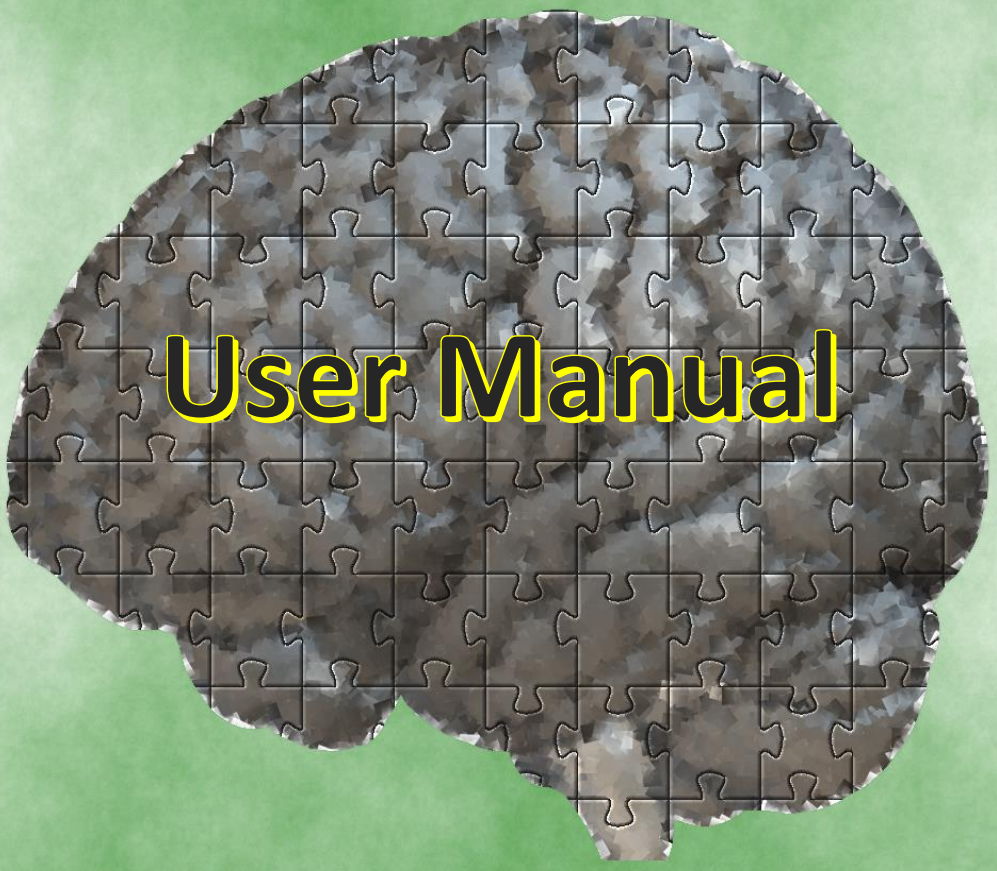
UF²C - User Friendly Functional Connectivity

University of Campinas - Neuroimaging Laboratory
Brazilian Institute of Neuroscience and Neurotechnology – BRAINN



Brunno Machado de Campos, Ph.D.
Raphael Fernandes Casseb, Ph.D.
Elise R. Facer-Childs, Ph.D.
Marina Weiler, Ph.D.

2017

UF²C: User Friendly Functional Connectivity

User Manual

Volume 1

Campinas
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UF²C is an open source software developed at the Neuroimaging Laboratory at Unicamp. The aims of UF²C are to simplify and organize functional connectivity studies in neuroimaging through a clean and validated methodology, without sacrificing quality.

UF²C Team:

Coordinator:
Brunno M. de Campos, Ph.D. (www.lniunicamp.com/brunno)

Developers:
- Brunno M. de Campos, Ph.D.
- Raphael Fernandes Casseb, Ph.D.

Theoretical Collaborators:
- Fernando Cendes, M.D. Ph.D. BRAINN Director (www.brainn.org.br/fernando-cendes)
- Marina Weiler, Ph.D.
- Elise Facer-Childs, Ph.D.

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1. How to Cite

UF²C was presented to the community with an original research paper at Human Brain Mapping Journal, 2016:

de Campos, B. M., Coan, A. C., Lin Yasuda, C., Casseb, R. F. and Cendes, F. (2016), Large-scale brain networks are distinctly affected in right and left mesial temporal lobe epilepsy. Hum. Brain Mapp., 37: 3137–3152.

Please use this paper for future references or when citing UF²C in your studies.

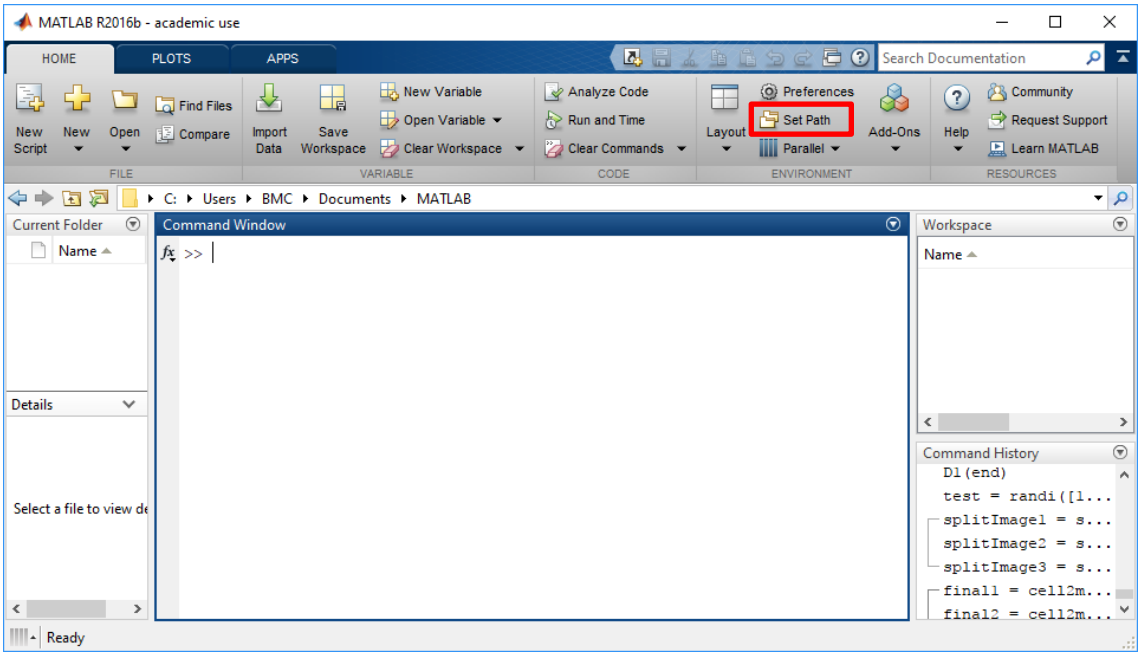
2. Requirements

UF²C is open source software, distributed under a BSD-style License. System requirements for **UF²C** are:

- Windows, Linux or Mac OS X operational systems
- SPM8-12 (Statistical Parametric Mapping, version 8 or 12)
- MATLAB (version R2010a or later, required by SPM)
- MATLAB Statistics toolbox
- MATLAB Signal Processing toolbox
- MATLAB Image Processing Toolbox (for advanced graphic results)

3. Installing UF²C

UF²C should to be added to the "**Matlab Path**". The recent versions of Matlab changed the toolbar design and have a direct icon on the main Matlab window:



Click on "**Set Path**" and add the **UF²C** folder extracted from the downloaded Zip file. Use the option "**Add with subfolders**". Now your MATLAB knows that **UF²C** exists in your machine.

If you are using Windows OS, you can run **UF²C** by running the **UF²C**.exe file in 'uf2c' folder.

4. Input format

UF²C uses the NIfTI format for input files. You can use the dcm2nii utility (MRIcron - www.mccauslandcenter.sc.edu/mricro/mricron/) or MRIcroGL to convert your files from many formats (e.g.: DICOM, PAR-REC…).

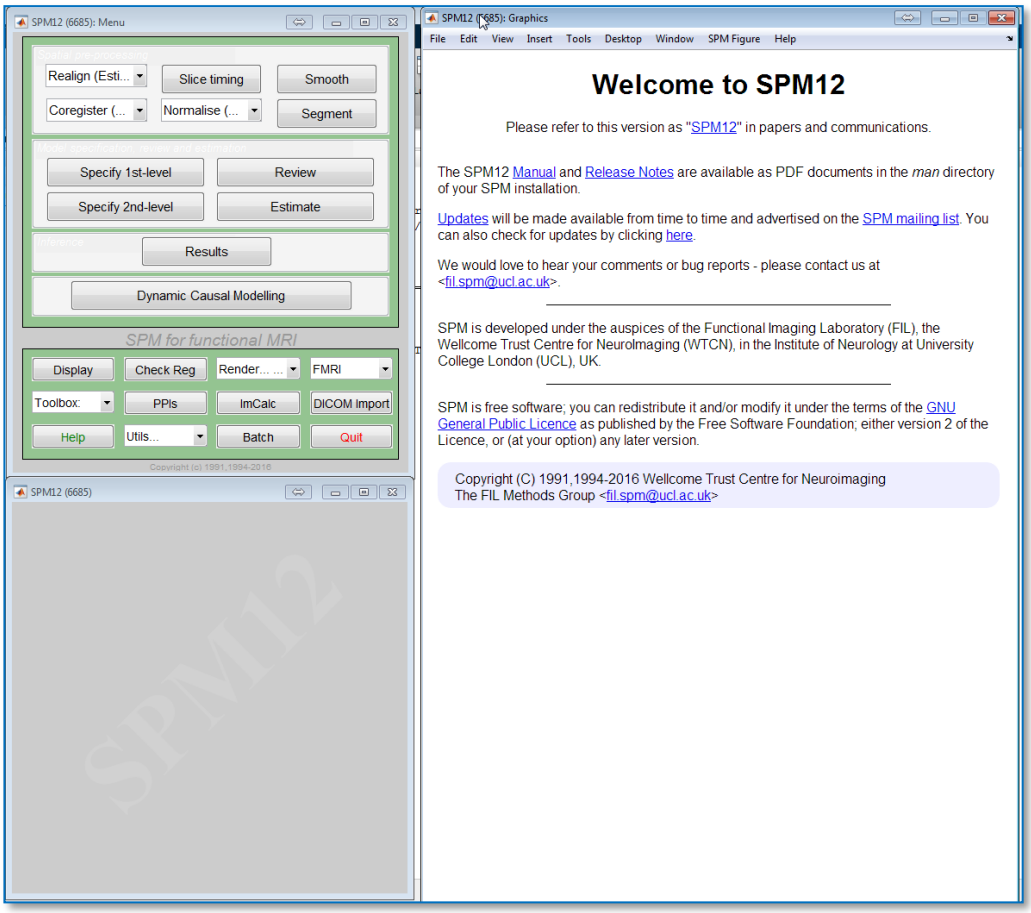
5. Starting

If you have already used SPM, you may know that defining the anterior commissure as the image origin (a pre-pre-processing step) improves SPM registration. SPM12 '**Display**' tool is used to perform this tedious step. **UF²C** will probably work without the anterior commissure set as the origin. However, in some cases, when the origin [0,0,0] is too far (more than ~3 cm away), the co-registration or normalization could lead to wrong deformations. There are some ways to set the anterior commissure as the origin automatically, but these methodologies need accuracy and repeatability in the FOV positioning during the MRI acquisition and are not recommended if you want to perform a high-quality study. Therefore, we suggest that the anterior commissure should be set for each image.

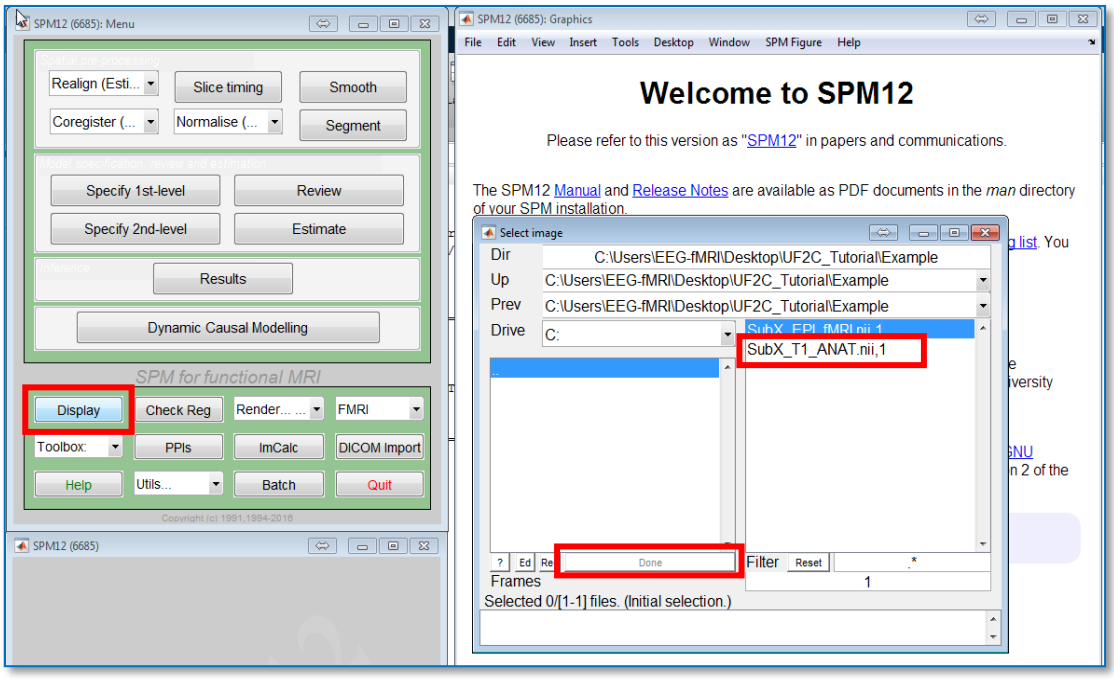
6. How to set the anterior commissure as the image origin (Reorientation)

1. Run Matlab.

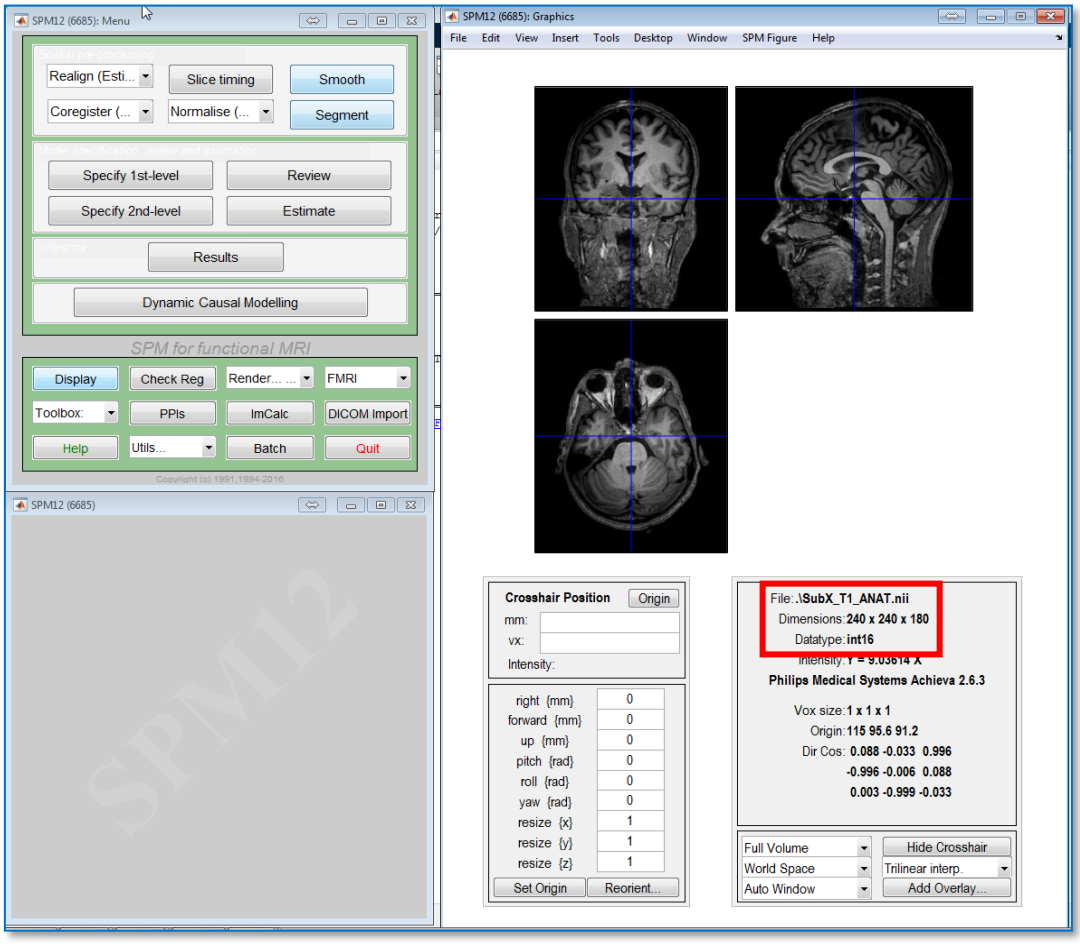
2. Run SPM (type "**spm fmri**" on the Matlab command window).



3. On the SPM8 or SPM12 Menu click on "**Display**".

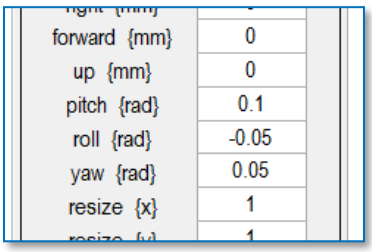


4. You will be asked to add an image. Add a structural (3D) file. Click once in the file of interest, and press "**Done**". You can make sure the correct file is selected by



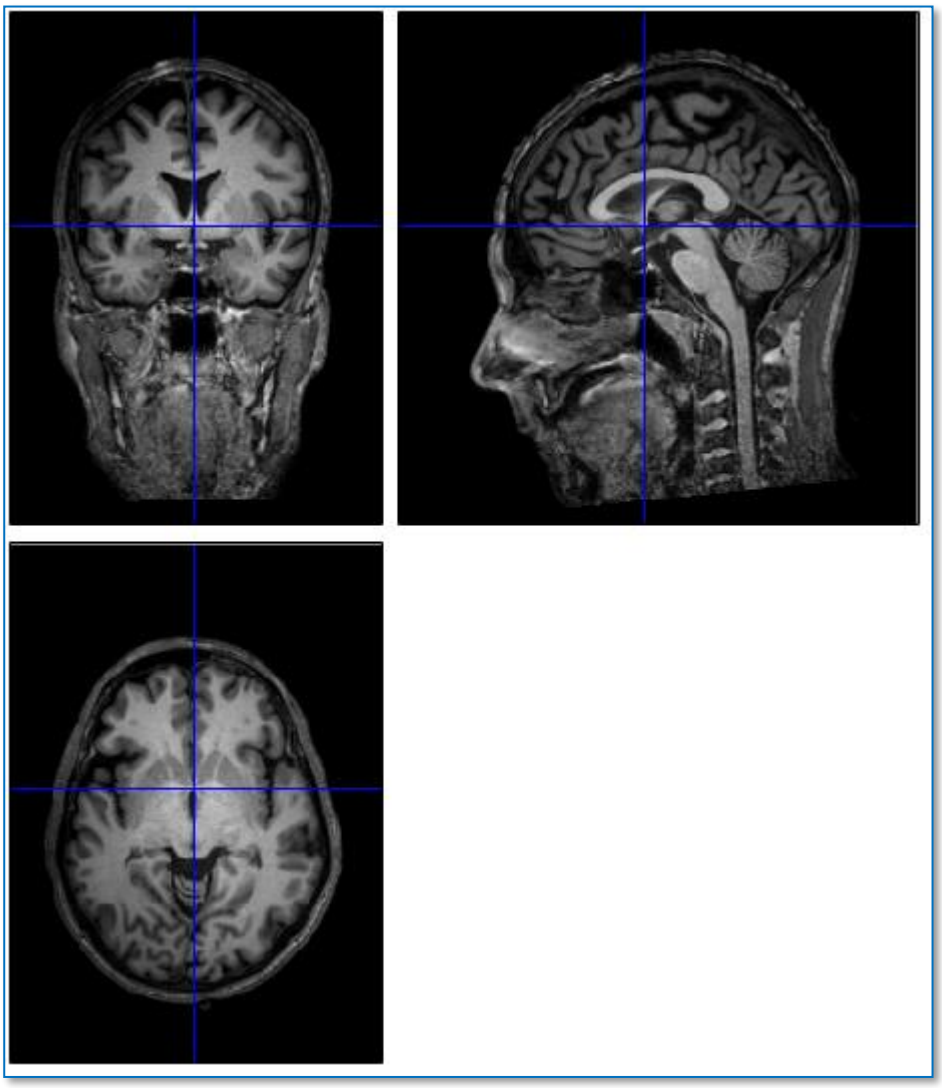
looking at the box at the bottom ("**Selected**" box in the figure above), and in the **Graphics** window as below:

5. Now you can use the fields "**pitch {rad}**", "**roll {rad}**" and "**yaw {rad}**" to correct the image orientations and rotations, using the anterior and posterior commissure as

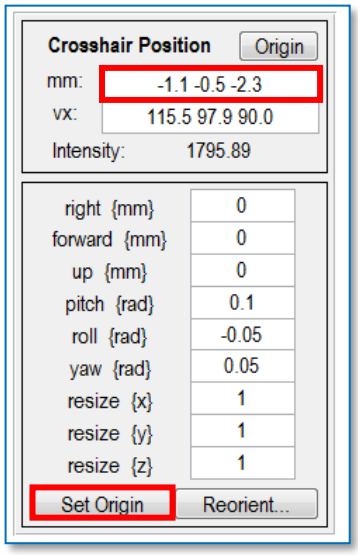


the horizontal reference and the inter-hemispheric fissure as the vertical reference.

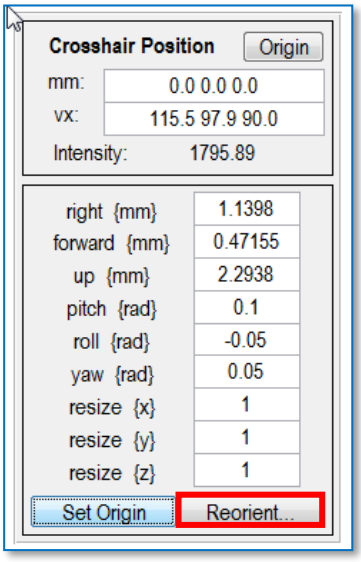
6. Now, you need to position the crosshairs on the anterior commissure.



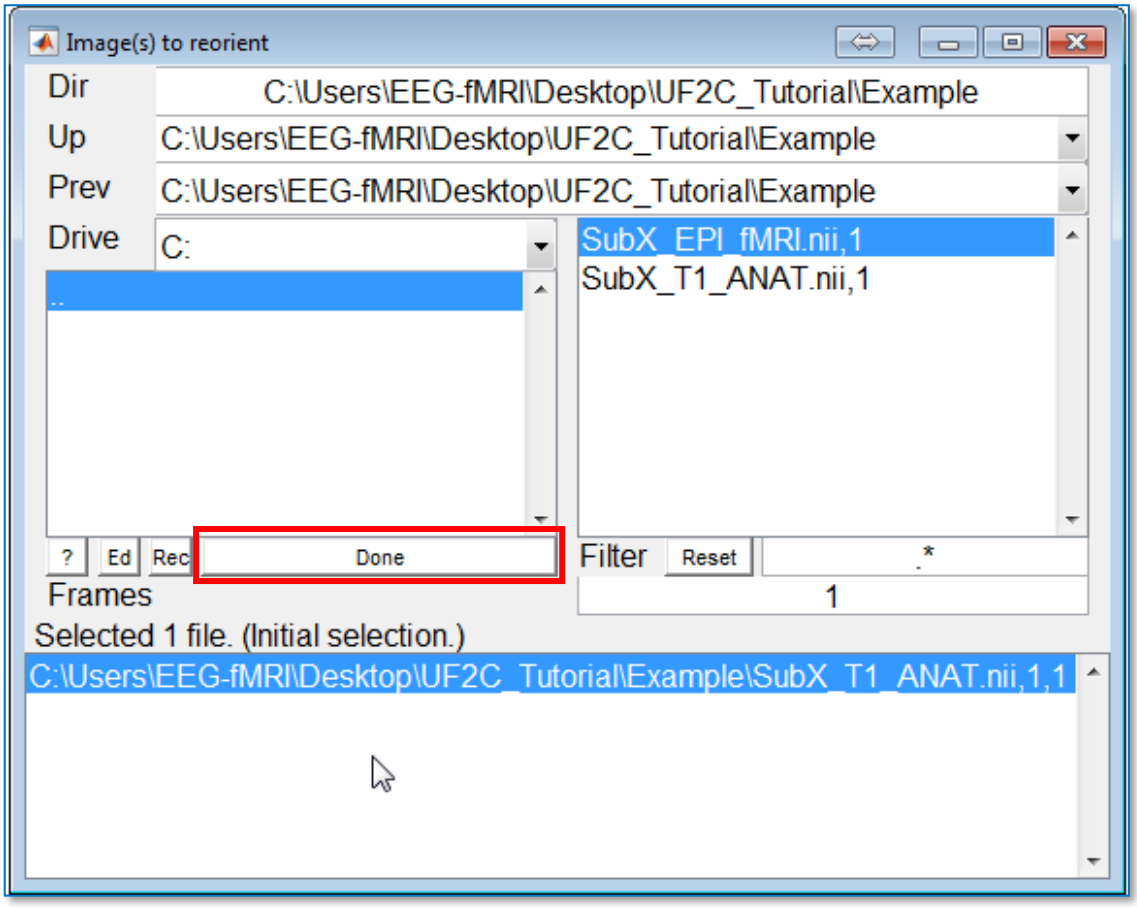
7. The numbers inside the first box "**Crosshairs Position**" are the coordinate offsets between current point, indicated by the crosshairs, and the actual origin.



8. Press "**Set Origin**" and the **"right {mm} ", "forward {mm}"** and **"up {mm}"** fields will be filled with the opposite number from the box above. Click on the "Reorient…" button.



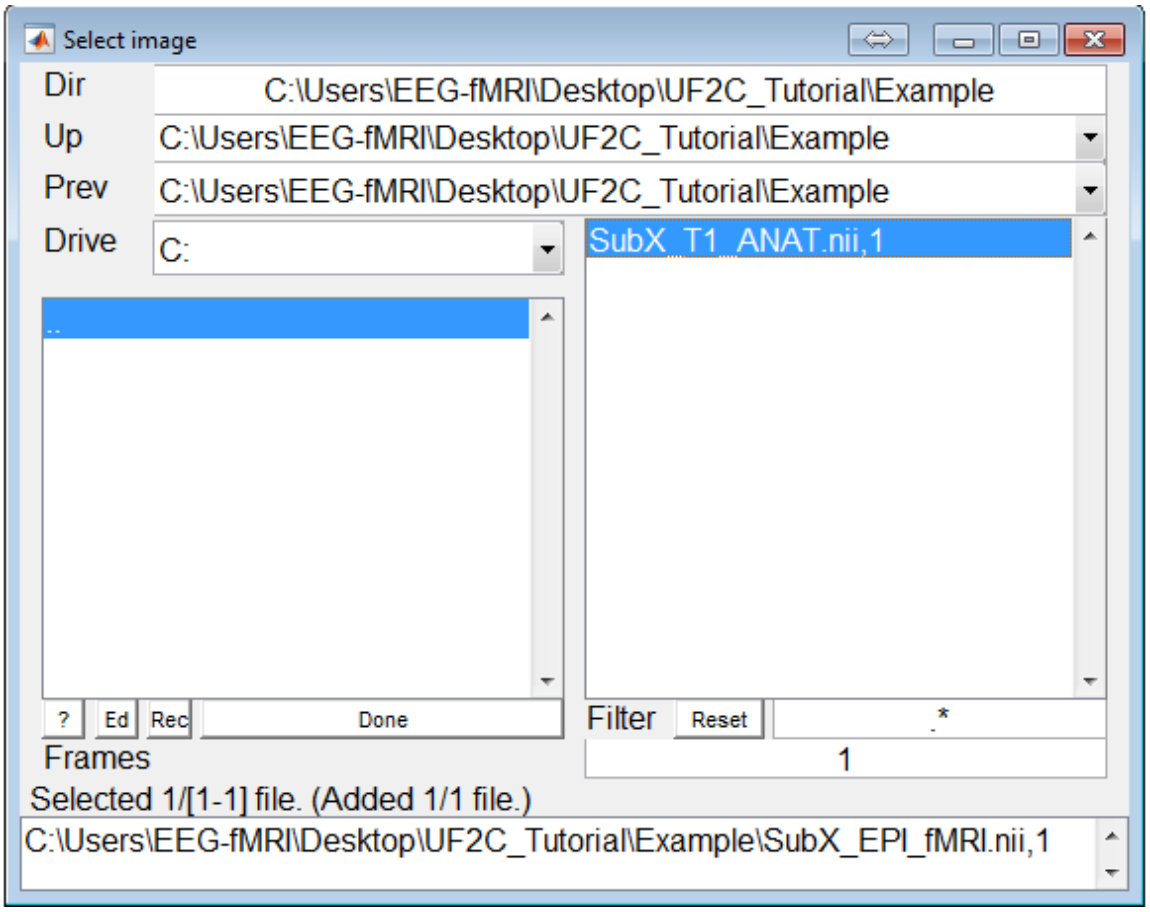
9. You will see the menu on the left-hand side of the screen display '**reorienting images**' and then the new reoriented image will be displayed. Just click "**Done**"!

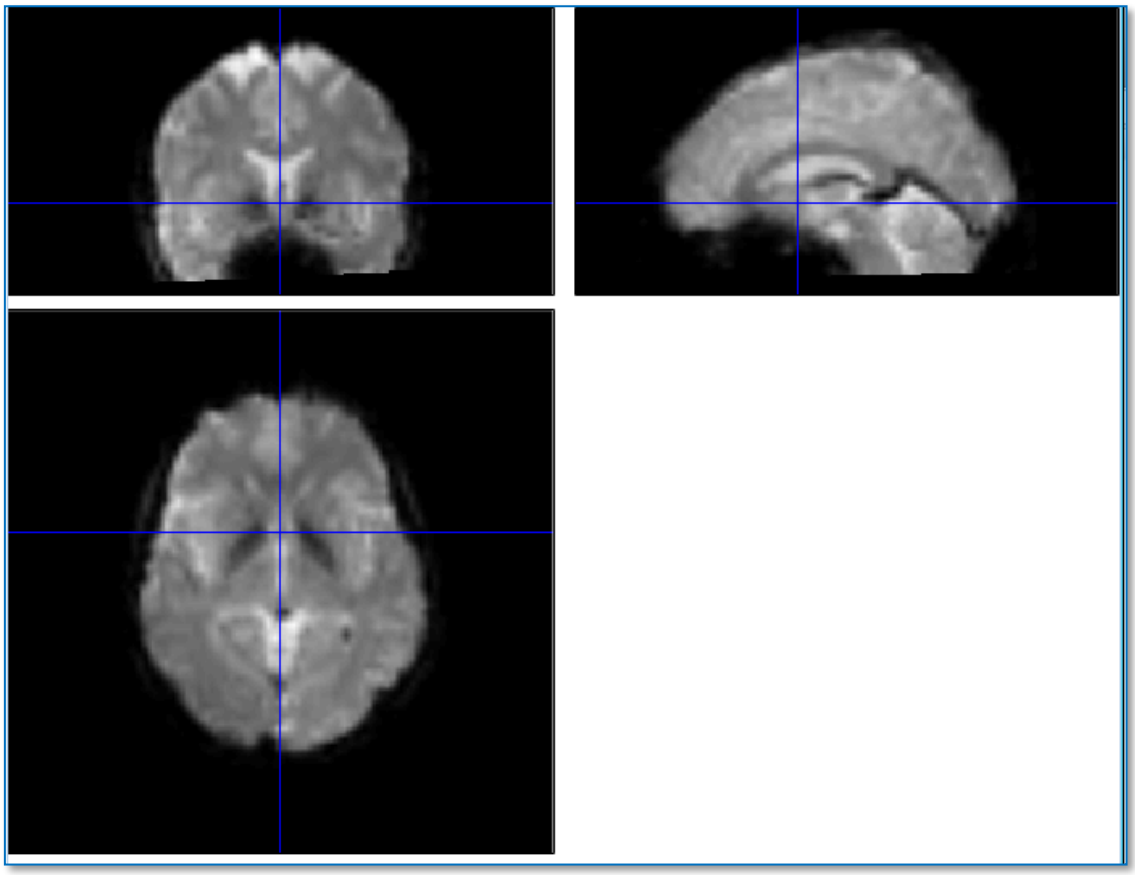


10. You need to repeat these steps for the functional image, but **pay attention**: **YOU NEED TO APPLY THE COORDINATES FOUND TO ALL VOLUMES (DYNAMICS)!**

11. Add the fMRI. Note the "*.nii**,1**". This ",1" means that you are adding the first volume ONLY.

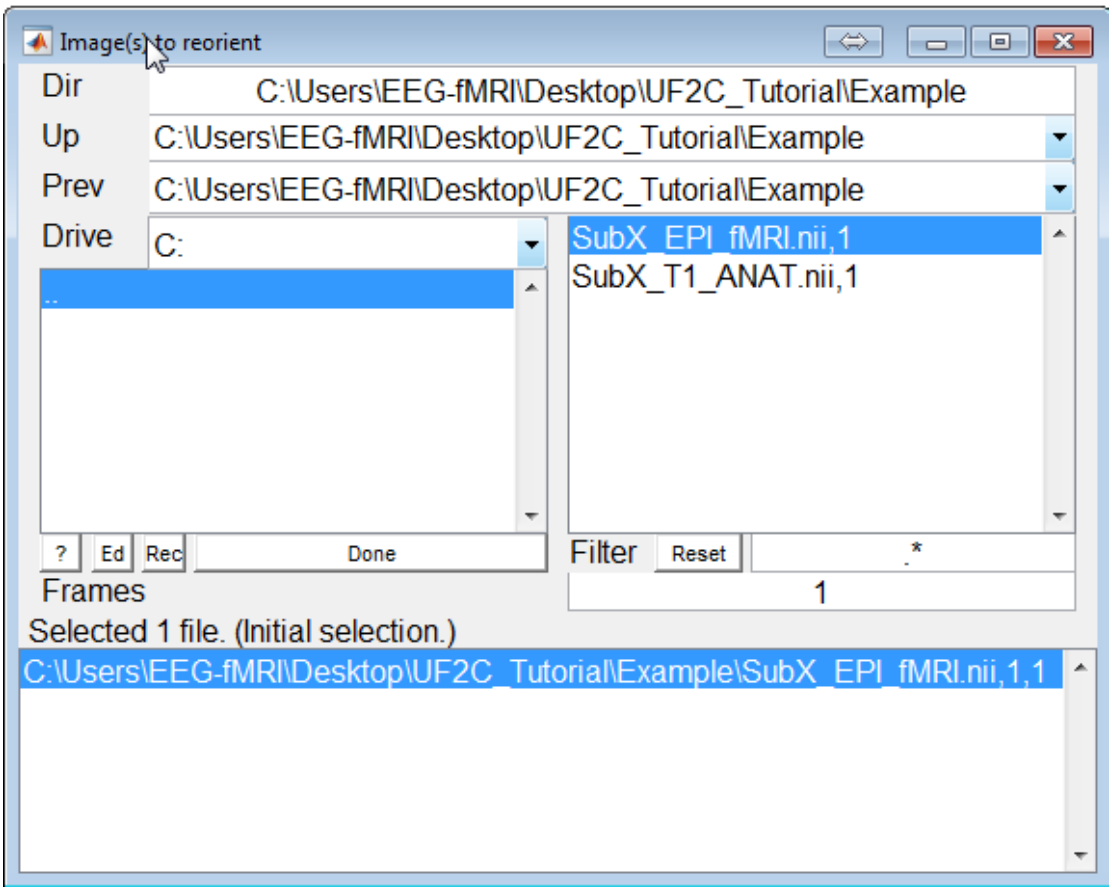
12. Use the same strategy to find the anterior commissure position as best you can. You can use the dark grey corpus callosum and even darker grey fornix as reference:


Select image
Dir
C:\Users\EEG-fMRI\Desktop\UF2C_Tutorial\Example
Up
C:\Users\EEG-fMRI\Desktop\UF2C_Tutorial\Example
Prev
C:\Users\EEG-fMRI\Desktop\UF2C_Tutorial\Example
Drive
C:
SubX_T1_ANAT.nii,1
..
?
Ed
Rec
Done
Filter
Reset
.*
Frames
1
Selected 1/[1-1] file. (Added 1/1 file.)
C:\Users\EEG-fMRI\Desktop\UF2C_Tutorial\Example\SubX_EPI_fMRI.nii,1

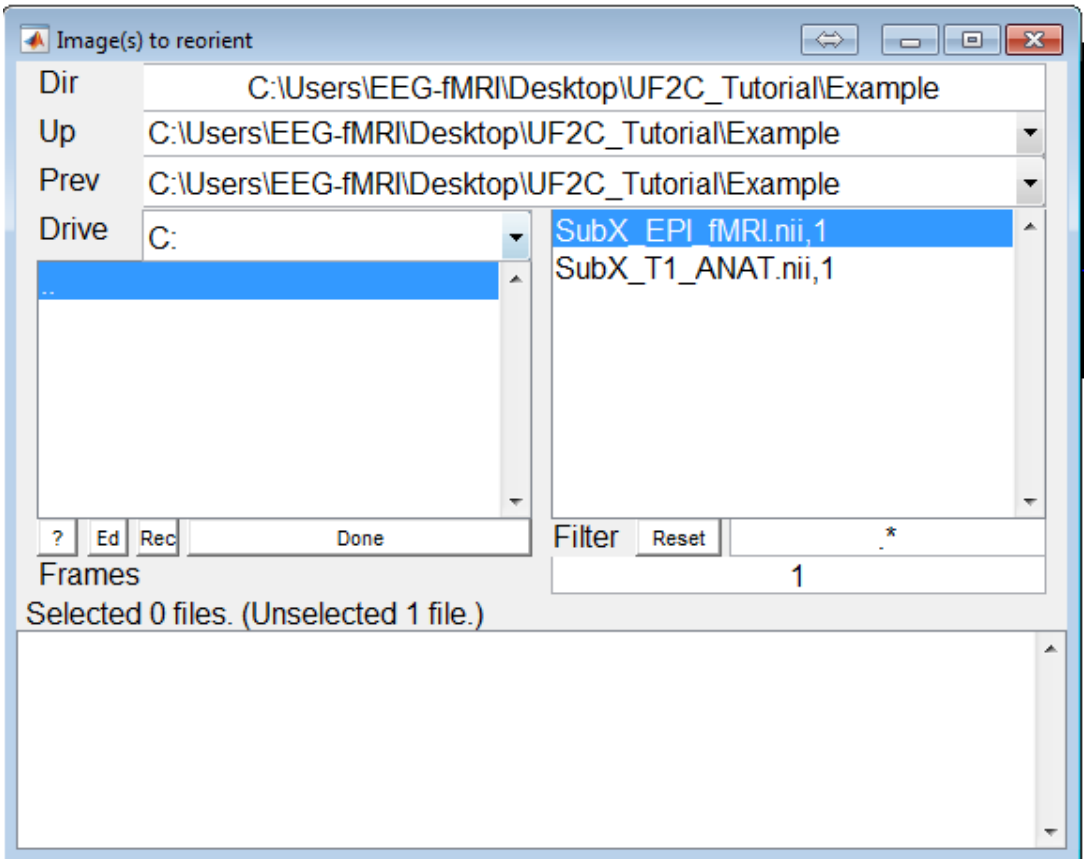


13. Repeat steps 5 to 9.

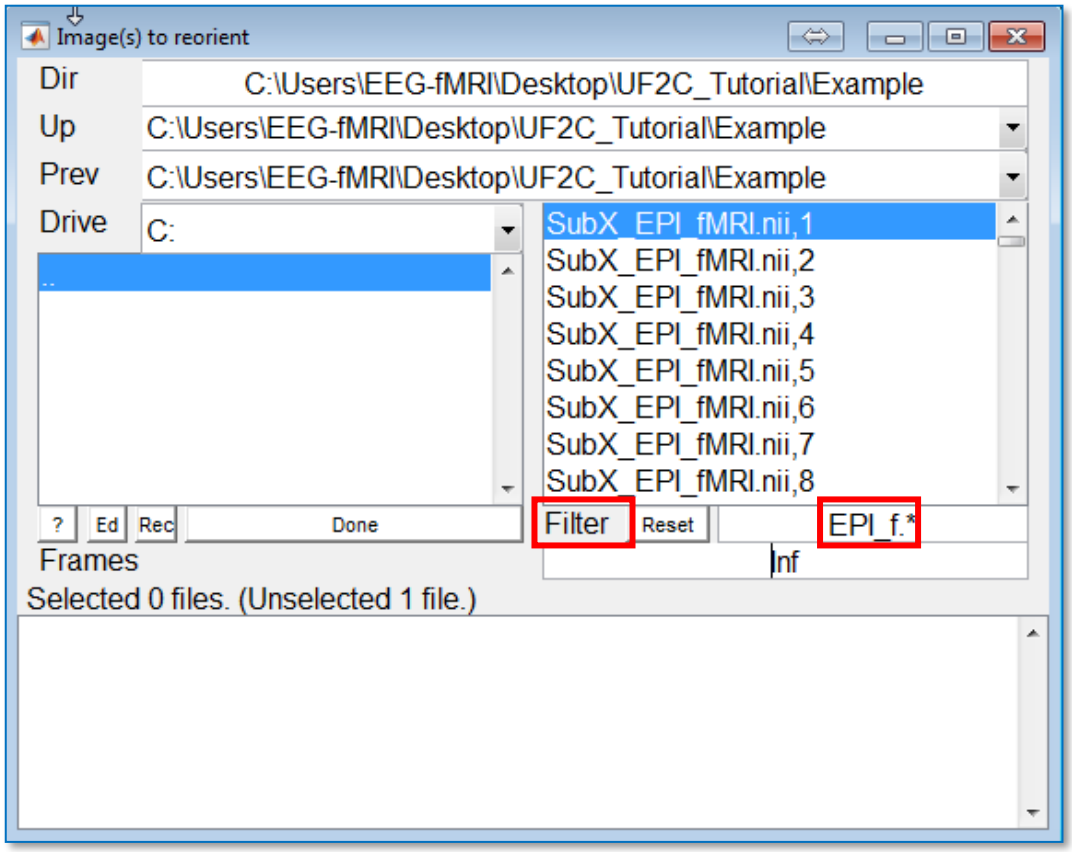
14. Once this is done you need to apply this reorientation to all dynamics:



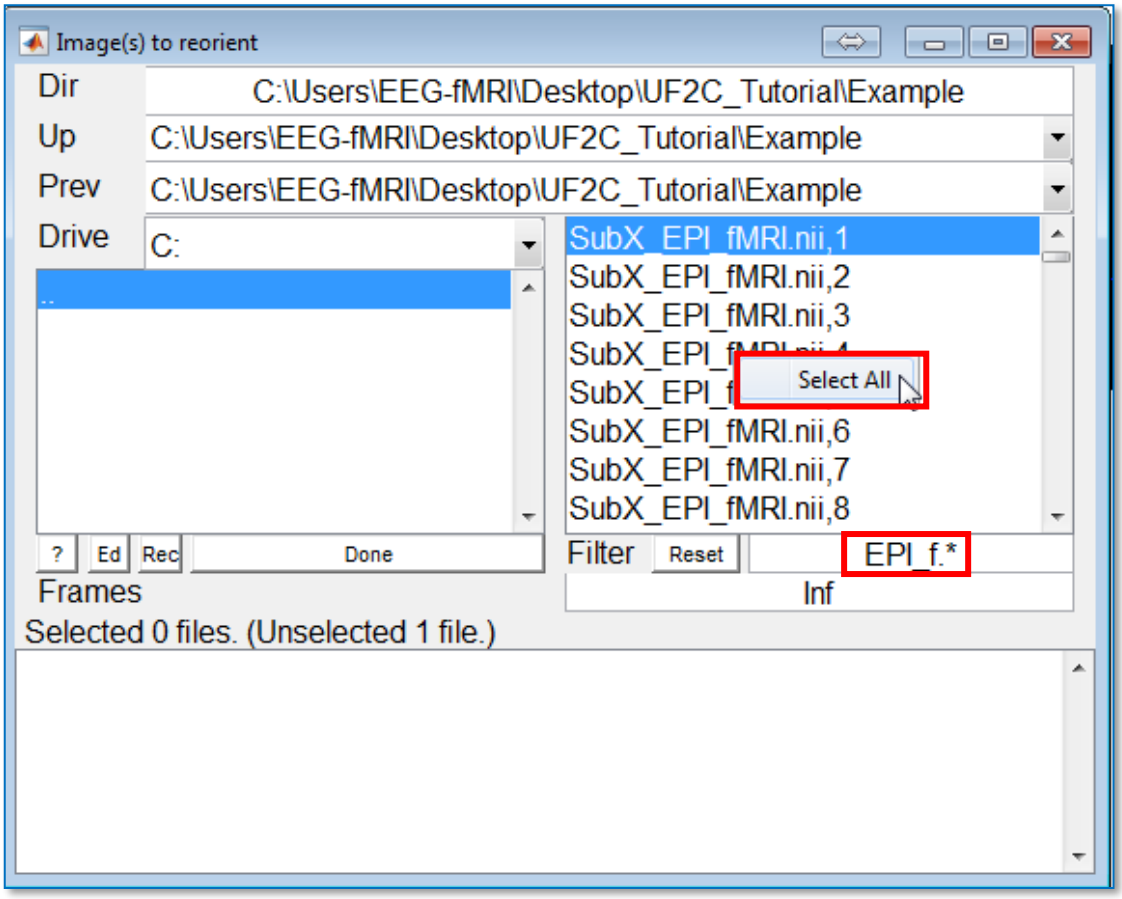
15. Remove the already added ",1" volume (left click on it once).



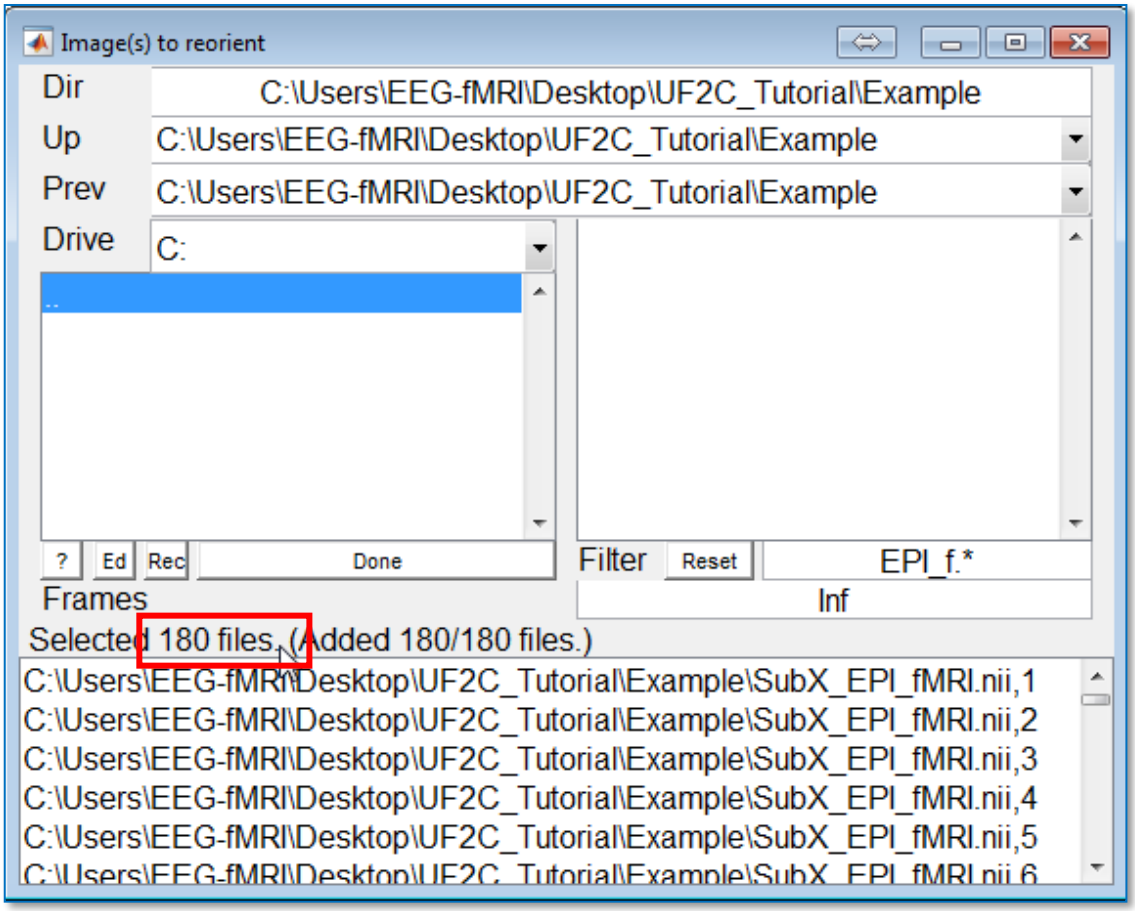
16. Use the field “Filter” to keep only the fMRI that you reoriented:



17. Type “**Inf**” replacing the number ‘**1**’. Press “**return**”. You will see all dynamics listed.



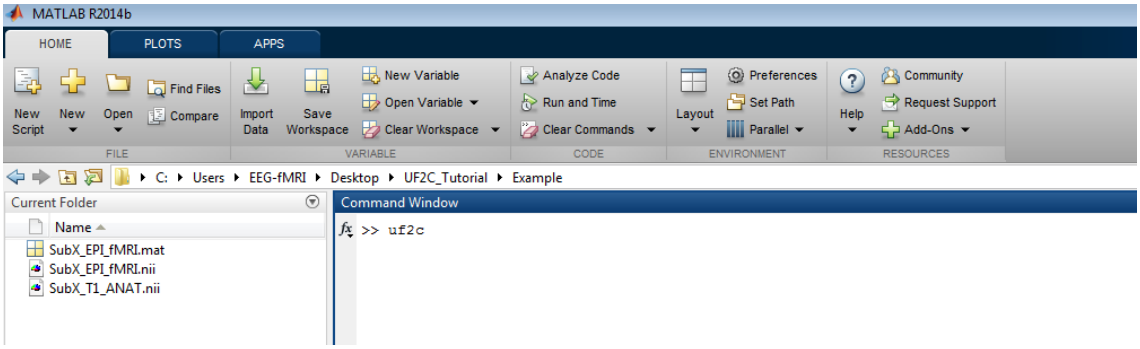
18. With the Right Mouse button, “**Select All**”:



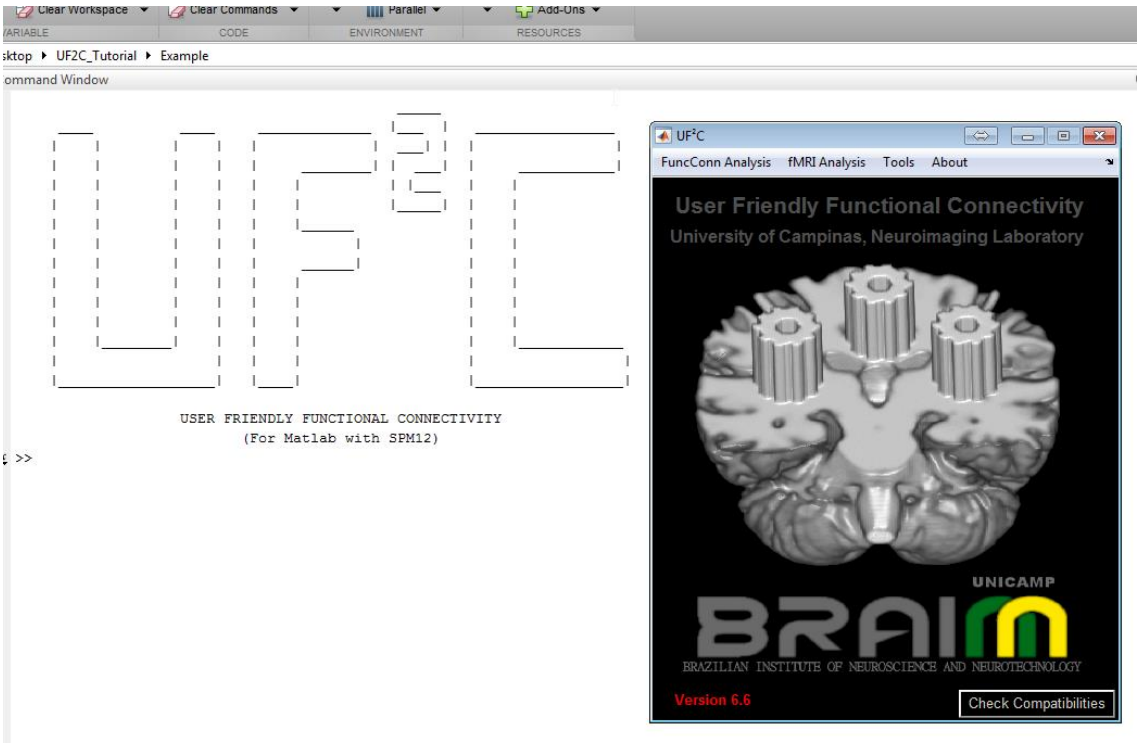
19. Check that the number of files added is correct (as highlighted above) and then click "**Done**".

7. Starting to use UF²C

With the **UF²C** folder added to the Matlab path, type **uf2c** in the Matlab command window.

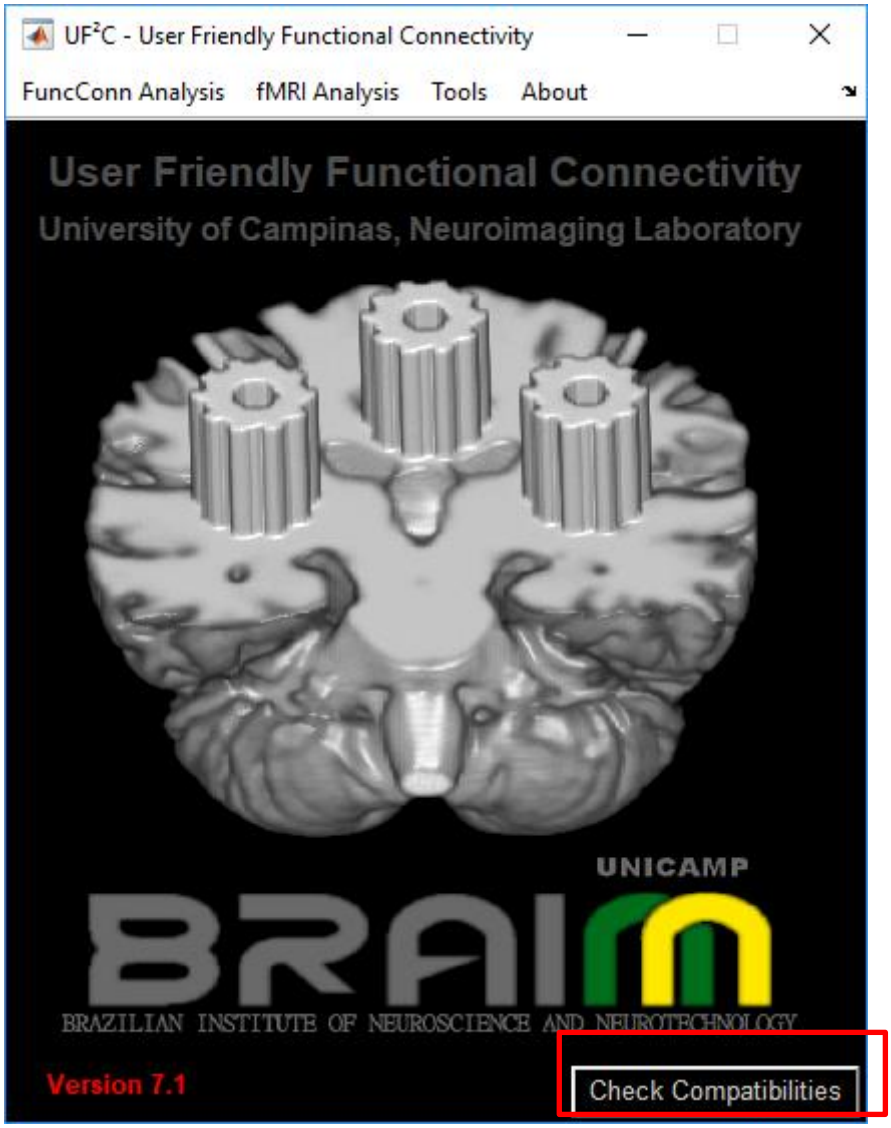


If you are using Windows OS, you can run **UF²C** running the **UF²C**.exe file in the uf2c folder.

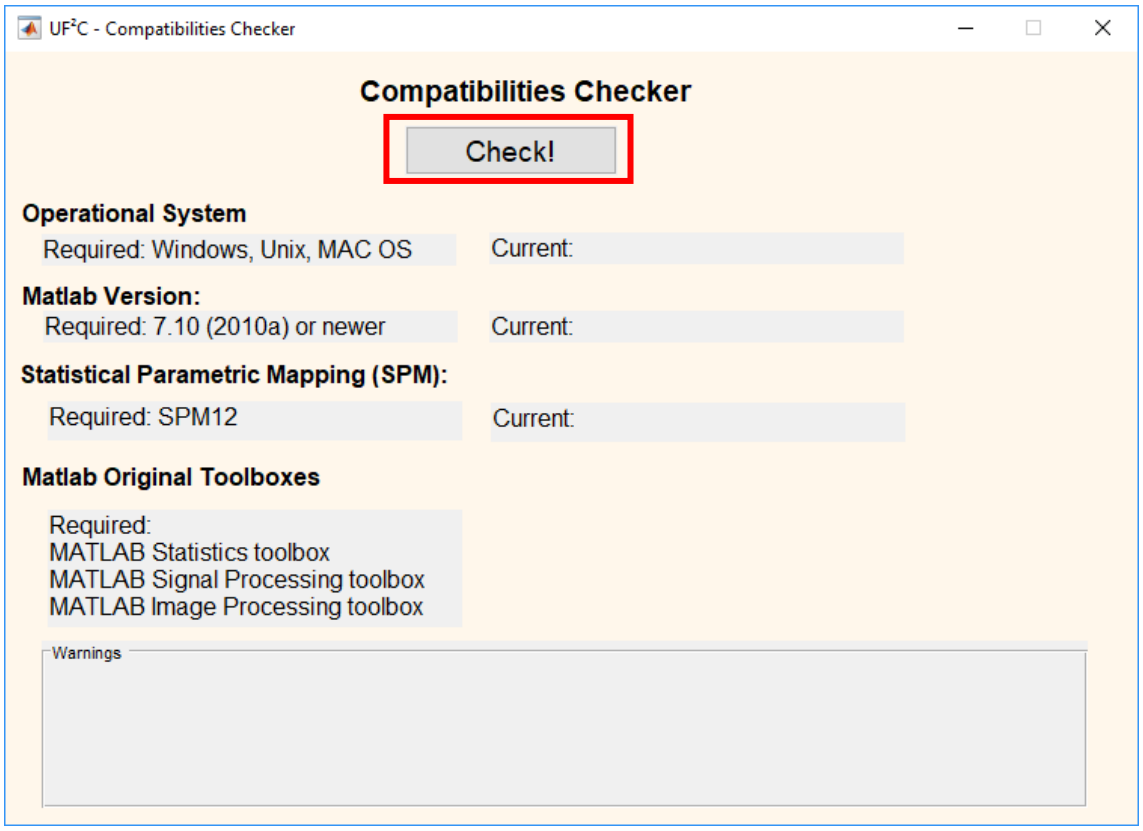


8. Checking compatibilities

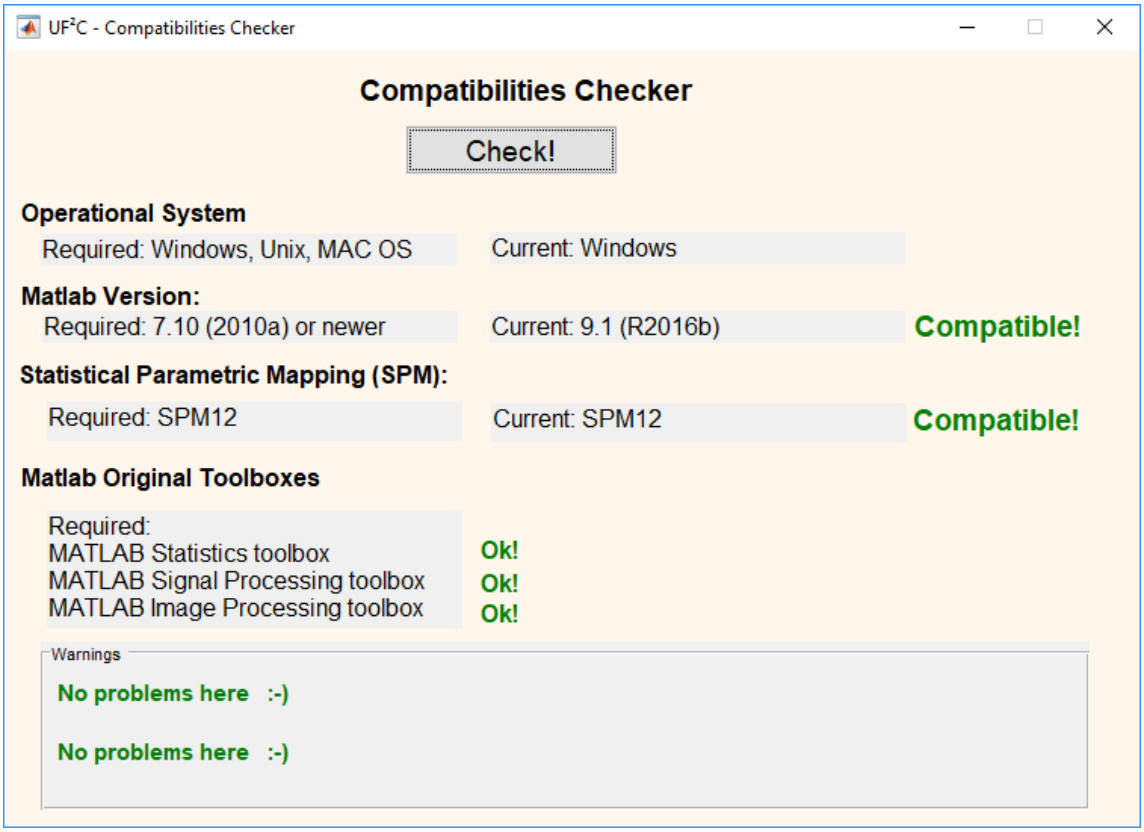
UF²C has a tool to verify the compatibility with your system, Matlab and SPM. Click on the "**Check Compatibilities**" Button on start screen:



Click on "**Check**" button.

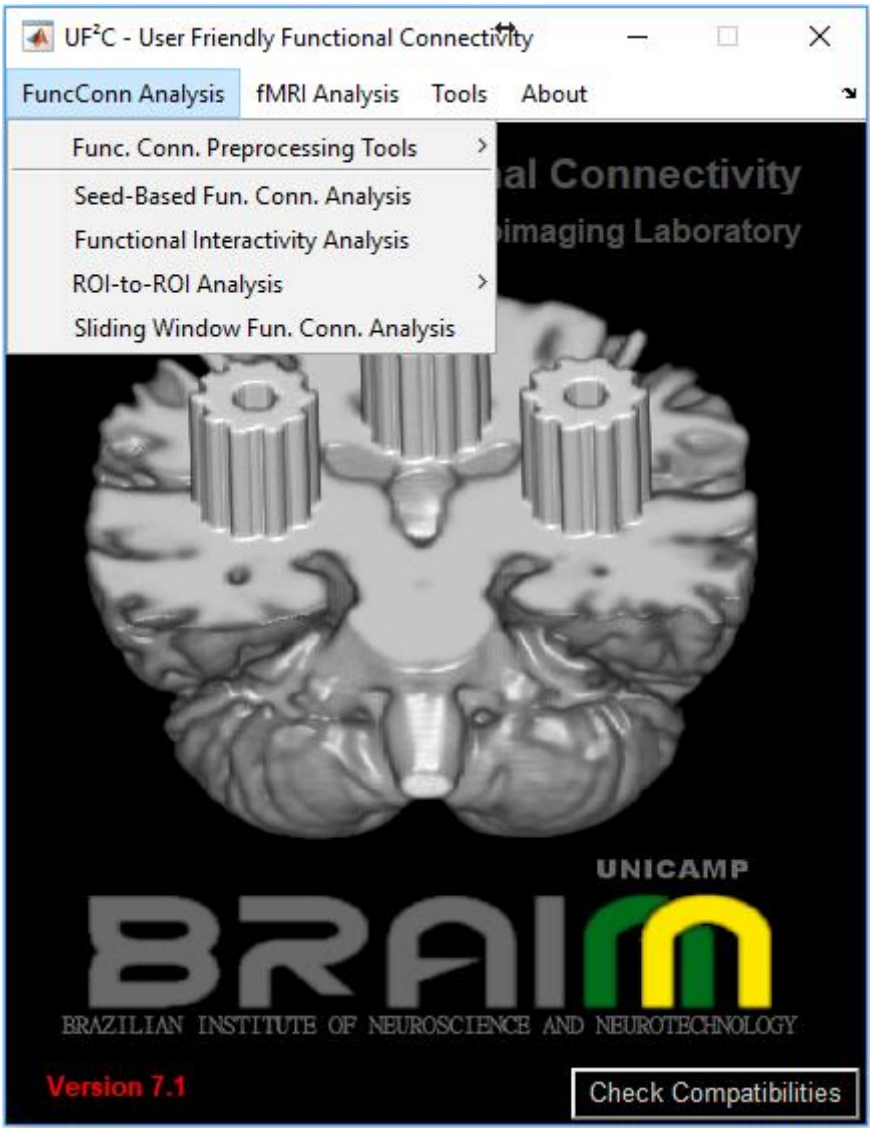


The **UF²C** will show in green compatible settings and in red if something is wrong or missing:



9. General Information

At this point, **UF²C** has **four** connectivity analysis modalities and TWO connectivity-pre-processing tools:



- "Func. Conn. Pre-processing Tools"

  - "Conventional **UF²C** Pre-proc" (**all analysis modules can also run this step!**)

  - "Pre-proc with NoVolEx" correction

1. Traditional "Seed-Based Fun. Conn. Analysis (ROI to whole brain)"

2. The concept analysis "Functional Interactivity Analysis" (ROIs to whole brain)

3. ROI-to-ROI Analysis

   i.ROI-to-ROI (first level)

ii.ROI-to-ROI Group Comp. (Sec. Level)

iii.ROI-to-ROI Correlation Test (Sec. Level)

iv.**UF²C** Outputs for NBC users

4. A sliding-window seed based functional connectivity (ROI to whole brain)

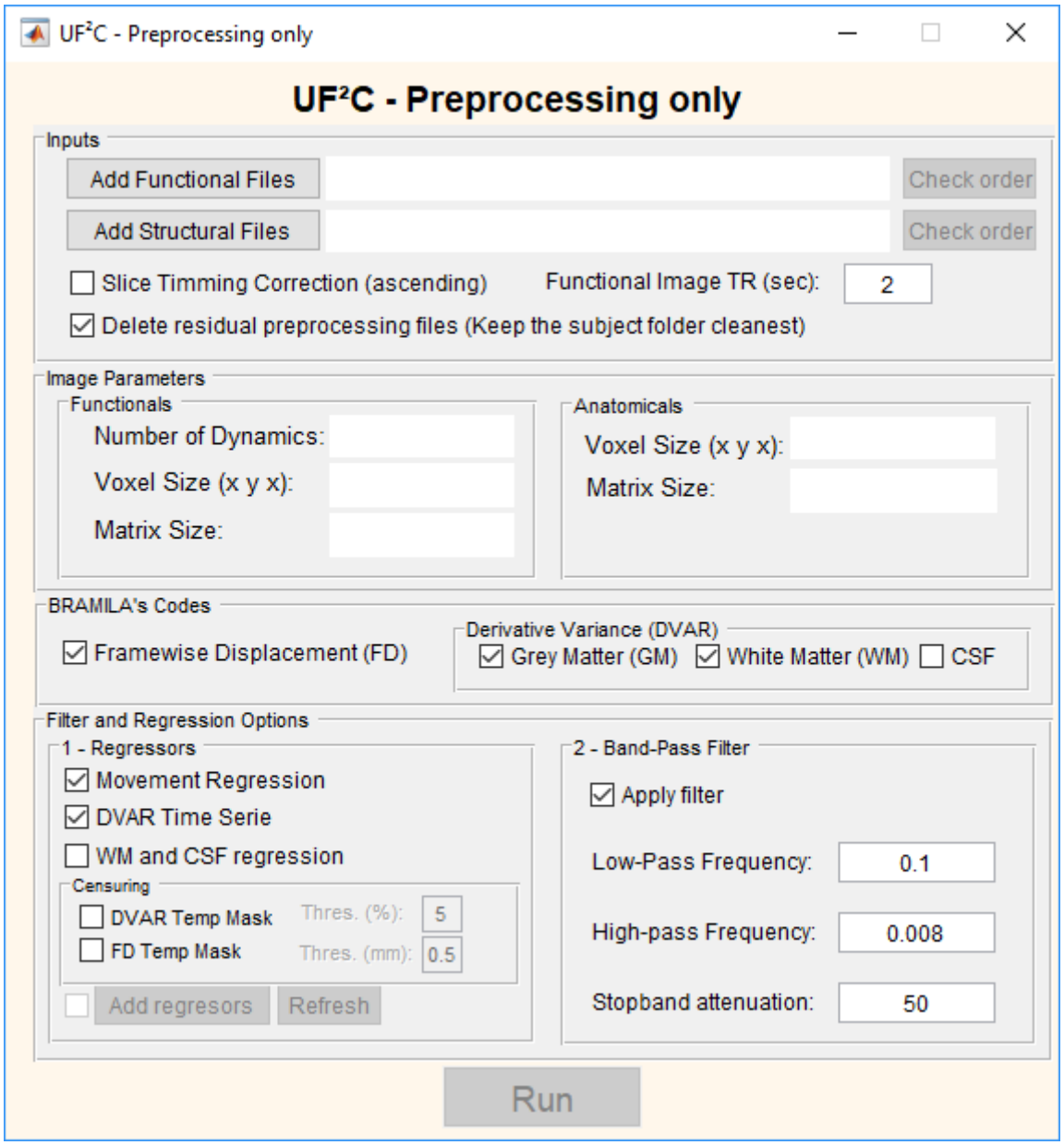
All modalities require functional (EPI) and structural (T1WI) images for all subjects and perform the same SPM PRE-processing pipeline:

- 1 - Functional Image Realignment: Realign: Estimate & Reslice
- 2 - Functional – Structural co-registration: Coregister: Estimate
- 3 - Structural image segmentation: Segment
- 4 - Structural image normalization: Normalise: Write
- 5 - Functional image normalization: Normalise: Write
- 6 - Functional image smooth: Smooth

For **UF²C** specific steps please see below.

All **UF²C** codes are open so you can check and modify all of the parameters. Otherwise, please respect the **UF²C** License.

10. Pre-processing only routine (Conventional UF²C Pre-proc)



Input Panel

The Funn. Conn. Pre-processing Tools (Conventional UF2C Pre-proc) allow you to pre-process your data without any statistical or connectivity inference. It is important to highlight that all **UF²C** analysis modalities are also able to do this process followed by the connectivity analysis. Once you have the post processed functional images (FiltRegrSW_****.nii; obtained from this tool or from any other **UF²C** modalities) you can quickly perform the connectivity analysis in any modality, skipping the pre-processing step (see below for instructions on how to do this).

With the button "**Add Functional Files**" you can add functional (4D) files of all subjects of your study. All functional files need to be in the same folder. With the button "**Add Structural Files**" you will be able to insert all structural (3D) files of all subjects in your study. Obviously, the number of functional and structural files should match. **UF²C will sort files in alphabetic order to match each functional file to their respective structural file, so it is extremely important that both files have similar name structures.** You can use the extra tool "**Filename Changer**" to modify and adjust your filenames, adding prefixes, suffixes or just removing name parts.

After the fMRIs and the T1 WI images are added, you can click on "**Check order**" to verify if all the functional and structural images are in the same order.

Please note that you can perform slice timing correction only when using the "pre-processing only" modality. The slice timing correction is more important in "event-related" analysis and is optional in FC studies. To avoid more data manipulation in resting-state analysis, we opted to not set the slice timing correction as a default here.

Define the functional image repetition time (TR). The checkbox "**Delete residual pre-processing files**" gives to you the option to save all the processing files or just the final version of each post processed file. By checking this box, the program will save just the realignment parameters (rp_*.txt file), the regressed-filtered-normalized-realigned functional file (FiltRegrSW*.nii) and the modulated-normalized structural file (wm*.nii file).

BRAMILA's Codes

UF²C added codes created by Dr. Enrico Glerean from the Brain and Mind Lab at Aalto University. These codes quantify the Framewise Displacement (FD) and the Derivative VARiance (DVAR) with the functional images, aiming to control the movement influence on the results. These parameters are discussed and were firstly presented on: Power et al. (Neuroimage, 2012), Satterthwaite et al. (Neuroimage, 2013), Yan et al. (Neuroimage, 2013), Power et al. (Neuroimage, 2014) and newer derived papers.

FD: The time series of the sum of the absolute values of the derivatives of the six realignment parameters extracted from the functional image realignment process. For the rotational parameters, **UF²C** uses a radius of 50 mm.

DVAR: The time series of the root mean square (RMS) of the derivatives of the time-series of all in-brain-masked voxels for each volume.

In this panel, you have the option to quantify the FD and the DVAR for different tissues. If you select more than one tissue for DVAR quantification, **UF²C** will also compute the average DVAR time-series (used for thresholding). Quantifying these parameters will allow you to perform temporal masks, censuring the supra-threshold volumes on the regression ("1 – Regressors" panel).

Theoretical observations

1 – Since the head movement occurs mainly due angular movements of the head, the DVAR of more external structures are naturally higher than structures close to the center of the brain. In this sense usually DVARs of: CSF>GM>WM. For thresholding, we suggest to always include GM and WM to estimate the average, and then create the temporal masks.

2 – Although the censuring is a well-described methodology to exclude noisy volumes, in practice, the inclusion of columns with ONES in the time points of the "bad" volumes gives zero value for all voxels of that volume. The number of censured volumes will influence the correlation values since time-points filled with ZEROS (whole volume) will be always a time point of full synchronicity between voxels.

3 - The suggested threshold for FD on **UF²C** is 0.5 mm, the same proposed in Power's papers. On the other hand, the DVAR threshold suggested on **UF²C** is 5% rather than 0.5% suggested in Power's papers. Because the DVAR value is strongly dependent on the pre-processing parameters, rigid body transformations and temporal normalizations, we believe that a threshold of 5% is compatible and adequate for UF²C pre-processing images. Thinking about this problem and in the growing demand for higher level counter-movement procedures, **UF²C** created and proposed a new pre-processing modality aiming to address all these issues (movement, thresholds,

variations on the number of removed volumes and etc…). Please see the section "**Preproc with NoVolEx correction**"

4 – The BRAMILA's quantifications are applied after the SPM standard PRE-PRE-processing (on page 26), and just before the Filtering and Regressions step.

Image Parameters

In this panel, relevant information about your functional and structural files will be shown. The first image of each type will be used as reference. **It is extremely important that all the files (for different subjects) have the same image parameters, such as voxel sizes, FOV, and number of dynamics.**

Filter and Regression Options (same for all UF²C modalities)

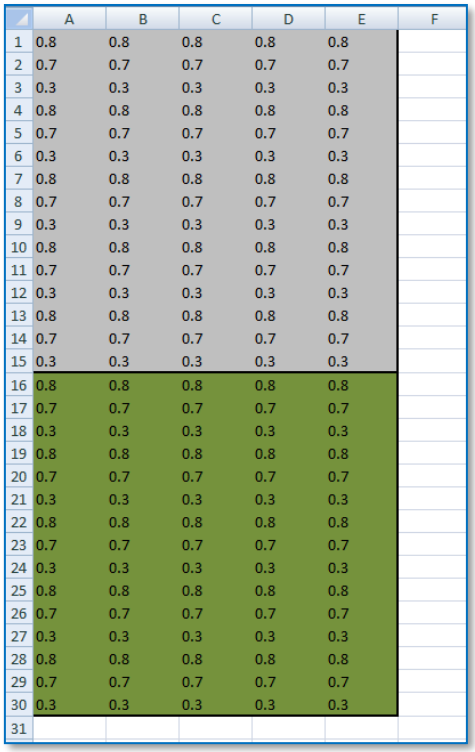
Regression

UF²C enables the user to perform multiple regressions. By default, the program automatically applies regression to 6 movement parameters (3 rotational and 3 translational). **UF²C also regresses** each time series to average signal fluctuation for white matter (WM) and cerebral spinal fluid (CSF). Both options can be disabled.

Additionally, the software allows you to add additional regressors by enabling and clicking the "**Add regressors**" button. This option is activated after adding the functional files.

Add regressors - instructions

In these instructions, we will use an example that included 5 subjects. The functional file will have 15 dynamics (just for illustration). Two additional regressors will be added. For details please see the next figure.



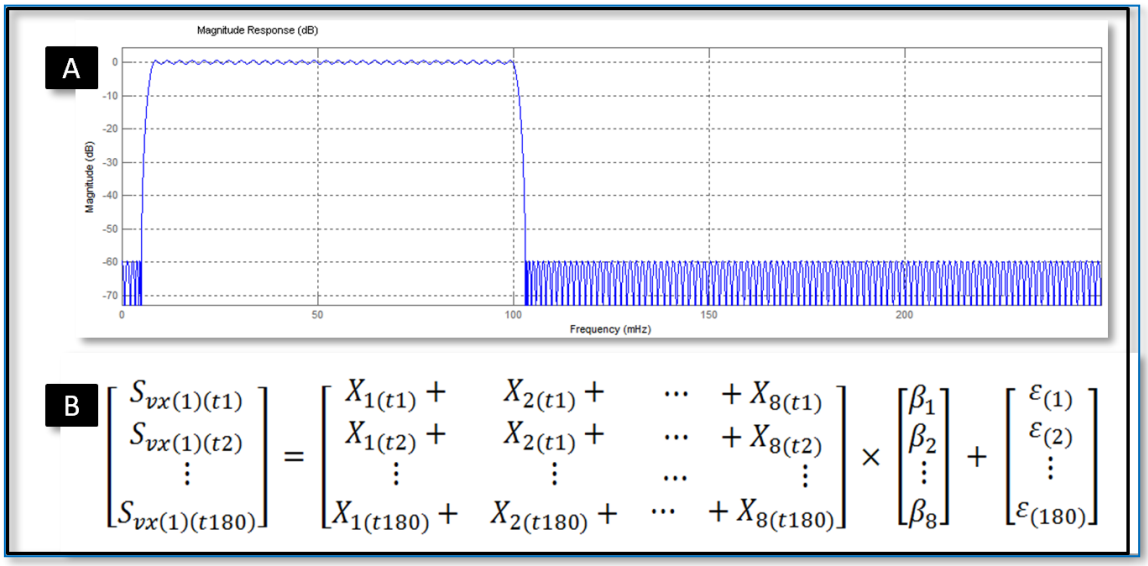
	A	B	C	D	E	F
1	0.8	0.8	0.8	0.8	0.8	
2	0.7	0.7	0.7	0.7	0.7	
3	0.3	0.3	0.3	0.3	0.3	
4	0.8	0.8	0.8	0.8	0.8	
5	0.7	0.7	0.7	0.7	0.7	
6	0.3	0.3	0.3	0.3	0.3	
7	0.8	0.8	0.8	0.8	0.8	
8	0.7	0.7	0.7	0.7	0.7	
9	0.3	0.3	0.3	0.3	0.3	
10	0.8	0.8	0.8	0.8	0.8	
11	0.7	0.7	0.7	0.7	0.7	
12	0.3	0.3	0.3	0.3	0.3	
13	0.8	0.8	0.8	0.8	0.8	
14	0.7	0.7	0.7	0.7	0.7	
15	0.3	0.3	0.3	0.3	0.3	
16	0.8	0.8	0.8	0.8	0.8	
17	0.7	0.7	0.7	0.7	0.7	
18	0.3	0.3	0.3	0.3	0.3	
19	0.8	0.8	0.8	0.8	0.8	
20	0.7	0.7	0.7	0.7	0.7	
21	0.3	0.3	0.3	0.3	0.3	
22	0.8	0.8	0.8	0.8	0.8	
23	0.7	0.7	0.7	0.7	0.7	
24	0.3	0.3	0.3	0.3	0.3	
25	0.8	0.8	0.8	0.8	0.8	
26	0.7	0.7	0.7	0.7	0.7	
27	0.3	0.3	0.3	0.3	0.3	
28	0.8	0.8	0.8	0.8	0.8	
29	0.7	0.7	0.7	0.7	0.7	
30	0.3	0.3	0.3	0.3	0.3	
31						

That is an example of ".xls" file that could be added to **UF²C**.

In this figure, 5 columns are shown, one for each subject (or functional file added). There are also 30 rows. The first 15 rows are from regressor one (shown in grey) and the next 15 rows are from regressor 2 (shown in green). The columns need to be in the same order of the subjects added. To check the subject order, click on the "subject list" button. Add the file on the "**Addreg**" window and click on the "Refresh" button to check if the number of regressors is Ok. Finally, click the "save and close" button to confirm.

The next figure shows the output design of the bandpass filter (“A”). In "**B**" the figure shows the multiple linear regression equation where:

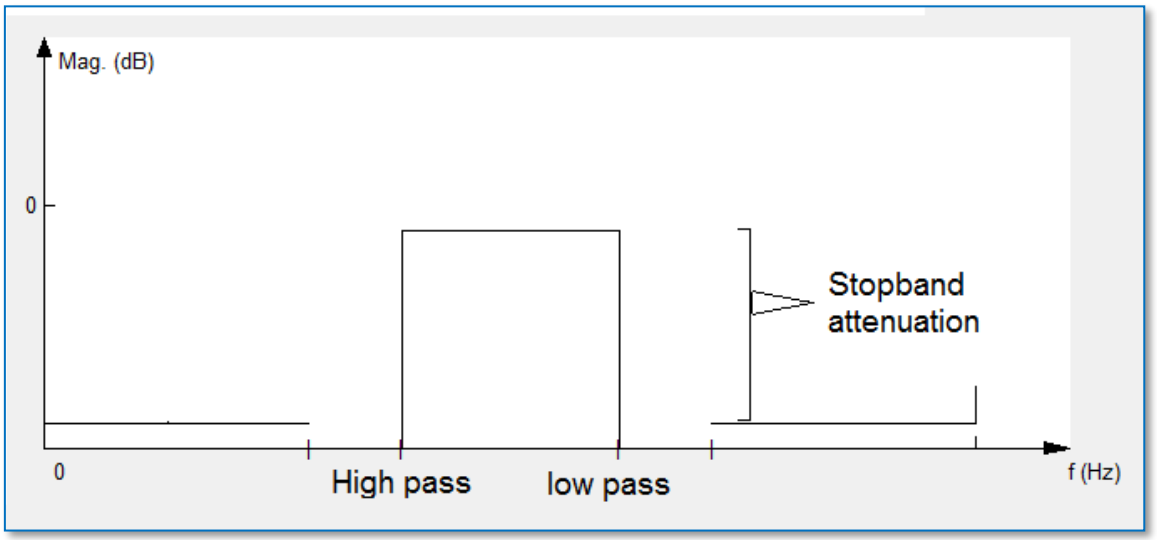
- "X(n)" are the regressors (in this case, we have 8)
- "t(n)" is the temporal variable (in this case, 180 time points)
- "Svx(n)(t)" is the variable that represents each voxel's temporal signal.



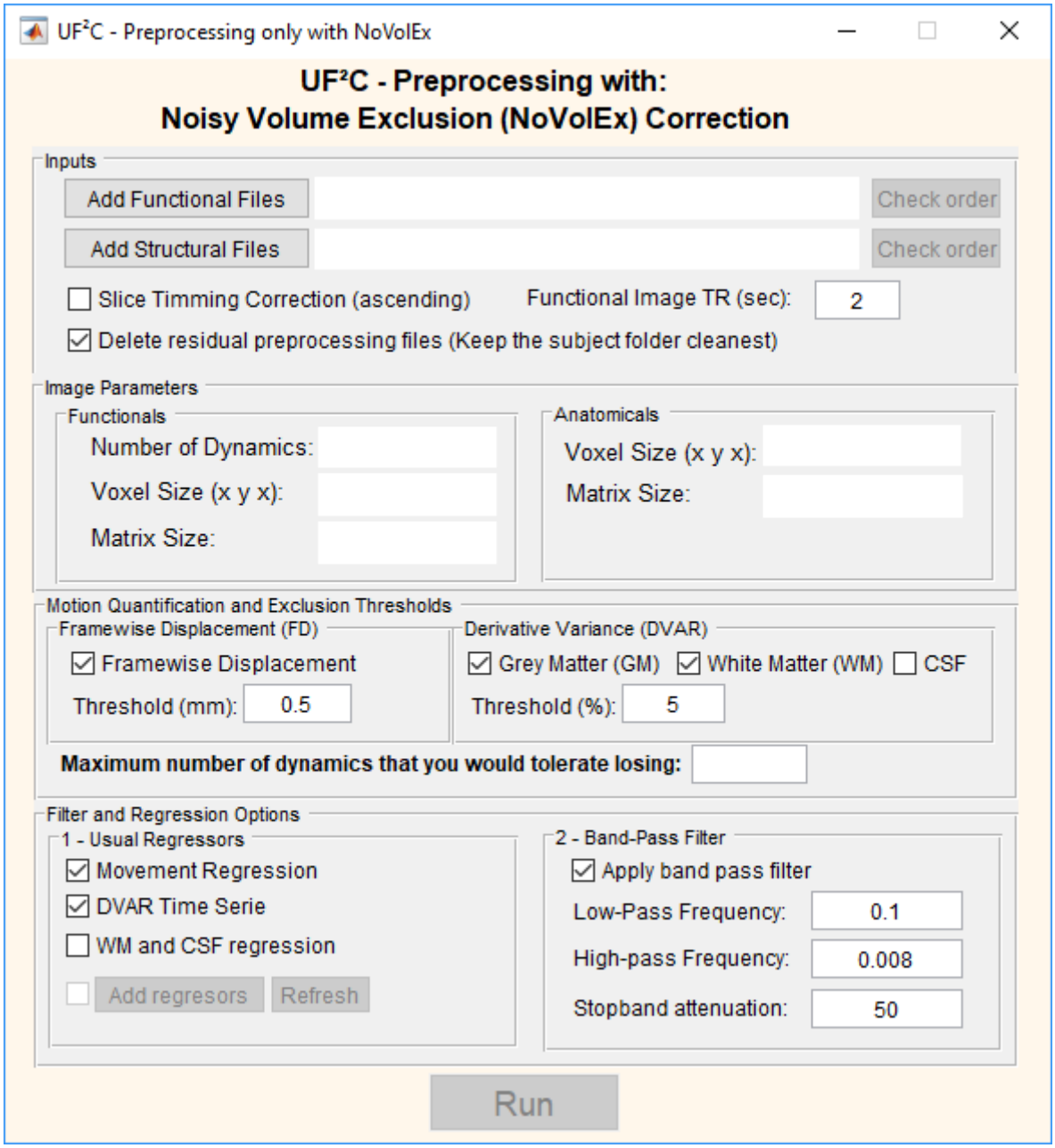
Band-Pass Filter (low-pass and high-pass filters)

In the check-box "**Apply band pass filter**", you can choose if you want to apply a filter to your temporal series. If the check box is selected, you will have the option to set low-pass and high pass frequency according to the next figure. The presented values are like the ones used in most resting-states studies.

The stopband attenuation option defines the value (in dB) of the magnitude difference of the pass band (0dB) and the stop band frequencies.



11. Pre-processing only routine with NoVolEx correction (Pre-proc with NoVolEx)



The Funn. Conn. Pre-processing (Pre-processing only), allows you to pre-process your data without any statistical or connectivity inference. Once you have the post-processed functional images (FiltRegrSW_****.nii; obtained from this tool or from any other **UF²C** modalities), you can quickly perform the connectivity analysis in any modality, skipping the pre-processing (see below for instructions on how to do this).

In this modality of pre-processing, you should add ALL IMAGES (controls and patients, for example) TOGETHER. This is important to ensure that all POST-processed images will have the same number of volumes at the end.

How does NoVolEx work?

The NoVolEx (Noisy Volumes Exclusion) correction method was developed to exclude volumes that have supra-threshold values of FD (on page 29) and/or DVAR (on page 29). The tool requires the definition of the FD and DVAR threshold and a limit of volumes that you would tolerate losing. With these inputs, the routine will quantify the motion of all included images, removing the supra-threshold volumes of each subject until the maximum number tolerated by the user.

1 – If I select DVAR for Grey Matter (GM) and Withe Matter (WM) how will the code define the threshold? R: The DVAR threshold will be always applied to the AVERAGE (between tissues) DVAR time series, although it will also print separate results for each tissue.

2 – What happens if I tolerate losing 30 volumes, but I have a subject that presents 33 bad volumes? R: The **UF²C** will suggest the exclusion of this volunteer (skipping his pre-processing).

3 – What happens if I tolerate losing 30 volumes, but my worst image/subject has only 17 bad volumes? R: The **UF²C** will remove only the 17 worst volumes of each volunteer and at the end, all processed images (of all subjects) will have 17 volumes removed too.

4 – What is the criteria to exclude volumes from a subject who does not have bad volumes? R: **UF²C** will rank the quality of each volume and will remove the worst ones, until it reaches the necessary number of volumes to keep all volunteers equals (in number of dynamics).

5 – Could I use NoVolEx to pre-process my images, and then perform a dynamic study (moving window etc…)? R: No.

Input Panel

With the button "**Add Functional Files**" you can add functional (4D) files of all subjects of your study. Note that all functional files should be in the same folder. With the button "**Add Structural Files**" you will be able to insert all structural (3D) files of all subjects in your study. Obviously, the number of functional and structural files should match. **UF²C will sort files in alphabetic order to match each functional file to their respective structural file, so make sure that both files have similar name structures.** You can use the extra tool "**Filename Changer**" to modify and adjust your filenames, adding prefixes, suffixes or just removing name parts.

After the fMRIs and the T1 WI images are added, you can click on "**Check order**" to verify if the list of functional and structural images is in the same order.

Please note that you can perform slice timing correction only when using the "pre-processing only" modality. The slice timing correction is more important in "event-related" analysis and is optional in FC studies. To avoid more data manipulation in resting-state analysis, we opted to not set the slice timing correction as a default option here.

Define the functional image repetition time (TR). The checkbox "**Delete residual pre-processing files**" gives to you the option to save all the processing files or just the final version of each post-processed file. By checking this box, the program will save just the realignment parameters (rp_*.txt file), the regressed-filtered-normalized-realigned functional file (FiltRegrSW*.nii) and the modulated-normalized structural file (wm*.nii file).

Image Parameters

In this panel, relevant information about your functional and structural files will be shown. The first image of each type will be used as reference. **Make sure that all the files (for different subjects) have the same image parameters, such as voxel sizes, FOV, and number of dynamics.**

Motion Quantification and Exclusion Thresholds

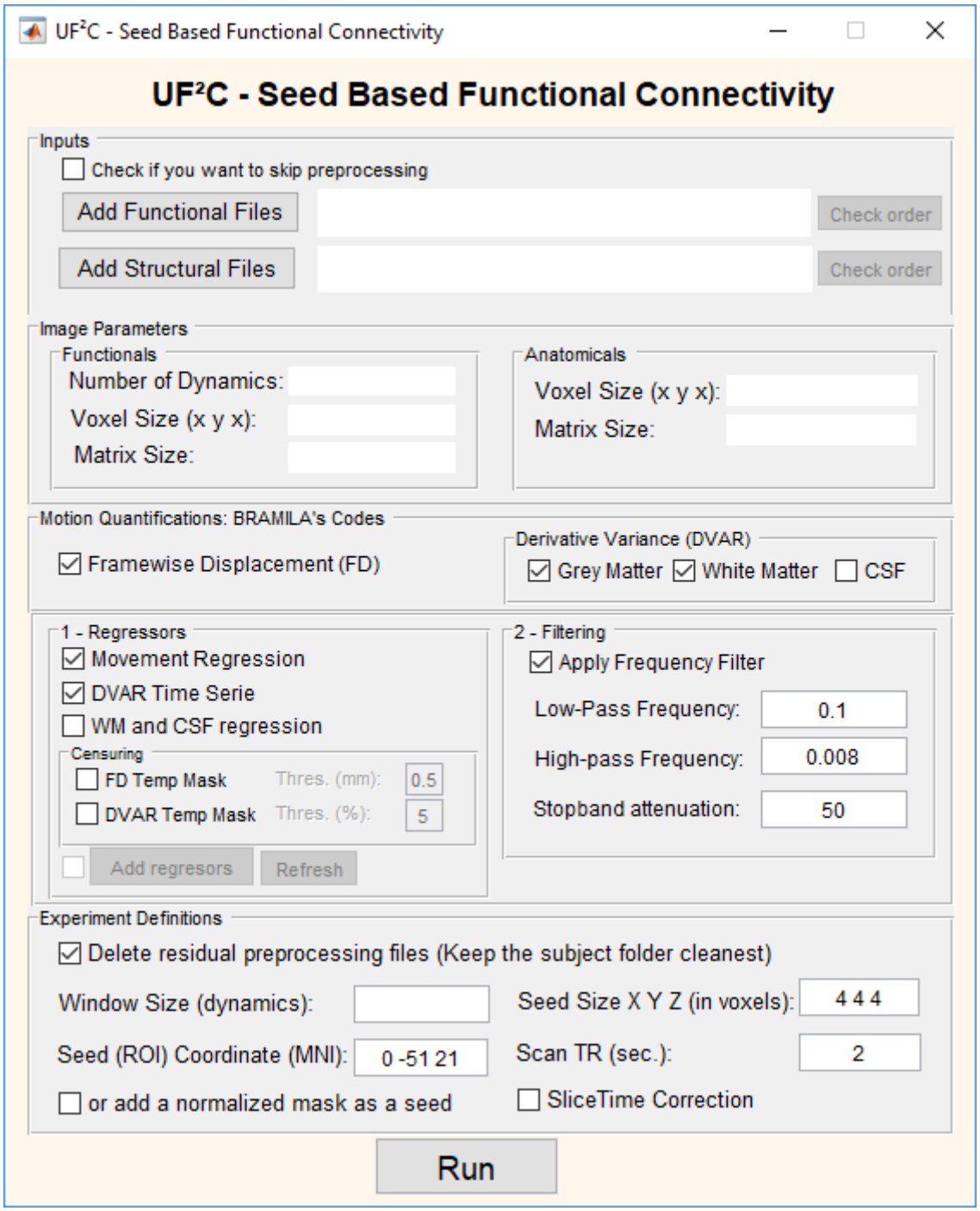
In this panel, you will define the parameters and the thresholds to exclude noisy volumes. You can enable the Framewise Displacement (FD) (on page 29) and the DVAR (on page 29) in different brain tissues. If you select more than one tissue for DVAR, the threshold will be applied to the average (time series) of the tissues.

The option: "**Maximum number of dynamics that you would tolerate losing**" will be automatically filled with 10% of the total number of dynamics. Here you will define how many volumes **UF²C** can remove according to the thresholds established. This process will follow the criteria mentioned and exemplified on page 34.

Filter and Regression Option

The same described on the "Pre-processing Only" routine (on page 32 and on page 30).

12. Modality 1 - Seed Based Functional Connectivity Analysis



Input Panel

In the "Input" panel, you have the option to "**Check if you want to skip pre-processing**". Click in the box if you already have the normalized post-processed functional image that you want to use in your analysis. We strongly recommend using

only the “FiltRegrSW” images generated by **UF²C** pre-processing pipelines. By checking this option, all pre-processing parameters and options will be disabled.

Using the button "**Add Functional Files**" you can add raw (reoriented) functional (4D) files of all subjects in your study. With the button "**Add Structural Files**" you can insert all structural (3D) files of all subjects in your study. Obviously, the number of functional and structural files should match. **UF²C will use alphabetic order to match each functional file to their respective structural file, so make sure that both files have similar names structures, e.g. Subj_XX1_fMRI.nii** and **Subj_XX1_T1.nii**. You can use the extra tool "**Filename Changer**" to modify and adjust your filenames, adding prefixes, suffixes or removing name parts.

After adding the fMRIs and T1 WI images, you can click on “**Check order**” to verify if the list of functional and structural images is in the same order.

Image Parameters

In this panel, relevant information about your functional and structural files will be shown. The first image of each type will be used as reference. **Make sure that all the files (for different subjects) have the same image parameters, such as voxel sizes, FOV, and number of dynamics.**

Experiment Definitions

In this panel, you can define important parameters that are relevant to you experiment. The first checkbox "**Delete residual pre-processing files**" gives you the option to save all the processing files or just the final version of each post-processed file. By checking this box, the program will just save:

- The realignment parameters (rp_*.txt file)
- The modulated-normalized-smoothed functional file (sw*.nii file)
- The regressed-filtered functional file (FiltRegrSW*.nii)
- The modulated-normalized structural file (wm*.nii file).

By default, the option "**Window Size**" will be filled with the total number of dynamics (volumes or temporal points of your functional file). When looking at dynamic connectivity, you can divide your temporal series by setting the size of these windows. For example, if you have functional data with 300 dynamics, and set the Window size to 60, you will divide your temporal series in 5 parts. This option gives information about the temporal variation in the connectivity during the acquisition period. **The window size should be divisible by the number of dynamics**. Remember that the reduction of the correlation time points, by the addiction of a large number of windows, will reduce the degrees of freedom of the correlation test and the significance of the r-values.

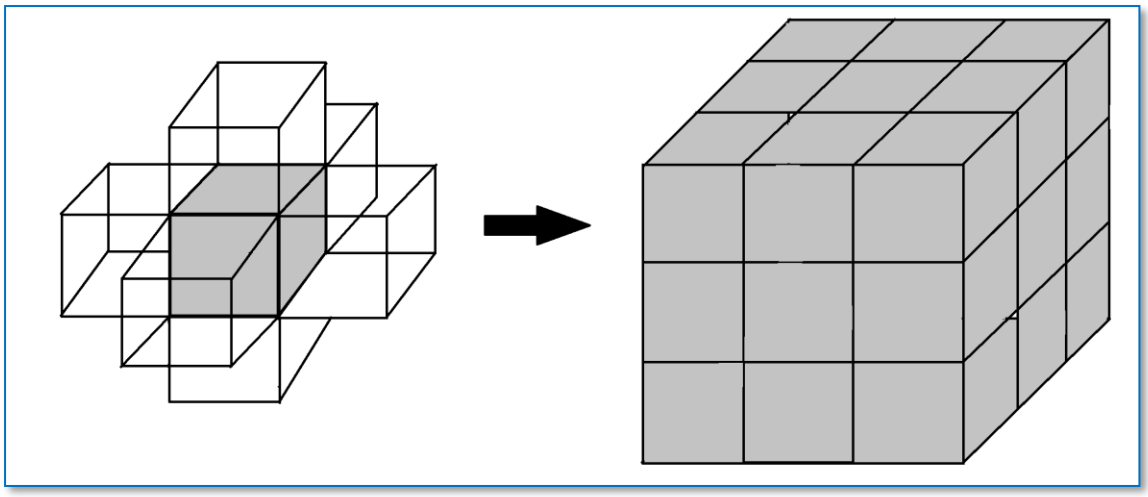
The correlation will be performed separately for each window. As a result, **UF²C** will plot a graph with the connectivity variation during these 5 time points. Also, the final connectivity map will be created as a 4D file in which each volume will be the statistical correlation r-map of each window.

Seed creation and definition

In the option "**Seed (ROI) Coordinate**", you should set the MNI (Montreal Neurologic Institute template) coordinate of the region that you want to use as seed. The seed will be the region from where **UF²C** will extract the reference time series. **UF²C** will use the seed's time series to calculate the correlations. In other words, the statistical values in the final map are always the correlation coefficient (r) between the time series of each cortex voxel and the seed's average time series. The coordinate set as default refers to the posterior cingulate cortex, commonly used as seed in Default Mode Network (DMN) studies.

The checkbox "**Add a normalized mask as a seed**" allows you to add a normalized (MNI-152) ROI image (NIfTI) as seed. The average time series will be generated by averaging all ROI voxels time series. In this case, the "**Seed (ROI) Coordinate (MNI)**" option and the "**Seed Size X Y Z (in voxels)**" option would be disabled.

The option "Seed size X Y Z" is used to increase the seed size. For example, 2 2 2 will add 1 voxel (2/2) for each side in each spatial axis. In this case the seed will be a cube with 3 voxels per edge or with 27 voxels total volume (illustrated in the figure below).



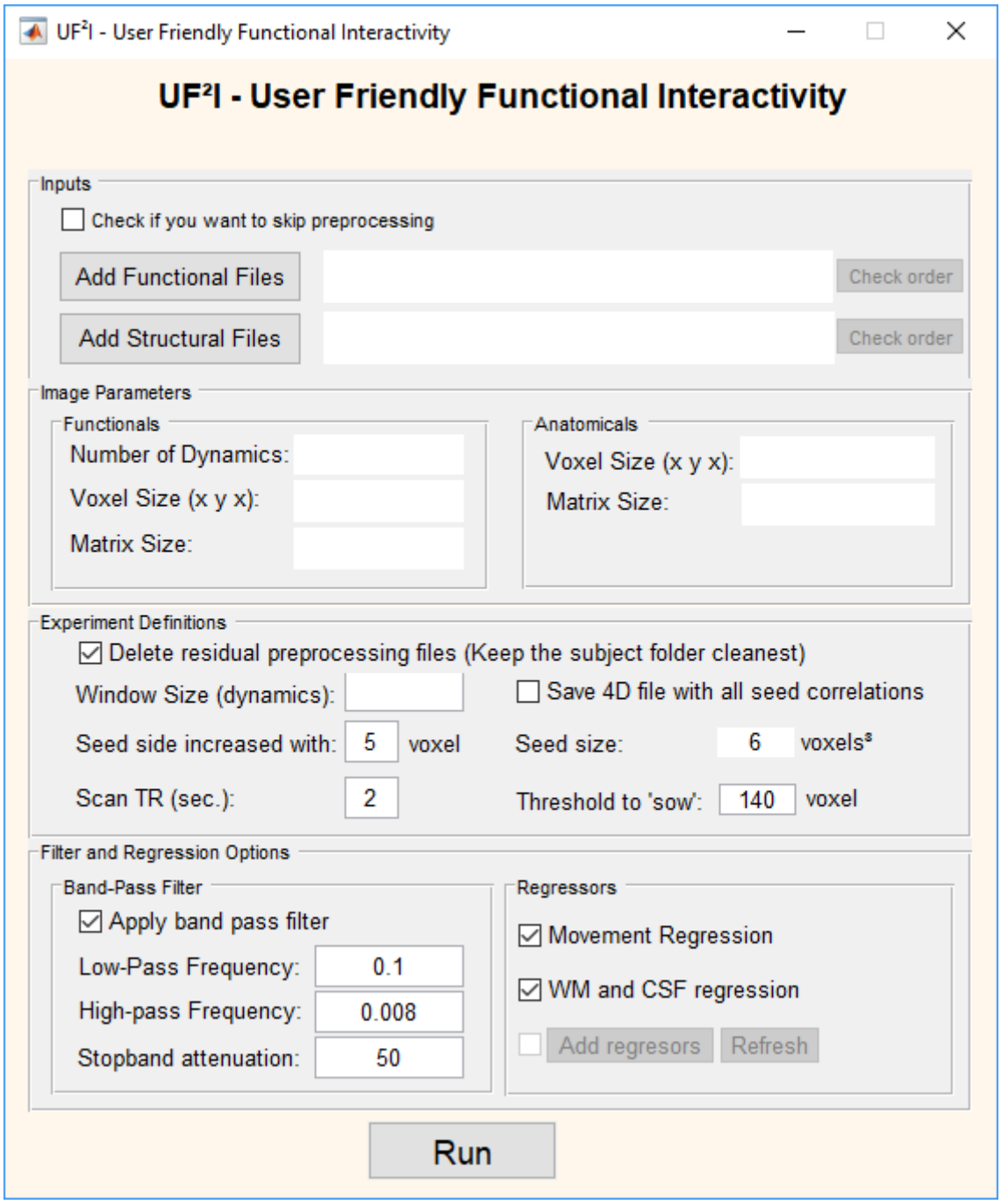
In this situation your seed is a VOI (volume of interest) with 27 voxels, so your reference time series will be the average time series between these voxels. This methodology to set the VOI size may seem unusual but is a safe way to keep the defined seed coordinates always in the center of the VOI.

Finally, the field Scan TR is used to define the repetition time (TR) in the functional acquisition. In this option, you need to set the TR in seconds, since this information is not correct or is missing in most NIfTI headers.

Filter and Regression Option

The same described on the “Pre-processing Only” routine (on page 32 and on page 30).

13. Modality 2 - Seed Based Functional Interactivity



Brief Introduction

Functional connectivity (FC) is an fMRI metric used to evaluate the synchrony of BOLD activity between two regions (also called seeds or ROIs) of the brain. FC analysis can describe brain interactional patterns without any cause-effect claims through the identification of varied functionally cooperative networks related to distinct brain states (Friston *et al.,* 1996). Different methods are used to access FC information from

fMRI data, which are divided between seed-based or data-driven methods (Liangsuo Ma; Suresh E. Joel; Kaiming Li). Seed-based FC is a modality that quantifies correlations throughout the whole brain time-series by using an ROI as reference and thereby allows the study of specific networks by controlling the seed position. The method enables an objective and straightforward analysis, although it provides results that depend on a precise initial assumption (the position of the ROI) and are restricted to those defined networks. On the other hand, independent component analysis (ICA) depends on the definition of the number of components to be isolated (also called *networks*), and produces a more complex set of results, which requires an exhaustive and bias susceptible task to separate study relevant components from misleading ones[1] (Liangsuo Ma; Calhoun VD, Adali T, Pearlson GD, Pekar JJ). A multi-seed FC analysis keeps the straightforward results provided by seed-based methods but also expands the networks of interest, giving extensive information about connectivity patterns and avoiding a strong initial assumption. The combination of the correlation maps generated for each seed could reveal distinct aspects of the functional networks organization, depending on how these maps were integrated or explored [The future of FMRI connectivity. Stephen M. Smith]. In this sense, the concept of functional interactivity (FI) is based on averaged FC maps to define areas more or less globally integrated, providing an exploratory view of the study and therefore allowing a generalized view of the brain connectivity patterns. [Inf Process Med Imaging. 2007; 20:147-59. Functional interactivity in fMRI using multiple seeds' correlation analyses--novel methods and comparisons. Wang YM1, Xia J.]

Inputs

The same described on the **Pre-processing Routine**.

Image Parameters

The same described on the **Pre-processing Routine**.

[1] Softwares like FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL) have automated methods to estimate the number of components to be isolated and each of them should be considered as noise components.

Experiment Definitions

The options "**Delete residual pre-processing files**" and "**Window Size (Dynamics)**" have the same functions described on the **modality 1 section**.

The FI analysis uses a cubic seed that varies in position. The option "**Seed side increased with:**" allows you to set your seed side size. For example: If you fill the option "**Seed side increased with:**" **3,** you will define a seed with **a** 4-voxel side and **64** voxels in total. By default, the "**Threshold to show**" option defines the number of overlapping voxels between the seed and voxels of GM, to use that seed position as an effective seed in the experiment. In the example, **UF²C** will place the cubic seed in all possible positions that retained at least 42 voxels overlapping with the GM. For each seed, an average time series will be extracted and the linear correlation will be estimated for all GM voxels, generating a statistical map for each seed position.

You can increase or decrease the "**Threshold to show**" value → lower values will result in a larger number of seed positions.

Filter and Regression Option

The same described on the "**Pre-processing Only" Routine**.

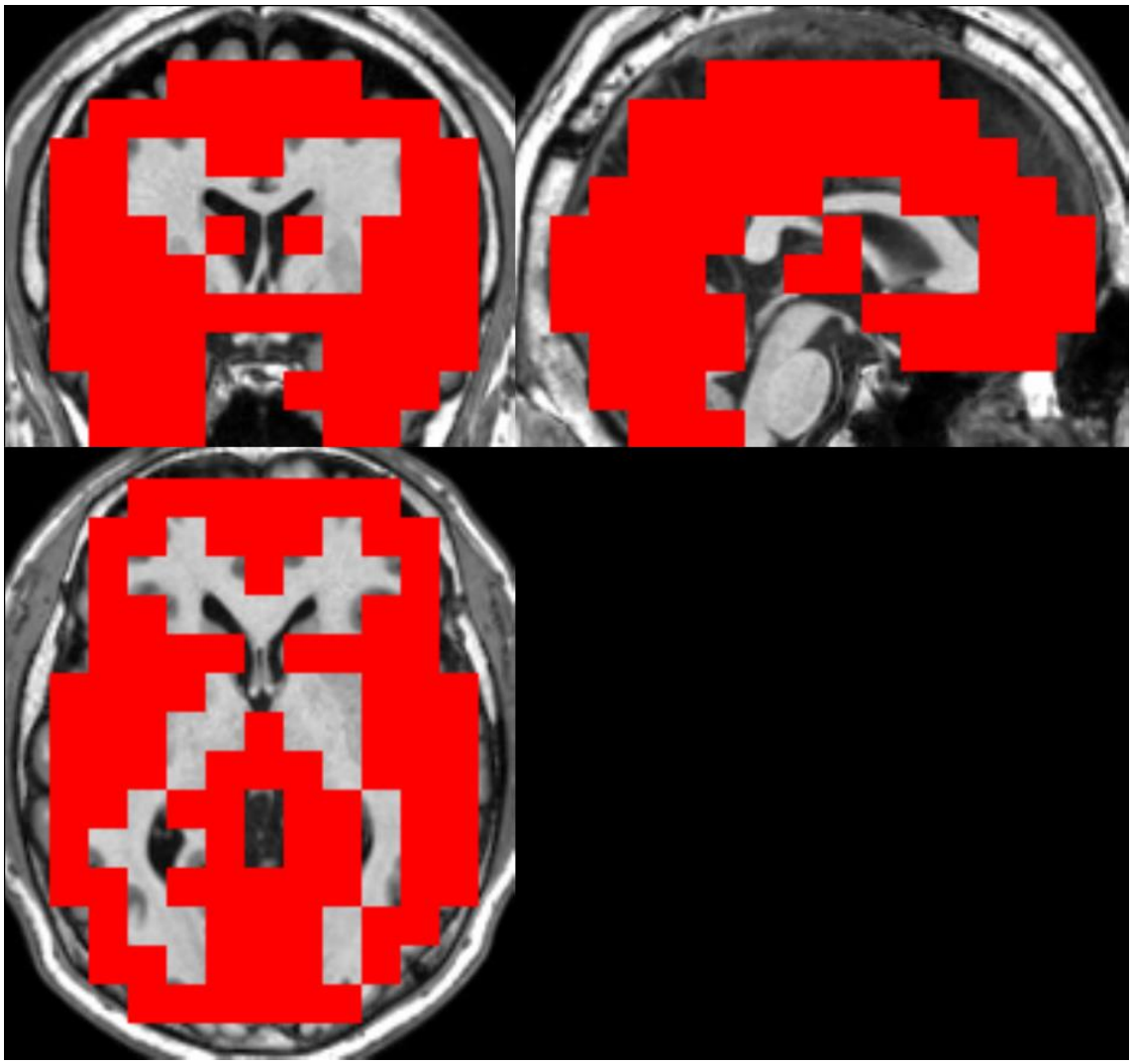
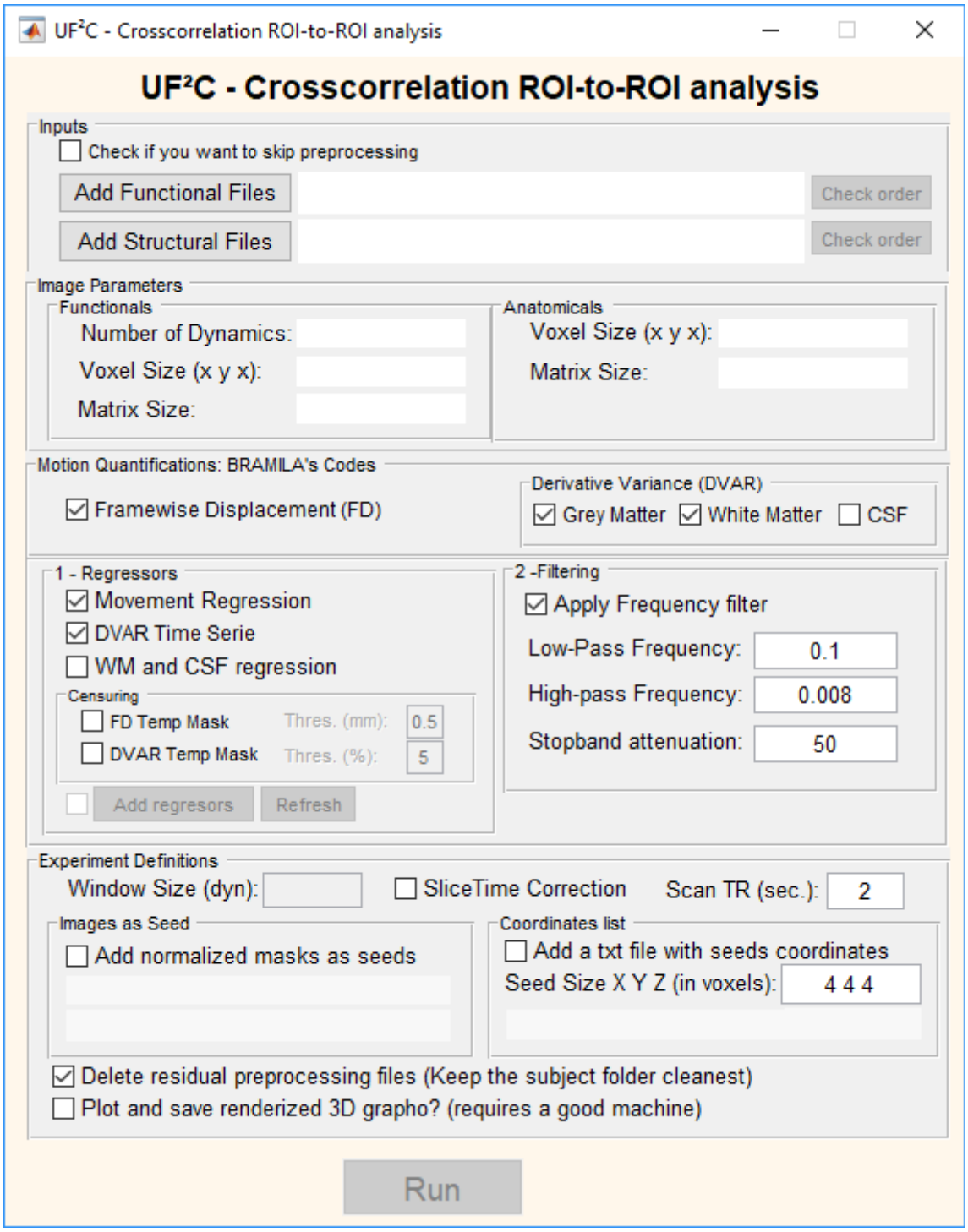


Fig: An example of mask of seeds generated by the code. The red cubes overlay all regions that were used as seeds.

14. Modality 3 – Cross-correlation ROI-to-ROI Analysis

a) First Level analysis (Individual)



Input Panel

In the "Input" panel, you have the option "**Check if you want to skip pre-processing**". Check this option if you already have the normalized post-processed functional image that you want to use in your analysis. We strongly recommend that you use only the

“FiltRegrSW” images generated by **UF²C** pre-processing pipelines. Checking this option, all pre-processing parameters and options will be disabled.
With the button "**Add Functional Files**" you can add raw (reoriented) functional (4D) files of all subjects of your study. With the button "**Add Structural Files**" you will be able to insert all structural (3D) files of all subjects in your study. Obviously, the number of functional and structural files should to match. **UF²C will use the alphabetic order to match each functional file to their respective structural file, so it is extremely important that both files have similar name structures, e.g.: Subj_XX1_fMRI.nii** and **Subj_XX1_T1.nii**. You can use the extra tool "**Filename Changer**" to modify and adjust your filenames, adding prefixes, suffixes or removes name parts.
After adding the fMRIs and the T1 WI images, you can click on “**Check order**” to verify if the list of function and structural images is in the same order.

Image Parameters

In this panel, relevant information about your functional and structural files will be shown. The first image of each type will be used as reference. **Make sure that all the files (for different subjects) have the same image parameters, such as voxel sizes, FOV, and number of dynamics.**

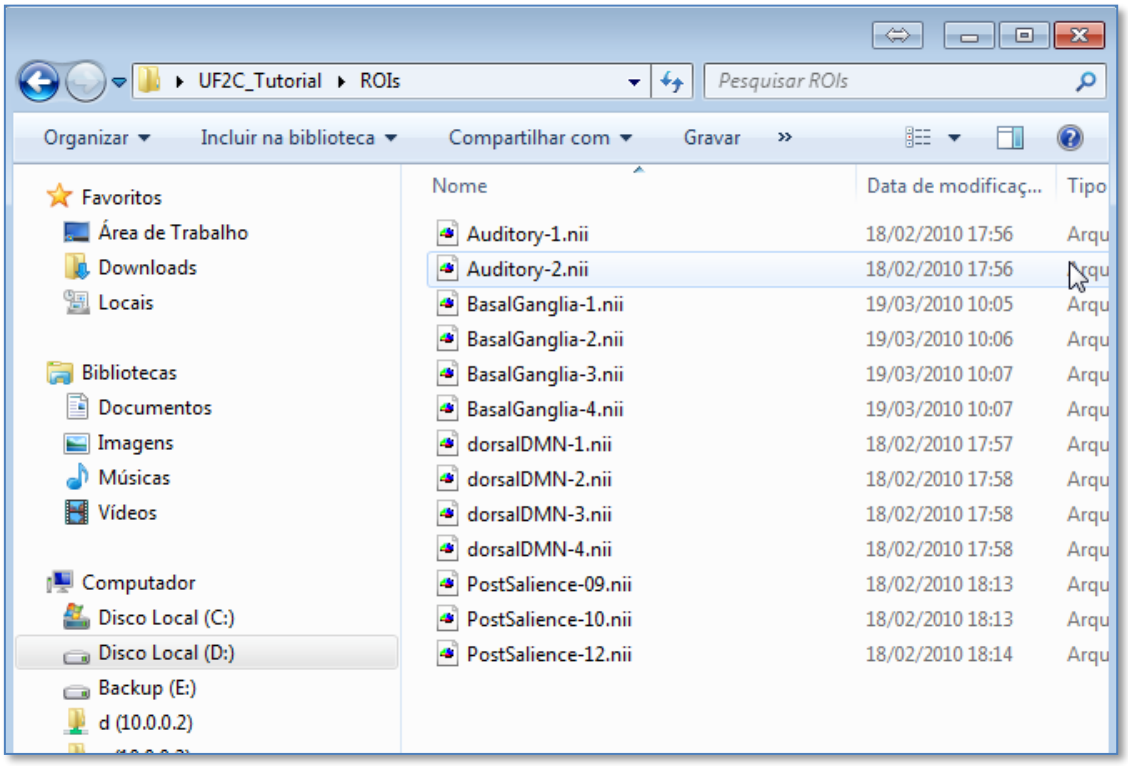
Experiment Definitions

In this panel, you will be able to define important parameters that will be extremely relevant to you experiment. The option "**Window Size**" will be filled by default with the total number of dynamics (volumes or temporal points of your functional file). You can divide your temporal series by setting the size of these windows. For example, if you have functional data with 300 dynamics, and set the Window size to 60, you will divide your temporal series in 5 parts. This option gives information about the temporal variation in the connectivity during the acquisition period. **The window size should be a divisor of the number of dynamics**. Remember that the reduction of the correlation time points, by the addiction of many windows, will reduce the degrees of freedom of the correlation test and the significance of the r-values. The correlation will be performed separately for each window. As a result, **UF²C** will plot a connectivity matrix for each of the five time points.

Finally, the field Scan TR is used to define the repetition time (TR) in the functional acquisition. In this option, you need to set the TR in seconds, since this information is not correct or is missing in most NIfTI headers.

To define the ROIs in which the **UF²C** will compute the ROI-to-ROI correlations, you have two options. In both options, **UF²C** can identify ROIs from a specific network and separate them into the analysis resulting in extra information. The network identification results in the concept of inter (between networks) and Intra (inside ROIs of a same network) connectivity. In the resultant images, like the resultant brain connectome images, the spheres that will represents the ROIs will be coloured according to the network organization (one color per network).

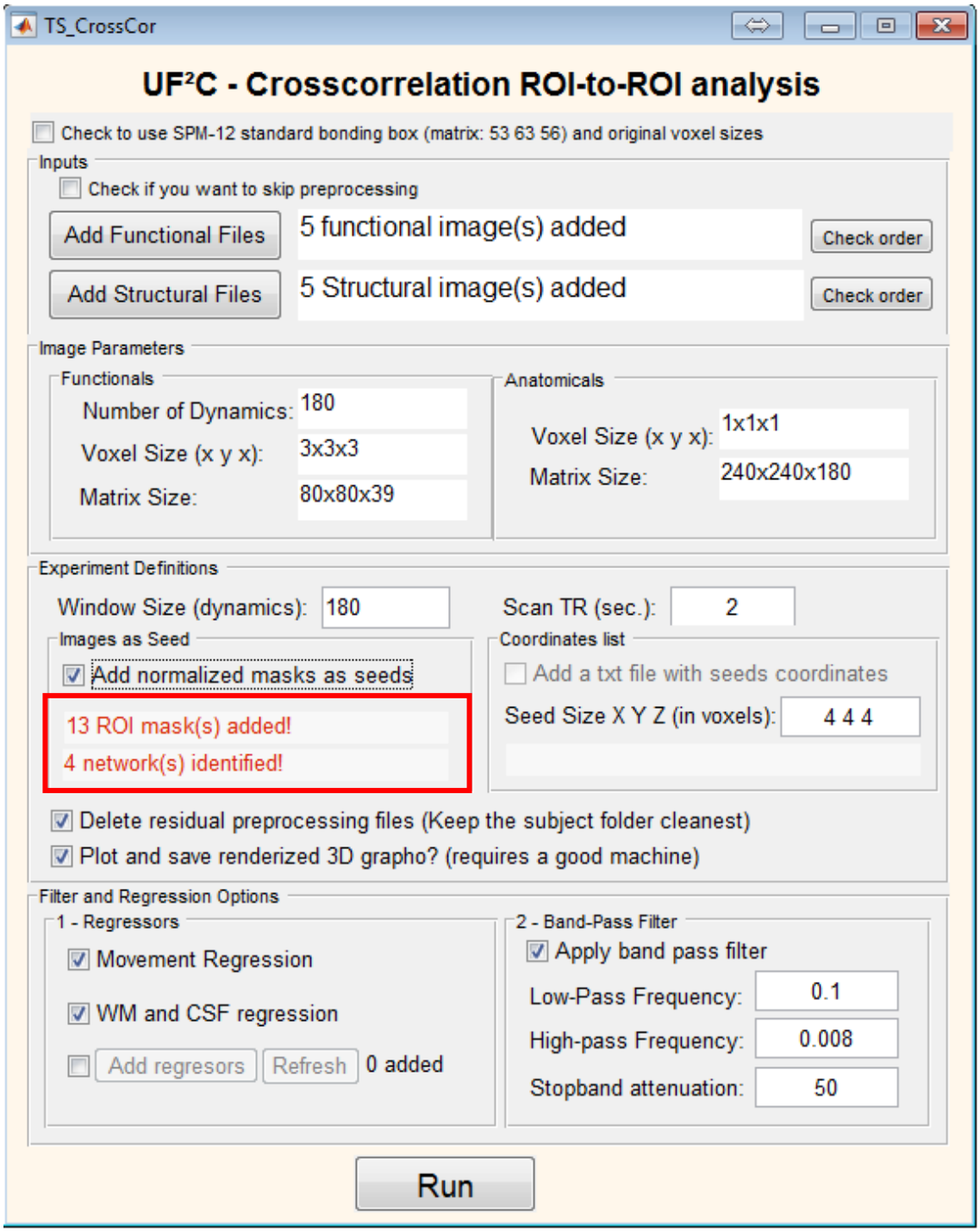
Option 1 – "**Add normalized masks as seeds**": By checking this option, an input window will open, and you will be able to add ROIs as binary NIfTI images. In this option, **UF²C** will identify the network organization among the files added, **using the first three filenames** characters:



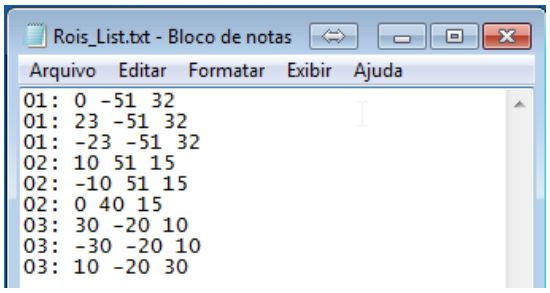
Considering these ROIs files, **UF²C** will identify 13 ROIs from 4 networks:

Network 1: Auditory (**Aud**); Network 2: basal Ganglia (**Bas**); Network 3 dorsalDMN (**dor**); Network 4: PostSalience (**Pos**).

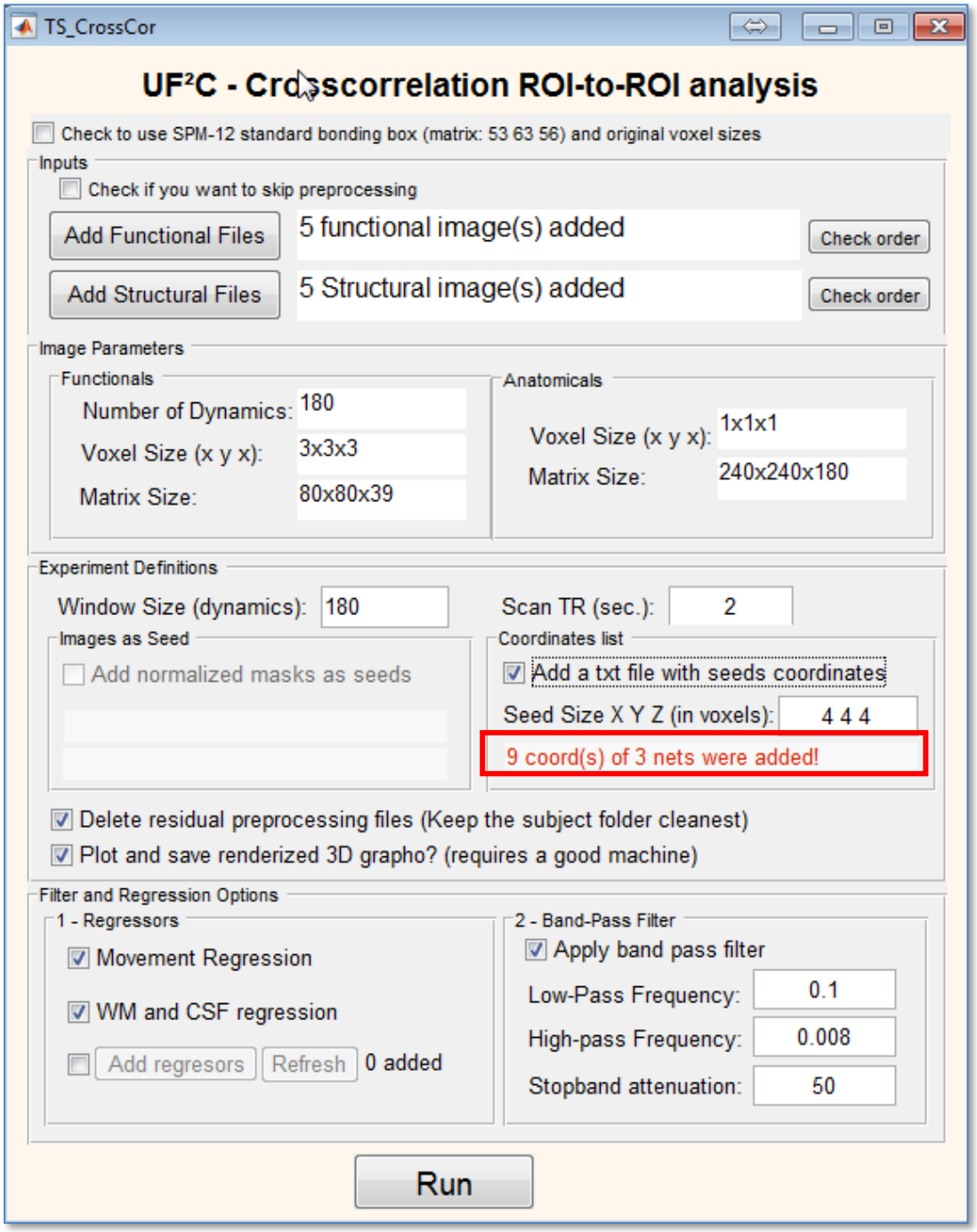
The **UF²C** GUI will show:



Option 2 - "**Add a txt file with seeds coordinates**": In this option, you will be able to add a text file (*.txt) with MNI coordinates of where you want the seeds. You will also be able to use networks distinction here. For this, you will need to format the text file as follows:



Considering this coordinate file, **UF²C** will identify 9 ROIs from 3 networks → Network 1: **01:**; Network 2: **02:**; Network 3: **03:**. The **UF²C** GUI will show:



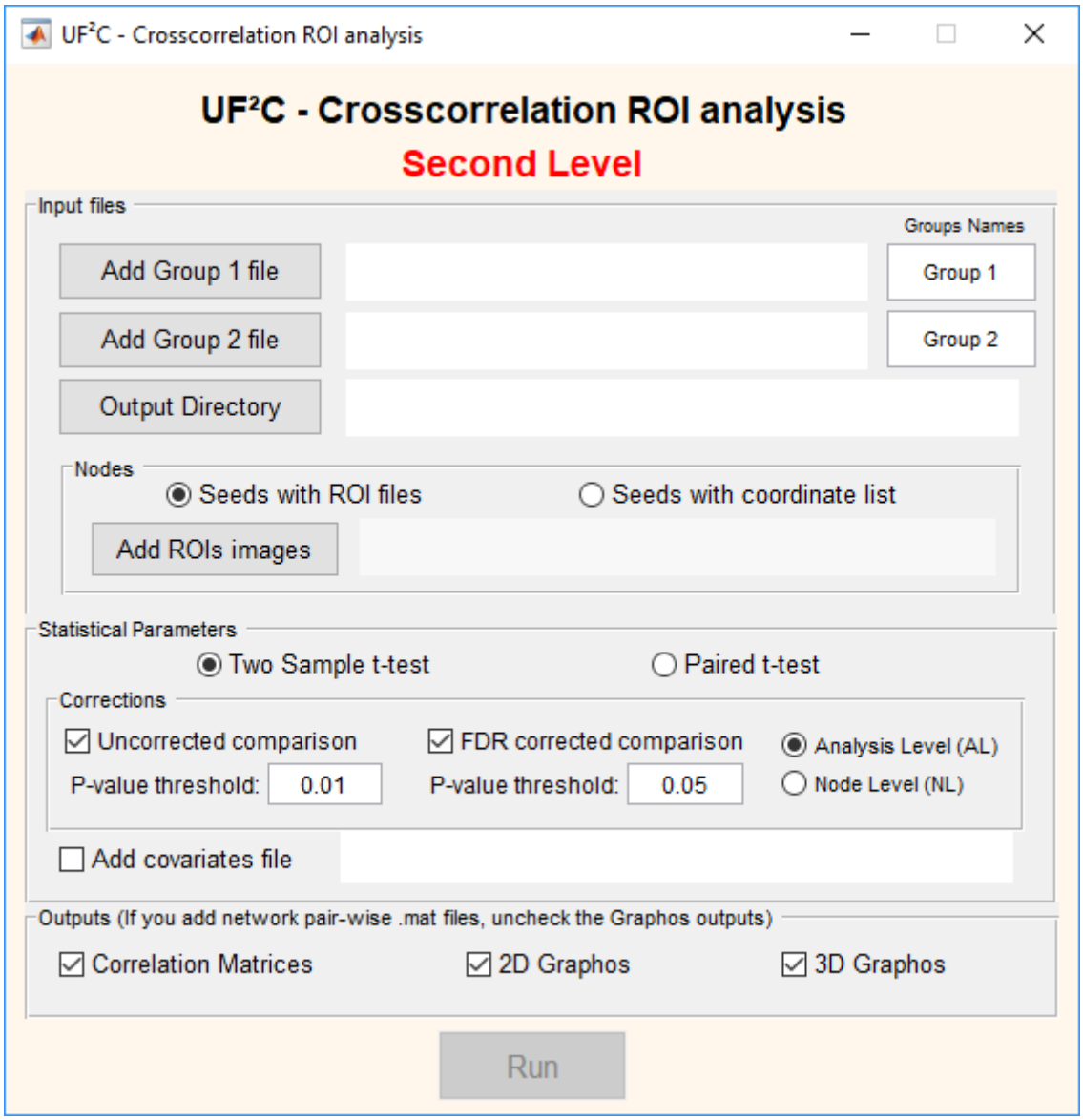
The checkbox "**Delete residual pre-processing files**" gives you the option to save all the processing files or just the final version of each post-processed file. By checking this box, the program will just save:

- The realignment parameters (rp_*.txt file)
- The modulated-normalized-smoothed functional file (sw*.nii file)
- The regressed-filtered functional file (FiltRegrSW*.nii)
- The modulated-normalized structural file (wm*.nii file).

Filter and Regression Option

The same described on the “Pre-processing Only” routine (on page 32 and on page 30).

b) Second Level analysis (Group inference)



This modality enables the analysis of group inference with the results of the first level analysis (**a**).

To do this, some **conditions** should be respected:

1. In the first level analysis (a), you need to add all images (fMRIS and T1s) of a group (control group for example) at the same time (running process). This procedure will create a general (group) resultant file called "**All_Subjs-VAR.mat**" inside the general folder "**Total_Log_*_*DATE/TIME*_***". This Matlab file will contain a **NxNxS matrix**, where **N** is the number of ROIs added in the first level analysis and **S** the number of subjects included in this group. **You need to have the All_Subjs-VAR.mat of at least**

two groups to perform the second level analysis, and these All_Subjs-VAR.mat needs to contain the information of all subjects of each group.

2. You need to use the same number of ROIs for all groups in the first level analysis to be able to compute the group comparison between then. So, in the **NxNxS matrix, the N should to be the same in both files (same number of ROIs) although the S (number of subjects) may differ between groups.**

3. Before adding the **All_Subjs-VAR.mat** to the second level analysis modality, we strongly recommend converting the **All_Subjs-VAR.mat** from r-score to z-score. You can use the **UF²C** tool **R-score to Z-score Transf**. to do that, creating the **Z_Transf_All_Subjs-VAR.mat file** that is ready to be added.

Input Files

In this panel, you can add group 1 and group 2 **Z_Transf_All_Subjs-VAR.mat** files clicking on the buttons **"Add Group 1 file" and "Add Group 2 file".** In the text box from the field **"Groups Names"** you can change the name of the groups, modifying how they will be shown in the results.

In the panel "**Nodes**", you should add the same ROIs files ("**Seeds with ROI files**") or the same text file ("**Seeds with coordinate list**") that you used on the first level analysis, respecting the condition **2.**

Statistical Parameters

In this panel, you can first choose if your test is transversal ("**two Samples t-test"**) or longitudinal ("**Paired t-test"**). In the sub panel corrections, you will be able to set the alpha levels ("**P-value threshold**") of your tests, choosing between "**Uncorrected comparisons"**, "**FDR corrected comparison**" or both.

To correct for Bonferroni, you just need to compute the number of multiple comparisons applied and change the uncorrected alpha ("**P-value threshold**").

How to compute the number of dependent tests?

$$\mathrm{NofTests} = \frac{((\mathrm{N} \times \mathrm{N}) - \mathrm{N})}{2}$$

Where **N** is the number of ROIs used. So, if you used 100 ROIs, the number of multiple tests would be:

$$\text{NofTests} = \frac{((100 \times 100) - 100)}{2}$$

$$\text{NofTests} = \frac{(10000 - 100)}{2}$$

$$\text{NofTests} = \frac{(9900)}{2}$$

$$\text{NofTests} = 4950$$

The alpha to correct by the Bonferroni methodology would be:

$$\text{BonferroniAlpha} = \frac{0.05}{4950} = 1{,}01 \times 10^{-5} \ (or\ 0{,}0000101)$$

Very restrictive!

Add a covariate file

If you selected the "**two Samples t-test**", you will be able to add covariates to the analysis, converting the statistical approach to an ANCOVA. To do this, you need to create a text file (*.txt) with columns that represent the covariates, and lines that represent the volunteers:

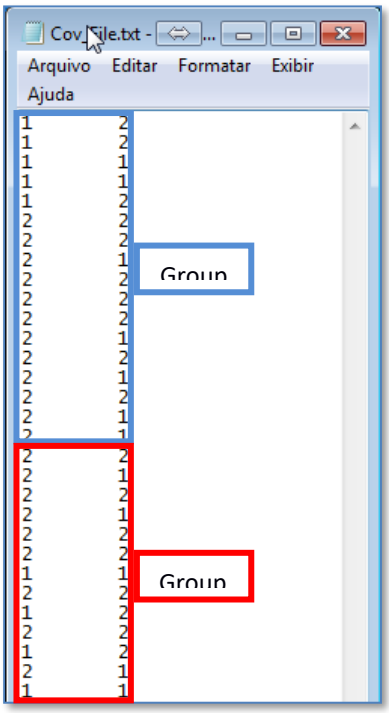
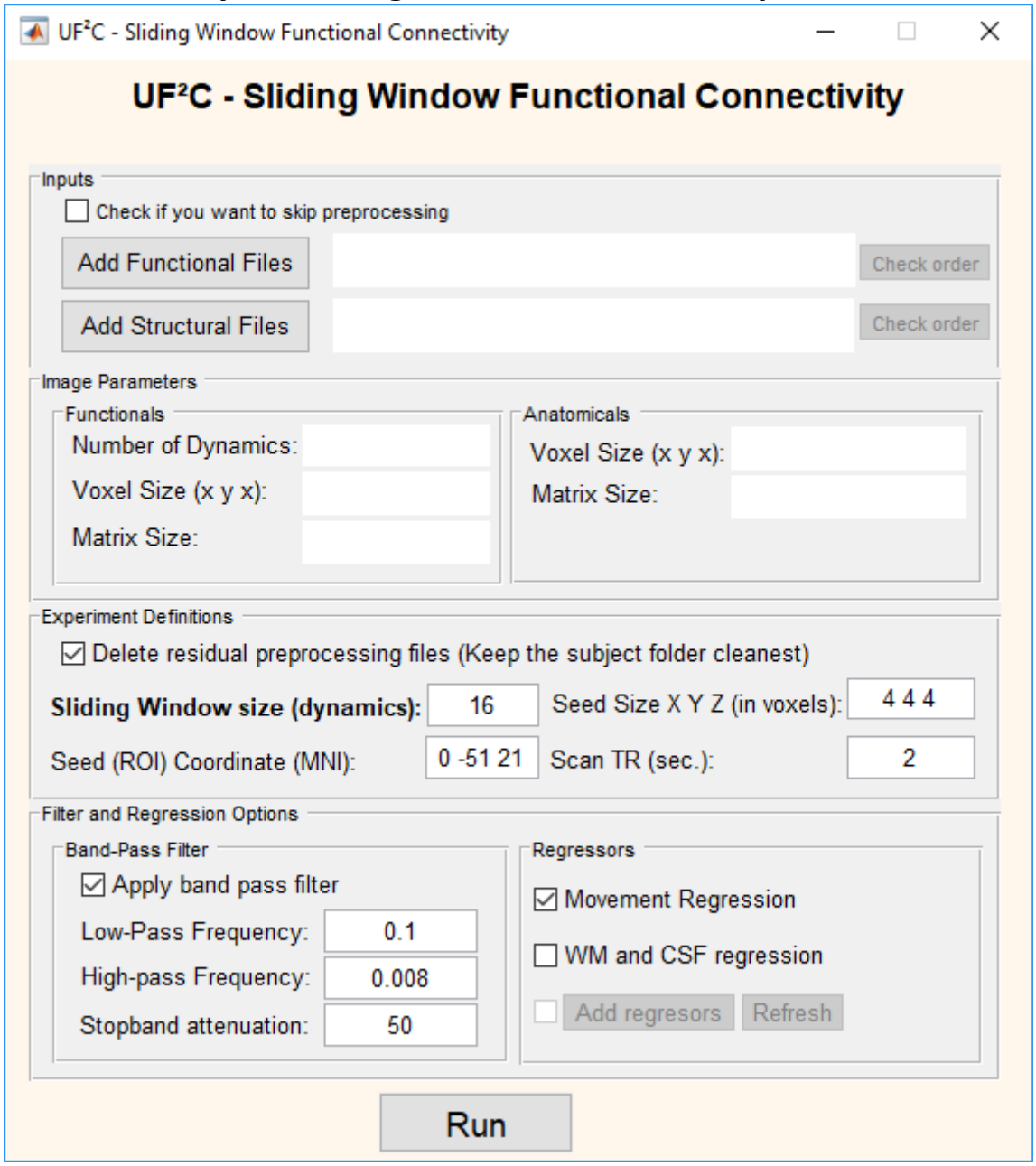


Figure: This figure represents a text file with 2 covariates (2 columns). In this example, we can see 30 rows divided into the two groups.

IMPORTANT: The order of the covariates should be the same as the order of each group added (Group 1 and 2), as well as the subject order inside each group. This is similar to the covariate option on the SPM interface.

15. Modality 3 – Sliding Windows Connectivity



Inputs

The same described on the **Pre-processing Routine**.

Image Parameters

The same described on the **Pre-processing Routine**.

Experiment Definitions

The options "**Delete residual pre-processing files**" and "**Window Size (Dynamics)**" have the same functions as described on the **modality 1 section**.

The sliding window analysis performs a correlation between a seed defined by a coordinate ("**Seed (ROI) Coordinate (MNI)**") and all cortical voxels. These correlations are calculated by each window separately. The windows sizes are defined in the "**Sliding Window size (dynamics)**" option. The final number of correlations are found by dividing the total number of volumes (dynamics, time points….) by the size of the time window (previously defined by you). **UF²C** will generate graphics and a 4D image for each volunteer, in which the 4th dimension is the 3D correlation map of each window.

The correlation window moves throughout the time series with a step of 1 time point.

The "**Seed Size**" is implemented equal to what is described in **Modality 1.**

Filter and Regression Option

The same described on the "Pre-processing Only" routine (on page 32 and on page 30).

16. Some considerations about “time series extraction”

Time-series are consistently extracted from each ROI of each subject. For a specific ROI, we used the average time series of all ROI voxels that matched two consecutive criteria:

a. Being included in the subject GM mask;

b. The UF2C correlates each single ROI voxel time series with the average ROI time series (GM-masked). The voxel will be included on the ROI mask (and to the average) if its correlation value is higher than the average minus the standard deviation of all correlations between the ROI-masked voxels;

This methodology was described on “**de Campos, B. M., Coan, A. C., Lin Yasuda, C., Casseb, R. F. and Cendes, F. (2016), Large-scale brain networks are distinctly affected in right and left mesial temporal lobe epilepsy. Hum. Brain Mapp., 37: 3137–3152”.**

An example of the effectiveness of this methodology in excluded residual non-GM voxels or non-functionally representative tissues can be seen in the following example: We performed an analysis using a **left-hippocampus** ROI, from the Shirer ROIs (**Shirer WR, Ryali S, Rykhlevskaia E, Menon V, Greicius MD (2012): Decoding subject-driven cognitive states with wholebrain connectivity patterns. Cereb Cortex. 22(1):158–165**). The subject included was a temporal lobe epilepsy patient, with left-hippocampus sclerosis. The hippocampus sclerosis may result in GM atrophy and gliosis:

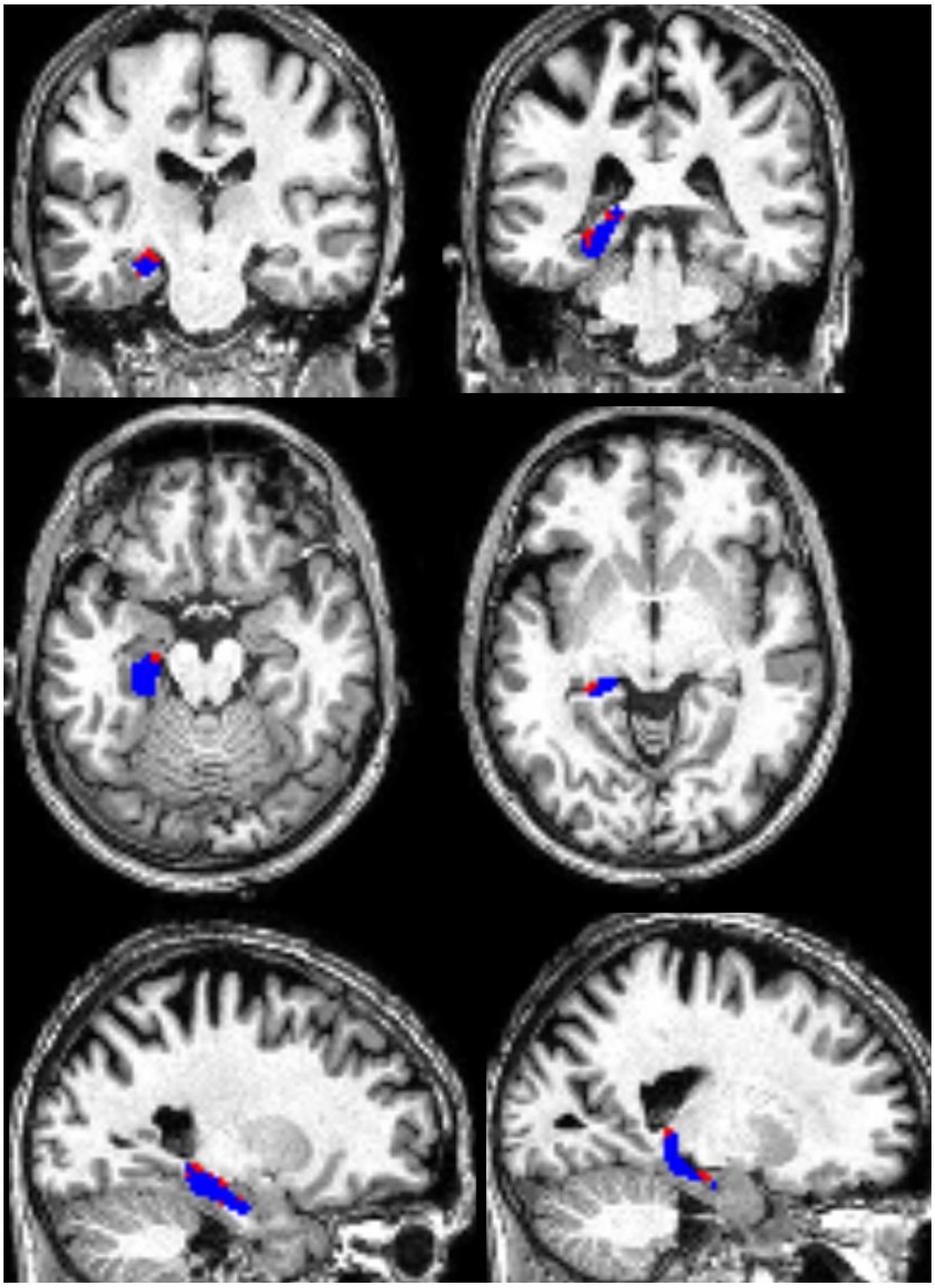
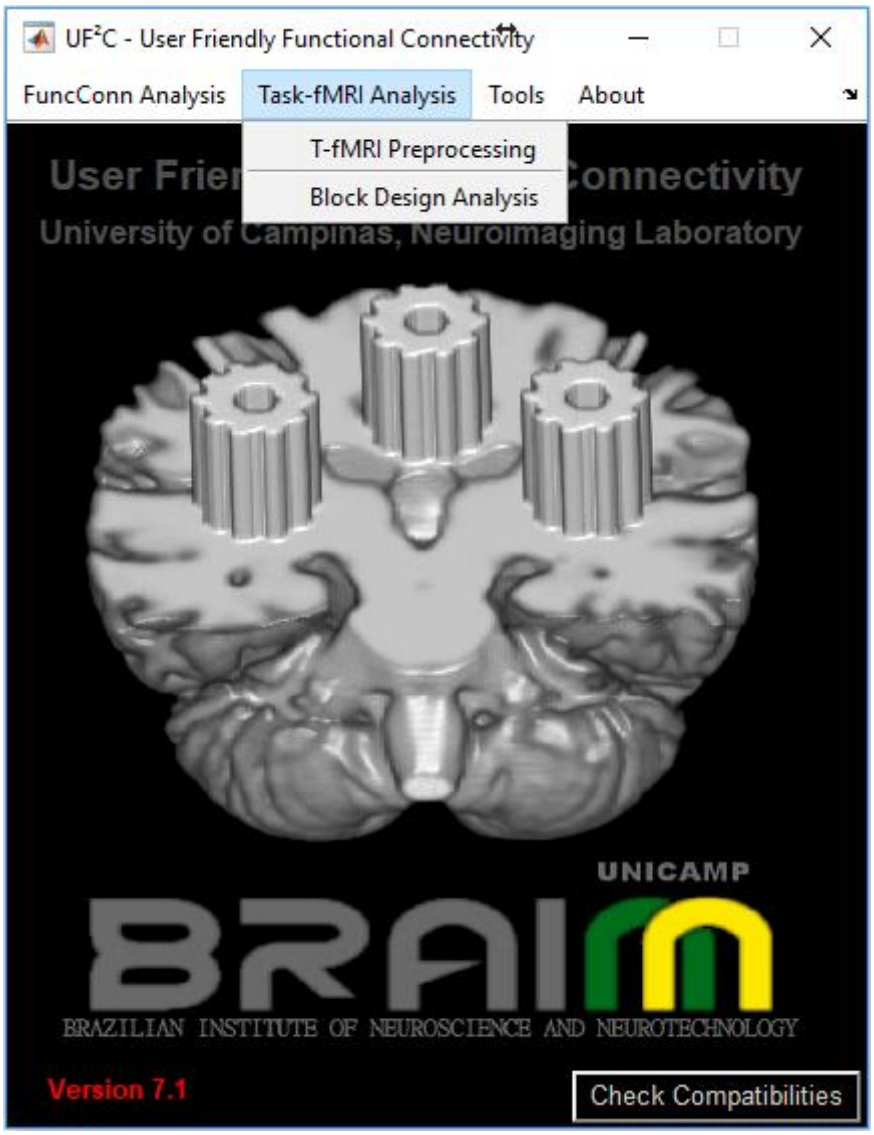


Figure: The ROI regions (shown in blue) that were included in the analysis and the voxels (shown in red) from the ROI (grey matter masked [criteria a.]) that was automatically excluded (only border voxels). The red voxels were excluded AFTER surviving to the GM masking (criteria a.), through the criteria b.
This result shows the effectiveness of the combined criteria a. and b. in providing a refined ROI mask and consequently a reliable ROI average time series.

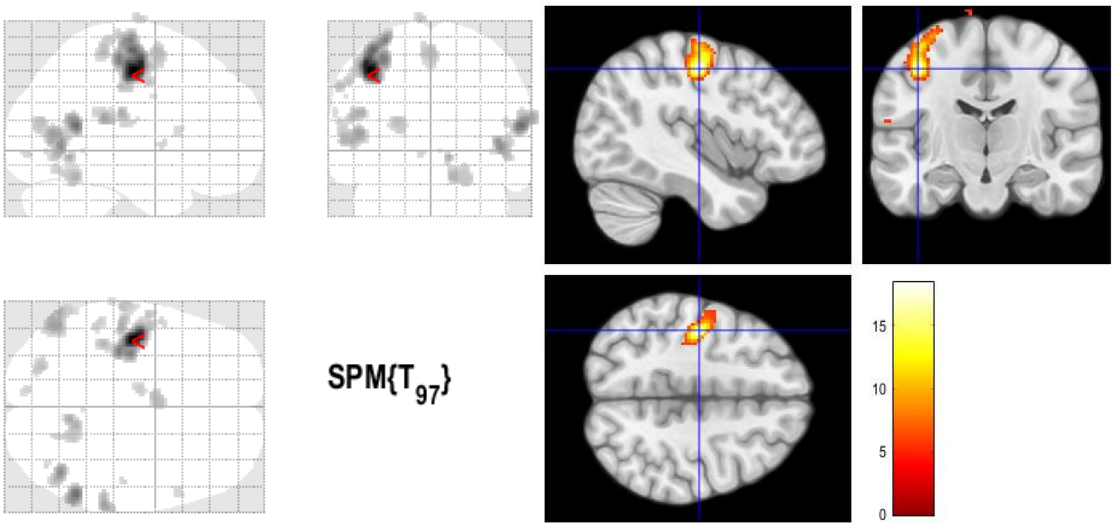
17. Task-fMRI Analysis



Analysis

Brief Introduction

Task fMRI is widely used nowadays to help researchers identify regions of the brain that are “active” when the participant is required to engage in a mental or physical task. For instance, you could ask the subject to think about words that start with the letter F, or to perform a finger-tapping movement. After the images are analysed, we can identify blobs of activation related to the task (like the ones below), which were obtained while the person performed a finger-tapping movement with the right hand.



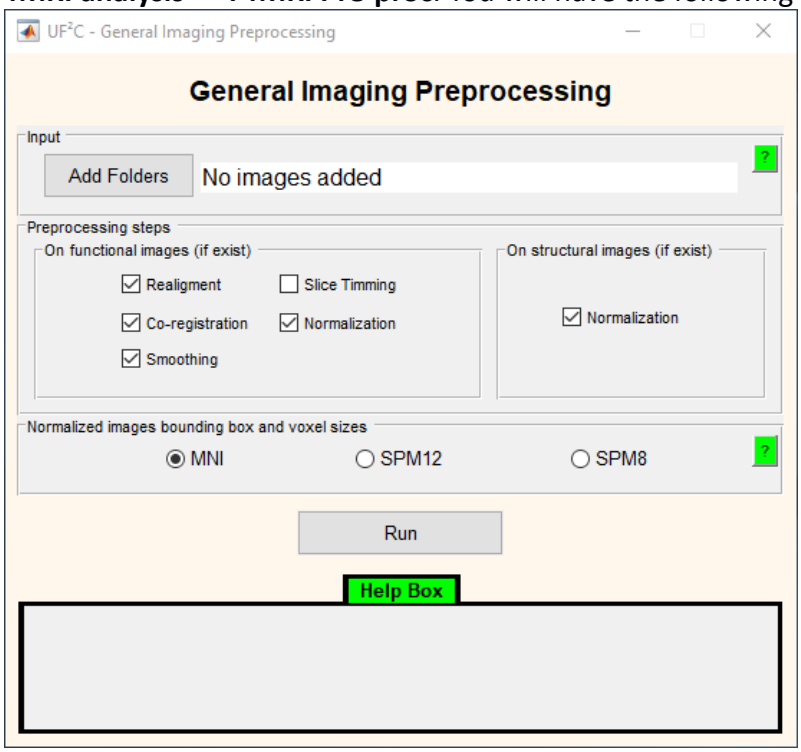
Several tasks may be investigated (motor, visual, auditory, cognitive) and have indeed been used to evaluate neural activation related to the task performance.

Instead of activation maps, however, it is more appropriate to say *statistical parametric maps*, since we have an indirect measure of activation derived from a very elaborated statistical procedure.

In this tutorial we will guide you through the analysis implemented in UF^{2}C, that covers only block designs (there is no module for event related or mixed (event + block) designs yet).

a) Pre-processing pipeline

Click on **Task-fMRI analysis -> T-fMRI Pre-proc**. You will have the following window:



Input Panel

Click on "**Add Folders**" to load the directories containing either:

b) 1 structural (3D) and 1 (or more) functional (4D) image(s); or

c) No structural (3D) and 1 (or more) functional (4D) image(s).

When there is more than one functional image, the software will consider it as a multisession experiment.

Pre-processing steps Panel

We normally set up the default options as the ones we think to be the most appropriate for the general case, but you have the final call. In this version, slice timing correction will be left unchecked[2].

[2] Slice timing correction seems to be more helpful for small blocks (~10s) and long TRs and is definitely necessary if you have event-related designs. As block designs are normally longer than 10 s, we have chosen to leave it unchecked. For more details, see http://andysbrainblog.blogspot.com.br/2012/11/slice-timing-correction-in-spm.html and Sladky, Ronald et al. "Slice-Timing Effects and Their Correction in Functional MRI." Neuroimage 58.2-2 (2011): 588–594. PMC. Web. 18 July 2017.

Normalized images bounding box and voxel sizes Panel

This option allows the choice of the voxel size and the amount of empty space around the brain, when the images are normalized. It is used for functional and structural images. The larger the bounding box, the larger the amount of empty space. The options are:

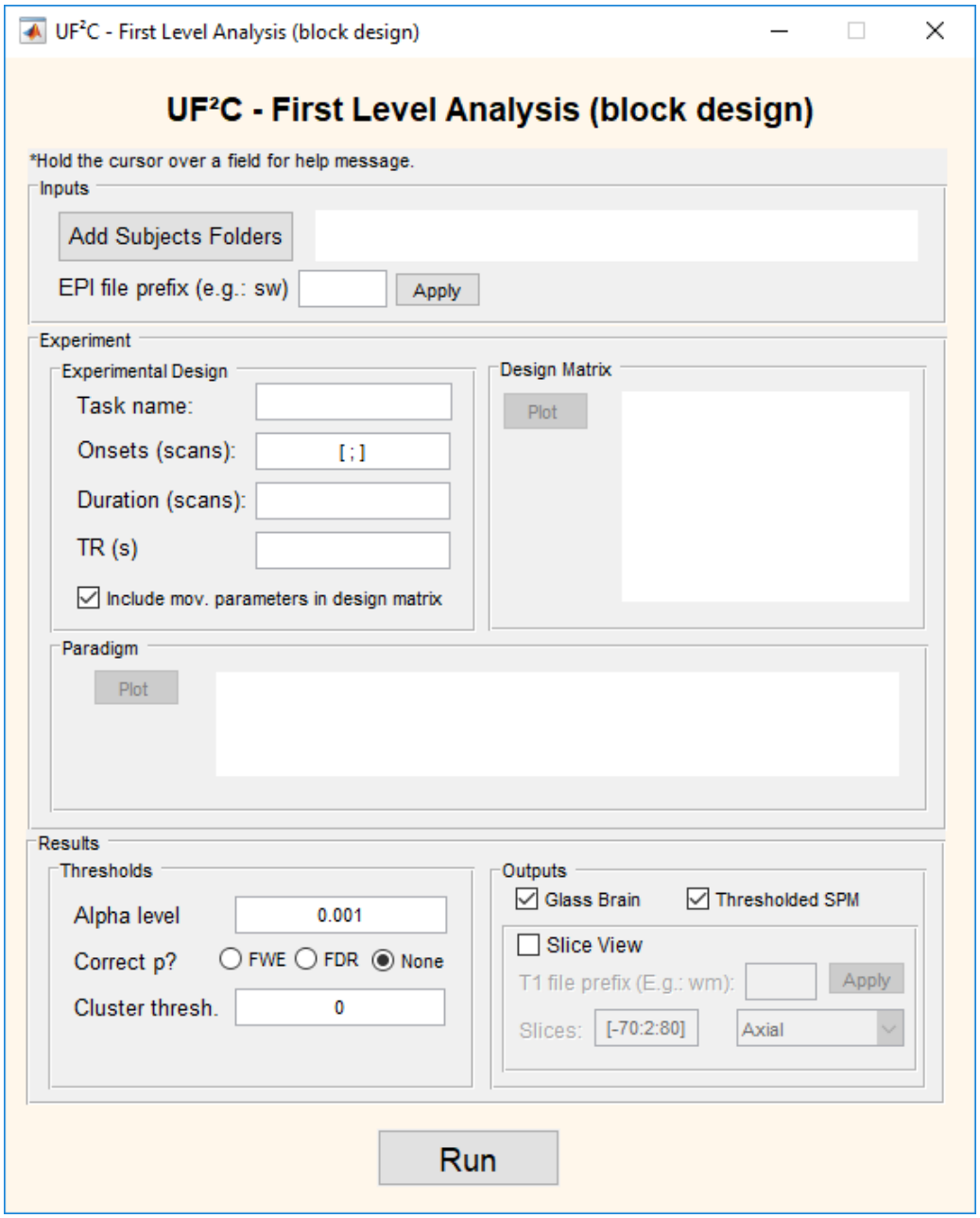
- MNI: yields normalized images with a matrix of 91x109x91 voxels, and voxel size of 2x2x2 mm^3.
- SPM12: yields normalized images with a matrix of 53x63x52 voxels and 157 189 156 for the functional and the structural images, respectively. Voxel sizes will not change.
- SPM8: yields normalized images with a matrix of 53x63x46 voxels and 157 189 136 for the functional and the structural images, respectively. Voxel sizes will not change.

The default option is “MNI”, since most studies are performed this way.

NOTE: any option will result in images being normalized to the MNI template. The only differences are the resolution and the amount of background of the resulting images.

b) Task-related activation analysis

Click on **Task-fMRI analysis -> Block Design Analysis**. You will have the following window:



Inputs Panel

Click on "**Add Subjects Folders"** and choose folders of the pre-processed images. Make sure they were pre-processed according to the steps from last session, **17a**, and not

from session **10** or **11**[3]. You can also pre-process them elsewhere and just plug them here.

Fill the box "**EPI file prefix (eg: sw)**" with the prefix of the pre-processed file. In case you used the default pre-processing steps, the final pre-processed file will start with *sw*. If you checked "**Slice Timing**", then it will start with *swa*. If you pre-processed them elsewhere, just be sure to fill this box appropriately.

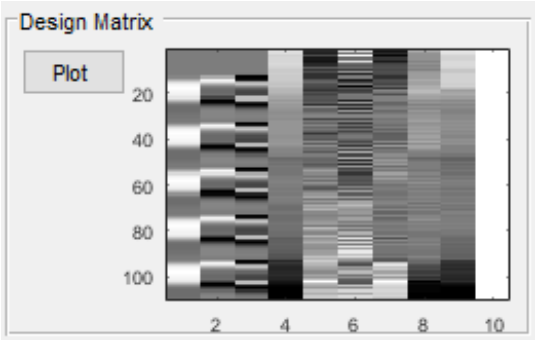
By clicking the "**Apply**" button, UF²C will check if all the added folders do have a sw*.nii (or whatever_prefix*.nii) file inside.

Experiment Panel

In the **Experiment Design** box, set:

- "**Task name**" (e.g.: Finger Tapping)
- "**Onsets**": the instants in which each task-block started (note that **scan**[4] is the temporal unit used, not seconds). You must input a nx1 vector. E.g.: [11;31;51;71;91]
- "**Duration**" of the task-blocks (also in **scan** units): in case all the blocks have the same duration, use a single scalar number (e.g.: 9). In case each block has a different duration use a nx1 vector to define durations (e.g.: [10;9;5;10;5])
- "**TR**" (repetition time): in seconds
- The box "**Include mov. parameters in design matrix**" is self-explanatory. The default is to use the movement parameters in the analysis (box checked).

After setting the options above, you will be able to plot the design matrix (figure), in the **Design Matrix** box, on the right of the **Experiment** panel. Just click the "**Plot**" button. If you change the **Experiment**



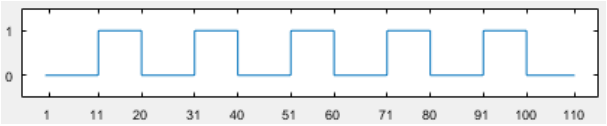
[3] Recall that sessions 10 and 11 describe the pre-processing procedure for resting-state images, which is divergent from the one for task-images.

[4] Each scan has the duration of one TR (repetition time). If your TR=2s, then each scan represents 2 seconds. Hence, if your first task-block started at the second 21, it is equivalent to say that it started at scan 11.

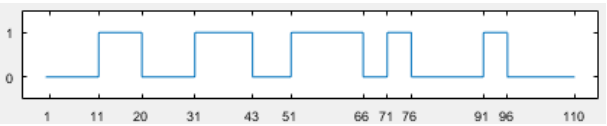
options (Onsets, Duration, TR, Include mov. parameters), click "**Plot**" again to update the design matrix.

The **Paradigm** box allows you to visualize task and rest blocks. Baseline represents the resting intervals, while the plateaus represent the periods of task. See paradigm plots below.

Blocks with same duration



Blocks with different durations



Results Panel

These options allow you to do simple activation analysis by contrasting task and rest conditions. UF²C automatically outputs statistical parametric maps of "activation" (Task>Rest) and "deactivation" (Task<Rest), based on your choices of statistical thresholds. You can also save figures illustrating results, like a "glass brain" and a panel of slices.

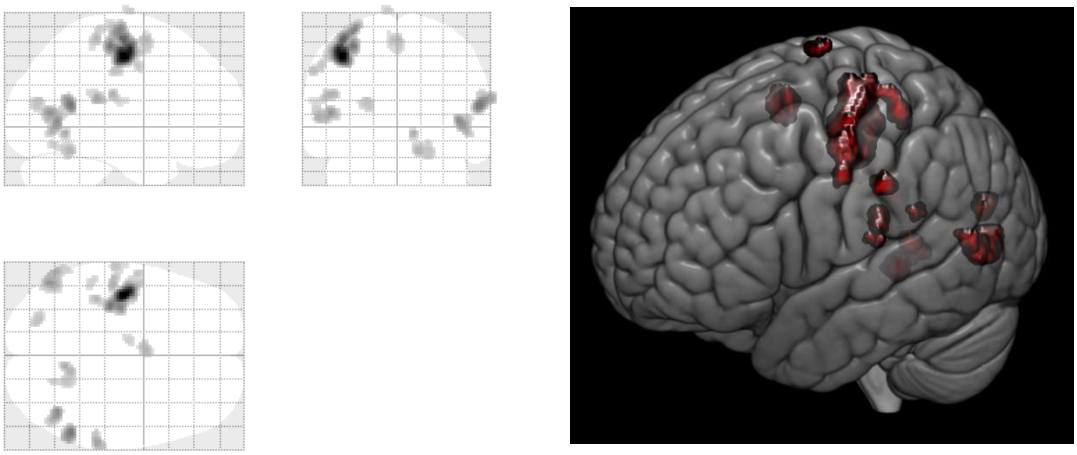
In the **Thresholds** box you are required to set:

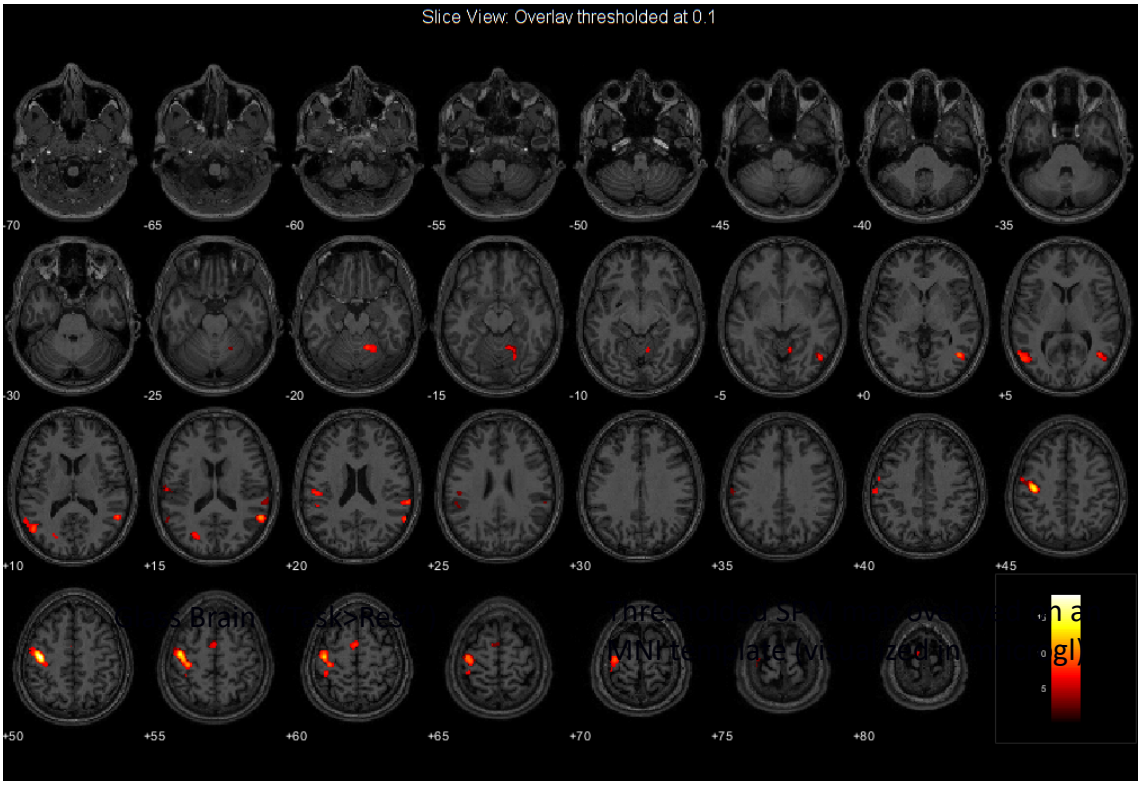
- "**Alpha level**": the significance level of your results (e.g.: 0.001 or 0.05).
- "**Correct p?**": defines whether you want to apply correction for multiple comparisons ("**FWE**" or "**FDR**") or not ("**None**"). Note that when you choose an option, the most common alpha level used with that option is suggested in the "**Alpha level**" space, but you can still change this value.
- "**Cluster thresh.**": defines the minimum amount of voxels that a cluster must contain to be considered in the results.

In the **Outputs** box, check whether you want to save a "**Glass Brain**" (figure below), an "**SPM threshold**" map (using the thresholds defined in the **Thresholds** box – figure below), and also a panel of slices ("**Slice View**"). You can check more than one. Only the glass brain of "Task>Rest" contrast is saved; "Rest>Task" is not.

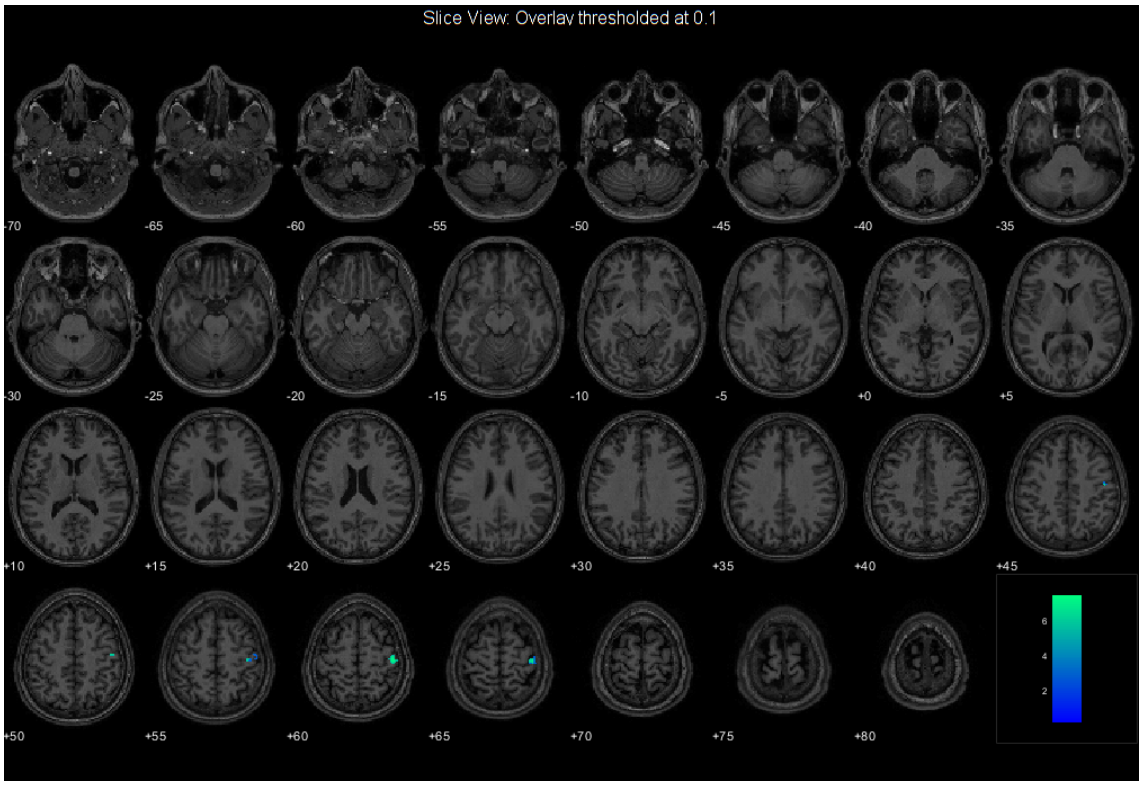
For the "**Slice View**" (figure below) you must inform the prefix of the anatomical normalized image ("**T1 file prefix (e.g.: wm)**"), which will be used as the underlay image in the slice panel. You must also specify the slices you wish to be shown

("**Slices**"), by informing the z coordinate (in mm) of: the bottom slice, the interval between slices and the top slice; e.g.: [-70:2:80], which means: z_{bottom} = -70 mm; z_{step} = 2 mm; z_{top} = 80 mm. You can also choose, in the drop-down menu, which plane you want ("**Axial**", "**Sagital**", "**Coronal**").





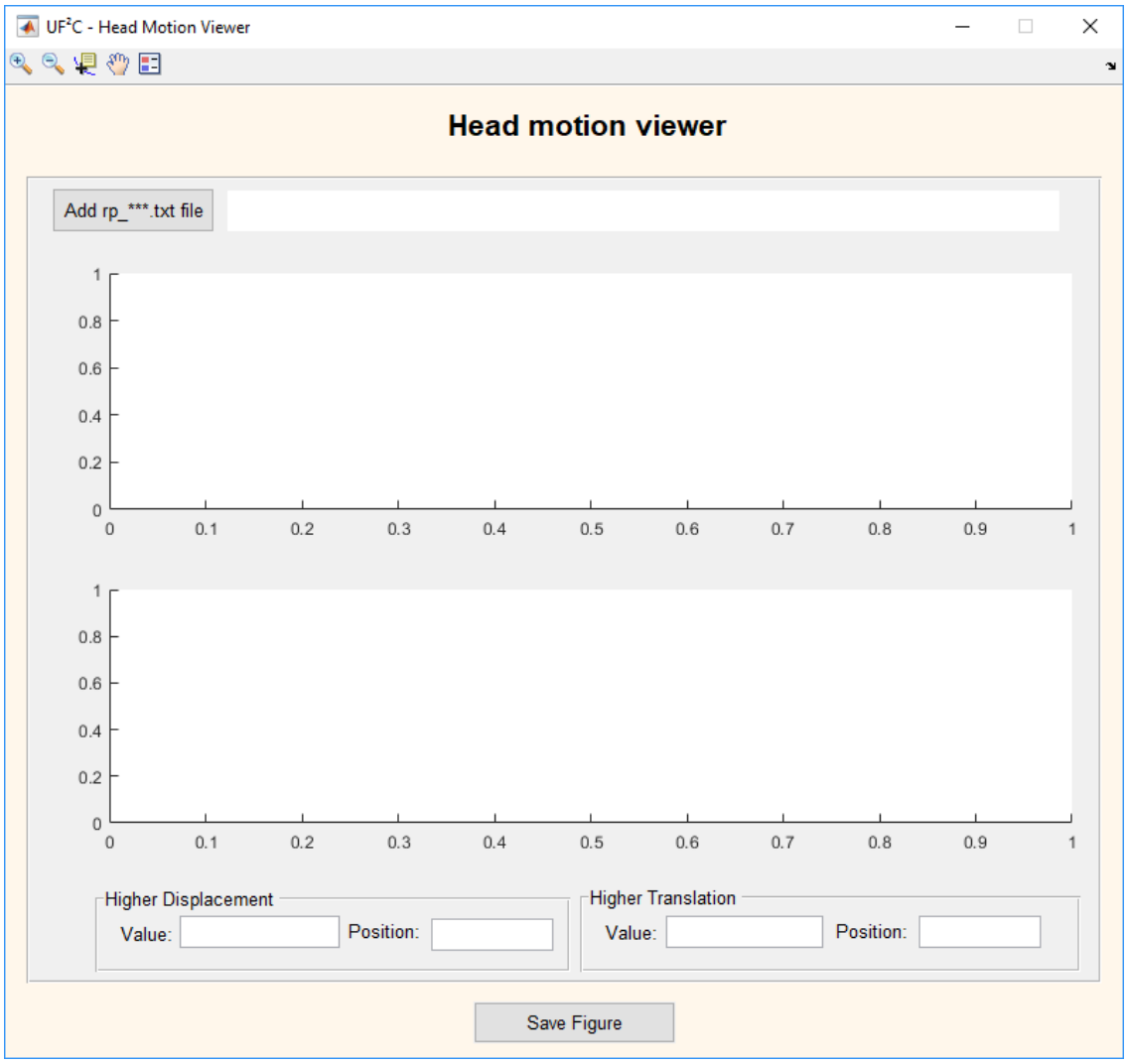
Slice View ("Task>Rest")



Slice View (“Rest>Task”)

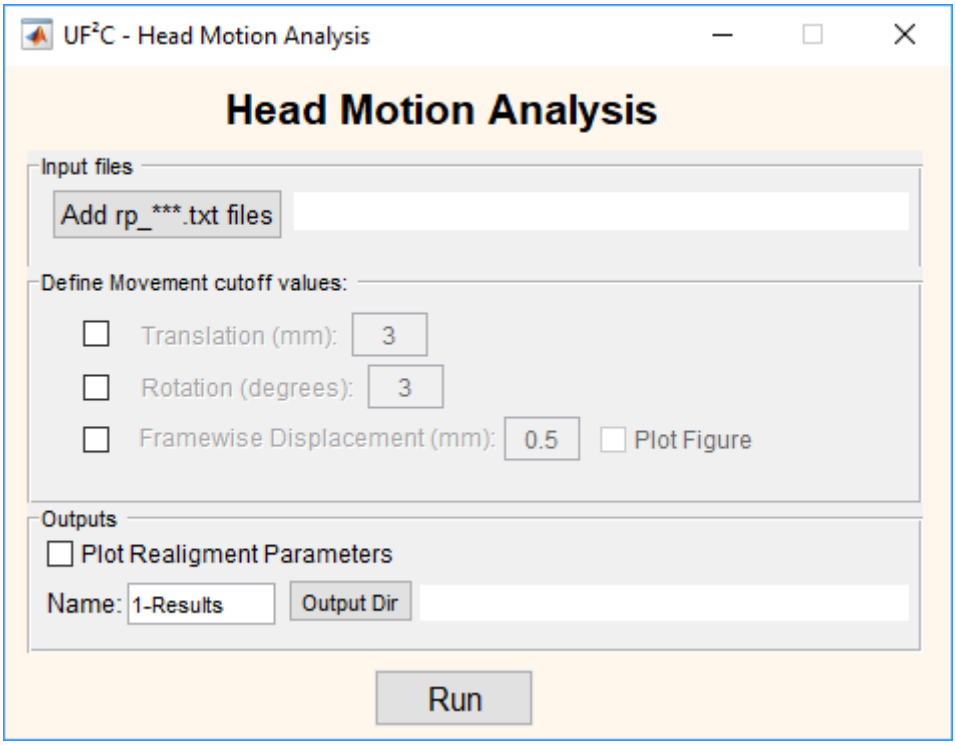
18. Tools

a) Movement Viewer



The **Movement Viewer** tool enable to check the graphic of movement generated by the realignment procedure on SPM. You only need to add the **rp_***.txt** file using the button "**Add rp_***.txt file**". The tool will plot the movement according to the realignment and will also show the time points and the values of higher displacement and higher translation.

b) Movement Analysis



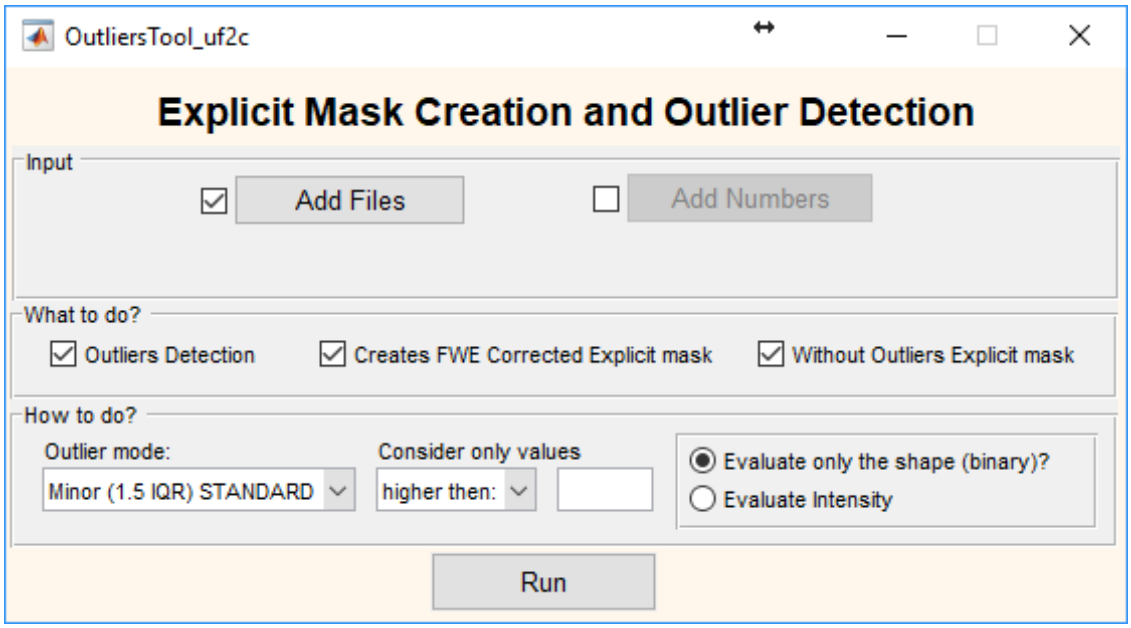
The **Movement Analysis** tool performs several quantifications using the **rp_***.txt** file generated by the realignment procedure on SPM. You can add one or several files. Additionally, you can define cut-off values for Translation (in mm), Rotation (degrees) and Framewise Displacement (mm) (on page 29).

In this tool you can also plot and save all individual plots by checking the option “Plot realignment Parameters”.

You should define the output directory where the tool will save a text file with all individual quantifications and the excluded cases (due the cut-off defined). UF²C text files ate tabulated and are always better visualised using an Excel® type software and “Import File” function.

c) Explicit Mask Creation and Outlier Detection

This tool has two purposes: 1 – to detect outliers in several types of files and 2 – to create an Explicit mask, based on the One-Sample T-test of the images added. This Explicit mask could be included in the Second Level analysis in SPM, aiming to restrict the statistical analysis only to consistent regions among subjects, reducing the multiple comparisons by the exclusion of noisy or inconsistent voxels.

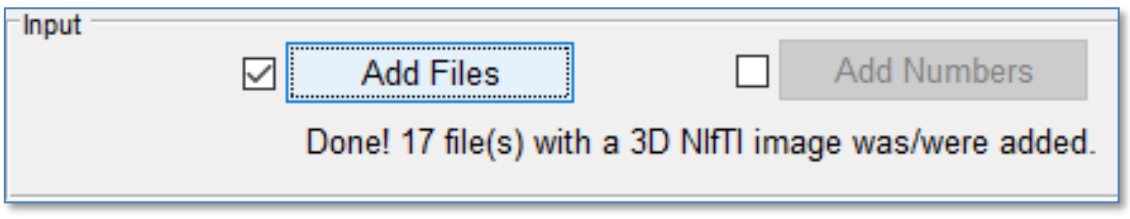


The Input panel has two options: Files or Numbers.

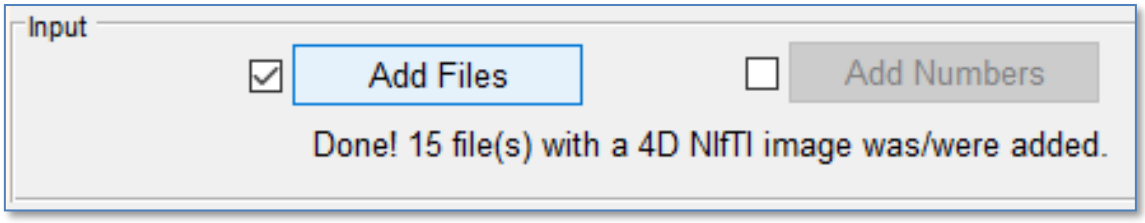
Checking and clicking in the option "**Add Numbers**", and input dialog window will appear, and you will be asked to add numerical values to simple compute the outliers.

Checking and clicking in the option "**Add Files**" you will be able to add NIfTI (*.nii) or MATLAB (*.mat) files. The NIfTI files can be 3D or 4D images of any nature:

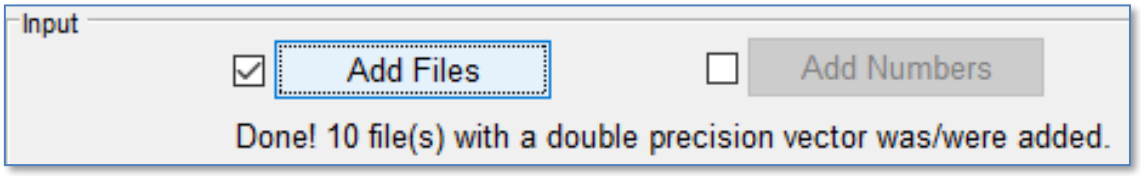
- If you add several 3D NIfTI files, the tool will perform the outlier detection comparing ALL images voxel-wisely.

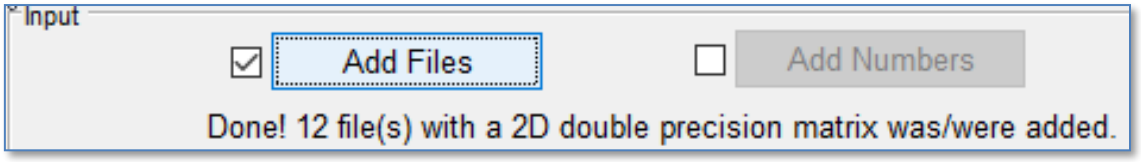


- If you add several 4D NIfTI files, the tool will compute the median volume among the 4th dimension (voxel-by-voxel) for each file, and then the outlier detection will be done comparing ALL median 3D volumes voxel-wisely.



- You can add *.mat files containing double precision variable inside. These variables can be vector, 2D matrix, 3D matrix or even 4D matrix. If you add several files of same nature, the tool will compute the outlier detection for each point separately. If you add *.mat file containing 4D matrix, the tool will do the same described for 4D NIfTI files, calculating the median 3D matrix and then the outlier detection.



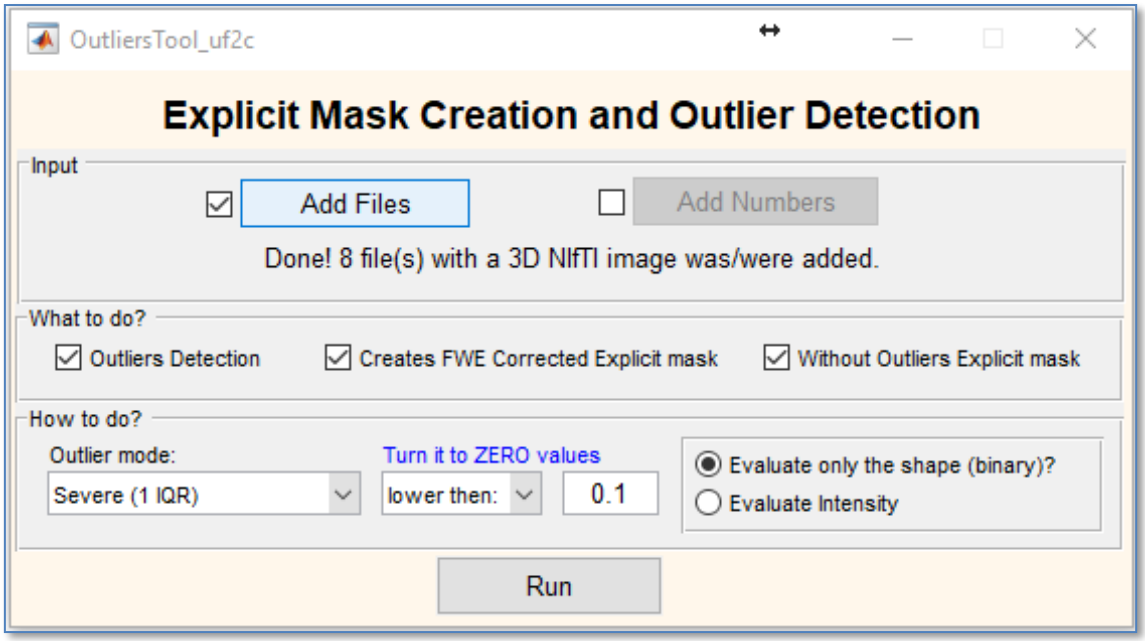


- If you add only one file (regardless of its nature), the tool will compute the INTRA-file outlier. If this singular file has 4 dimensions, the tool will also calculate the median 3D volume for them, and then calculate the INTRA-file outliers.

Panel "What to do?" and "How to do?":

After adding the files, you will have some options to define, which can vary according to the type and number of files added.

- **Case 1: Adding more than one NIfTI file.**

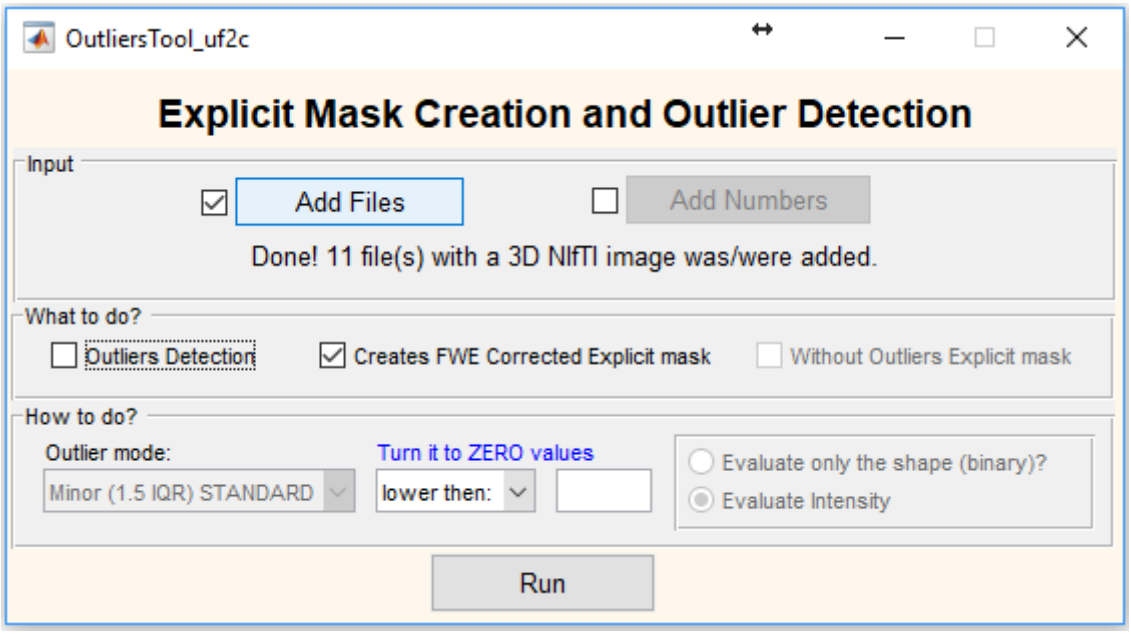


If you add some NIfTI files (3D or 4D) you will have the option to calculate INTER-files outliers, by checking the option "**Outlier Detection**" on the "**What to do?**" panel. Also, you will be able to create an explicit mask based on the One-Sample T-test FWE-corrected statistical map, performed with all input images (option "**Creates FWE Corrected Explicit mask**" on the "**What to do?**"). If the option "**Outlier Detection**" is checked, you will be able to compute also the same explicit mask, but automatically excluding Outlier images, if they exist. Just check the option "**Without Outliers Explicit mask**" on the "**What to do?**". This mask is also based on the One-Sample T-test FWE-corrected statistical map.

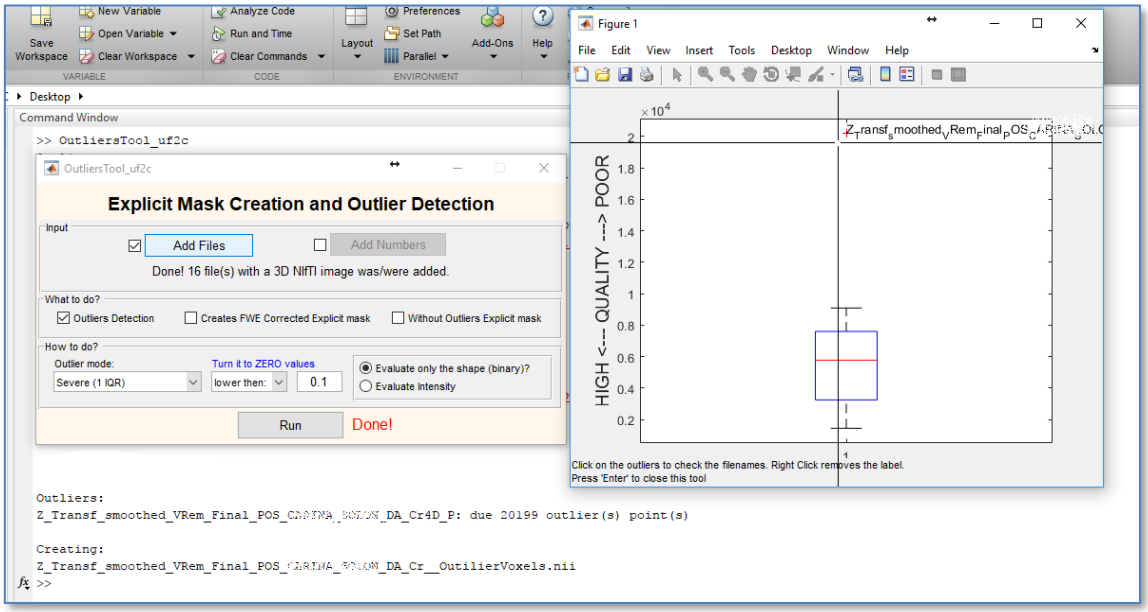
By checking the "**Outlier Detection**" option, you will need to choose the outlier detection severity on the "**Outlier Mode**" menu. The options are: "**Severe**" which consider as outlier all values out of the interquartile range; "**Minor**", the statistics standard, which consider as outlier all values out of 1.5 times the interquartile range; "**Major**" which consider as outlier only values out of 3 times the interquartile range.

Adding some NIfTI files (3D or 4D) will can also define a threshold, turning values under this threshold to zero. To do this, use the option "**Turn it to ZERO, values lower then:**". If you leave it empty, no thresholds will be applied. Finally, you have the option to evaluate outliers only considering the shape, '*binarizing*' the images (all values distinct from zero will be ones) or evaluate outliers considering the image intensities. Note that usually the intensity option is also sensible to shape alterations.

If you uncheck the option "**Outlier Detection**", then several options will be disabled, and you be able to only select "**Creates FWE Corrected Explicit mask**", defining or not a threshold.



In this case, the output will be the outlier file printed in Matlab command window, showing the filename and the number of outlier voxels. An outlier mask will be created in the file folder, with the same filename and suffix **"***__OutlierVoxels.nii**".

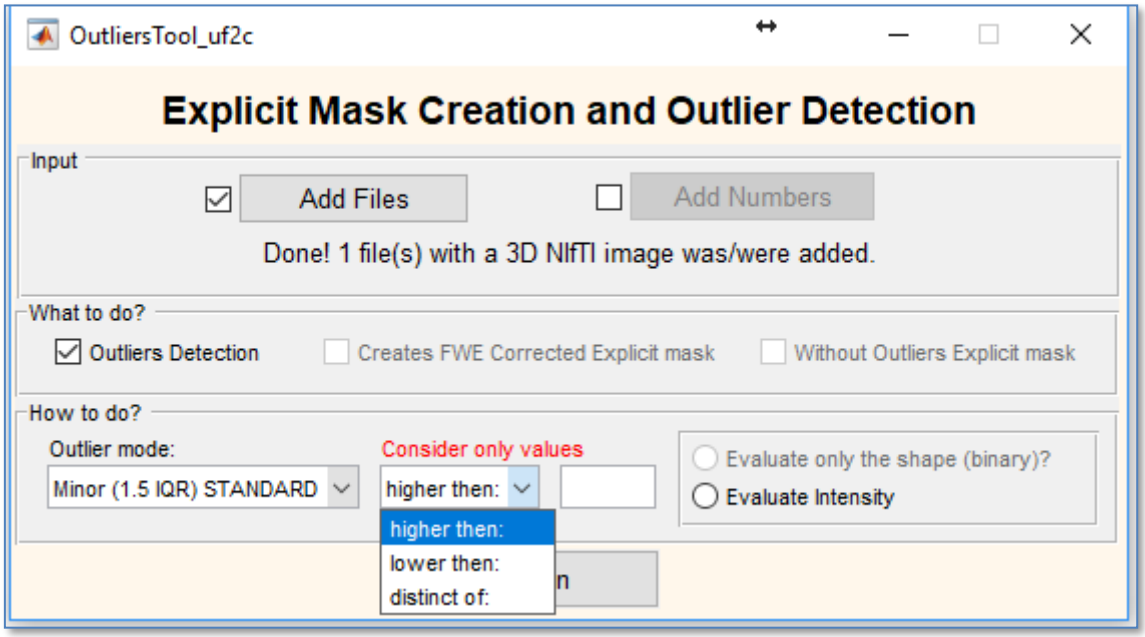


If you checked the "**Create FWE Corrected Explicit mask**" and/or "**Without Outliers Explicit mask**", a folder called "**Masks**" will be created in the first image folder. Inside the folder "**Mask**" you will find the Masks with statistical T-values ("**Mask_Original.nii**" and/or "**Mask_OutlierRemmoved.nii**"), and the binary version

("**BinaryMask_Original.nii**" and/or "**BinaryMask_OutlierRemmoved.nii**"). The binary masks are the ones suggested to be used as "**explicit mask**".

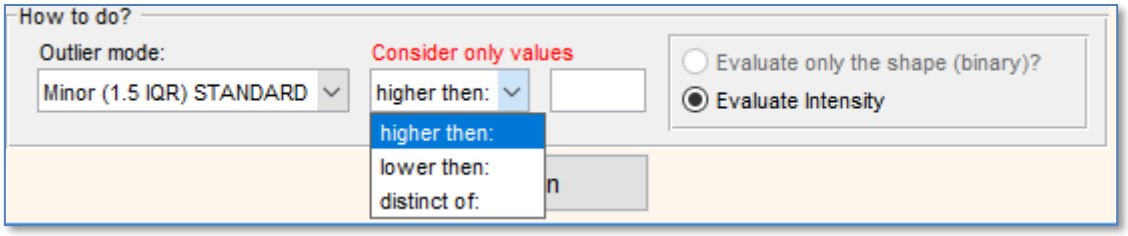
At the end, a BoxPlot will popup. If you have some outliers, they will be presented as a red X in the plot. You should consider only upper outliers as a problem. You can click in the red X to see the filename.

- **Case 2: Adding only ONE NIfTI file.**

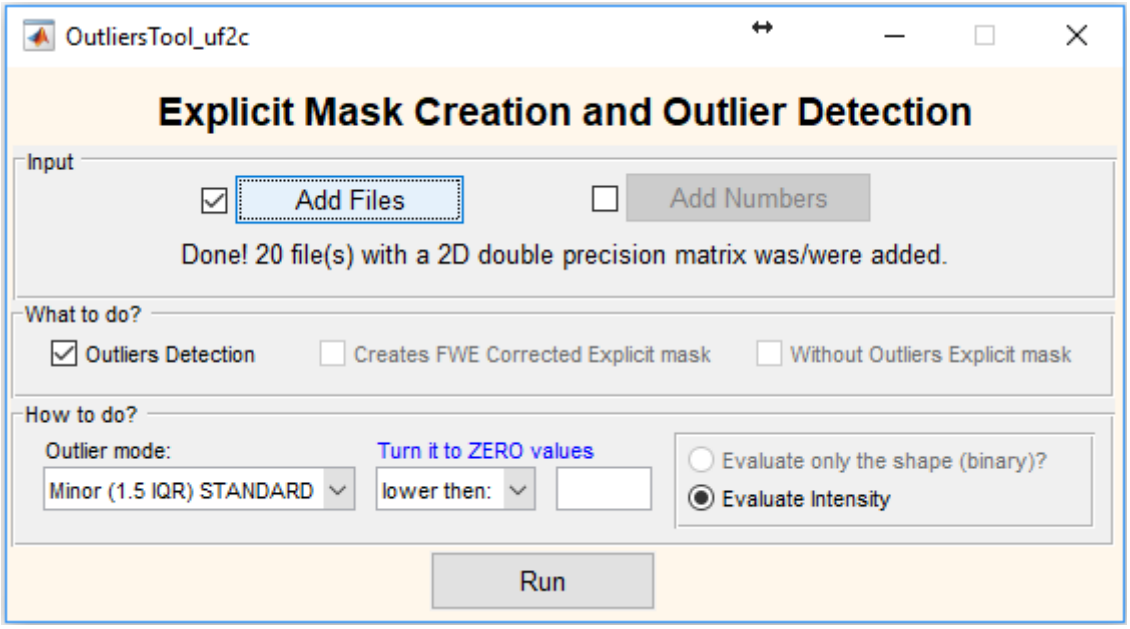


If you add only one NIfTI file, you cannot evaluate INTER-files outliers, create statistical Maps, neither evaluate only the shape of your image. However, the tool will compute the INTRA-file analysis, evaluating outliers within the voxels intensities. The outlier's options would be the same, but you have two more options for thresholding:

1. "Consider only values higher then:" → You will define a value and only the upper values will be included into the outlier's analysis.
2. "Consider only values lower then:" → You will define a value and only the lower values will be included into the outlier's analysis.
3. "Consider only values distinct of:" → You will define a value and all the values excluding the one defined will be included into the outlier analysis. This is useful to exclude the image background, for example (setting to 0). If you are adding a *.mat file with a connectivity matrix, you could set to 1, excluding from the analysis the diagonals.



- **Case 3: Adding more than one *.MAT files.**



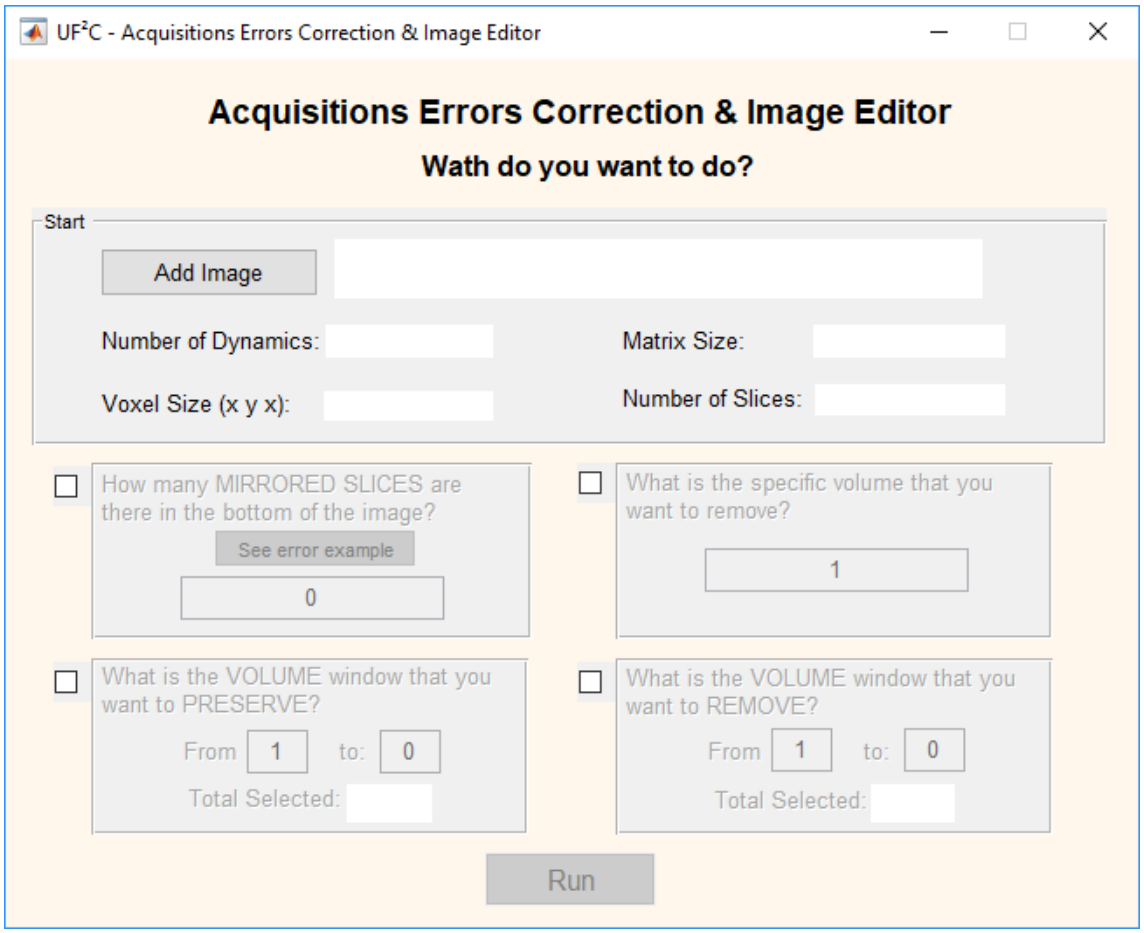
The addition of Matlab files with double precision variables inside will result in a similar process as described in **Cases 1.** The outliers will be shown in the Matlab command window and the BoxPlot will be created. The processes for 4D matrices is the same, the median 3D matrix will be used to inter-files analysis. Note that with Matlab files you cannot create the masks nor choose to evaluate only the shape of your image.

- **Case 4: Adding only ONE *.MAT files.**

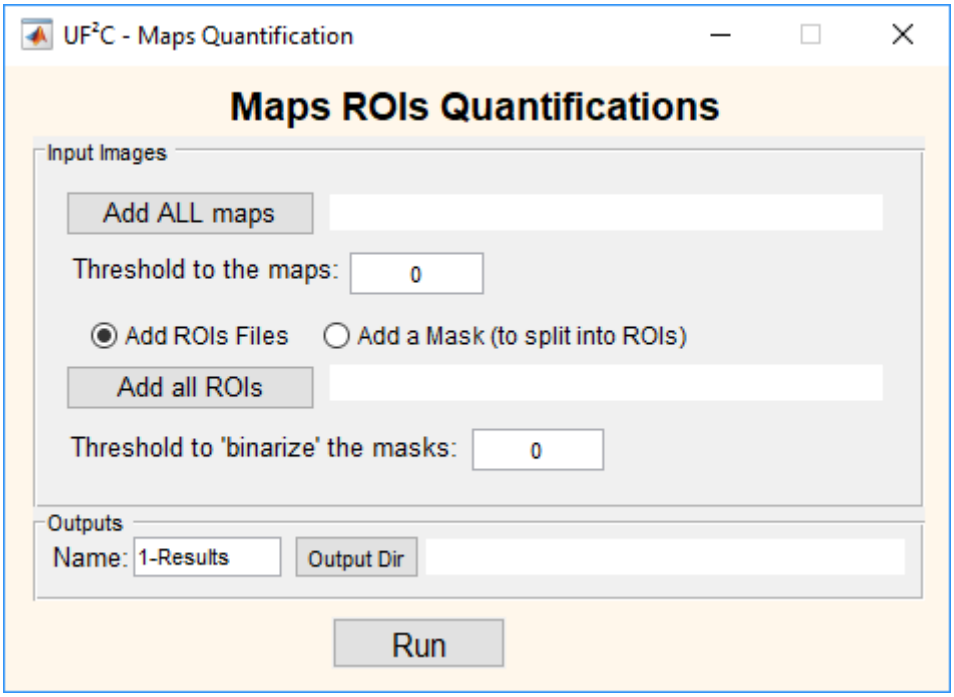
If this is the case, the tool will compute the INTRA-file outliers, the same way as described in **Case 2.**

d) Image Editor

The **Image Editor Tool** enables the correction and the edition of functional images. Besides to the correction of a specific reconstruction error (mirrored slices), you can also edit the functional image 4th dimension, removing a specific volume or intervals of volumes. You can add several files and apply the alterations for all of them.



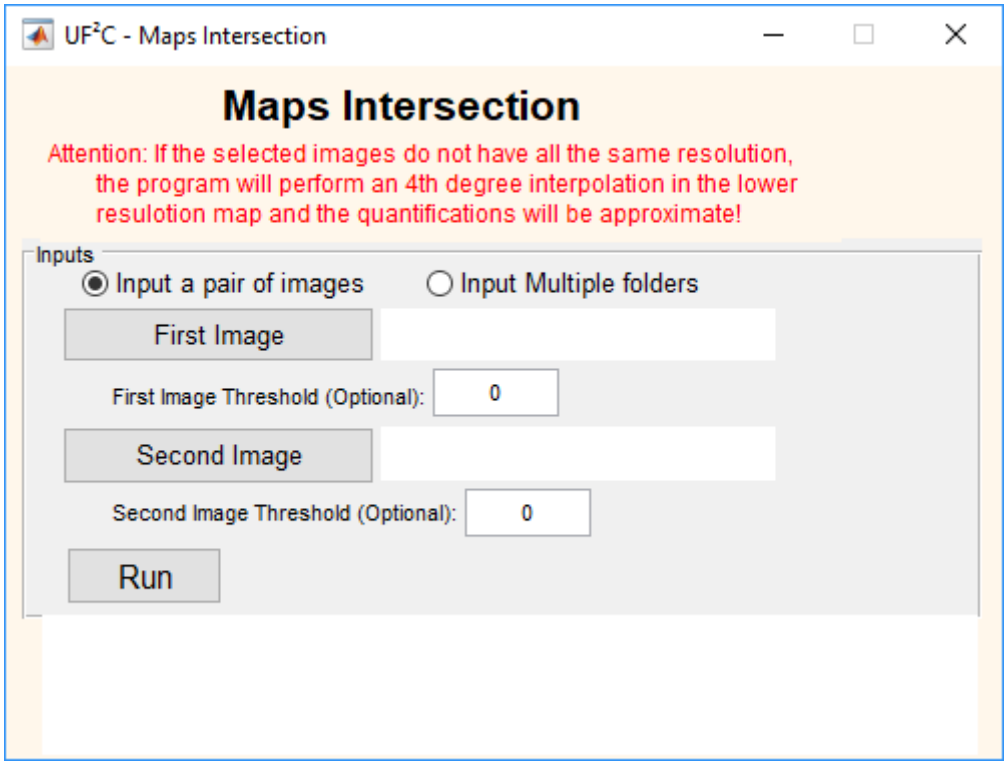
e) ROIs Maps Quantifications



The **ROI Maps Quantification Tool** compute the average value of ROIs from maps added. You can add several maps and several ROIs in the same space using the option "**Add ROIs Files**". You also have the option to add a mask of any nature. This mask will be "binarized" (if not already) using the Threshold defined on "**Threshold to 'binarize' the masks:"** field. The binary mask will be split into clusters and the averages for each cluster will be estimated.

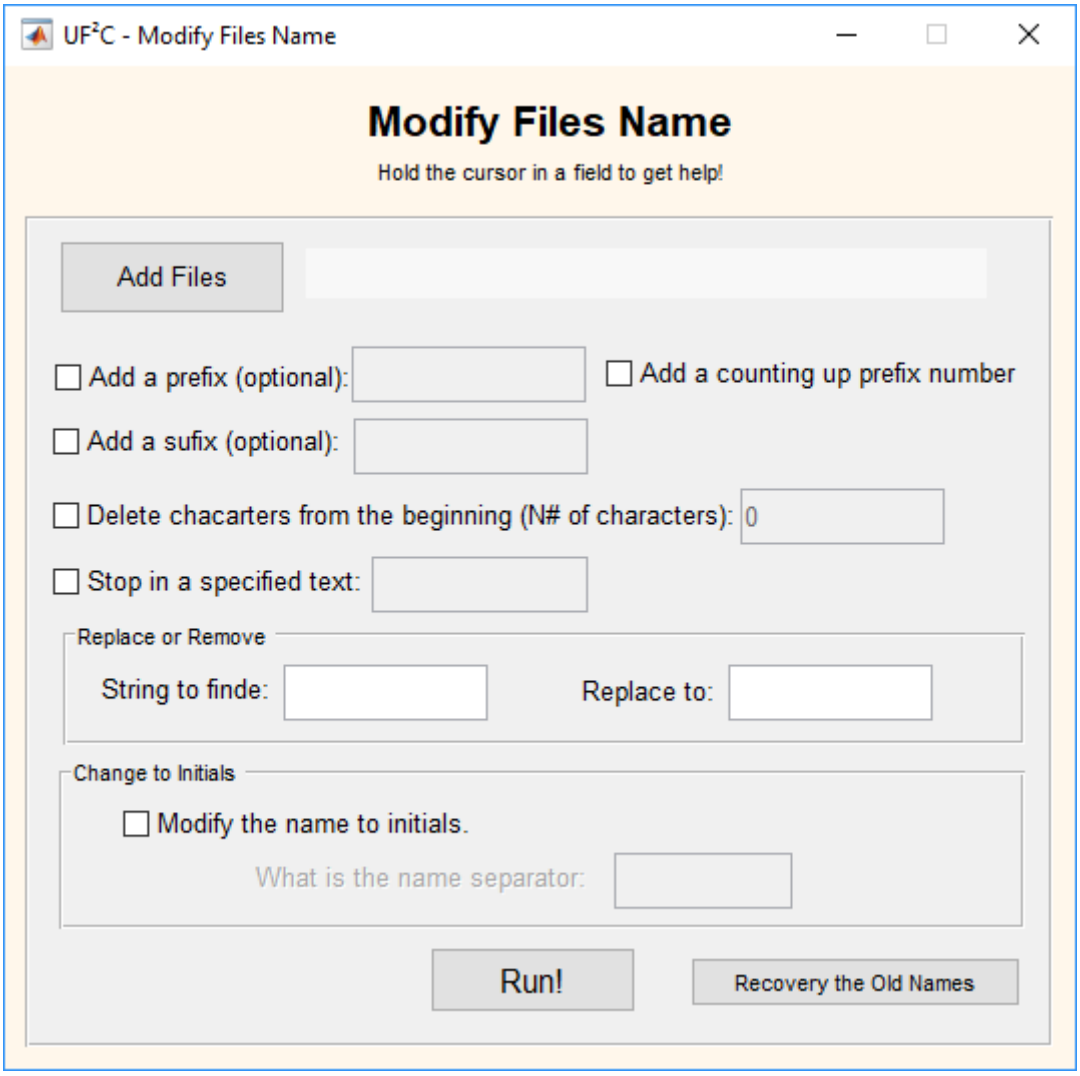
Finally, you should define the name and the output directory for the resultant text file with the values.

f) Maps Intersection



The **Maps Intersection Tool** performs the intersection of maps resulting in quantitative values as number of voxels and percentage of intersection. Using the option "**Input a pair of Images**", you will need to add two images, "**Fist Image**" and "**Second Image**". You can define thresholds for the images in the subsequent Threshold fields. If the images have distinct resolutions, the "second image" will be interpolated and registered to the first image parameters → This process can introduce inherent errors. In the same direction, by choosing the option "**Input Multiple folders"**, you will be required to add folders with a pair of images inside each one. The intersection will be done for each folder and the results will be saved into a text file created in the first folder added.

g) Modify Files Names

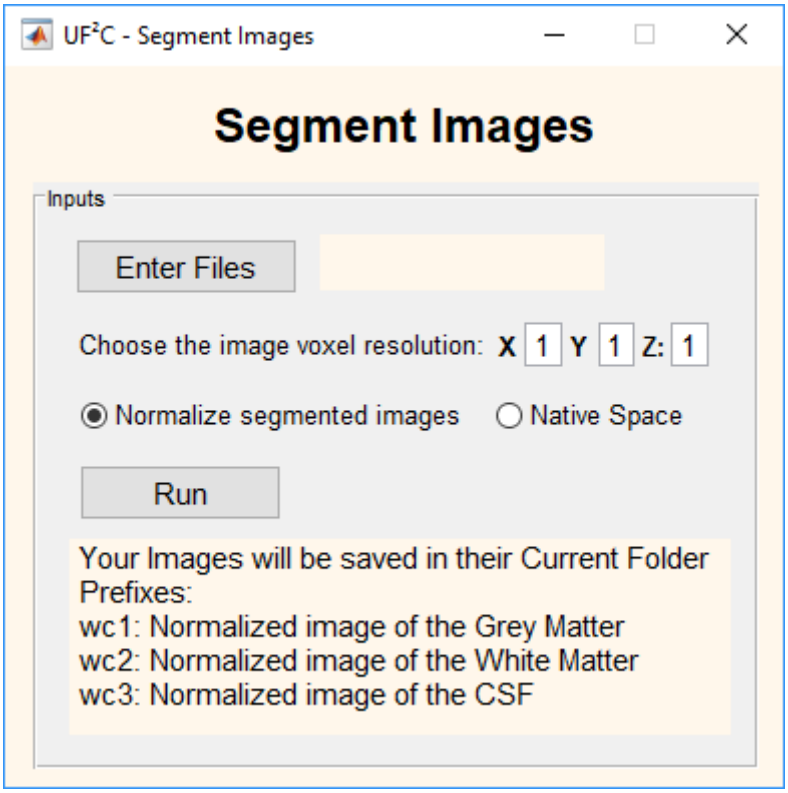


This it is a simple and powerful tool to rename files of any nature. If you have several files with the same name structure, you can add them all to modify according to several options. You can:

a) add prefix and/or a counting up prefix (e.g.: 1-, 2-,3-….)
b) add suffix
c) delete a specific number of characters starting from the first
d) stop the files names until a specific pattern of characters
e) replace a pattern for another (or leave the “Replace to” field empty to just remove the “String to find” pattern)
f) change the filename to initials (adding the name separator).

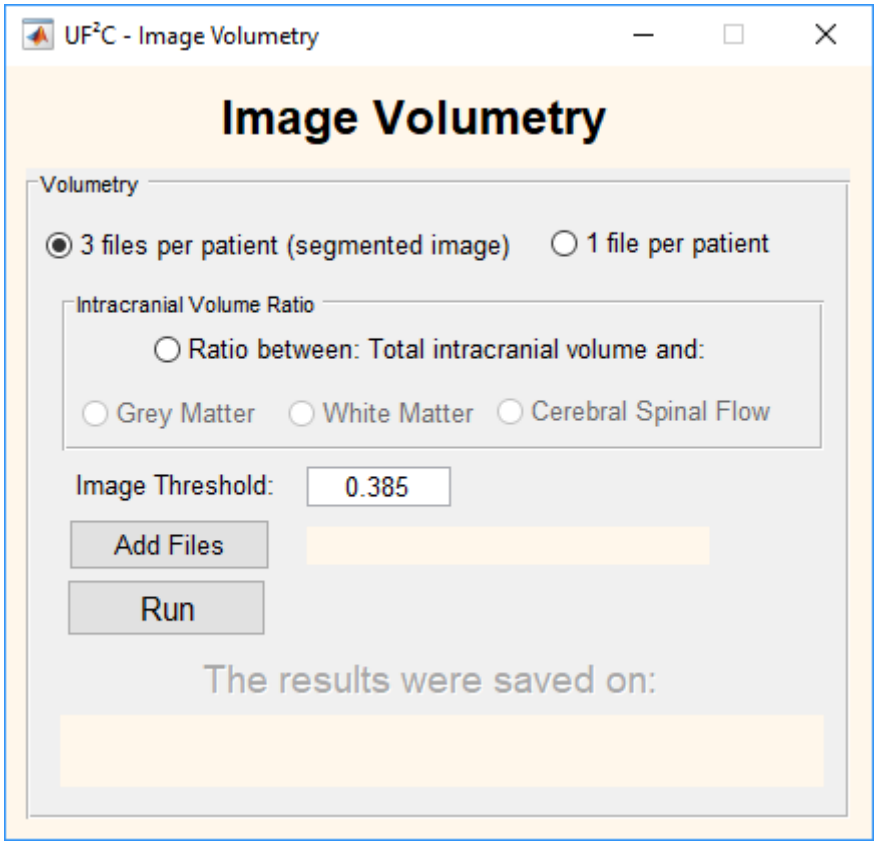
You can mix the options and the tool will apply then in a smart order. For each time that you “Run!” the tool, two files will be saved: a **text file** with the before and after of each file, and a ***.mat file**. You can use the *.mat file to recover (**Recovery the Old Names**) the old filenames. If several Runs are performed, several *.mat files will be generated. Just add them (using the **Recovery the Old Names**), from the newest to the older, to recover the original filenames.

h) Segment Images

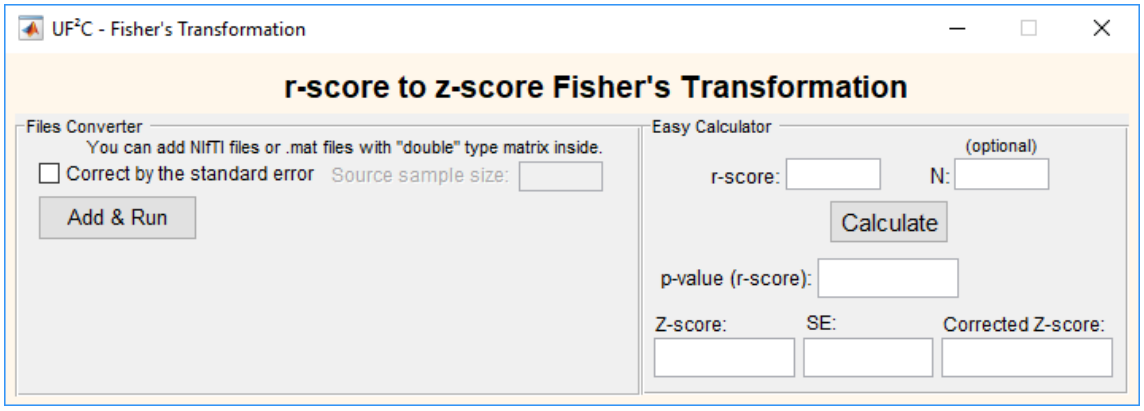


This tool is a simple shortcut to the SPM segmentation tool. Add the files, choose if you want them on native or standard space and “Run”.

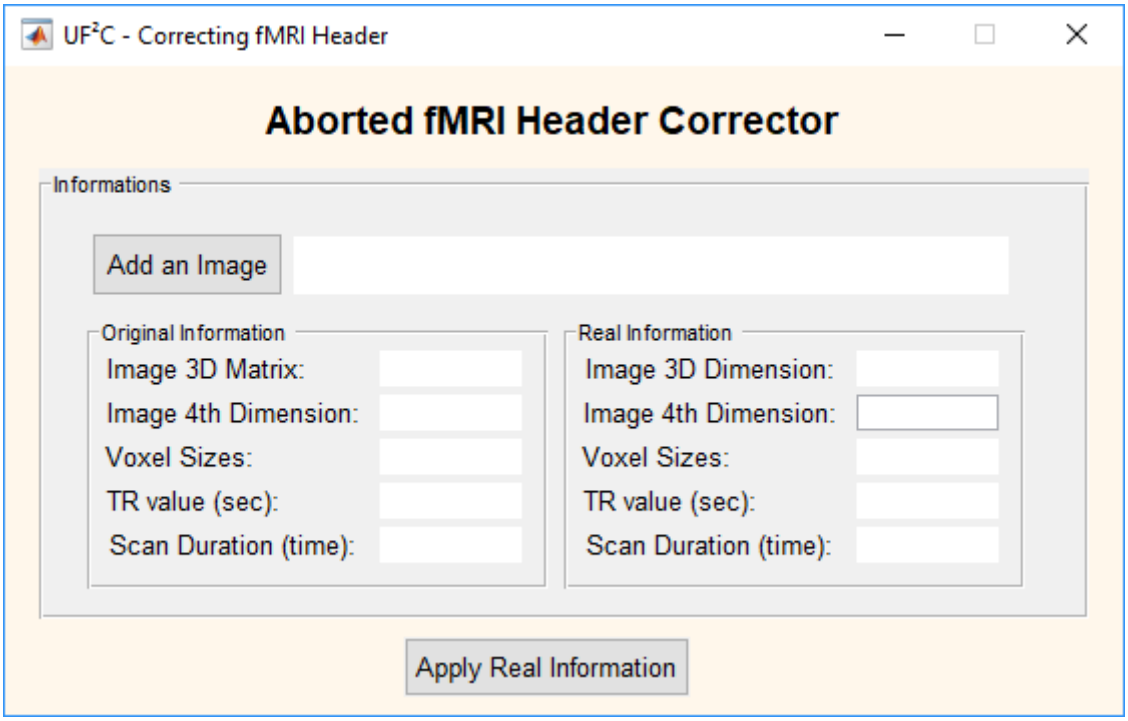
i) Images Volumetry



j) R-score to z-score Fisher's Transformation



k) Aborted fMRI Header Repairer



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