ENIGMA Whole Brain VBM Protocols
Version 1.0

These protocols have been developed by the ENIGMA-Ataxia working group for voxel-based morphometry of whole brain grey and white matter using inhouse scripts and the Computational Anatomy Toolbox (CAT12) on T1-weighted anatomical MRI images.

These methods are intended for use in normative samples and in cohorts with cortical atrophy. It is anticipated that they would be appropriate for 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 both the CAT12 toolbox and SPM12.

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Version Control

Version 1.0 October 2018 Ian Harding, Sid Chopra
- Processing pipeline for CAT12 segmentation, normalisation, modulation and quality assurance. for whole brain grey and white matter.

Software & Setup

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

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

3. CAT12 toolbox (http://dbm.neuro.uni-jena.de/cat/index.html#DOWNLOAD)
   - The ENIGMA-Ataxia VBM pipeline was designed using version CAT12.5 (r1363) released on 02/09/2018.
   - Unzip and place the 'cat12' 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_cat12" 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

If you have not previously done so (i.e. for the SUIT analyses or any other ENIGMA analysis), Create a directory/folder called "enigma", with a "input" subdirectory which will contain your unprocessed data and will be used as the starting point in all ENIGMA analysis (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.

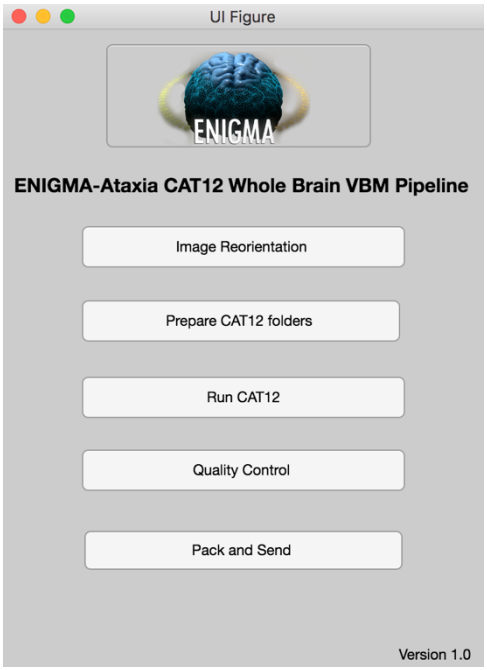
Ensure subject names are all the same length: *4chars_2nums.nii.gz*

Note: This naming convention differs slightly from a previous version of an ENIGMA-Ataxia pipeline (Cerrebellar-SUIT VBM Version 1.0); "_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 CAT12 Graphical User Interface (GUI):

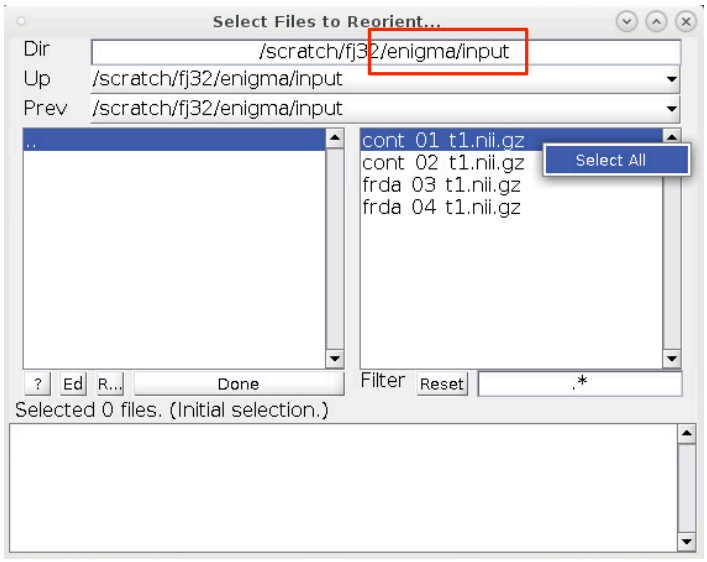
In MATLAB, type: ENIGMA_cat12GUI
(If you get an error, check your path setting as described above)



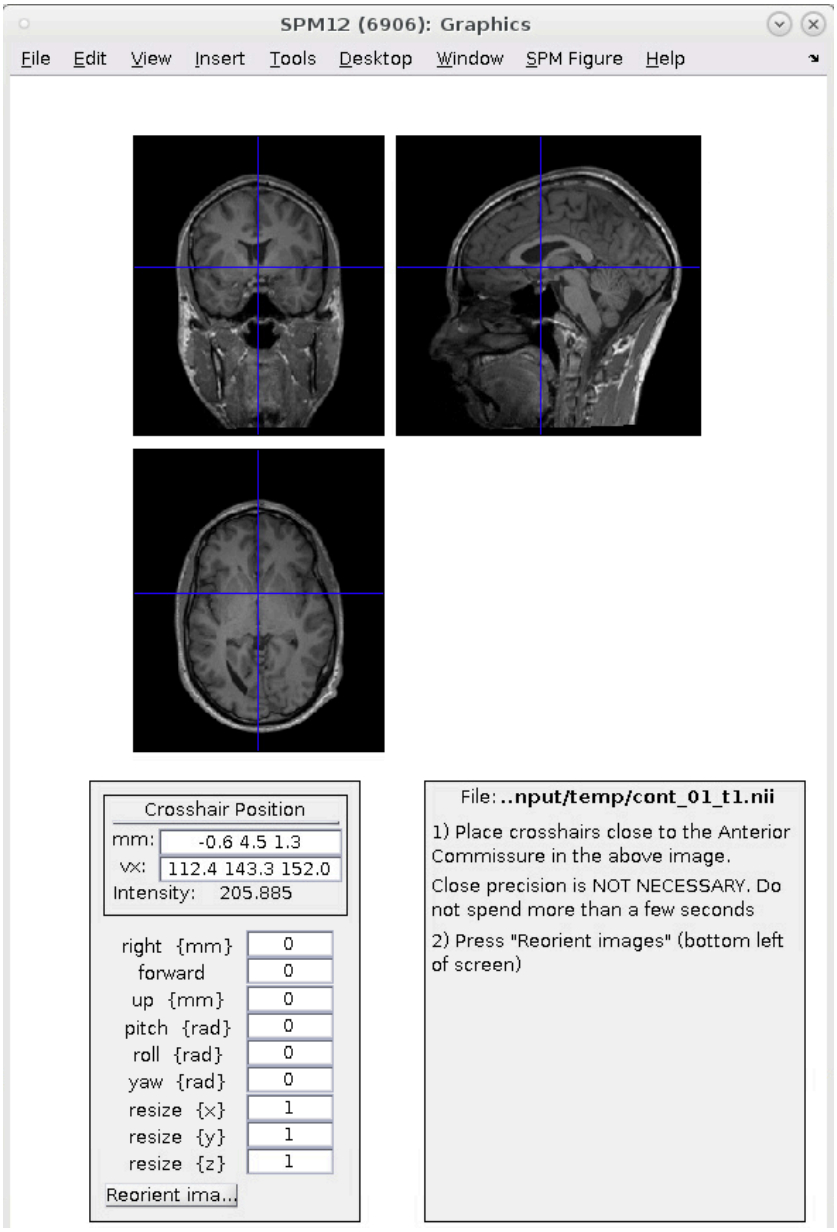
2) Reorient images

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.

This step only needs to be done once. If your images have already been reoriented from a different pipeline (e.g., ENIGMA_suit), 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 after a 5-10s delay.

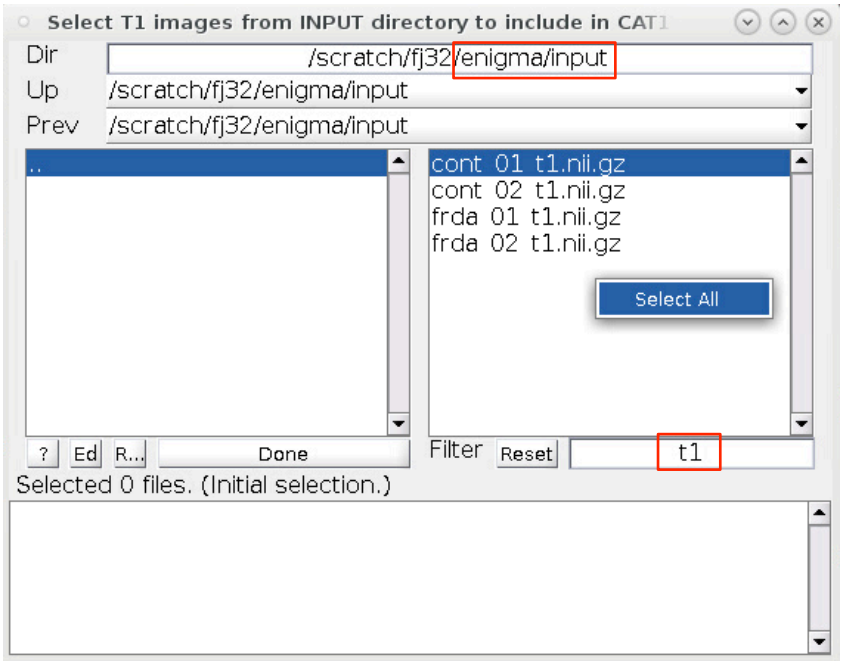
```
Error using matlab.ui.Figure/set
Functionality not supported with figures created with the uifigure function. For more information, see Graphics
Support in App Designer.
```

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 affect the image reorientation.

3) Prepare CAT12 Folders (~10s per subject)

Relaunch the ENIGMA-cat12 GUI if necessary (in MATLAB, type: ENIGMA_cat12GUI).

Click the ***Prepare CAT12 Folders*** button, select the **T1** images for your 'input' directory for the subjects that you would like to include in your analysis, and press 'Done'. The script will create a cat12 folder in the input directory and within it a subfolder for each subject, containing the T1 image in the correct format.



When complete, the message "CAT12 folders have been prepared" will be displayed in the command window.

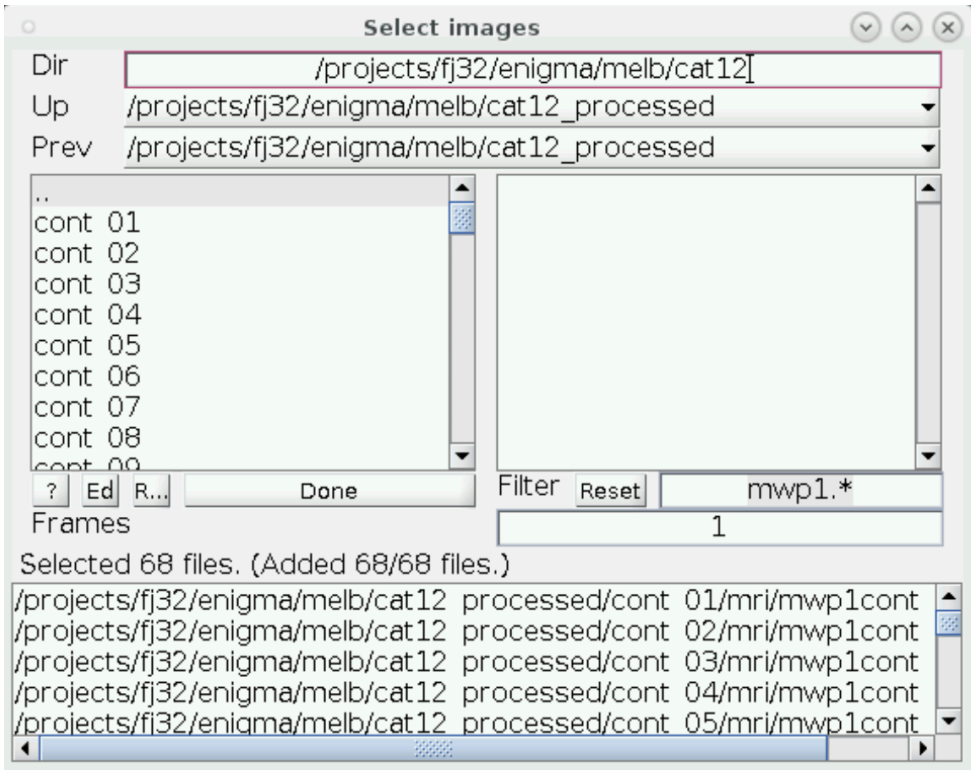
4) Running CAT12: Segmentation, Normalisation & Modulation (20-40mins per subject)

Clicking the Run CAT12 button will open up a dialog box prompting you to select all the T1-weighted (.nii) files from your **enigma/cat12** folders (not the 'input' folder this time) to include in the analysis. Once again, you can either click the "Rec" button to recursively select all scans (.nii) within the cat12 folders or manually select the scans from each subject directory. Clicking done will sequentially run CAT12 (using the ENIGMA-Ataxia settings) on each scan **(requiring up to 40mins per scan).**

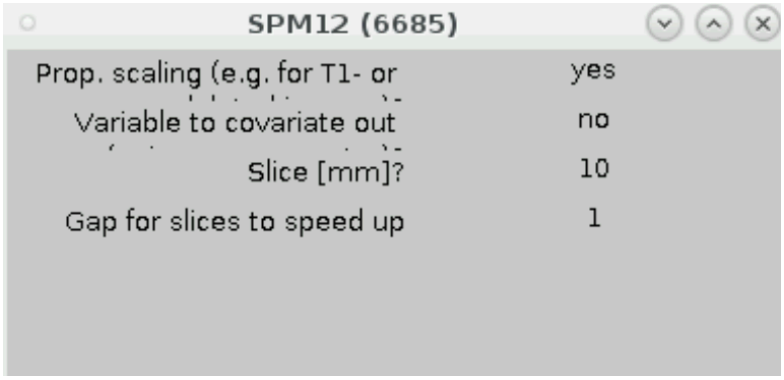
CAT12 Output: When the CAT12 pre-processing is complete, it will have generated two folders, mri and report, within your individual subject folders (for example: enigma/cat12/frda_01/mri). Inside with mri folder should be two files: mwp1*.nii and mwp2*.nii. These are the segmented, normalised and modulated grey matter (mwp1) and white matter (mwp2) images. Inside the report folder should be three files: cat_*.mat, cat_*.xml and catreport_.pdf.

5) Outlier Detection and Quality Control

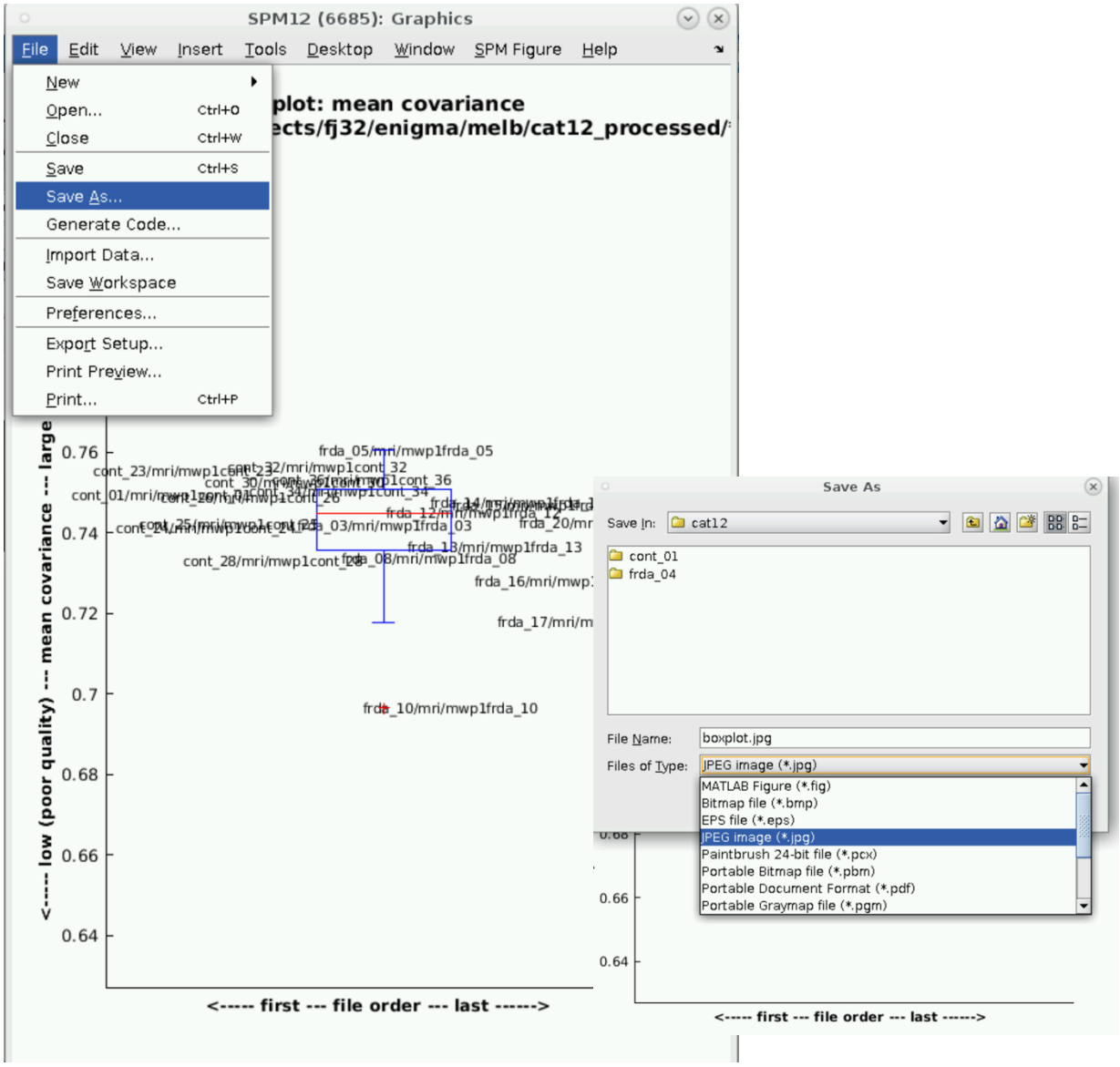
*Check Covariance***.** Reload the GUI (type: ENIGMA_cat12GUI), click ***Quality Control > Check Covariance,*** and Manually select the "mwp1*" images from the subfolders, or navigate to your 'cat12' directory, insert "mwp1.*" in the Filter box (no quotations) and press the 'Rec' button.



Select the following options when prompted:



The output may take some time to generate (up to 5 mins depending on the number of scans selected). 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), this data should be retained in the sample. Otherwise, this data should be excluded.

Save the boxplot of the final sample in your 'cat12' directory as a JPEG file: boxplot.jpg

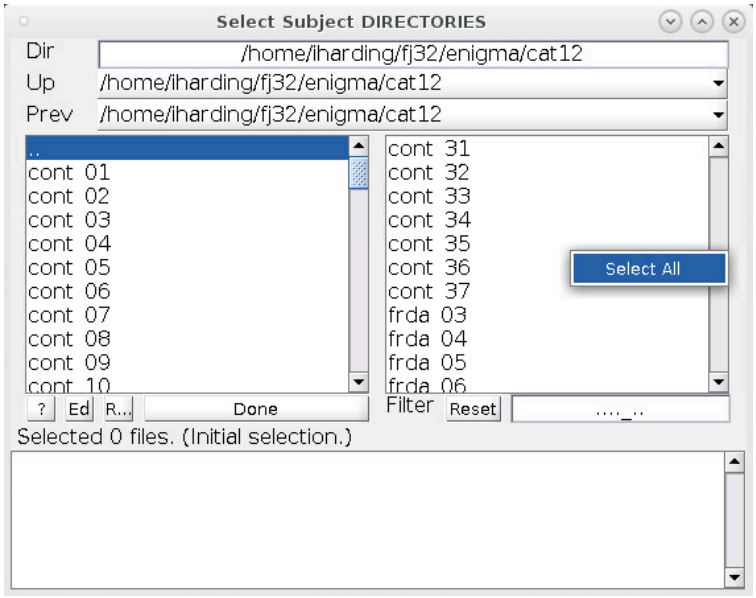
6) Visual Inspection

Click the ***Quality Control > Display Timeseries*** button, and select the "mwp1*" 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.

7) Package and Send

Click the ***Pack and Send*** button and again select the subject directories from your 'cat12' directory that you would like to include in your final cohort.



This script will collate and zip both the 'mri' and the 'report' folders and containing final processed images for each subject, plus the **boxplot.jpg** file (as created in previous steps). This archive will be labelled "CAT12_Final_All.tar" and written to your 'cat12' directory.

Please contact the project head to arrange for transfer of the 'CAT12_Final_all.tar' file.