

# ENIGMA Cervical Spinal Cord Protocol

## Version 1.0

These protocols have been developed by the ENIGMA-Ataxia working group to quantify the cross-sectional area of cervical spinal cord using in-house scripts and the Spinal Cord Toolbox (SCT) applied to T1-weighted anatomical MRI images.

The Spinal Cord Toolbox (SCT) is a validated, state-of-the-art software package designed to process spinal cord multimodal MRI data (De Leener et al, 2017). SCT is compatible with any scanner brand, it uses images in NIfTI format and is designed to accept a variety of sequences, modalities and contrasts. Being based on Python language, it is free and open source.

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 and citation of the constituent software (SCT).

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### **Version Control**

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-Processing pipeline for SCT segmentation, quantification and quality assurance for cervical spinal cord.

### **Software & Setup**

1. MATLAB (has been tested on version 2016a, 2018a, and 2019a);
2. SPM12 (<https://www.fil.ion.ucl.ac.uk/spm/software/download/>);
3. Python 3.0 for Unix or iOS (generally this is already preinstalled on your operating system)
4. Spinal Cord Toolbox: <https://github.com/neuropoly/spinalcordtoolbox/releases/tag/4.2.2>  
The ENIGMA-Ataxia SCT pipeline was designed using version SCT **4.2.2** (see below for installation instructions)
5. ENIGMA-Ataxia Script Package: <https://drive.google.com/drive/folders/1Rn1huVfJo-tPsVDLu9KCmdV4HCJt4Y4I?usp=sharing>

- a. Unzip and place the ENIGMA\_Ataxia folder in your MATLAB path. To check or edit path, type “pathtool”, click “Add with Subfolders” and select your ENIGMA\_Ataxia folder. Important, name the folder of this script as ENIGMA\_sct. In addition, remove from your MATLAB path the secondaries paths from fieldtrip tool inside SPM12 toolbox.

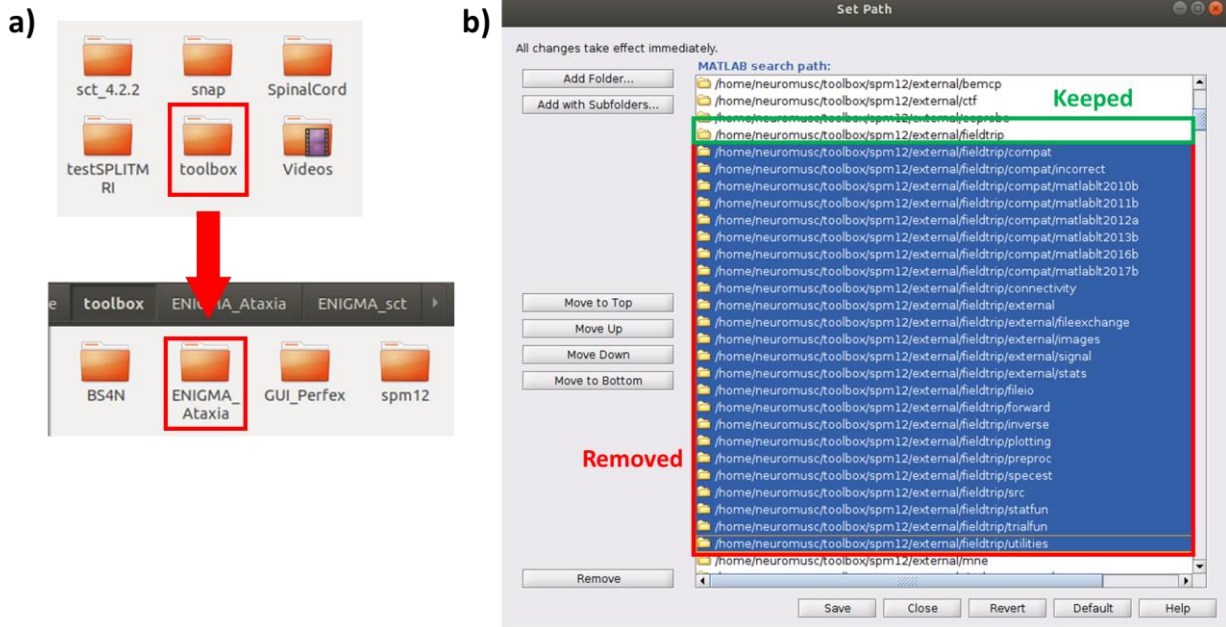


Figure 1: Setting path to Enigma\_sctGUI pipeline. a) Structure of folders to set MATLAB path. b) Secondary paths of fieldtrip tool that must be removed from MATLAB path.

### Install SCT (10-15mins)

SCT was built to work in Mac and Linux platforms. Any flavor of Linux should be fine, but Ubuntu is recommended if you have the option, because this operating system is more user friendly and SCT is tested on it. Windows users can use a virtual machine, but set-up is quite complicated, and we recommend this option only as a last resort – contact us for further details. Furthermore, SCT only runs in the ‘bash’ shell. To check your shell type, in your terminal type “echo \$0”. If the answer is not “-bash”, then type “/bin/bash”.

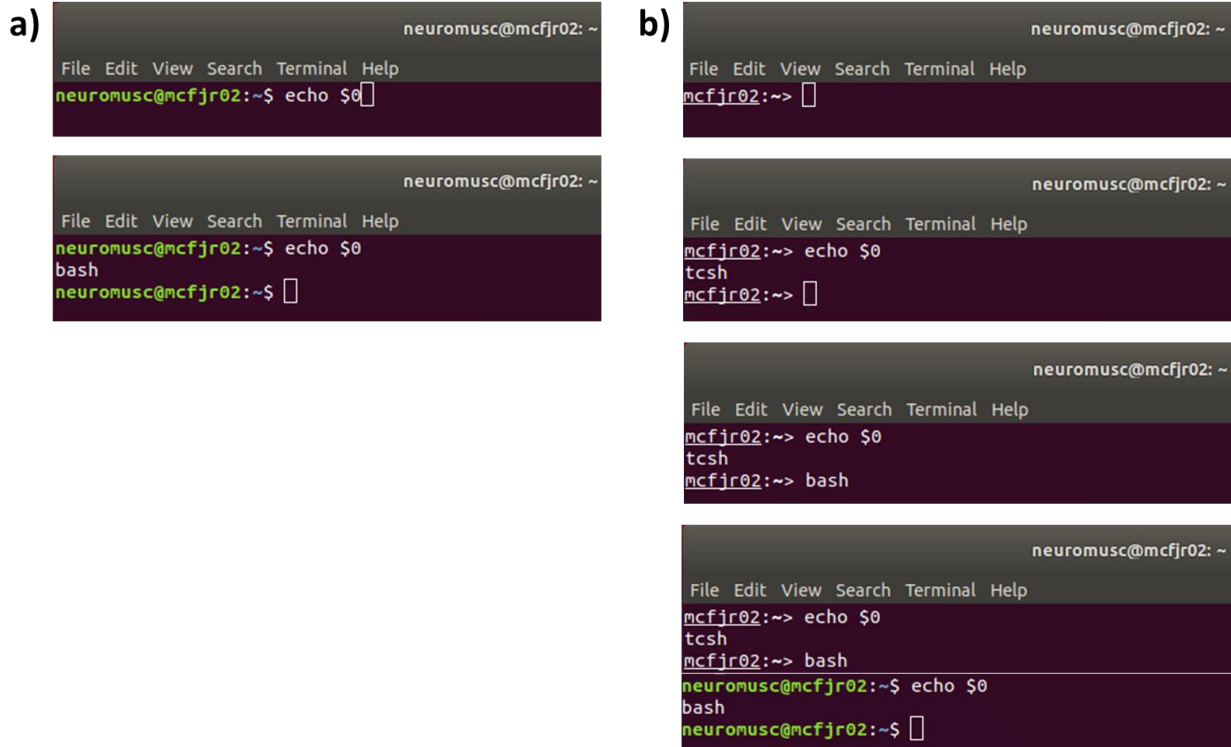


Figure 2: Verifying if terminal is running in bash shell. a) expected answer. b) not expected answer and how to solve it.

To install SCT enter in the following link:

<https://github.com/neuropoly/spinalcordtoolbox/releases>

Download the **version 4.2.2**. Unzip the file, open the terminal, and type:

1. `cd /path/to/sct/spinalcordtoolbox-4.2.2/`

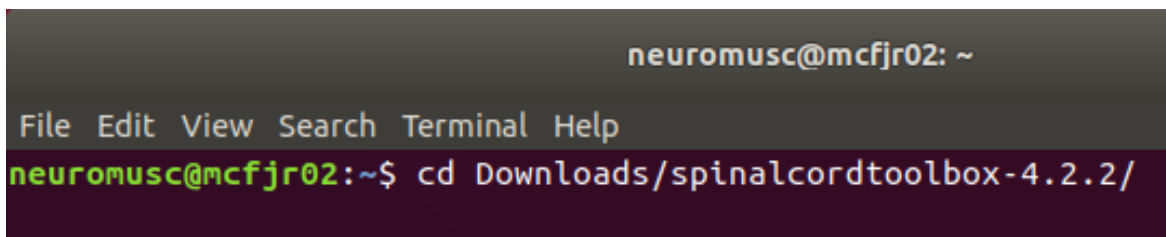
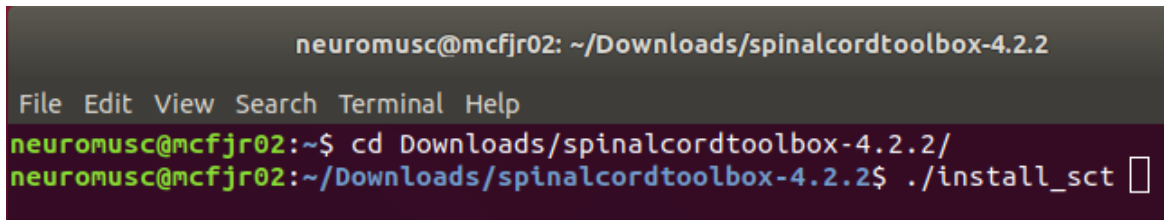


Figure 3: Installing SCT, step 1.

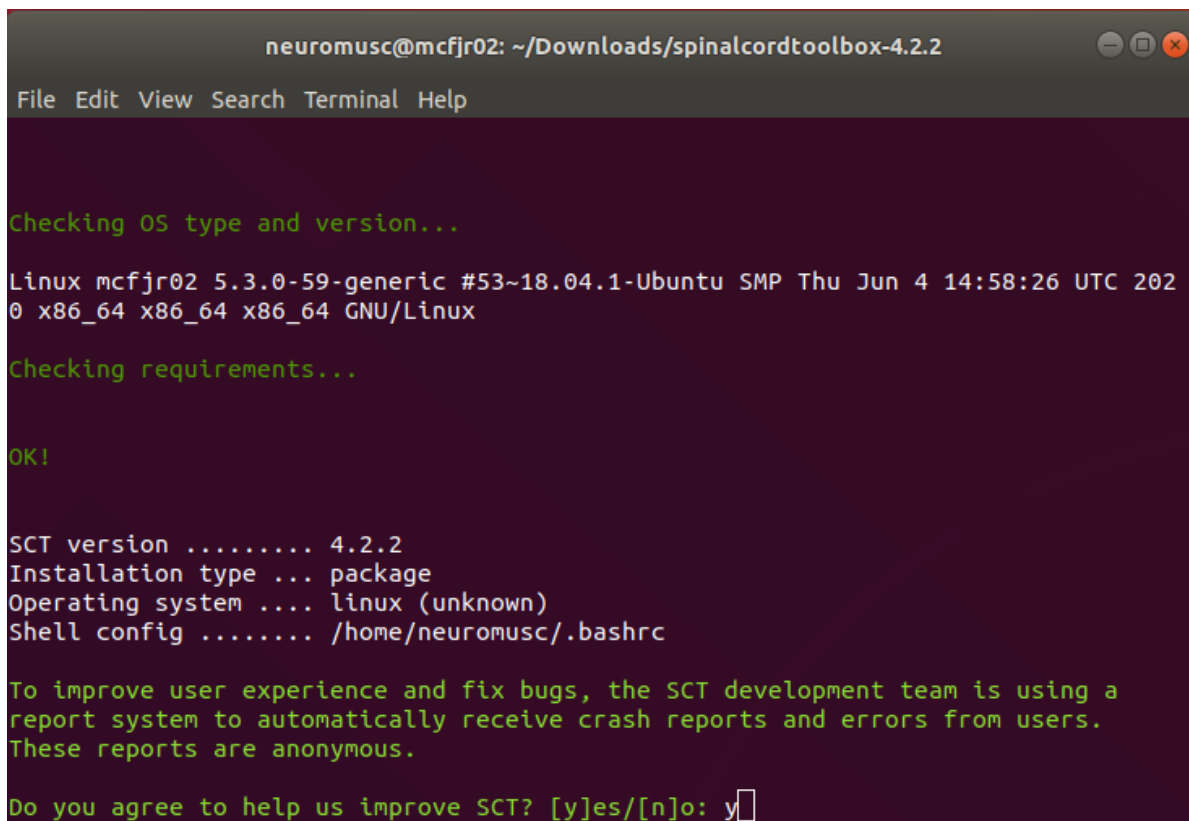
2. `./install_sct`



```
neuromusc@mcfjr02: ~/Downloads/spinalcordtoolbox-4.2.2
File Edit View Search Terminal Help
neuromusc@mcfjr02:~$ cd Downloads/spinalcordtoolbox-4.2.2/
neuromusc@mcfjr02:~/Downloads/spinalcordtoolbox-4.2.2$ ./install_sct
```

Figure 4: Installing SCT, step 2.

2.1. I recommend agreeing in share information about bugs with the SCT team.



```
neuromusc@mcfjr02: ~/Downloads/spinalcordtoolbox-4.2.2
File Edit View Search Terminal Help

Checking OS type and version...

Linux mcfjr02 5.3.0-59-generic #53~18.04.1-Ubuntu SMP Thu Jun 4 14:58:26 UTC 202
0 x86_64 x86_64 x86_64 GNU/Linux

Checking requirements...

OK!

SCT version ..... 4.2.2
Installation type ... package
Operating system .... linux (unknown)
Shell config ..... /home/neuromusc/.bashrc

To improve user experience and fix bugs, the SCT development team is using a
report system to automatically receive crash reports and errors from users.
These reports are anonymous.

Do you agree to help us improve SCT? [y]es/[n]o: y
```

Figure 5: Installing SCT, command 3.

2.2. Choose the local directory where SCT will be installed (or agree with the path suggested by SCT). If you are installing on a cluster or where there are other admin constraints, SCT can be installed in your Home or Documents folder (ie., a central install is not required).

```
neuromusc@mcfr02: ~/Downloads/spinalcordtoolbox-4.2.2
File Edit View Search Terminal Help

Checking requirements...

OK!

SCT version ..... 4.2.2
Installation type ... package
Operating system .... linux (unknown)
Shell config ..... /home/neuromusc/.bashrc

To improve user experience and fix bugs, the SCT development team is using a
report system to automatically receive crash reports and errors from users.
These reports are anonymous.

Do you agree to help us improve SCT? [y]es/[n]o: y

--> Crash reports will be sent to the SCT development team. Thank you!

SCT will be installed here: [/home/neuromusc/sct_4.2.2]

Do you agree? [y]es/[n]o: y
```

Figure 6: Installing SCT, command 4.

2.3. I strongly recommend to agree with SCT in save all SCT scripts in your path.

```
neuromusc@mcfr02: ~/Downloads/spinalcordtoolbox-4.2.2
File Edit View Search Terminal Help

--
Spinal Cord Toolbox (4.2.2)

Trying URL: https://osf.io/t97ap/?action=download
Downloading 20190117_c2c3_disc_models.zip...
Status: 100%|#####| 8.94k/8.94k [00:00<00:00, 29.9MB/s]

Create temporary folder (/tmp/sct-20200615162922.132899-09v_s2v1)...

Unzip data to: /tmp/sct-20200615162922.132899-09v_s2v1

Check if folder already exists on the destination path...

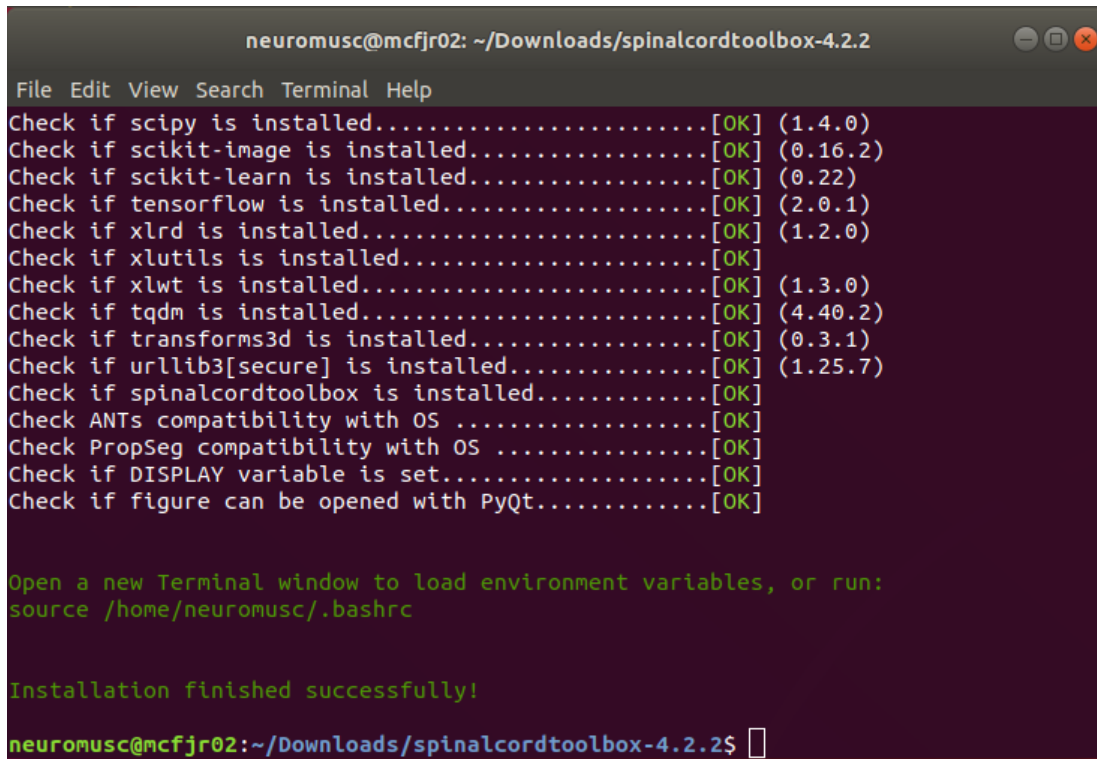
Remove temporary folders...
Done!

In case an old version SCT is already installed (4.0.0-beta.1 or before), remove
'sct_env' declaration in RC file

Do you want to add the sct_* scripts to your PATH environment? [y]es/[n]o: y
```

Figure 7: Installing SCT, command 5.

2.4. The installation is finished.



```
neuromusc@mcfjr02: ~/Downloads/spinalcordtoolbox-4.2.2
File Edit View Search Terminal Help
Check if scipy is installed.....[OK] (1.4.0)
Check if scikit-image is installed.....[OK] (0.16.2)
Check if scikit-learn is installed.....[OK] (0.22)
Check if tensorflow is installed.....[OK] (2.0.1)
Check if xlrd is installed.....[OK] (1.2.0)
Check if xlutils is installed.....[OK]
Check if xlwt is installed.....[OK] (1.3.0)
Check if tqdm is installed.....[OK] (4.40.2)
Check if transforms3d is installed.....[OK] (0.3.1)
Check if urllib3[secure] is installed.....[OK] (1.25.7)
Check if spinalcordtoolbox is installed.....[OK]
Check ANTs compatibility with OS .....[OK]
Check PropSeg compatibility with OS .....[OK]
Check if DISPLAY variable is set.....[OK]
Check if figure can be opened with PyQt.....[OK]

Open a new Terminal window to load environment variables, or run:
source /home/neuromusc/.bashrc

Installation finished successfully!

neuromusc@mcfjr02:~/Downloads/spinalcordtoolbox-4.2.2$
```

Figure 8: Installing SCT, command 6.

3. Close the current terminal, open a new one to start to use SCT.

### Directory Structure and Data Set-Up

If you have not previously done so (i.e. for other ENIGMA-Ataxia analyses), 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.

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	...

Ensure subject names are all the same length: *4chars\_2nums.nii.gz*

Note that the folder structure and naming convention we use for the ENIGMA-Ataxia analysis is quite rigid (deviations from this format will result in pipeline failures). This greatly aids management of the data during mega-analysis by the project PI, facilitates our ability to provide technical support, and mitigates a number of potential data handling errors.

### Launch the ENIGMA SCT Graphical User Interface (GUI):

Launch MATLAB and open the GUI by typing:

```
> ENIGMA_sctGUI
```

(if you get an error, check your path setting as described above).

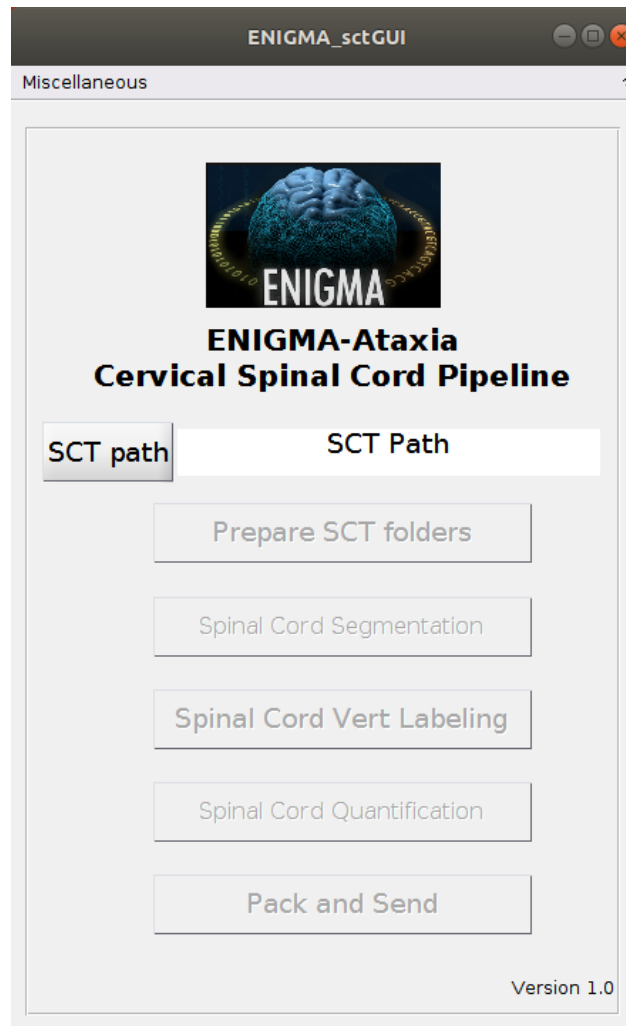


Figure 9: Enigma SCT GUI.

### SCT Path

To ensure that ENIGMA SCT GUI works properly, we need to provide the SCT path. This is a fundamental step, because SCT was made in Python and we are running it using MATLAB. Then, click the **SCT path button** and select the installation folder of SCT.

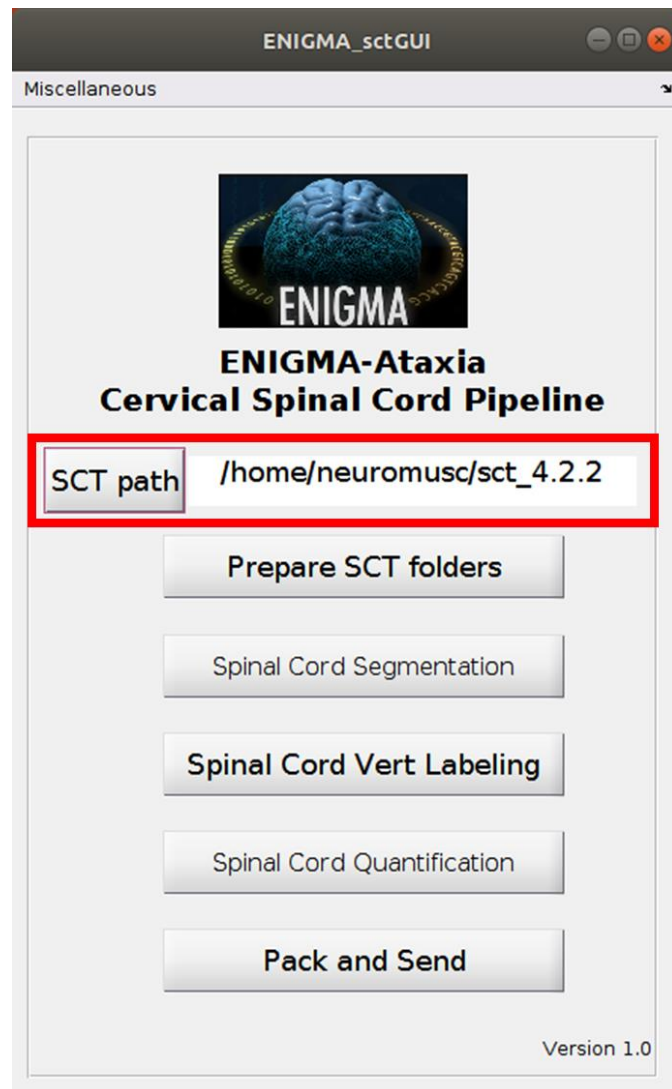


Figure 10: Setting SCT path in the ENIGMA GUI script.

### Prepare SCT folders (< 1min)

This step will organize the images in folders in order to guarantee that you are checking or handling the correct files for each subject, containing the T1 image in the correct format. Click the **Prepare SCT folders** button and select the T1 images from your “input” directory.

When complete, the message “The processing is done.” will be displayed in the MATLAB command window.



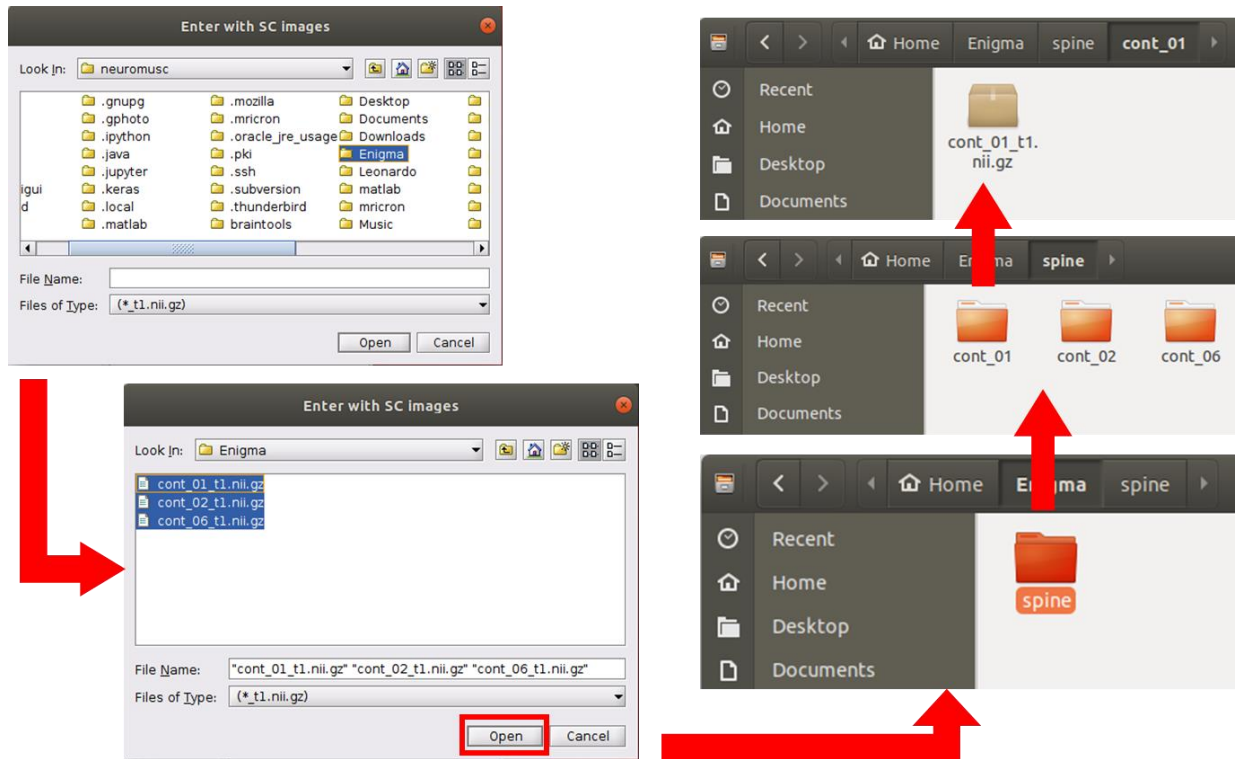


Figure 11: Prepare SCT folders

### Spinal Cord Segmentation (2-3mins per image)

Press the “Spinal Cord Segmentation” button. This will launch SCT and ask you to select the subject directories from your “/enigma/spine/” folder that you would like to include in the analysis (NOT individual files from the input directory, as in the previous step).

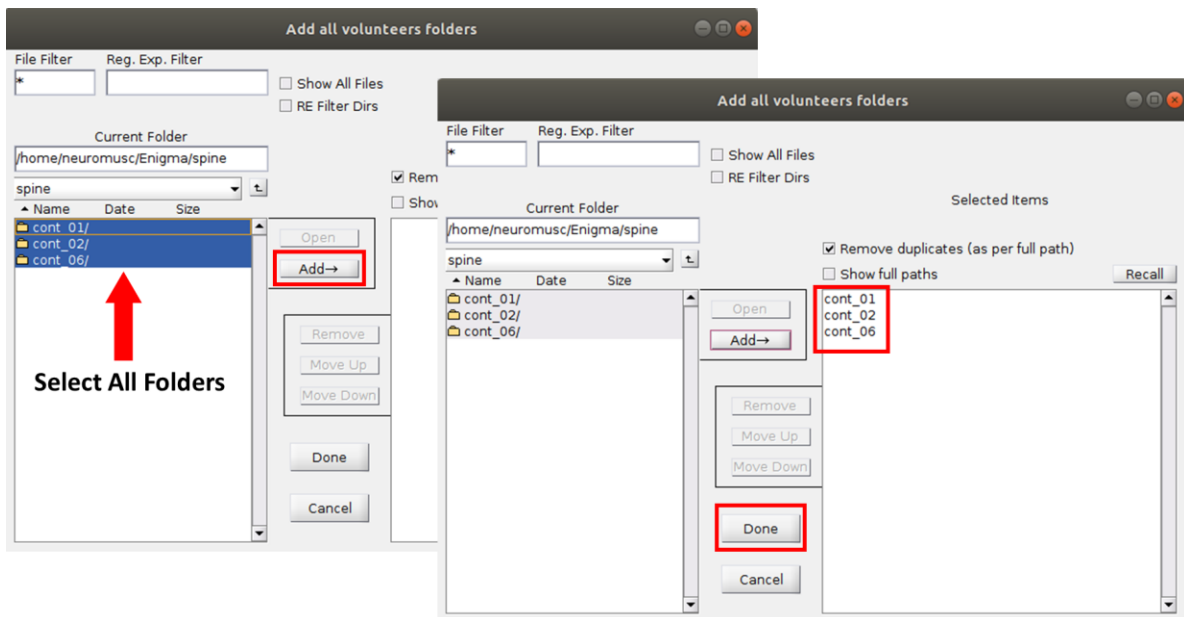


Figure 12: Input of spinal cord segmentation.

When completed, the message “The processing is done. Please, check the segmentations and, if necessary, perform manual correction.” A number of files will be output for QC.

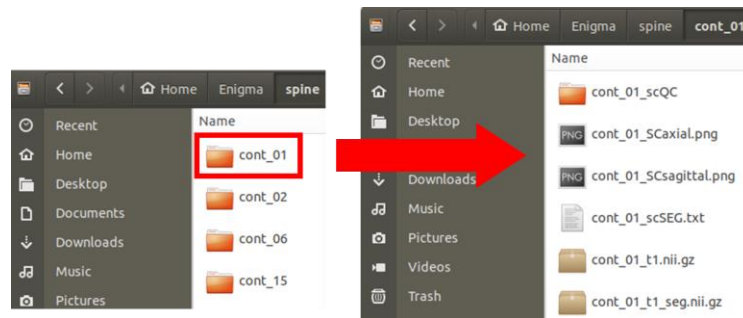


Figure 13: Output files for spinal cord segmentation pipeline.

### Quality Control, Manual Inspection and Correction of Spinal Cord Masks

Quality control (QC) in multicenter studies is a critical issue. To facilitate this process, the pipeline creates and saves two PNG files (axial and sagittal) of the spinal cord segmentation overlaid on the T1-weighted image (Figure 14).

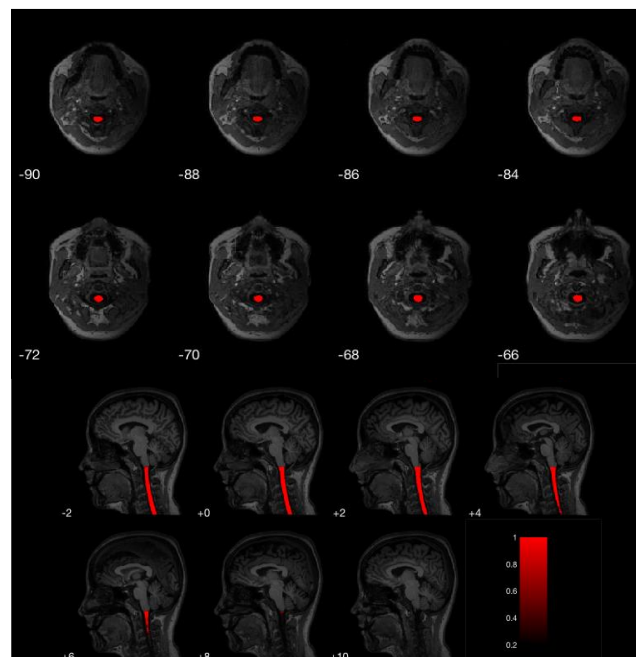


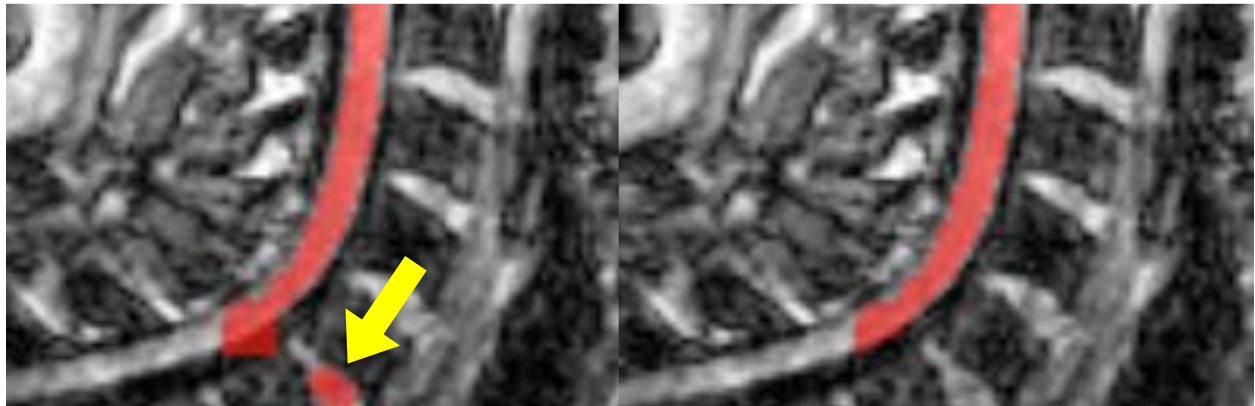
Figure 14: Axial and Sagittal view of the spinal cord segmentation and saved by the script.

These images should be visually inspected to identify any obvious errors in the segmentation. In our experience, segmentation errors are rare, and when present, generally occur close to the bottom or top of the segmentation mask. Minor errors – particularly over-inclusion of non-spinal tissue – can be manually corrected with relative ease, and the image can be retained

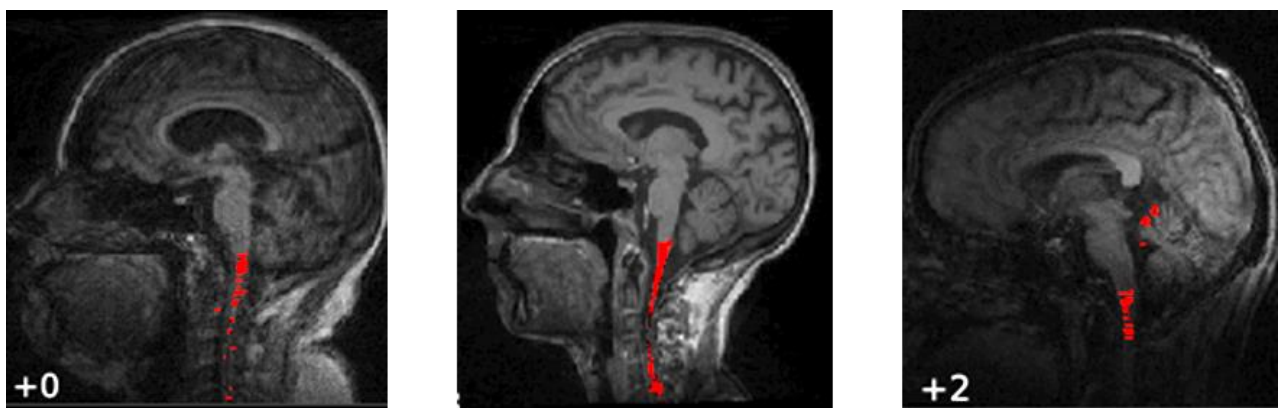
(Figure 15). In the event of more extensive segmentation failures (often from low neck signal or extensive movement artefact), the image should be excluded (Figure 16).

If edits are required, any image viewer/editor can be used, but we highly recommend FSLeyes (do NOT use FSLview – include in FSL version 5.0.9 or previous due to known bugs):

1. Overlay the <subj>\_t1\_seg.nii.gz mask onto the <subj>\_t1.nii.gz anatomical image;
2. Edit the mask (e.g., use the eraser tool in FSLeyes: [https://users.fmrib.ox.ac.uk/~paulmc/fsleyes/userdoc/latest/editing\\_images.html#](https://users.fmrib.ox.ac.uk/~paulmc/fsleyes/userdoc/latest/editing_images.html#));
3. Save the corrected mask with the same name (<subj>\_t1\_seg.nii.gz), overwriting the previous version.



*Figure 15: Example of slight segmentation error that can be corrected (bottom of the mask).*



*Figure 16: Example of images with low SNR or high level of movement that lead to segmentation failures which cannot be corrected.*

### Spinal Cord Vertebral Labeling (~30s per image)

Press the “Spinal Cord Vert Labeling” button. As in the previous step, this will launch SCT and ask you to select the subject directories from your “/enigma/spine” folder that you would like to include in the analysis.

This function opens a dialog box and the user must identify manually the C2 and C3 intervertebral discs. After the dialogue box shown in Fig 17 opens, use the keyboard arrows find a sagittal slice that shows C2 and C3 discs clearly (preferentially a slice close to the mid-sagittal line) and click with the left button **at the posterior tip of each intervertebral disc** (Figure 17). As indicated at the top of the box, the mouse can also be used to Zoom or alter the image contrast/brightness. After you mark the C2 and C3 discs, click *Save and Quit button*. If you marked a wrong place, click *Undo button* and mark again.

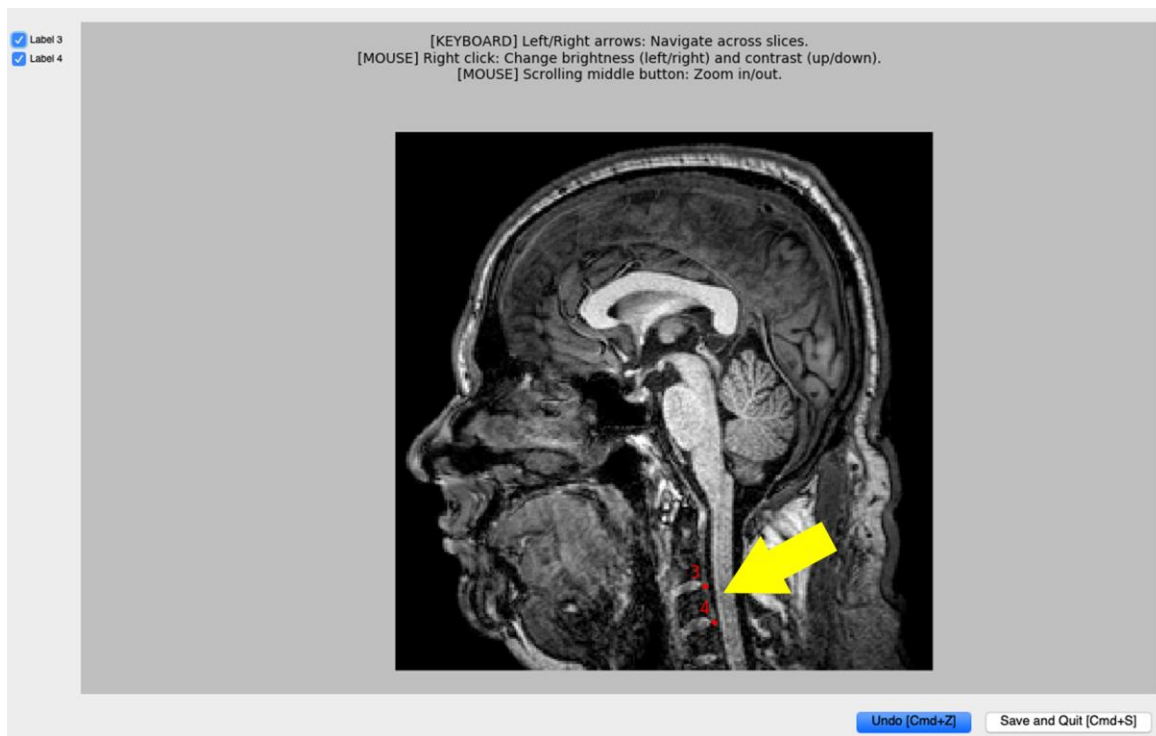


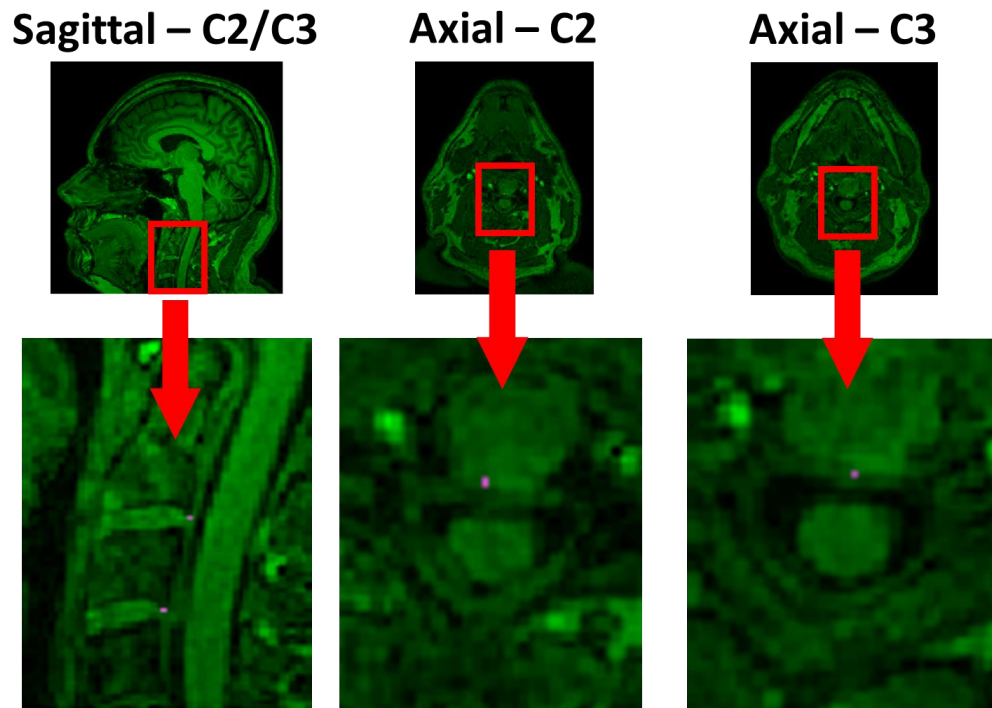
Figure 17: Manual identification of C2 and C3 intervertebral discs.

In some cases, depending of the size head and FOV of the image, the user will only be able to mark C2 (Fig 18). In that case, the registration will be done considering only one label (C2) and no crash will be caused in the script. Beware that this situation is an exception and wherever possible, both C2 and C3 should be marked. This is a critical step to guarantee the quality of the imaging processing and, hence, the computation of the spinal cord cross-sectional area.



*Figure 18: Manual identification of C2 intervertebral disc.*

The script saves three pictures showing the marked labels (Figure 19) and their coordinates (subs\_discCORD.txt). These files are principally for the project PI to check that vertebral labelling was correctly marked, and do not need to be reviewed. When completed, the message “The processing is done” will be displayed in the MATLAB command window.



*Figure 19: Quality Control for vertebral labelling.*



### Spinal Cord Quantification (15-20mins per image)

Press the “Spinal Cord Quantification” button. As in the previous step, this will launch SCT and ask you to select the subject directories from your “/enigma/spine” folder that you would like to include in the analysis.

This function performs multiple steps necessary to quantify the cross-sectional area of the cervical spinal cord. When completed, the message “The processing is done. ‘N’ subjects were successfully processed and ‘M’ failed.” will be displayed in the MATLAB command window. The main output files of this step consist in the PAM template wrapped into subject image space and a text file with all subjects that SCT failed to run (failSUBJECTS.txt). If any subjects failed, **check this .txt file for the subject labels and delete them from your ‘spine’ directory** (they will be excluded).

The final QC step involves checking that the registration between the template and the subject images is correct. To accomplish that, inside each subject folder there is a picture named as “subj\_regisQC” showing the overlay of the template on the T1w image in the sagittal plane (Figure 20). In case of misregistration (focus on whether the spinal cord in blue is a direct continuation of the brainstem; Figure 21), it is advised to re-run this imaging again reviewing the vertebral labelling carefully.

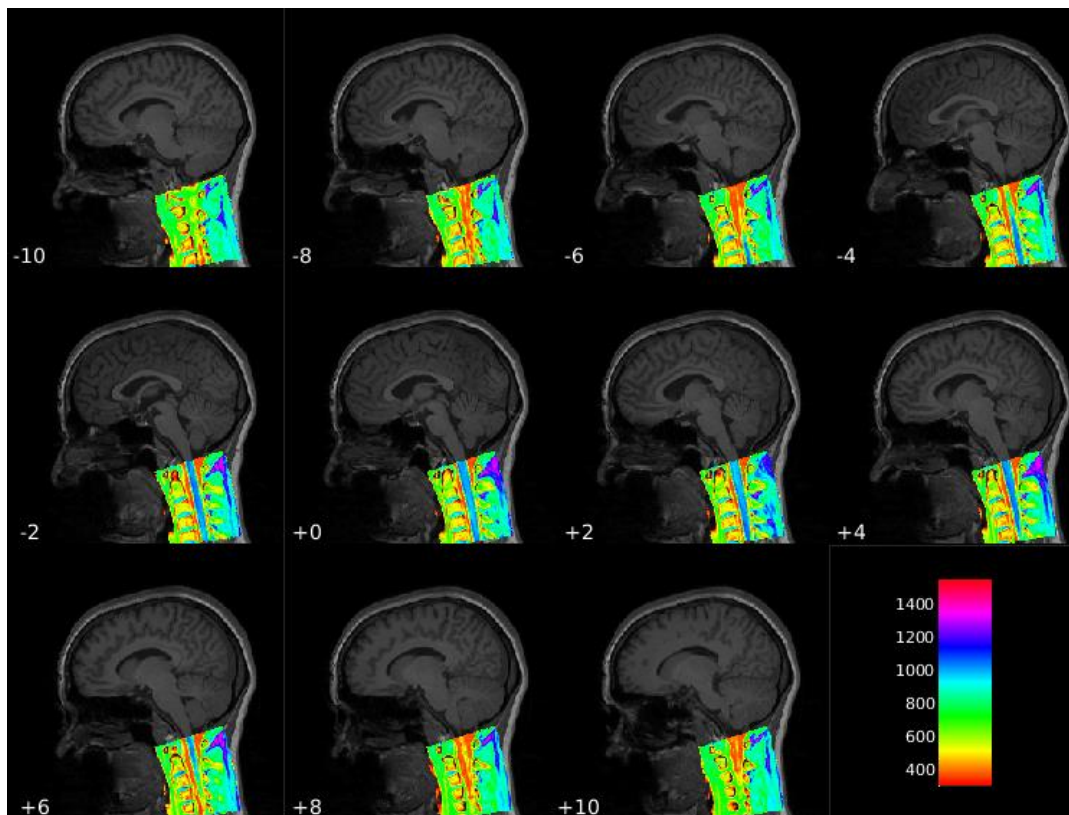


Figure 20: Quality control of template registration.

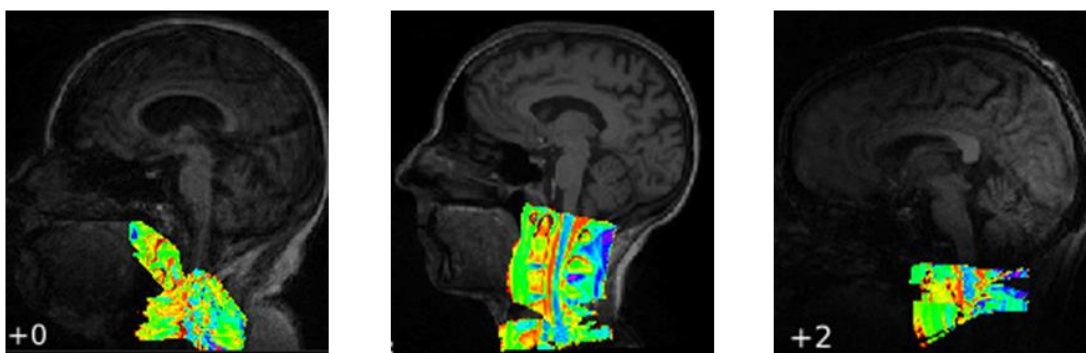


Figure 21: Examples of failed template registration.

The imaging processing is now complete. If you want to visualize the images and output files, please see the section below on “Visualizing Outputs” for some tips.

### Package and Send

Click the **Pack and Send button** and again select the subject directories from your “input” directory that you would like to include in your final cohort. This script will zip all the final processed images, plus the QC files (axial and sagittal slice views and text files). This archive will be labelled “filesPACKED.zip” and written to your “/enigma/spine” directory. The raw images are not included in the packaged files. After you finish this step, please contact the project head to arrange for transfer of the “filesPACKED.zip” file.

### Miscellaneous

The miscellaneous menu bar contains 2 tools that may be useful for your own research: Data Extraction and Tutorial (Figure 22). The first tool computes the CSA for each subject, extracting these values in a single csv table. The second, opens the tutorial to assist the user.

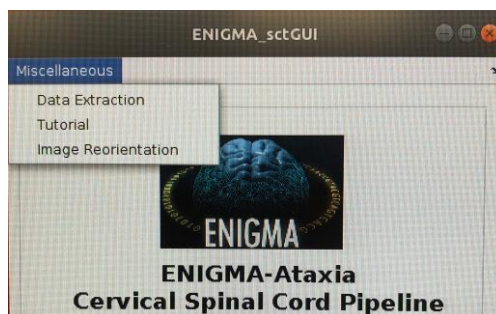


Figure 22: Miscellaneous menu bar.

To launch the Data Extraction pipeline, click in the miscellaneous menu bar and then Data Extraction. To compute the CSA, click the **SCT path button** and select the installation folder of SCT and select the subject folders as previous described in the **Figure 11** clicking the **Subj. Folders button**, then click in the **RUN button**. After the script finishes the processing, a csv table named “subject\_CSA.csv” is found inside the respective subject folder (Figure 23).

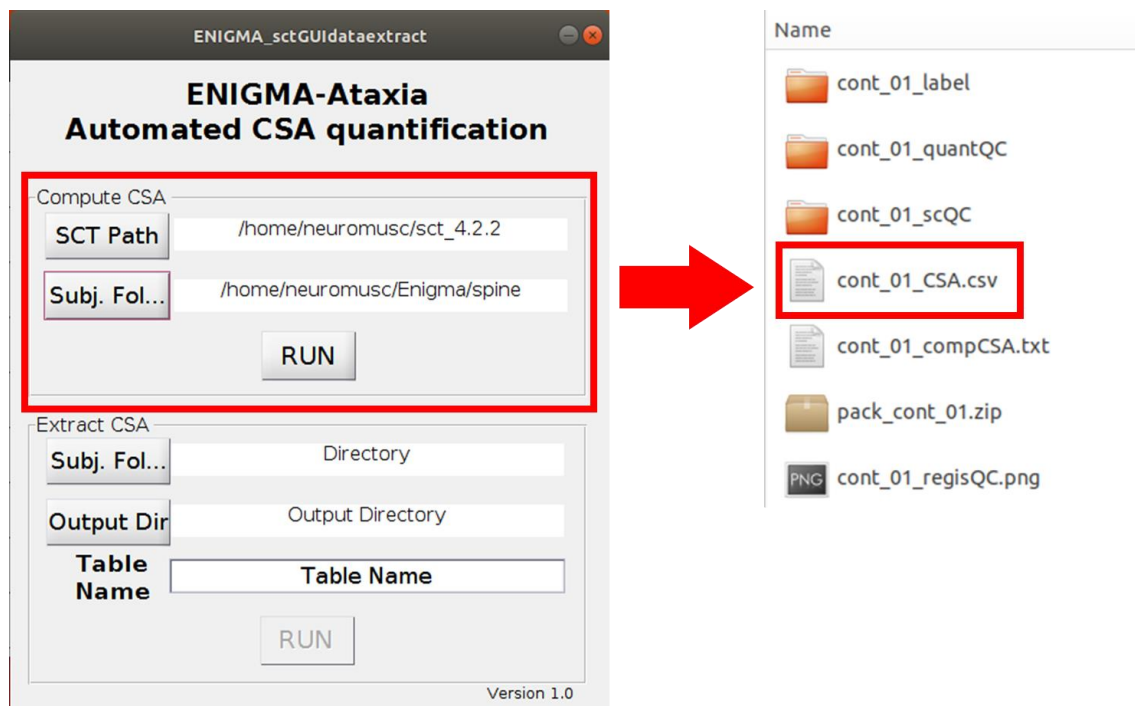


Figure 23: Computing CSA of the cervical spinal cord.

To extract and merge all CSAs, click the **Subj. Folders button** and select the subject folders as previous described in the **Figure 11**. Select the output directory and the table name. Next, click the **RUN button** and, then, 2 excel tables will be created, being one for C2 vertebra and other for C3 vertebra (Figure 24).



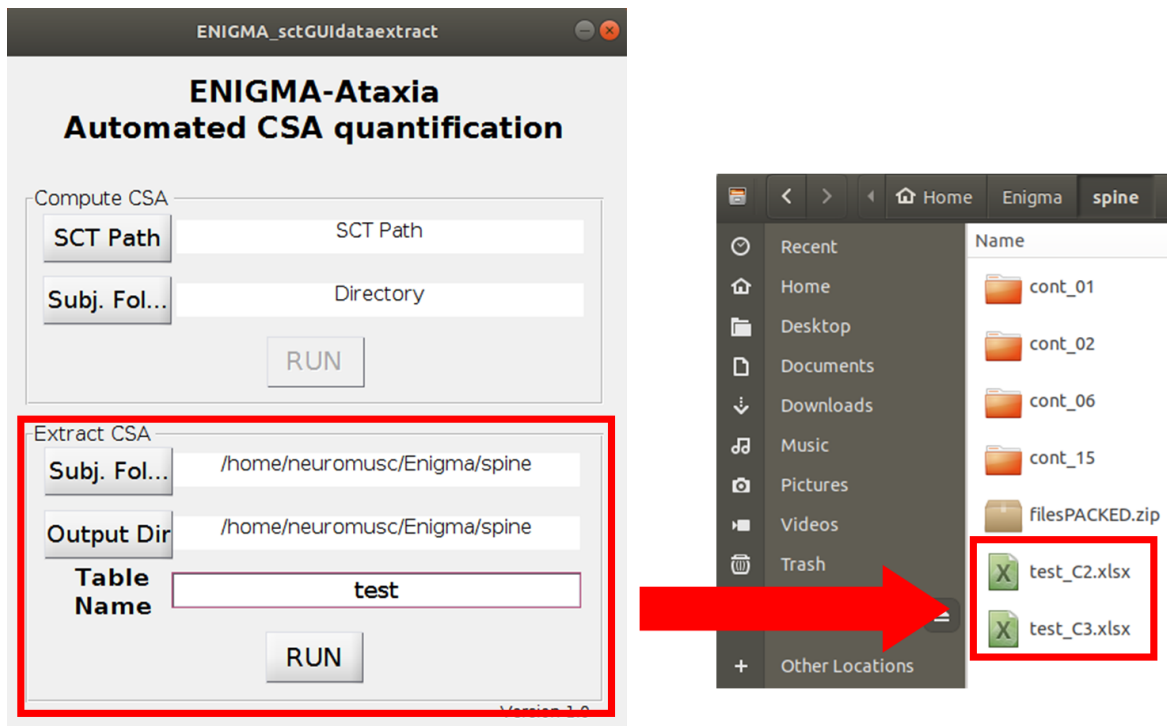


Figure 24: Extracted CSA values for all subjects.

## Image Reorientation

To ensure that QC images cover the whole imaging, your original images will need to be in rough alignment with anterior commissure. 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\_CAT12 or ENIGMA\_SUIT), this step does not need to be repeated.

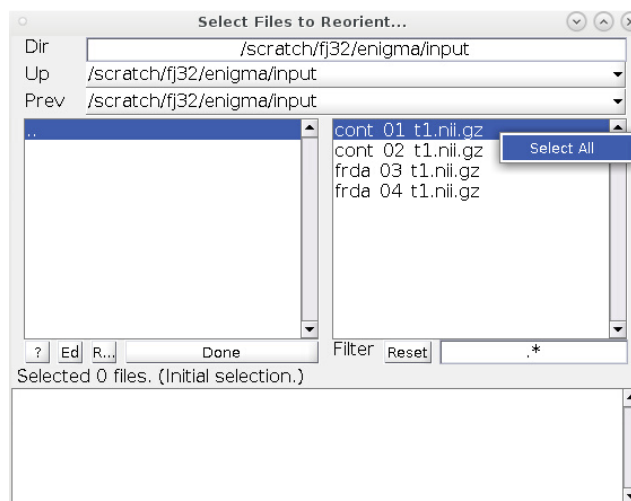


Figure 25: Input data to image reorientation.

Clicking 'Done' will open the interactive Image Reorientation GUI:

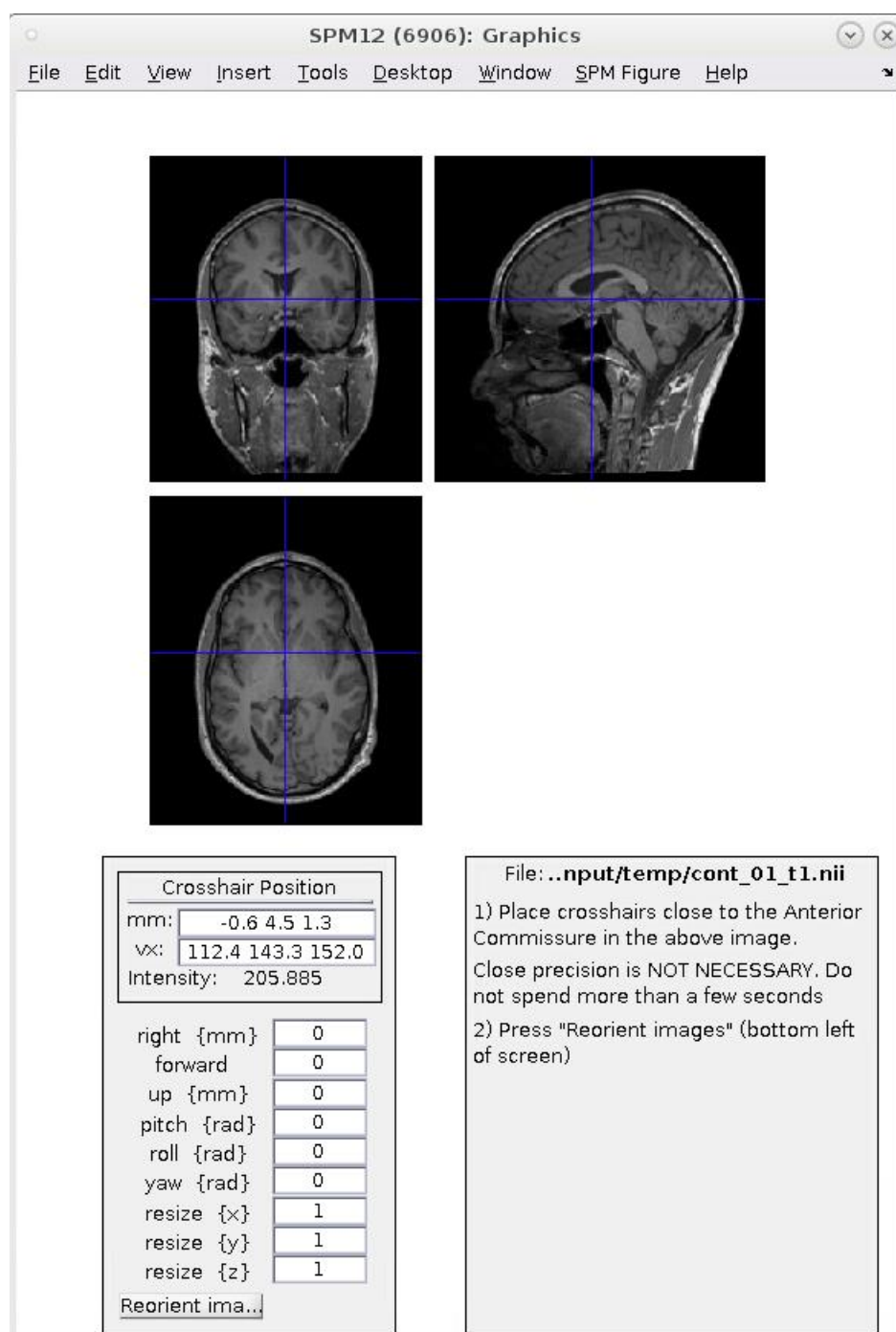


Figure 26: Manual identification of the anterior commissure.

Then, click on the Anterior Commissure so that the blue crosshairs are aligned with it. It does not have to be exact (i.e. do not spend more than a few seconds on this). 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. 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.

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

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](#).

## Visualizing Outputs

### 1. Spinal Cord Segmentation

```
fslview /Users/thiago_admin/Documents/testEnigma_2/cont_03.nii.gz -l Greyscale  
/Users/thiago_admin/Documents/testEnigma_2/cont_03_seg.nii.gz -l Red -t 0.7 &
```

or

```
fsleyes t1.nii.gz -cm greyscale t1_seg.nii.gz -cm red -a 70.0 deepseg/t1_seg.nii.gz -cm green -a  
70.0 &
```

### 2. To visualize the vertebral labels

```
fslview /Users/thiago_admin/Documents/testEnigma_2/cont_03.nii.gz -t 1  
./cont_03_seg_labeled.nii.gz -l MGH-Subcortical -t 0.5 &
```

### 3. To Visualize Registration

```
fslview cont_03.nii.gz template2anat.nii.gz &
```

### 4. To visualize the Atlas Registration

```
fslview cont_03.nii.gz -l Greyscale -t 1 cont_03_label/template/PAM50_t2.nii.gz -l Greyscale -b  
0,4000 -t 1 cont_03_label/template/PAM50_gm.nii.gz -l Red-Yellow -b 0.4,1 -t 0.5  
cont_03_label/template/PAM50_wm.nii.gz -l Blue-Lightblue -b 0.4,1 -t 0.5 &
```