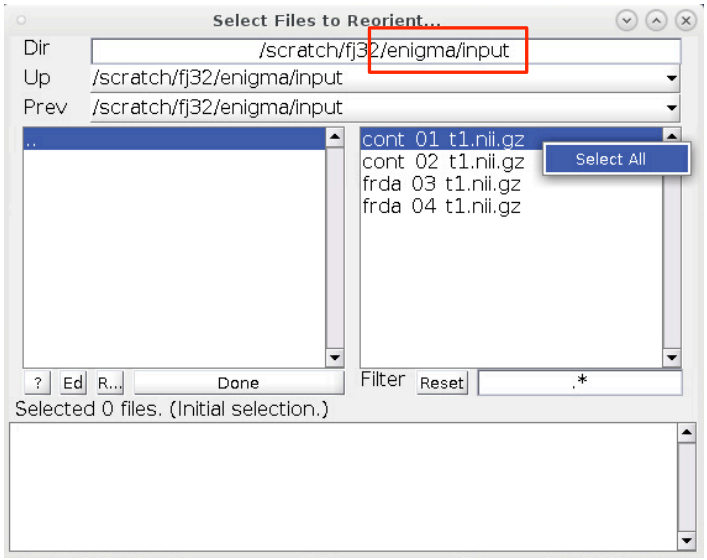
```
images=spm_select(inf,'any','Select images to rename');
for i=1:size(images,1);
new=strrep(images(i,:),'.nii.gz','_t1.nii.gz');
movefile(images(i,:),new);
end
```

1) Launch the ENIGMA SUIT Graphical User Interface (GUI):
In MATLAB, type: ENIGMA_suit (If you get an error, check your path setting as described above)

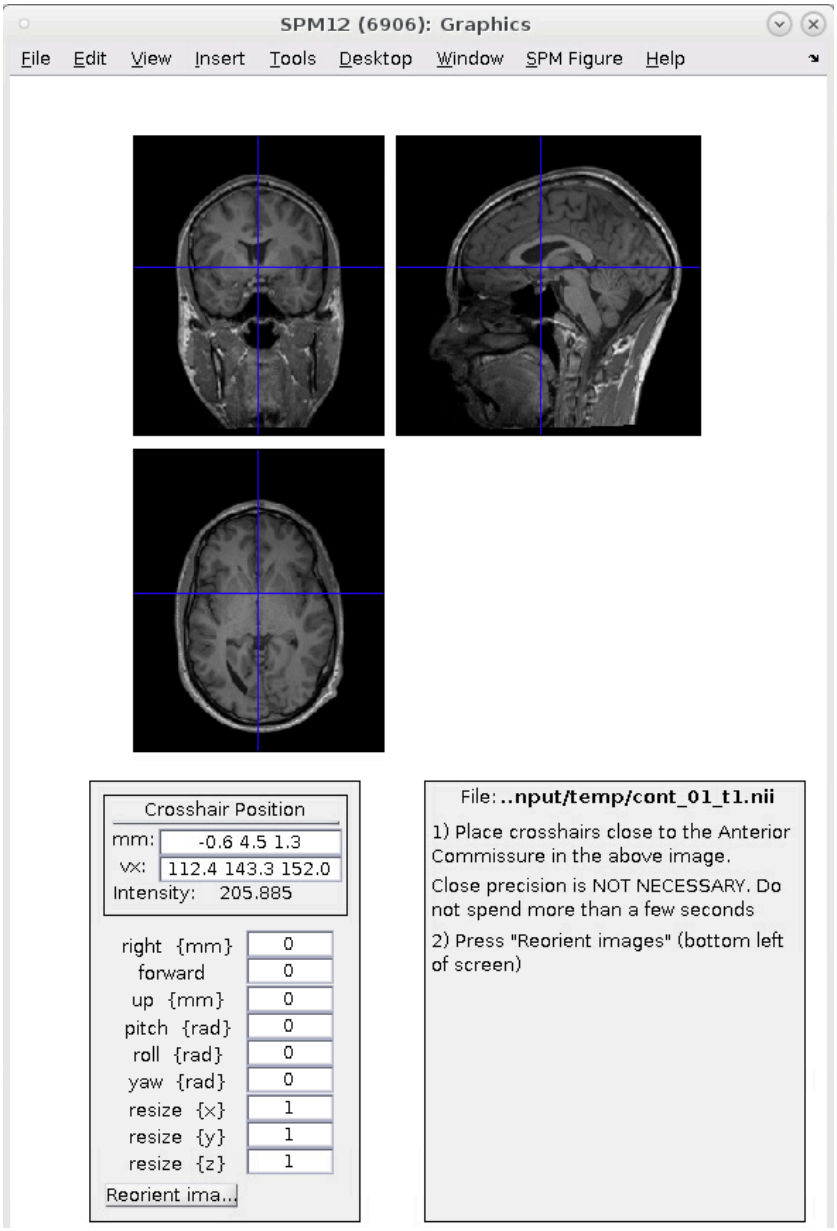


2. Reorient images
To ensure that segmentation proceeds smoothly, your original images will need to be in rough alignment with MNI space. Click the ***Image Reorientation*** button and select all the image files from your 'enigma/input' folder you want to include in the analysis. Include both your T1-weighted and T2-weighted images if you are including the latter. This keeps the two images in rough alignment to aid later coregistration.

This step only needs to be done once. If your images have already been reoriented from a different pipeline (e.g., ENIGMA_CAT12), this step does not need to be repeated.



Clicking ‘Done’ will open up the interactive Image Reorientation GUI:



2.1 Click on the Anterior Commissure so that the blue crosshairs are aligned with it. It does not have to be exact (i.e. don’t spend more than a few seconds on this)

2.2 If there are large rotations (usually pitch-up), try playing with the pitch, roll, or yaw settings (start with +/- 0.1) until the image is closer to AC-PC orientation.

2.3 Press the “Reorient Image” Button, which will translate your image by the required amount. If you selected more than one image for reorientation, the next image will then appear.

```
Error using matlab.ui.Figure/set
Functionality not supported with figures created with the uifigure function. For more information, see Graphics
Support in App Designer.
```

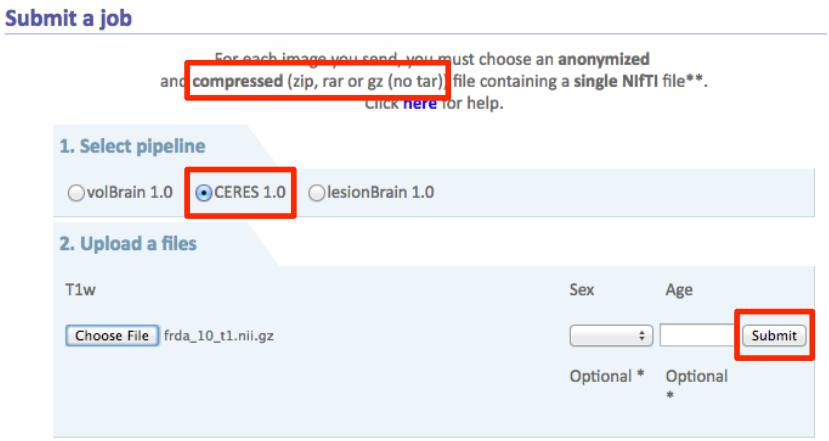
Troubleshoot note: Some versions of Matlab have issues running figure manipulations like the image reorientation function through a GUI. **The above error (or anything similar) can be ignored.** It does not effect the image reorientation.

3) Cerebellum Isolation

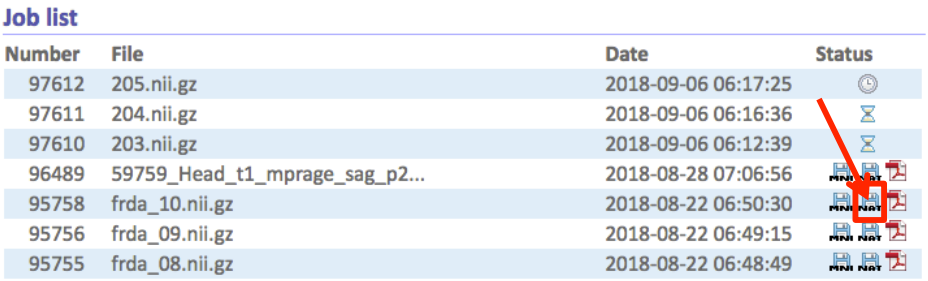
This step is optional. To improve the quality and consistency of the SUIT cerebellar isolation included in SUIT, and minimise the need for manual edits in Step 6, we currently recommend incorporating the cerebellum masks generated by the CERES pipeline. This step is not included in the ENIGMA_SUIT scripts, and is undertaken outside of MATLAB using a web portal. Omitting this step is equivalent to applying a standard SUIT approach and will not impact the validity of the results. However, manual corrections to the masks will likely be more burdensome. Please see the Appendix for further instructions if you choose to omit this step (or use an alternate approach to CERES), as the following steps expect to find the CERES mask.

IMPORTANT: Ensure you have completed Image Reorientation (previous step) BEFORE running CERES, or your masks will be out of alignment with your T1 images.

Register and sign-in at (http://volbrain.upv.es/). Submit T1-weighted images from your 'input' folder one at a time (inputting sex and age is not necessary). Processing only takes ~15mins per subject, but **volBrain only allows 10 images to be submitted per day**. While this is a huge inconvenience, it is still the best approach we have come across for now. I recommend signing up for multiple accounts using different email addresses, or asking students/colleagues to register for an account so you are not delayed by multiple days.



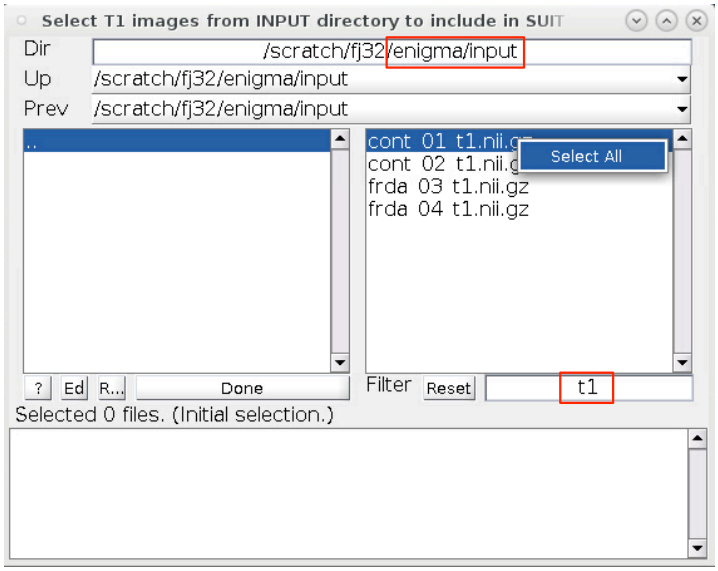
After CERES processing is completed, you will get an email with the segmentation report. But you need to go back to the volBrain site to download the necessary images. Create a 'ceres' folder in your 'enigma' directory (i.e., /enigma/ceres/), and download the "NAT" zip files for each subject into this folder. Do not unzip these files or create subfolders for each subject.



NOTE: If you use the CERES 1.0 outputs for your own work, beware that it drastically over-estimates CrusI volume (as the transverse sinus is often not properly excluded). The developers advise that an improved version (CERES2) is due out in late 2018.

4) Prepare SUIT Folders (~10s per subject)
Relaunch the ENIGMA-SUIT GUI if necessary (in MATLAB, type: ENIGMA_suit).

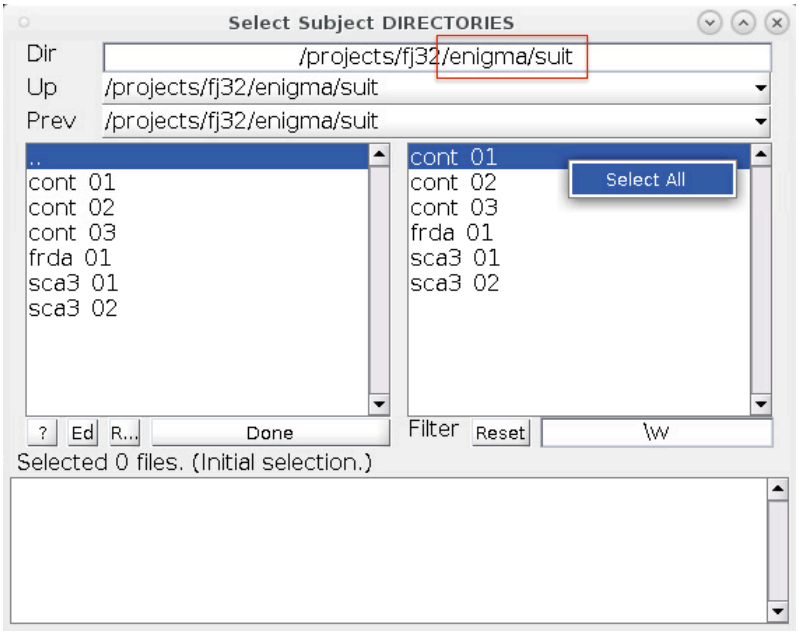
Click the ***Prepare SUIT Folders*** button, select the **T1** images from your 'input' directory for the subjects that you would like to include in your SUIT analysis, and press 'Done'. The script will create a folder in the 'suit' directory for each subject, containing the T1, T2 (if available and named as <subj>_t2.nii.gz in your 'input' directory), and CERES images (if included in the 'ceres' directory as per the previous step) in the correct format.



When complete, the message "SUIT folders have been prepared" will be displayed in the command window.

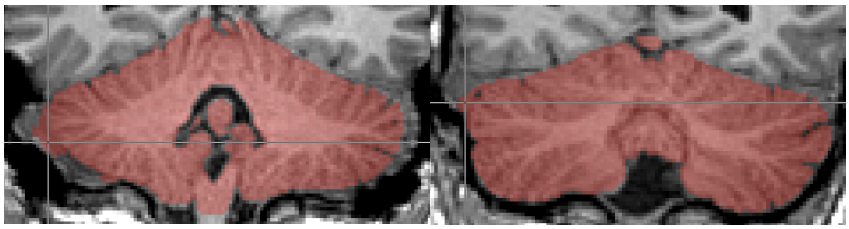
5) Tissue Segmentation & Mask Refinement (10-15mins per subject)
If you have included T2-weighted scans, click the "Segment (T1w & T2w)" button. Otherwise, use the "Segment (T1w only)" option.

This will launch SPM12, and ask you to select the subject DIRECTORIES from your '/enigma/suit' folder that you would like to include in the analysis (NOT individual files from the input directory, as in previous steps).



6) Manual Inspection and Correction of Cerebellum Masks

For T1-only processing, the cerebellar isolation may have problems fully excluding the transverse sinuses (see image), even with the CERES mask, and manual deletions of these inclusions from the "<subj>_t1_pcereb.nii" image will be necessary. When using a T2-weighted image, it is expected that manual edits will not be required, although the masks should still be checked for accuracy. Corrections to the mask outside of the cerebellum (i.e., brainstem and midbrain) are not necessary.



In rare instances, areas of the lateral hemispheres may also be excluded from the mask, particularly in abnormally shaped cerebella. These regions cannot be recovered (even if added back into the mask) and the subject may need to be excluded. It is difficult to provide blanket advice on such exclusions – hopefully you'll get a sense of what is normal across your sample and the masking failures will be very obvious. But do not hesitate to send us screen shots if you are unsure of necessary corrections or exclusions. Note that variability of coverage in the vermis is expected, and would generally not warrant exclusion.

Any image viewer/editor is fine for inspection and editing, but we highly recommend FSLeyes (which has replaced FSLview from FSL ver 5.0.10):

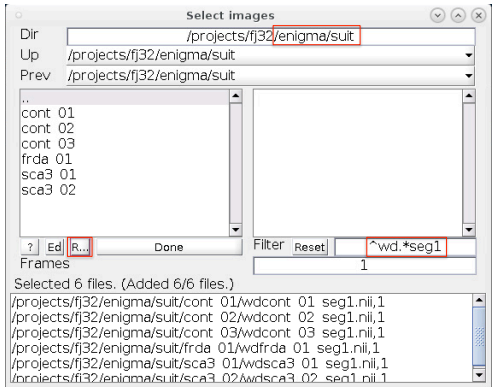
(1) Overlay the "<subj>_t1_pcereb.nii" mask onto the "<name>_t1.nii" anatomical image
(2) Edit the mask (e.g., use the eraser tool in FSLeyes:
https://users.fmrib.ox.ac.uk/~paulmc/fsleyes_userdoc/editing_images.html)
(3) Save the corrected mask with the same name ("<subj>_t1_pcereb.nii"), overwriting the original.

7) Normalise and Reslice (~5mins per subject)

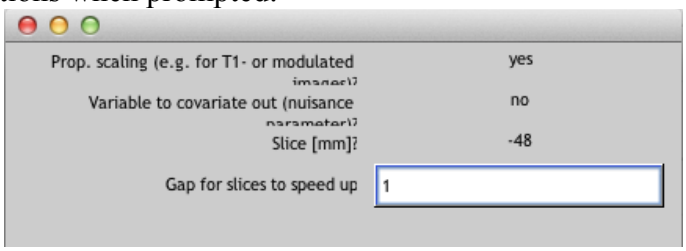
In the ENIGMA-SUIT GUI (reload if necessary by typing: ENIGMA_suit), click on the ***SUIT Normalise and Reslice*** button and again select the desired subject directories from the '/enigma/suit/' folder (as in Step 5).

8) Outlier Detection and Quality Control

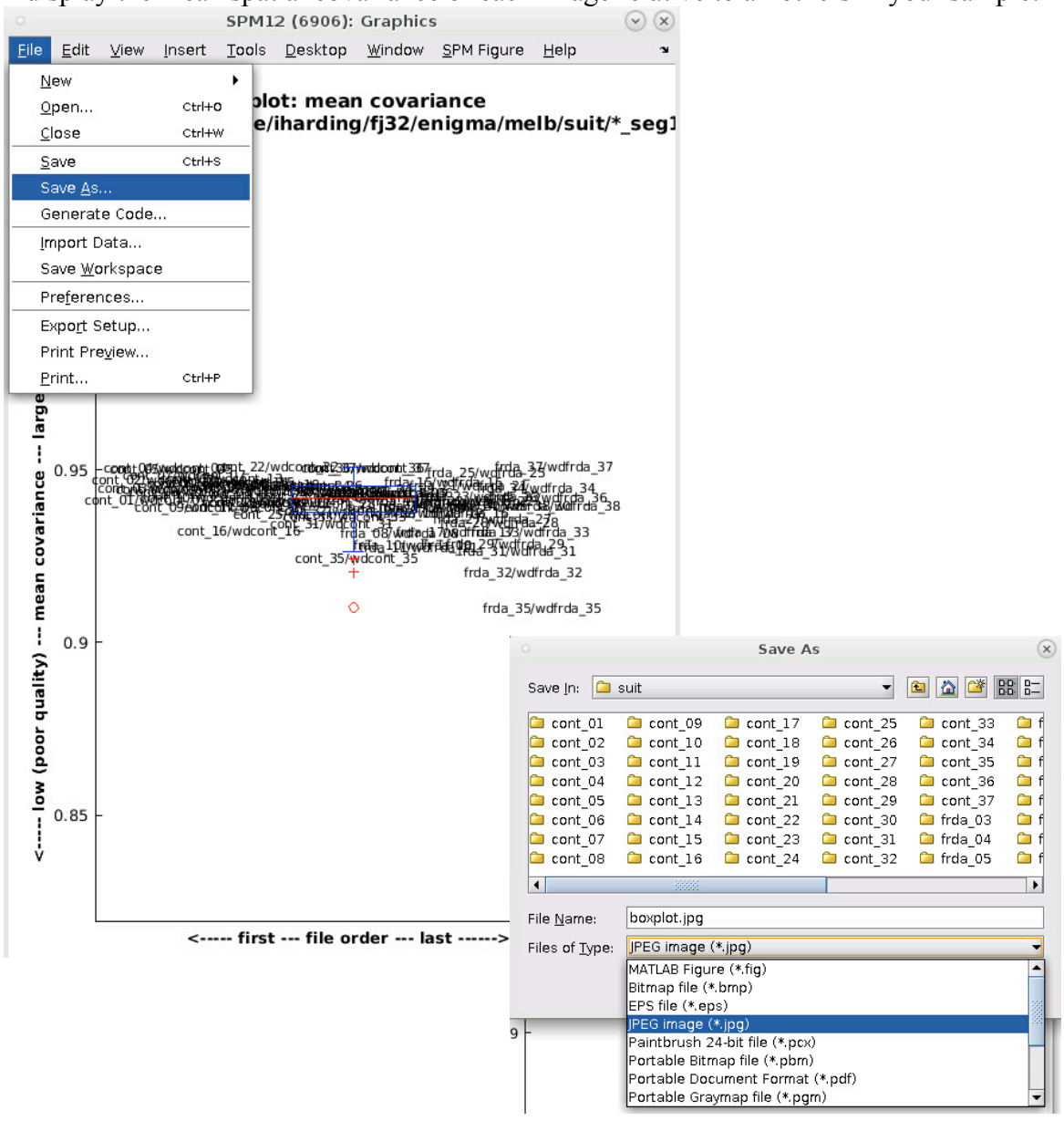
*Check Covariance***.** Reload the GUI (type: ENIGMA_suit), click ***Quality Control > Check Covariance,*** and manually select the "wd*seg1" images from the suit subfolders, or navigate to your 'suit' directory, insert "^wd.*seg1" in the Filter box (no quotations) and press 'Rec' button.



Select the following options when prompted:



While there will be several outputs you can explore, the important output is the boxplot, which will display the mean spatial covariance of each image relative to all others in your sample:



Any data points that are >2s.d. below the mean will be identified in the MATLAB command window and will fall below the lower arm of the boxplot. The original images should be closely inspected for artefacts (motion, anatomical abnormalities, etc.) or other image quality issues. Normalisation or data processing failures will be inspected in the next step. If you are confident that the image quality is fine, and the outlier is due to a real effect (i.e., extreme cerebellar atrophy in late disease stages), retain this data in the sample. Otherwise, this data should be excluded.

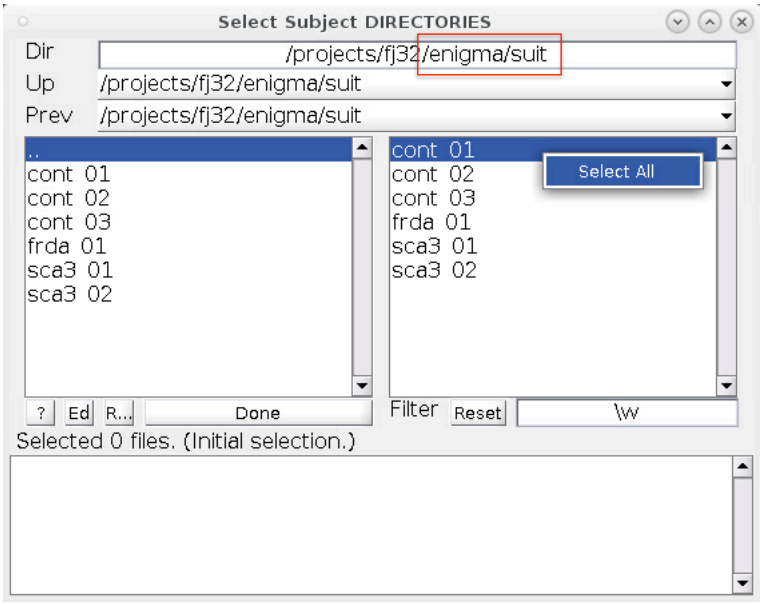
Save the boxplot of the final sample in your ‘suit’ directory as a JPEG file: boxplot.jpg

8.2 *Visual Inspection.* Click the ***Quality Control > Display Timeseries*** button, and select the "wd…seg1" images as in the previous step.

Scroll through your images to ensure that they are all well-aligned. The anatomy across subjects should be very similar at this stage, with only the intensity values varying. This will help you scrutinise outliers identified in the previous step.

9) Package and Send.

Click the ***Pack and Send*** button and again select the subject directories from your 'suit' directory that you would like to include in your final cohort.



This script will collate and zip all of the final processed images (**the "wd" files**), plus the **boxplot.jpg** file (as created in previous steps). This archive will be labelled "SUIT_Final_All.tar" and written to your 'suit' directory.

Please contact the project head to arrange for transfer of the 'SUIT_Final_all.tar' file.

APPENDIX

i. Processing without an External Cerebellum Mask (i.e., CERES)

The use of third-party cerebellar isolation is intended to improve and further automate the native SUIT pipeline, limiting the amount of manual intervention required to correct the cerebellum masks prior to SUIT normalisation. In particular, SUIT can often over-estimate the cerebellum-cerebrum boundary, and erroneously include areas of the transverse sinus. Conventionally, these errors are manually corrected by the experimenter. Our 'optimised' approach seek to improve automation of this process by taking the intersection of the SUIT mask and a third-party mask that is less prone to these errors.

However, you may choose to omit a third-party mask (i.e. CERES) and follow a more conventional SUIT procedure. Simply skip Step 3, and use the following code at Step 5. Corrections to the mask will likely be required (Step 6).

Step 5, alternative T1w-only segmentation:

Copy the following into your MATLAB window:

```
%SELECT ALL THE SUBJECT DIRECTORIES IN THE 'SUIT' FOLDER (NOT THE INDIVIDUAL IMAGES)
clear all
spm fmri
dir_list=spm_select(inf,'dir','Select Subject DIRECTORIES in the SUIT
folder',{},pwd,'\w');
for i=1:size(dir_list,1)
[~,name,~,~]=spm_fileparts(dir_list(i,:));
t1 = [dir_list(i,:),'/',name,'_t1.nii'];

%Run SUIT Segment
suit_isolate_seg({t1});

%Clean-up Files to Conform to SUIT GUI
movefile([dir_list(i,:),'/c_',name,'_t1_pcereb.nii'],[dir_list(i,:),'/',name,'_t1_pcereb.ni
i'])
delete([dir_list(i,:),'/c_',name,'_t1.nii']);
end
```

Step 5, alternative T1w & T2w segmentation:

```
%SELECT ALL THE SUBJECT DIRECTORIES IN THE 'SUIT' FOLDER (NOT THE INDIVIDUAL IMAGES)
clear all
spm fmri

%Select Directories
dir_list=spm_select(inf,'dir','Select Subject DIRECTORIES in the SUIT
folder',{},pwd,'\w');
for i=1:size(dir_list,1)
[~,name,~,~]=spm_fileparts(dir_list(i,:));
t1 = [dir_list(i,:),'/',name,'_t1.nii'];
t2 = [dir_list(i,:),'/',name,'_t2.nii'];
```

```
rt2 = [dir_list(i,:),'/r',name,'_t2.nii'];

%Coregister T2 --> T1
matlabbatch{1}.spm.spatial.coreg.estwrite.ref = {t1};
matlabbatch{1}.spm.spatial.coreg.estwrite.source = {t2};
matlabbatch{1}.spm.spatial.coreg.estwrite.other = {''};
matlabbatch{1}.spm.spatial.coreg.estwrite.eoptions.cost_fun = 'nmi';
matlabbatch{1}.spm.spatial.coreg.estwrite.eoptions.sep = [4 2];
matlabbatch{1}.spm.spatial.coreg.estwrite.eoptions.tol = [0.02 0.02 0.02 0.001
0.001 0.001 0.01 0.01 0.01 0.001 0.001 0.001];
matlabbatch{1}.spm.spatial.coreg.estwrite.eoptions.fwhm = [7 7];
matlabbatch{1}.spm.spatial.coreg.estwrite.roptions.interp = 4;
matlabbatch{1}.spm.spatial.coreg.estwrite.roptions.wrap = [0 0 0];
matlabbatch{1}.spm.spatial.coreg.estwrite.roptions.mask = 0;
matlabbatch{1}.spm.spatial.coreg.estwrite.roptions.prefix = 'r';
spm_jobman('run',matlabbatch);
clear matlabbatch

%Run SUIT Segment
suit_isolate_seg({t1
    rt2});

%Clean-up Files to Conform to SUIT GUI
movefile([dir_list(i,:),'/c_',name,'_t1_pcereb.nii'],[dir_list(i,:),'/',name,'_t1_pcereb.ni
i'])
delete([dir_list(i,:),'/c_',name,'_t1.nii']);
end
```

ii. Alternative Approach to External Cerebellum Masking

After testing multiple approaches, we have found CERES to provide a balance between ease of use and quality. However, it is theoretically possible to use any third-party masking approach that provides reliable delineation of the cerebellar borders.

Note that Freesurfer and FSL-FIRST do **NOT** provide suitably accurate cerebellum isolation for this purpose.

If you are using an alternative cerebellar isolation approach, skip step 3 and include your cerebellum mask in each of your '/enigma/suit/<subj>/' directories, labelled as '<subj>_ceresmask.nii'. The mask does not need to be binarised, but any non-cerebellar voxels should have values of 0 or NaN.

WARNING: Ensure you segment the cerebellum after Image Realignment in Step 1. If this is not possible (i.e., your cerebellum masks have already been generated), skip the Image Realignment procedure, but examine your data for segmentation failures during QA (e.g., Step 8.2) – most commonly indicated by an empty image following normalisation.

Please advise the project head if you are using an alternative masking approach.