

WhiFuN Manual



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Introduction

We present the White Matter Functional Networks (WhiFuN) Toolbox for automated preprocessing of WM and GM fMRI, robust construction of WM and GM Functional Networks (FN), computation of WM-FC and GM-FC, and FC analysis modules. WhiFuN is based on SPM12 preprocessing and contains statistical tools for group-level analyses. WhiFuN provides an intuitive graphical user interface allowing users to execute all steps from preprocessing to final group level analyses and does not require prior knowledge of computer programming. However, familiarity with programming may enhance the user experience and provide more flexibility in customizing workflows.

WhiFuN was developed using MATLAB (version R2022b) under the Windows environment. The WhiFuN GUI was developed using MATLAB App Designer. In addition to custom-written code, several functions from SPM12 (<https://www.fil.ion.ucl.ac.uk/spm/>) were used for preprocessing and quality control. WhiFuN saves quality control plots to assess the quality of preprocessing steps. Users may select the data corresponding to participants (using the *uigetfile_n_dir* function developed by Tiago (Tiago, 2024)) for which the preprocessing was not done as desired and discard them from further analysis. Visualization of the WM and GM-FNs was performed in conjunction with the BrainNet viewer toolbox (Xia et al., 2013).

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1. Getting started

1.1 Download MATLAB Toolboxes

- i) Run MATLAB
- ii) Make sure that you are in the Home Tab. In the Environment section click on *Add-Ons* (See Figure 1-1).

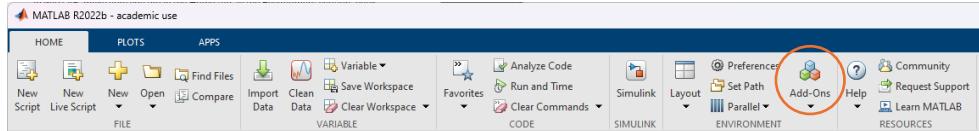


Figure 1-1: MATLAB Add-Ons in Home Tab

It opens the *Add-Ons* explorer (Figure 1-2).

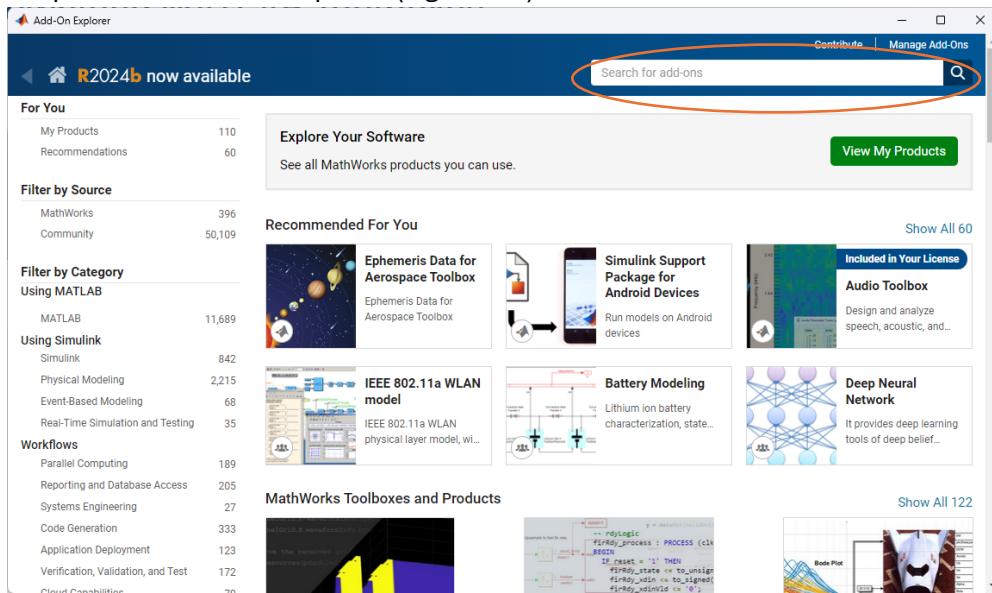


Figure 1-2: MATLAB Add-Ons Explorer

- iii) From the search bar, type the names of the following toolboxes and install them in MATLAB.
 - 1) *Bioinformatics Toolbox*
 - 2) *Image processing Toolbox*
 - 3) *Signal Processing Toolbox*
 - 4) *Statistics and Machine Learning Toolbox*

1.2 Download SPM 12 and add path to MATLAB

- i) Download *SPM12* toolbox from (<https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>).
- ii) Add path of *SPM* toolbox using
 - 1) MATLAB Set path (**Recommended**) (*you will have to do this only once*)

In MATLAB, make sure you are in the Home tab. In the *Environment* section, click on *Set path* (**Figure 1-3**).

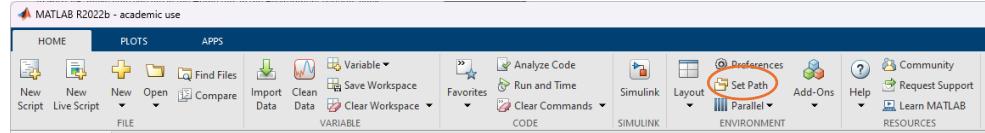


Figure 1-3: MATLAB set path in Home Tab

Set Path window opens (**Figure 1-4**).

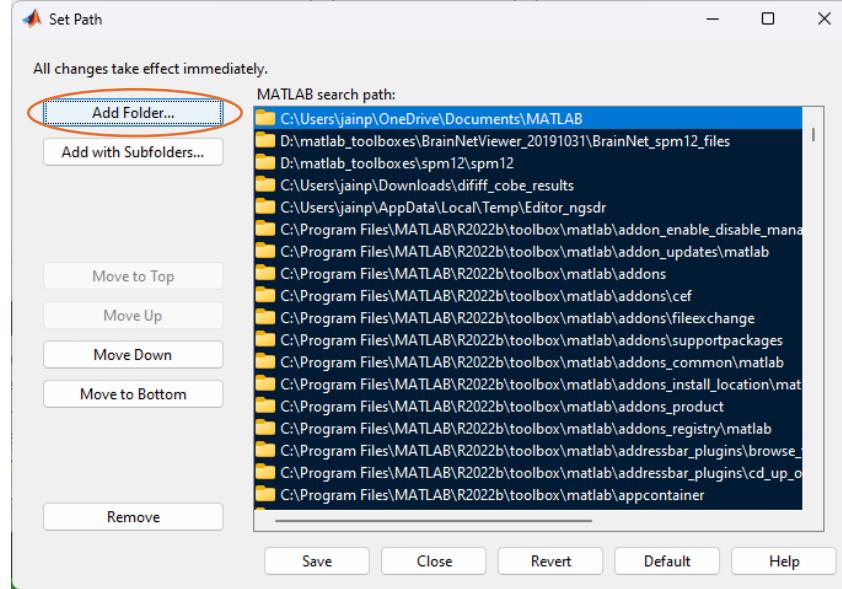


Figure 1-4: MATLAB Set Path window.

Click on add folder and select the SPM folder that has the *spm.m* code (**Figure 1-5**)

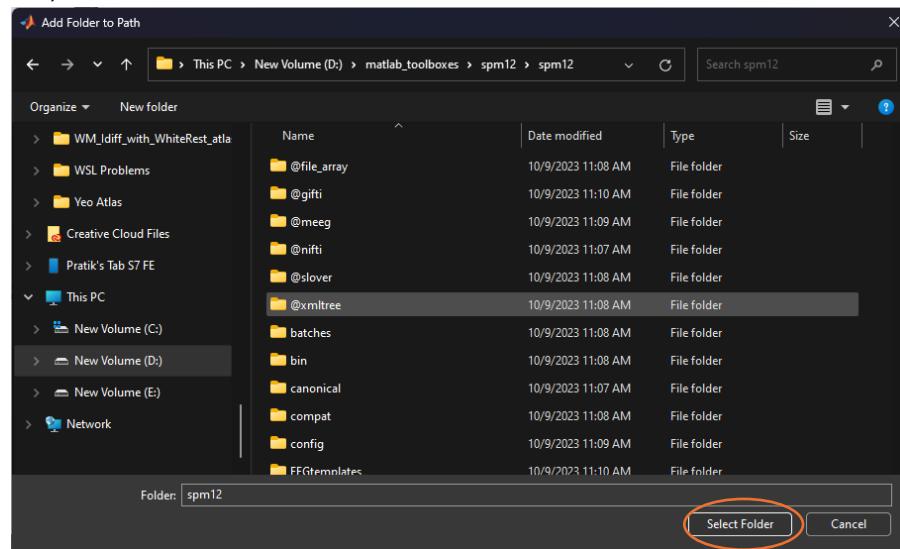


Figure 1-5: MATLAB, Add Folder to path window

You will see the folder added to the path. You should *save* and *close* the *set path* window.

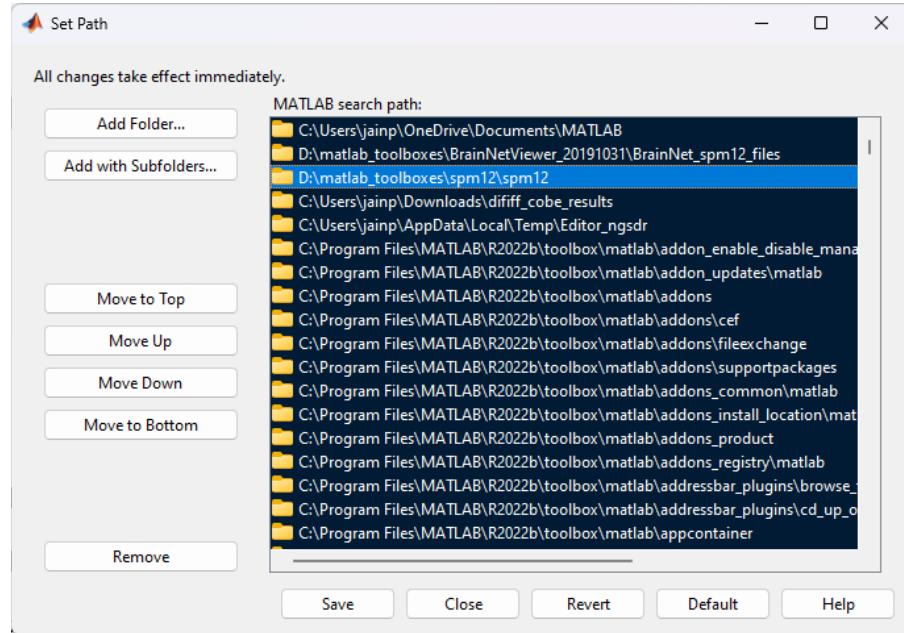


Figure 1-6: New folder added to the MATLAB *Set Path*

or

- 2) By MATLAB Command (***you will have to do this every time MATLAB is restarted***)

In MATLAB command window type

```
addpath('X:\ ... \spm12\spm12')
```

where

'X:\ ... \spm12\spm12' is path of the SPM 12 toolbox on your machine.

Eg.

```
addpath('D:\matlab_toolboxes\spm12\spm12')
```

1.3 Download *WhiFuN* and add path to MATLAB

- i) Download *WhiFuN* from <https://github.com/Brain-Connectivity-Lab/WhiFuN> (click on the green code button and then download zip, *Figure 1-7*)

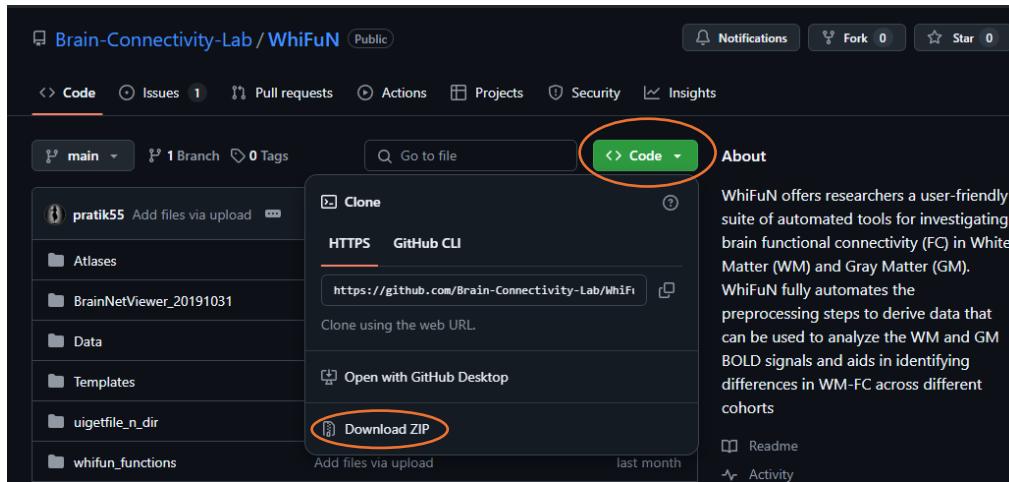


Figure 1-7: Download WhiFuN from GitHub repository

- ii) Unzip the contents and add path to MATLAB using MATLAB *Set path (Recommended)*

In MATLAB, make sure you are in the Home tab. In the *Environment* section, click on *Set path* (Figure 1-8).

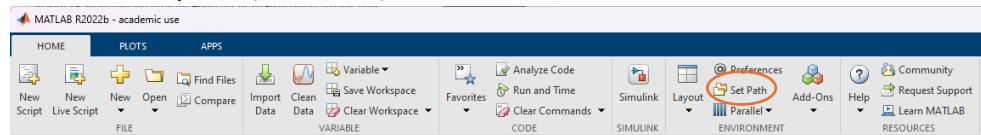


Figure 1-8: MATLAB set path in Home Tab

Set Path window opens (Figure 1-9).

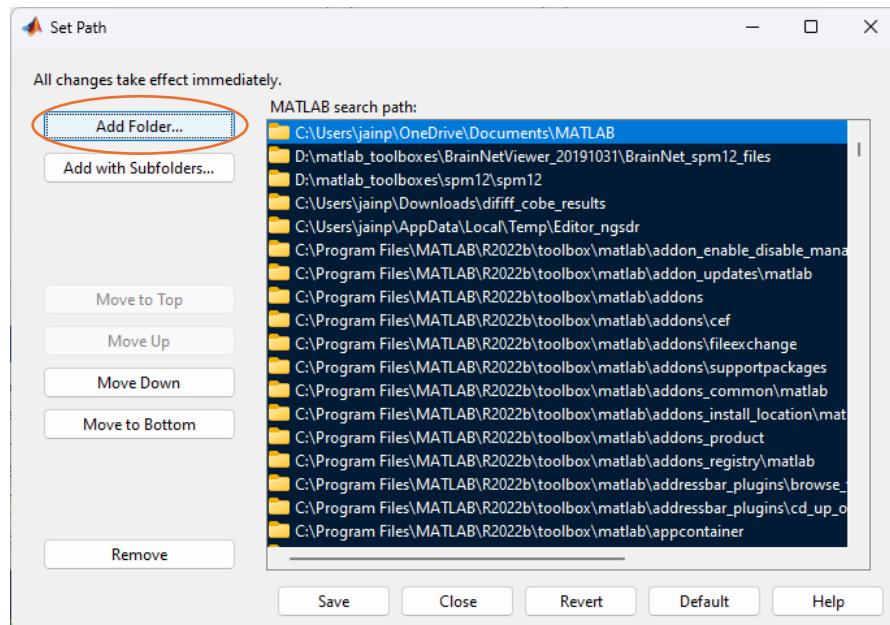


Figure 1-9: MATLAB *Set path* window.

Click on *Add folder* and Select the *WhiFuN* folder that has the *whifun.m* code (**Figure 1-10**)

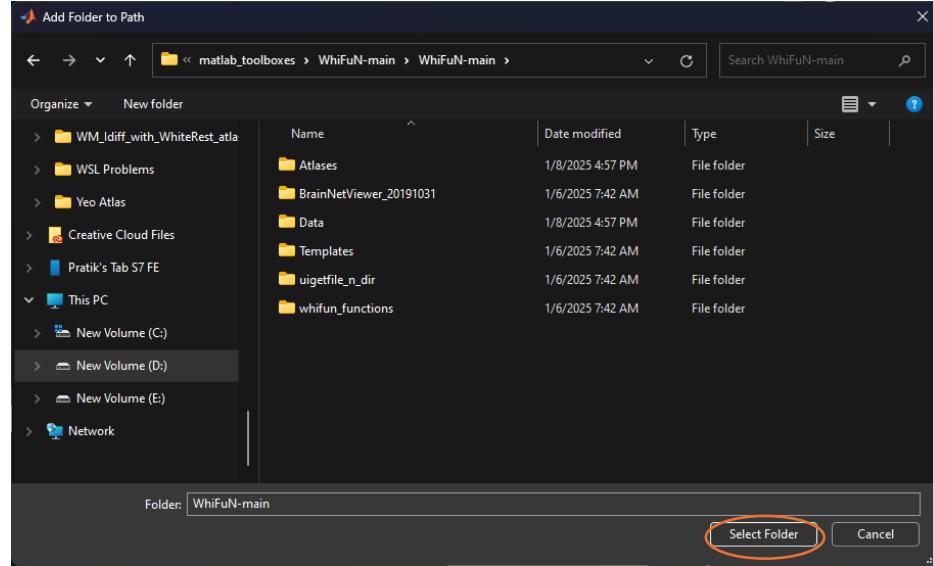


Figure 1-10: MATLAB, Add Folder to path window

You will see the folder added to the path. You can *save* and *close* the *set path* window (**Figure 1-11**).

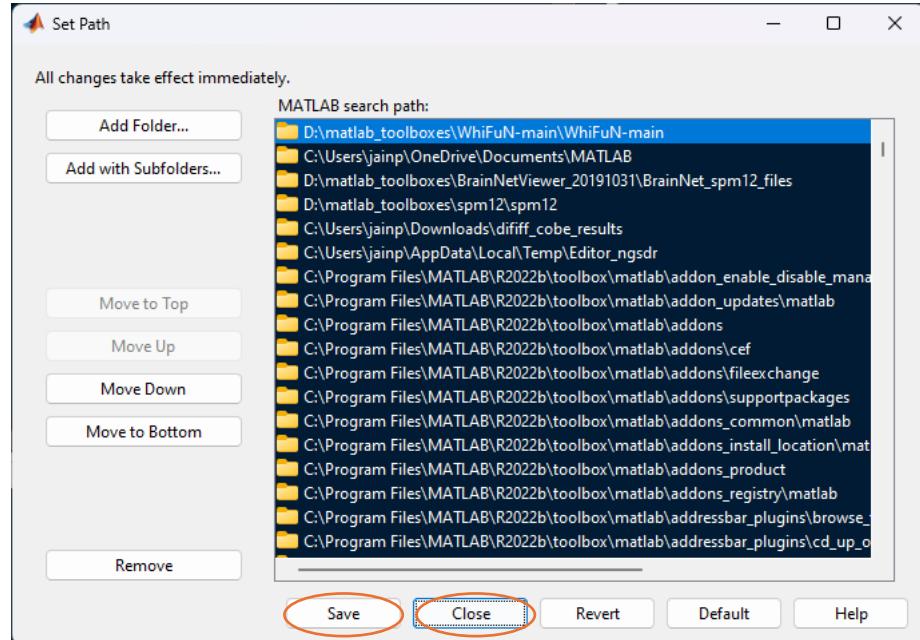


Figure 1-11: New Folder Added to MATLAB path

Once all the paths are added, type *whifun* in the MATLAB command window and the *WhiFuN* GUI will pop up (**Figure 1-12**).

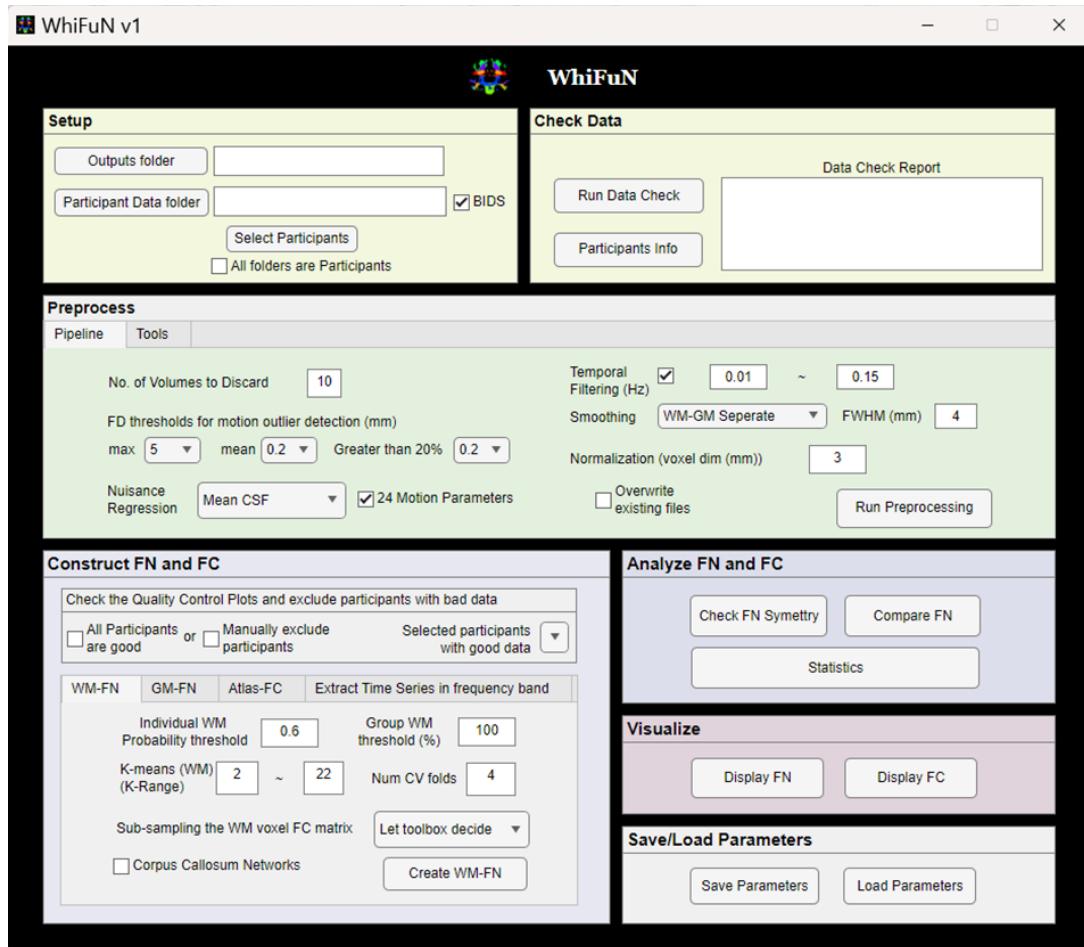


Figure 1-12: *WhiFuN* GUI

2. Setup

The Folder paths for where the MRI data is stored and where the outputs of *WhiFuN* will be stored are provided in the *Setup* (Figure 2-1).

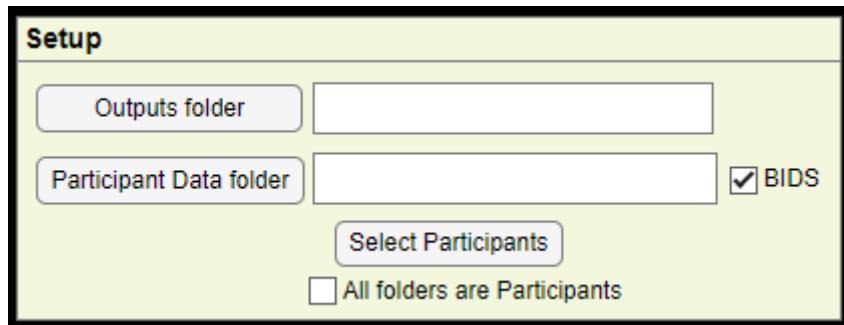


Figure 2-1: *WhiFuN* Setup module

2.1 Output Folder

Output Folder is an empty folder where the results and the quality control plots of *WhiFuN* will be stored.

Click the *Outputs folder* button or alternatively paste the path of the *Outputs folder* in the text box besides the *Output folder* button. This will be referred to as *Output_folder_path* for the rest of the manual.

2.2 Participant Data Folder

Participant Data Folder is where the raw MRI data is present. This folder should contain subfolders that correspond to data of Participants. Every subfolder should contain anatomical MRI scan and the fMRI scan.

Similar to the *Output folder*, the *Participant Data Folder* path can be specified by clicking the *Participant Data folder* button or by pasting the path in the text box besides the *Participant Data Folder* button. Once the *Participant Data Folder* is selected, *WhiFuN* will tell the number of folders identified in the *Participant Data Folder*.

If all the folders inside the *Participant Data Folder* correspond to a participant, then check the *All folders are Participants* checkbox. Else, the *Select Participants* button can be used to select the folders corresponding to the Participants. Once the participants are selected, *WhiFuN* will show the total number of participants selected.

As an example, the practice data (uploaded [here](#)) can be used to understand the paths to be given to *WhiFuN*.

2.3 Data Structure

Most fMRI datasets use the Brain Imaging Data Structure ([BIDS](#)). If the dataset that is to be preprocessed and analyzed is in BIDS format, then the checkbox besides the *Participant Data Folder* called *BIDS* should be checked.

If, however, the dataset is not in BIDS format then uncheck the *BIDS* checkbox. That will open *Participant Data Folder Details* window. By default, the fields here are fields for the BIDS format. To use any other data format these fields have to be changed.

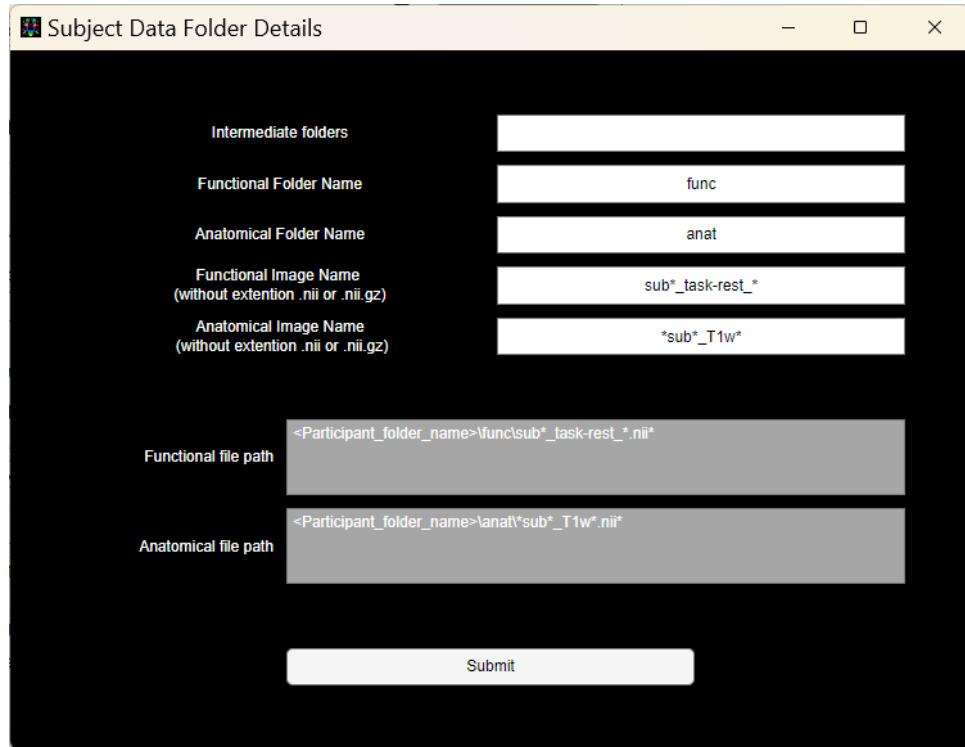


Figure 2-2: WhiFuN Participant Data Folder Details GUI

WhiFuN can process the following non-BIDS format datasets:

1. The dataset to be processed should have participant folders and every participant folder should have an anatomical folder which contains the anatomical MRI scan (e.g. T1 weighted scan in nifti format) and a functional folder which contains the functional MRI scan (e.g. Resting state scan in nifti format).
2. The anatomical and the functional folder should be in the same folder.

WhiFuN will not be able to process any dataset that deviates from the above-mentioned format.

We demonstrate how the deviations from BIDS can be handled by WhiFuN using an example of practice [ABIDE](#) dataset that can be downloaded from [here](#). It has the following folder structure

Example Data format 1:

	<i>Participant Data folder</i>
practice_nyu_abide	
0050952	<i>Participant Folder</i>
session_1	<i>Intermediate folder</i>
anat_1	<i>Anatomical folder</i>
rest_1	<i>Functional folder</i>
0050953	
session_1	
anat_1	
rest_1	
0050954	
session_1	
anat_1	
rest_1	
0050955	
session_1	
anat_1	
rest_1	

Fields of *Participant Data Folder Details*

Intermediate folders: If there are folders between the *participant folder* and the folder containing the *anatomical* and *functional* images then those should be specified here.

In the practice dataset example (example format 1), the *intermediate folder* field will be

Intermediate folders: - session_1

Note that if there are differences in the intermediate folders across participants then the wild card can be used. (For e.g. session*).

If there are multiple folders, then the complete path between the *participant folder* and the folder that contains the *anatomical* and the *functional folder* should be provided.

For example, if the data has the following structure

Example Data format 2

Practice_nyu_abide

0050952

MRI

3T

session_1

anat_1

func_1

0050953

MRI

3T

session_1

anat_1

func_1

For example, data format 2 the *Intermediate folders* field should be

Intermediate folders: - MRI/3T/session_1 (for windows)

or

Intermediate folders: - MRI\3T\session_1 (for Mac or Linux)

Users can also use wild cards (*).

Functional Folder Name: the folder name where the functional file is present should be specified.

For example: for both the example data formats, the *functional folder name* will be

Functional folder name: - func_1

Anatomical folder name: Specify the folder name where the anatomical file is present.

For example: for both the example data formats, the *anatomical folder name* will be

Anatomical folder name: - anat_1

Functional/Anatomical Image name: Specify the name of the *functional/anatomical image* here. Since most datasets have the participant ID included in the *functional image name*, one should use the wild card (*) for these cases.

While most datasets have the compressed files in the form *.nii.gz*, some have the *.nii* file extension. Do not specify the extension in the field, *WhiFuN* will automatically detect the file and if zipped files with *.nii.gz* file extension is found, it will uncompress it and process the file.

Once all the fields are set, the path of the functional and anatomical images can be seen in the *functional and anatomical file path* fields. The user must check these paths and then click submit. When the user clicks submit, *WhiFuN* program will check if the given anatomical and functional path works for the first participant. If it does not execute properly, it will say which field it feels is giving the error. The user can then fix the error and try again.

3. Check Data

Once the *Setup* is complete, *WhiFuN* will check all the paths provided and inform the user of any participant that has missing anatomical or functional files. Additionally, the anatomical and functional voxel sizes, number of time points and Time resolution (TR) of the functional image will also be checked.

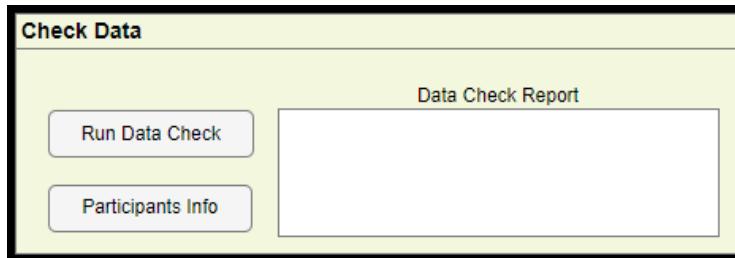


Figure 3-1: *WhiFuN* Check Data module.

3.1 Run Data Check

Once the *Setup* is complete the user must click *Run Data Check* button to start the data check (See *Figure 3-1*). This might take some time depending on the number of participants. Once the check is done, two plots will be saved in the folder
<Output_folder_path>/Quality_Control

Scanning Parameters

Q1a_scanning_parameters: A bar plot of the number of fMRI/functional images/volumes, TR of the functional image, and voxel sizes of the functional and structural image is saved. The user must check that the number of fMRI images/volumes, the TR of the fMRI images and the voxel sizes must be same across the participants.

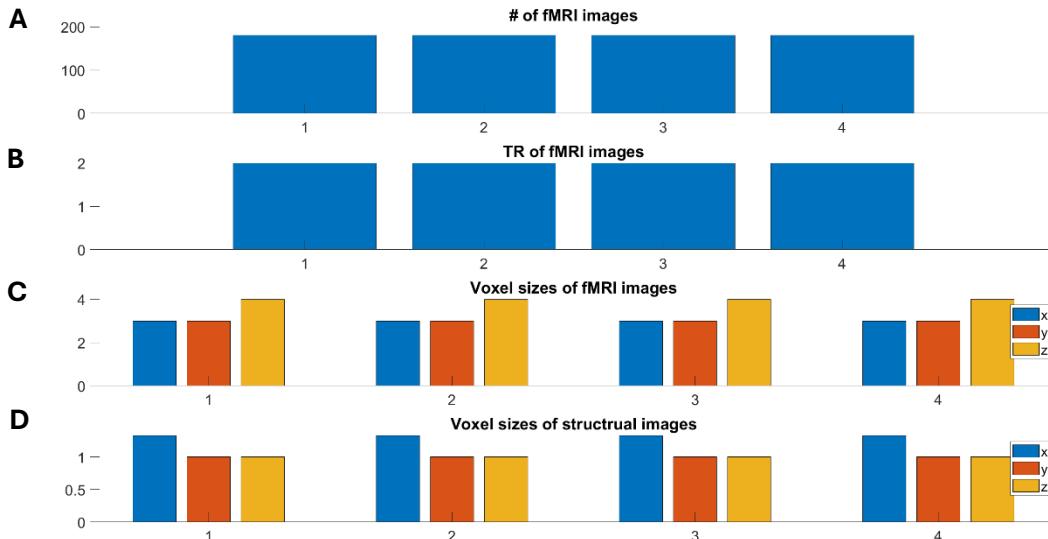


Figure 3-2: Q1a_scanning_parameters.jpg saved when the practice data set was used. The x axis denotes the participants. A: Number of fMRI images/volumes B: TR C: Voxel size of fMRI images D: Voxel sizes of structural images, across all participants.

Scanning parameters Histogram

If the number of participants is a large (>1000 participants), then the Scanning parameters histogram would be a better choice to see if the MR parameters are consistent across the participants. Then, the user must check that there should be just one histogram bar for every plot. If there is more than one bar for a histogram (e.g. *Figure 3-3 D*, voxel x dimension of structural MRI image) that means, that parameter is not consistent across participants. In this case the difference is very small (1.329995 and 1.330005) users may ignore this as the difference is very small. But if the difference is large then that particular participant may be discarded.

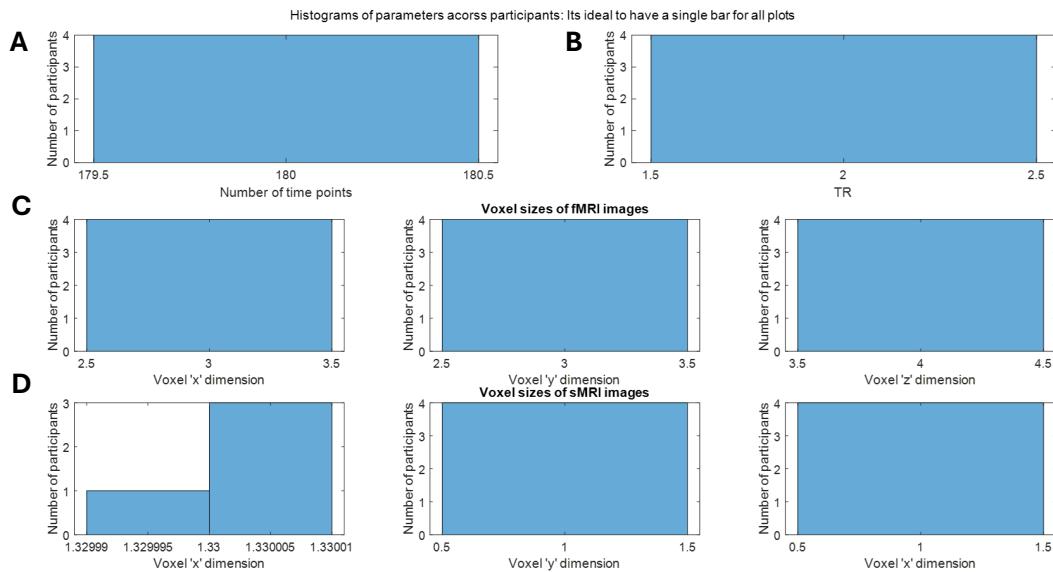


Figure 3-3: Q1a_scanning_parameters_histogram.jpg saved when the practice data set was used. Histogram of A: the number of timepoints/volumes/images in the fMRI image. B: TR C: Voxel sizes of fMRI images D: Voxel sizes of structural images.

Data check Report

The *data check report* mentions the information of any missing files. If the check is completed without any problems, *Data Check report* will state that the *Data Check was completed successfully*.

WhiFuN reads the TR value from the nifti header. There may be instances where the nifti header is corrupted or the TR value is not present in the nifti header. *Data check report* will notify the user that the TR corresponding to Participant <Participant ID> is not found and the user will have to manually specify the TR for those participants.

3.2 Participant Info

Once the Data check is performed, the user can look at the information extracted from *WhiFuN* using the *Participant Info* button.

Subject Information

name	folder	error	manual_ex	motion_ex	nt	x_func	y_func	z_func	x_Anat	y_Anat	z_Anat	TR
0050952	C:\Users\krish\Box\practice_NYU_abide	0	0	0	180	3	3	4	1.3300	1	1	2
0050953	C:\Users\krish\Box\practice_NYU_abide	0	0	0	180	3	3	4	1.3300	1	1	2
0050954	C:\Users\krish\Box\practice_NYU_abide	0	0	0	180	3	3	4	1.3300	1	1	2
0050955	C:\Users\krish\Box\practice_NYU_abide	0	0	0	180	3	3	4	1.3300	1	1	2

All Subjects Subjects good to preprocess save changes

Figure 3-4: Participant Information extracted from Data check.

The fields in the *Participant Info* (shown in *Figure 3-4*) are as follows:

- 1) *Name*: The Participant name/ID same as the *Participant Folder name*.
- 2) *Folder*: The *Participant Data Folder*
- 3) *Error*: If any error was found for a particular participant, this field will be 1 corresponding to that participant else it will be shown 0.
- 4) *manual_ex*: If any participant was manually excluded from preprocessing during the setup stage then *manual_ex* will be 1 for that participant, else it will be shown 0.
- 5) *motion_ex*: During Preprocessing if a particular participant does not meet the motion criteria, then *motion_ex* will be 1 for that participant else 0.
- 6) *nt*: Number of time points/ volumes in the functional image.
- 7) *x_func*, *y_func*, *z_func*: The x, y, z voxel dimension of the functional image.
- 8) *x_anat*, *y_anat*, *z_anat*: The x, y, z voxel dimension of the anatomical image.
- 9) *TR*: The temporal resolution (TR) of the functional image extracted from the Nifti header.

Participants good to Preprocess

If the *Participants good to preprocess* button is clicked, only the participants that are to be processed will be displayed. The participants that were manually excluded (*manual_ex = 1 || motion_ex = 1 || error_ex = 1*) will not be displayed.

Manual corrections and Save Changes

If the *Data Report* says the TR was not identified for a participant, then then user can make changes by clicking on the TR column of that participant and manual enter the TR value. Once the TR value is entered click on *save changes* and the TR value will be stored.

4. Preprocess

WhiFuN uses a combination of inhouse MATLAB scripts and *SPM12* toolbox to preprocess the functional images. Briefly, the following steps are executed for preprocessing the images (See *Figure 4-1*). Each of the functional volumes in a time series are realigned with reference to the first time point and the corresponding motion parameters are computed. Framewise displacement criteria is then used to identify the scans that have excessive motion. Then, the realigned functional images are co-registered with the anatomical image, and the anatomical images are segmented. The segmentation generates a CSF tissue probability image from which a CSF mask is generated and used to identify the CSF voxels in the functional images. The mean CSF signal is regressed from the entire brain along with Friston's 24 motion parameters. After regression, a bandpass filter with a default range of 0.01 – 0.15 Hz filters every voxel time series. The brain's GM and WM regions are then smoothed separately and are finally normalized to standard MNI space. Quality control measures are saved as image files for every participant, which can be used to check if the processing was correct.

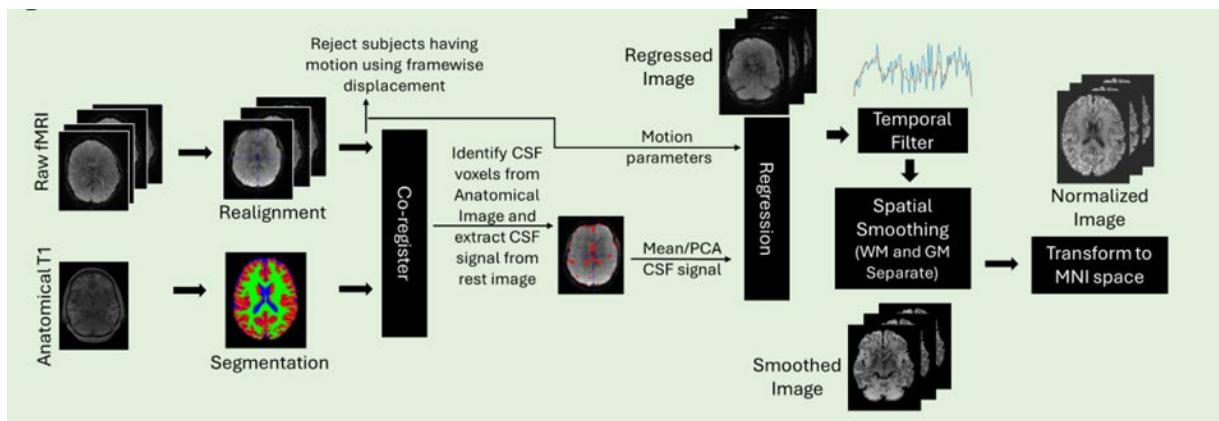


Figure 4-1: *WhiFuN* Preprocessing Pipeline.

Figure 4-2 shows the Preprocessing GUI of *WhiFuN*. The parameters related to the different preprocessing steps can be tuned here.

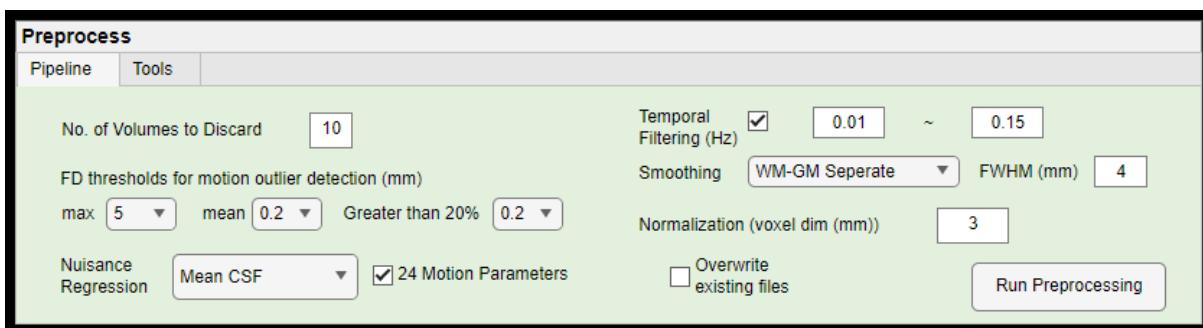


Figure 4-2: *WhiFuN* Preprocessing GUI

In the following sections we will go through the preprocessing pipeline in detail and discuss these parameters in detail.

4.1 Overwrite Existing files

If preprocessing is interrupted by the user or due to an issue such as a system shutdown or MATLAB crash, *WhiFuN* allows users to resume from where it stopped. Before restarting, *WhiFuN* checks for previously generated preprocessing files. If the required files are found, *WhiFuN* skips the completed steps and proceeds to the next stage. However, if the user wishes to restart preprocessing from the beginning, they must select the "*Overwrite Existing Files*" option.

If *overwrite existing files* checkbox is unchecked, prior to preprocessing, *WhiFuN* will check if any preprocessing files already exist and skip preprocessing for these files.

If *overwrite exiting files* checkbox is checked, *WhiFuN* will delete any previously created preprocessing files (if they exist) and start the preprocessing from the start for every participant.

4.2 Unzipping and Discarding initial Volumes

The preprocessing pipeline starts by ‘*gun zipping*’ any .gz file and discarding initial volumes (default is 10) from the functional images, allowing for the magnetization to stabilize to a steady state (Caballero-Gaudes & Reynolds, 2017).

No. of Volumes to Discard → Expects positive integer values including 0.

If the user specifies 0, No initial volumes will be discarded.

Output of Discarding initial Volumes

- 1) c_<functional image name>.nii :- Image with the initial volumes discarded as specified by the user. This file will be stored in the folder that contains the functional image.

If 0 initial volumes were specified to be discarded by the user then, no new image will be generated.

4.3 Realignment and Framewise Displacement

The raw functional volumes are realigned to the first image using *SPM*’s ’*Realign (estimate and reslice)*’. The head motion associated with each volume is estimated by evaluating how much each volume is transformed to match the first volume.

The following files will be stored in the folder that has the functional file after Realignment is successfully completed.

Outputs of Realignment

- 1) r<cut functional image name>.nii :- Realigned image generated after discarding the initial volumes.

- 2) `rp_<cut functional image name>.mat` :- Motion parameters namely the translations of each volume in x, y, and z directions in millimeters and the rotations of each volume in pitch, roll, and yaw in radians in a text file.
- 3) `mean<cut functional image name>.nii` :- Mean functional image is a 3D image with every voxel representing the mean value of the BOLD timeseries.

Framewise Displacement

Using the motion parameters obtained from realignment, the Framewise Displacement (FD) is calculated using the equation proposed by Power and colleagues (Power et al., 2012).

$$FD = (x_t - x_{t-1}) + (y_t - y_{t-1}) + (z_t - z_{t-1}) + 50((\alpha_t - \alpha_{t-1}) + (\beta_t - \beta_{t-1}) + (\gamma_t - \gamma_{t-1}))$$

Where x_t, y_t, z_t is the translation motion (in cm) in the x, y and z direction and $\alpha_t, \beta_t, \gamma_t$ are the rotational motion parameters (in radians) at timepoint t . Here, it is assumed that the head is a sphere with a radius of 50 cm and that the axis of rotation passes through the center of the head. This gives $nT - 1$ FD value for where nT is the total number of time points/volumes in the functional image.

Outlier volumes are identified and corresponding scans are excluded if the maximum FD is greater than a threshold (default is 5mm), if the overall mean FD is greater than a threshold (default 0.2 mm), or if more than 20% of volumes are greater than a threshold (default 0.2 mm) (Parkes et al., 2018).

Once the preprocessing is completed for all participants, based on the distribution of the FD values WhiFuN allows the user to set the above-mentioned thresholds based on how much motion can be tolerated for a particular cohort (See Section 6.2).

4.4 Segmentation, Co-registration and Mask Extractions

After realignment, *WhiFuN* performs segmentation and skull striping on the anatomical image. Next, the functional images are co-registered to the skull-stripped anatomical image to ensure spatial alignment. A cerebrospinal fluid (CSF) mask is then generated, and the BOLD time series within the CSF mask is extracted from the functional images. There are no parameters that the user must define for these steps.

Output of Segmentation

Segmentation generates the following files in the folder that has anatomical image.

Tissue Probability Maps in Participant Space

- 1) `c1<anat image name>.nii` :- The GM tissue probability map in participant space. It is a 3-dimensional image with the same size as that of the anatomical image, with voxel values representing the probability of it belonging to GM.
- 2) `c2<anat image name>.nii` :- The WM tissue probability map in participant space. It is a 3-dimensional image with the same size as that of the anatomical image, with voxel values representing the probability of it belonging to WM.
- 3) `c3<anat image name>.nii` :- The CSF tissue probability map in participant space. It is a 3-dimensional image with the same size as that of the anatomical image, with voxel values representing the probability of it belonging to CSF.

Tissue Probability Maps in MNI Space

- 4) `wc1<anat image name>.nii` :- The GM tissue probability map in MNI space. It is a 3-dimensional image with the same size as that of the MNI template, with voxel values representing the probability of it belonging to GM.
- 5) `wc2<anat image name>.nii` :- The WM tissue probability map in MNI space. It is a 3-dimensional image with the same size as that of the MNI template, with voxel values representing the probability of it belonging to WM.
- 6) `wc3<anat image name>.nii` :- The CSF tissue probability map in MNI space. It is a 3-dimensional image with the same size as that of the MNI template, with voxel values representing the probability of it belonging to CSF.

WhiFuN also saves the modulated versions of the above mentioned images (`mwc*<anat image name>.nii`, where * = 1,2 or 3 for GM, WM or CSF).

Files required for Normalization to MNI space.

- 7) `y_<anat image name>.nii` :- Deformation field. This file contains the transformation parameters needed to warp images from the participant's native space to standard MNI space. It is used for normalizing any image to the MNI template.
- 8) `iy_<anat image name>.nii` :- Inverse Deformation field. This file contains the inverse transformation parameters, allowing images in MNI space to be mapped back to the participant's native space.

Bias Regularized Image

- 9) `m<anat image name>.nii` :- bias regularized image. Removes the smooth spatially varying artifact that modulates the intensity of the image (bias).

Outputs of Skull Stripping

- 1) `b<anat image name>.nii` :- Skull stripped Anatomical image.

Output of Co-registration

The header of the realigned functional image is changed. No new image is generated.

- 1) `<Realigned functional image name>.mat` :- A MATLAB variable file, having the new transformation matrix information after the co-registration is done.

Outputs of Masks Extraction

- 1) `anat_mask.nii` :- Binary mask in participant space (same size as that of the original anatomical image) where voxels inside the brain are labeled as 1 and those outside as 0.

- 2) `wanat_mask.nii` :- Binary mask in MNI space (same size as that of the MNI template) where voxels inside the brain are labeled as 1 and those outside as 0.
- 3) `CSF_MASK0.95.nii` :- Binary mask identifying the CSF voxels in participant space. Voxels with CSF probability greater than 0.95 are labeled as 1, while others are 0.

The following file is stored in the folder where the functional file is present.

- 4) `Covariance_csf_REST.mat` :- This is a .mat file that contains the mean/PCA of the CSF BOLD signal which is to be regressed during the Nuisance regression step.
- 5) `rest_mask.nii` :- Binary mask in the participant space for the functional image where voxels inside the brain are labeled as 1 and those outside as 0.

4.5 Nuisance regression

To reduce noise, WhiFuN regresses out unwanted signals from every voxel in the brain. Users can choose between regressing the mean CSF signal for a faster computation or using principal component analysis (PCA) to regress the dominant CSF signal components, which is more effective but computationally slower. Alternatively, users may opt to skip CSF signal regression altogether.

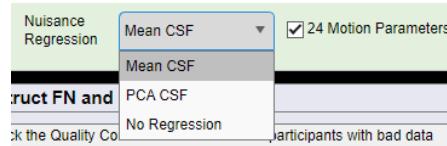


Figure 4-3: Options for Nuisance regression.

Besides the dropdown for the Nuisance regression, is the check box for regressing out the Friston's 24 motion parameters (Friston et al., 1996; Yan et al., 2013). Friston's 24 parameters are the three translational and three rotational parameters, the squares of them, the derivatives of them, and the squares of the derivatives. Regression is performed to remove the nuisance signals from the rsfMRI time series. If this box is unchecked the motion parameters won't be regressed out.

If the user decides to use the PCA of CSF signals, then WhiFuN will prompt an additional popup asking for the number of PCA components to regress.

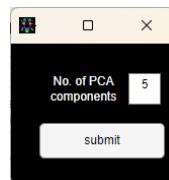


Figure 4-4: Number of PCA components to regress.

Output of the Nuisance Regression

- 1) Reg_<realigned functional image name>.nii :- Image with the specified confounds regressed.

4.6 Temporal Filtering

After Regression, the voxel time series from every voxel is filtered using a Butterworth bandpass filter of second order in the range specified by the user (default 0.01 to 0.15 Hz). The user may choose to skip the filtering step, by unchecking the check box besides Temporal filtering (Hz).



Figure 4-5: Filter frequency range

Output of the filtering

- 1) f<regressed functional image name>.nii :- filtered functional image within the specified frequency bands.

4.7 Smoothing

Smoothing is a very important step for WM analysis of BOLD signals. Some criticism in the field is that the GM BOLD signals mix into the WM signals. To completely avoid that WM and GM signals are separately smoothed. *WhiFuN* also allows the user to use the predefined smooth module for *SPM* that smooths the WM and GM together, but this is not recommended for WM analysis. Smoothing is performed using a Gaussian kernel with Full width at half maximum (FWHM) that can be specified by the user. Moreover, the users may choose not to perform the smoothing step.

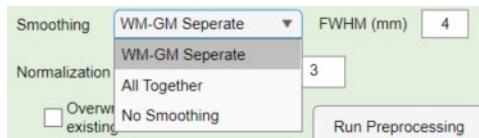


Figure 4-6: Options for Smoothing

Output of the smoothing

- 1) s<filtered functional image name>.nii :- Smoothed Image

4.8 Normalization to MNI space

Normalization is performed after smoothing, where images are transformed from the participant's native space to the standard MNI space with a voxel size specified by the user (default: 3 mm). A 3 mm voxel size is commonly used because after preprocessing, *WhiFuN* constructs Functional Networks using a voxel-level FC matrix. Using a smaller voxel size (e.g., <3 mm) increases the number of voxels, which may lead to excessive memory requirements, making voxel-level FC computation challenging.

The deformation field generated during segmentation is used for normalization. The normalization step has a bounding box parameter that determines the field of view of the

image with respect to the anterior commissure. The default bounding box parameter set in SPM is slightly expanded to [-90 -126 -72; 90 90 108] to ensure that all brain tissue regions are included.

A screenshot of a software interface showing a text input field labeled "Normalization (voxel dim (mm))" containing the number "3".

Figure 4-7: Normalization voxel dimensions.

Output of the Normalization

- 1) w<smoothed functional image name>.nii :- Image normalized in the MNI space with the voxel size specified by the user. Stored in the folder where the functional image is stored.
- 2) w<skull stripped anatomical image name>.nii :- Anatomical Image normalized in the MNI space. Stored in the folder where the anatomical image is stored.

5. Quality Control

Once the preprocessing is complete, the user is recommended to go through the quality control plots generated after most preprocessing steps. These quality control plots are saved in <Output_folder_path>/Quality_Control.

5.1 Initial Check

The raw anatomical and functional images are stored as <Participant_ID>_anat.png, and <Participant_ID>_func.png files respectively in Initial_check folder with the image contours on the standard MNI reference template for every participant. The user must check the initial position and orientation of the images with that of the MNI space. If the anatomical image is far from the MNI template or orientated differently, then the user must reorient the image manually to the template direction and reset the origin to the anterior commissure (Di & Biswal, 2023).

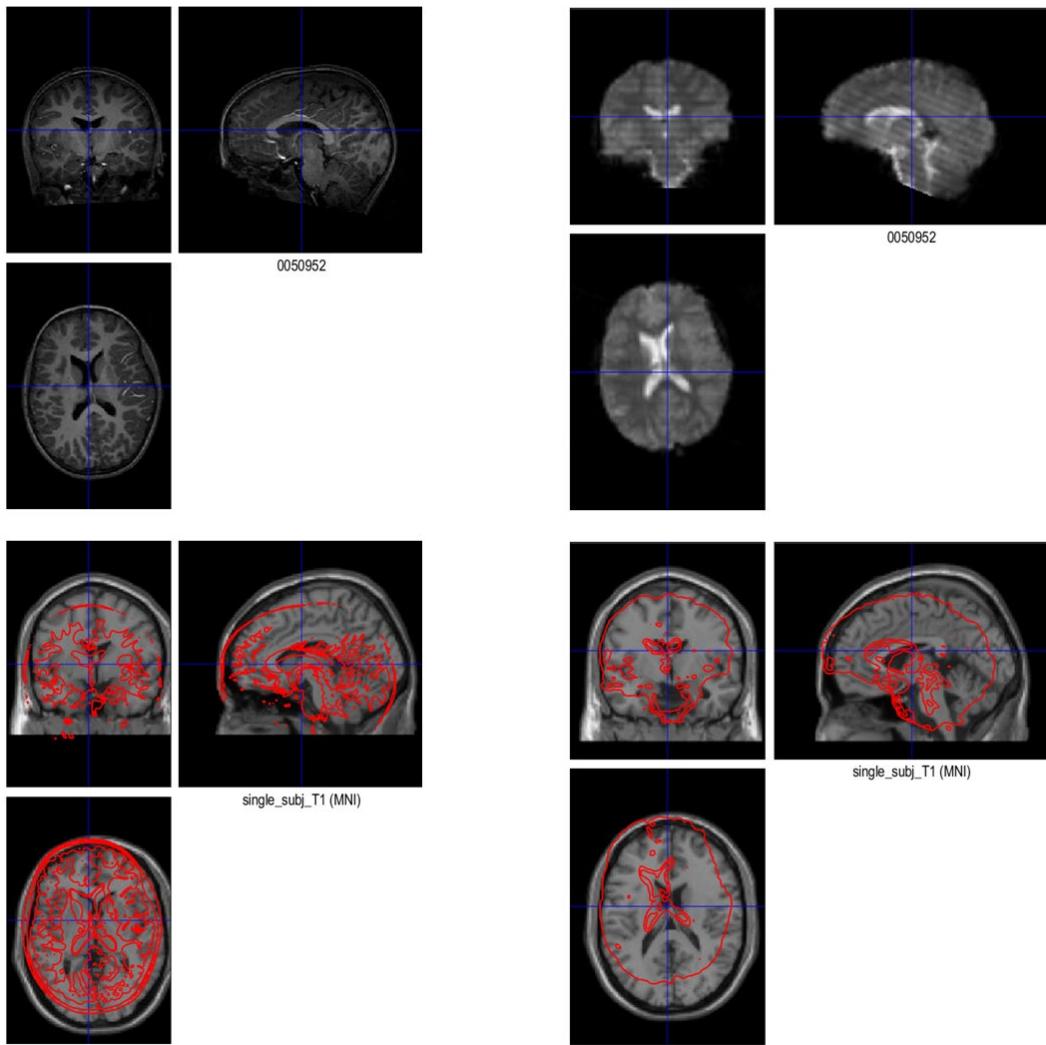


Figure 5-1: Initial Check Quality Control. The raw anatomical and functional image and their contours plotted on the standard MNI template

5.2 Head Motion

For each participant, a <Participant_ID>.png file is saved in the Head-Motion folder. This image contains a subplot with the following visualizations (See *Figure 5-2*):

- 1) Global BOLD Signal – Displays the overall BOLD signal across time.
- 2) Motion Parameters – Shows the translational and rotational motion parameters.
- 3) Pairwise Variance of the Global BOLD Signal – Illustrates variations in the global signal over time.
- 4) Framewise Displacement – Computed using the equation provided in *Section 4.3*, this plot quantifies participant movement between consecutive volumes. This plot also shows the thresholds used for head motion.

These plots allow the user to understand as to why *WhiFuN* rejected certain participants. By default, *WhiFuN* automatically excludes participants with excessive motion, requiring no additional action from the user.

It can be observed from the *Figure 5-2* that the spikes in the global signal correspond to the spikes in the motion parameters. This is the reason why the motion parameters are regressed out from every voxel time series (See section 4.5).

Additionally, an `excluded_<Participant_ID>.txt` file is saved for each rejected participant, indicating the specific FD time points that exceeded the threshold, leading to the rejection.

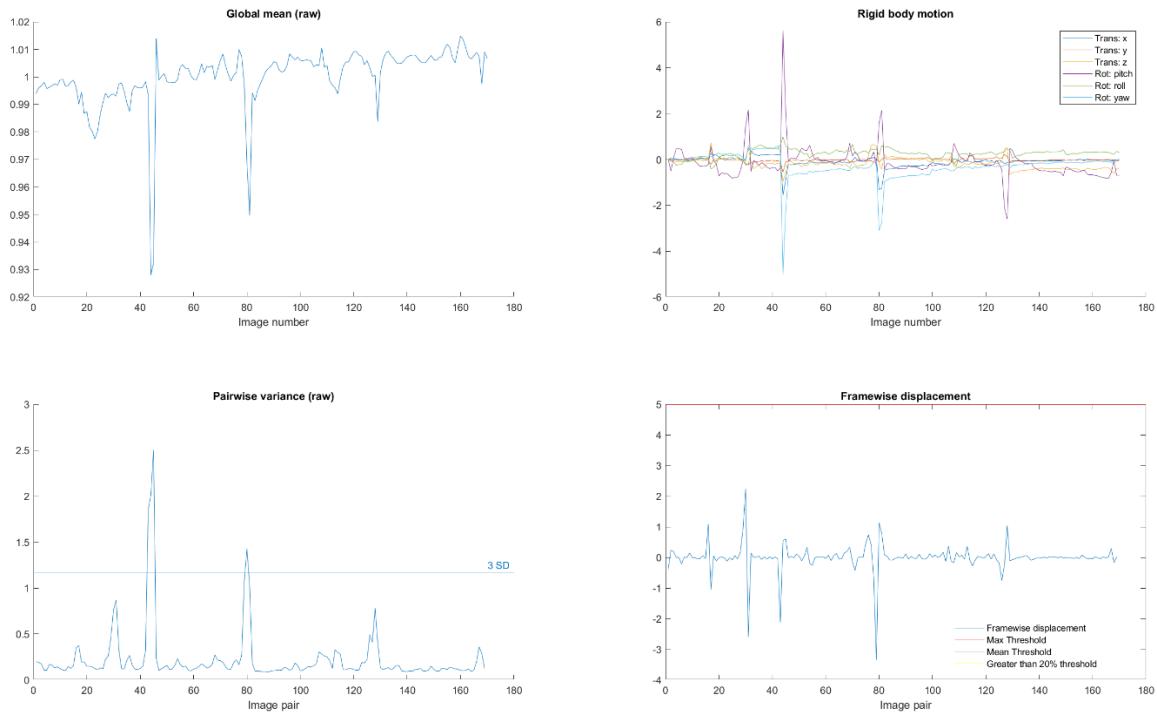


Figure 5-2: Head motion quality control plot.

5.3 Segmentation

After segmentation the GM, WM and CSF images are overlapped. *WhiFuN* saves the segmentation outputs with the GM, WM and CSF probabilities overlapped on the same image in red, green and blue colors respectively. It also draws the contour of the segmentation output on the reference MNI space to check if the alignment of the normalized anatomical image with respect to the MNI template is correct. Users must observe the images for each participant and note any misclassification of the tissues. If any of the tissues are misclassified, then that participant may be discarded. Also, if brain lesions or image quality issues are noticed in the anatomical image, this participant's data should be discarded (*Figure 5-3*).

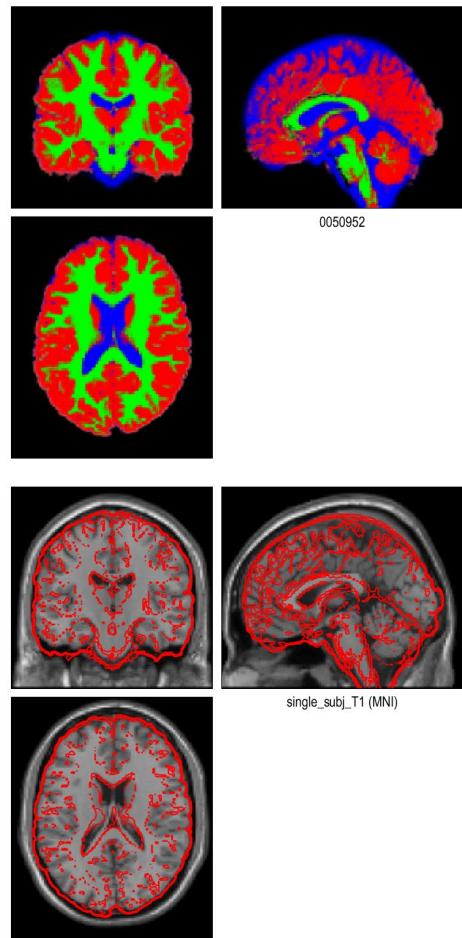


Figure 5-3: Segmentation Quality Control Plot. Gray Matter (Red), White Matter (Green) and Cerebrospinal Fluid (Blue) overlapped in the MNI space and its contour plotted on the standard MNI template.

5.4 Coregistration

The anatomical image is shown with the functional image, and the contour of the anatomical image is overlaid on the functional image in red. The user must check the orientation and alignment of the anatomical and the functional images. (The user should check if the images are not aligned, the user can then manually align them using the *manually coregister participants* module (See Section 6.2).

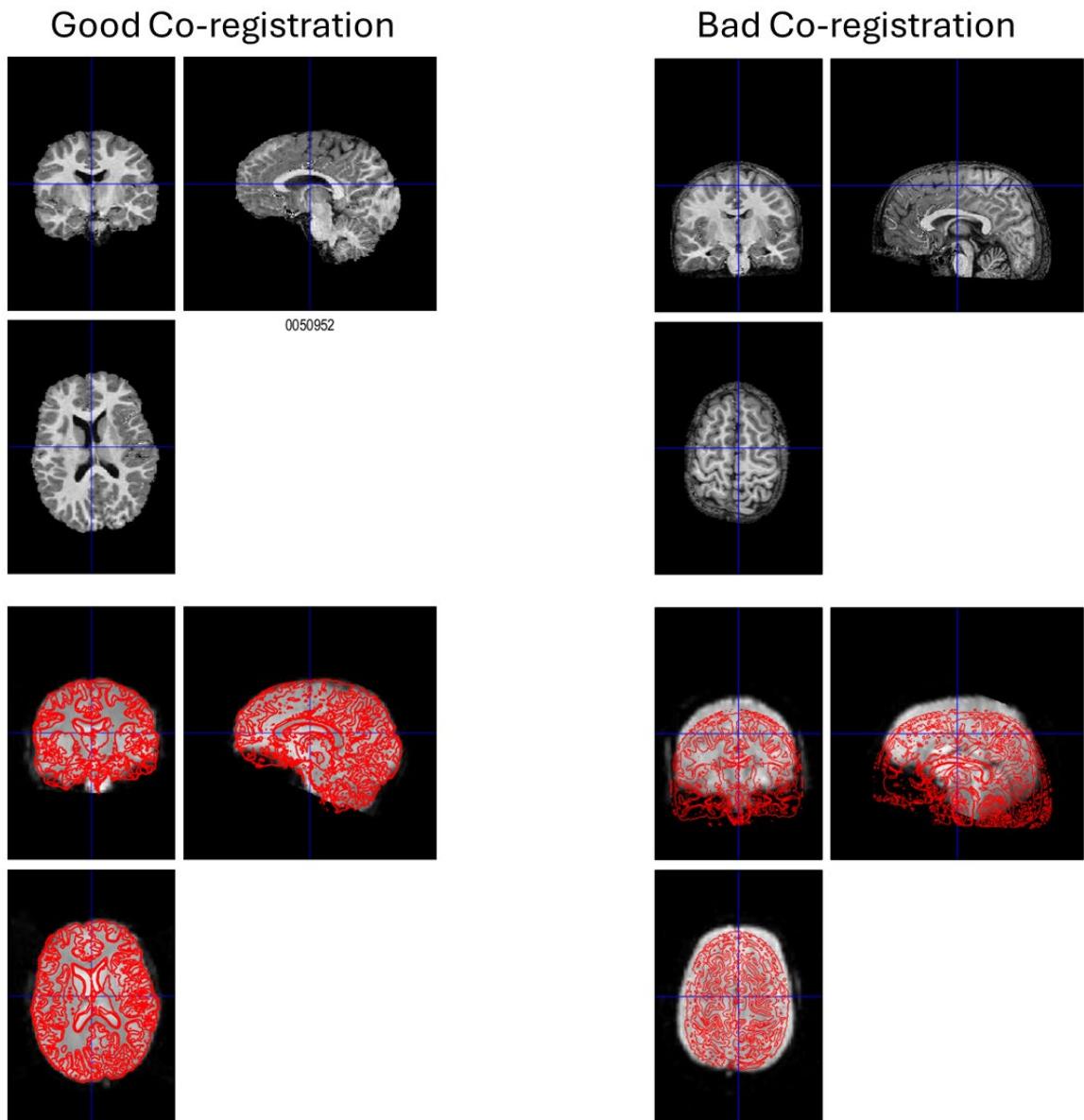


Figure 5-4: Coregistration Quality plot. Left, example of good coregistration Quality Control image. Right Example of a bad coregistered image. If the user observes an image on the right, he/she should go to section 6.2 to manually Coregister the image.

5.5 Cerebrospinal Fluid Masks

Here, the CSF mask extracted using a threshold of 0.95 on the tissue probability map of CSF is plotted with the functional image along with the contour of the CSF mask on the rsfMRI image. The user must check the alignment of the CSF mask and the rsfMRI image. It is observed in some cases that no voxels satisfy the 0.95 threshold. In such cases, the anatomical image must be rechecked, and the user may discard this participant.

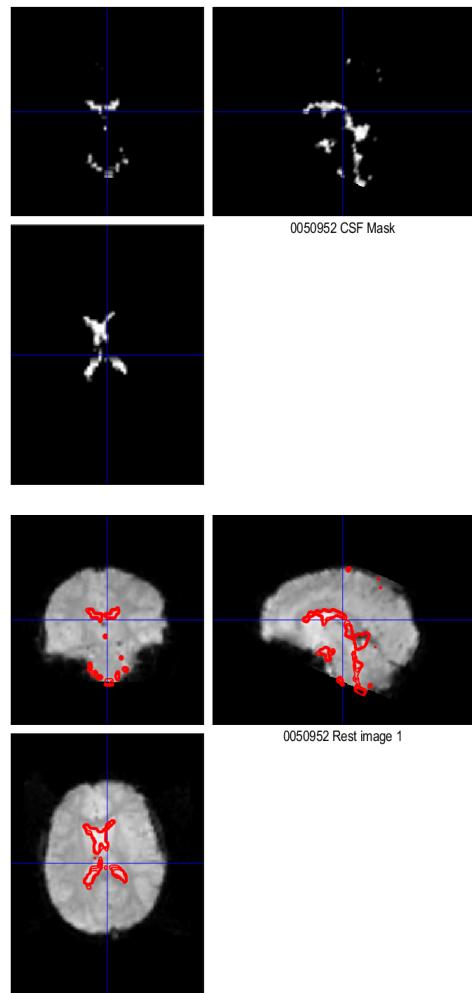


Figure 5-5: CSF mask plotted, and the contour of the CSF mask overlapped on the functional image.

5.6 Effects of Regression and Temporal filtering

The global (average) signal before and after regression of the motion parameters and the CSF signals is plotted. The user may observe that any upward or downward trends and artifacts due to motion are removed after regression.

Here, the user may observe how very high or low-frequency signals are removed during filtering. These plots are used primarily for observing the effects of regression and temporal filtering on the global signals. The user does not need to take any additional steps here.

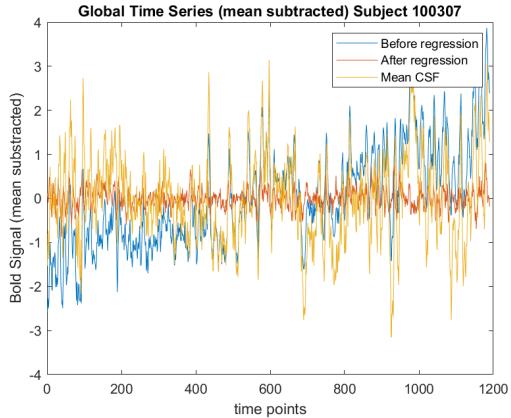


Figure 5-6: Regression Quality Control plot

5.7 Effects of Smoothing

Based on the options chosen by the user, either Smooth GM-WM separately (Recommended for WM Analysis) or Smooth all together, WhiFuN stores the smoothed image. One may observe that when the GM and WM voxels are smoothed separately, the cortex gets a number of small holes as these voxels were neither identified as GM nor WM. The accuracy of segmentation depends on the resolution of the anatomical and the rsfMRI images. If the user observes a large number of holes in the cortex, the participant may have to be discarded.

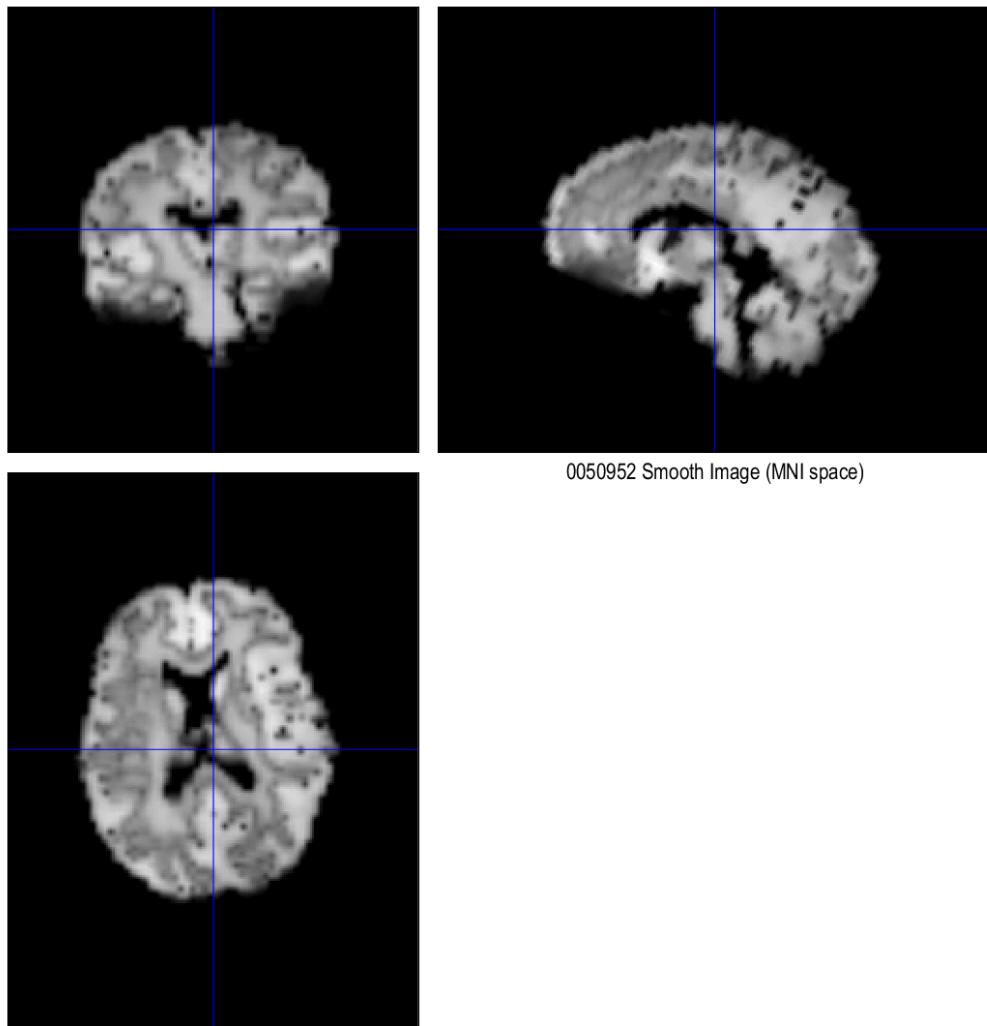


Figure 5-7: GM-WM smooth separately QC image

5.8 Normalization QC plot

The normalized functional image is plotted with the reference MNI template image with the contour of the normalized rsfMRI on the reference MNI template. The user must ensure that the spatial alignment of the normalized rsfMRI image with that of the MNI template is correct.

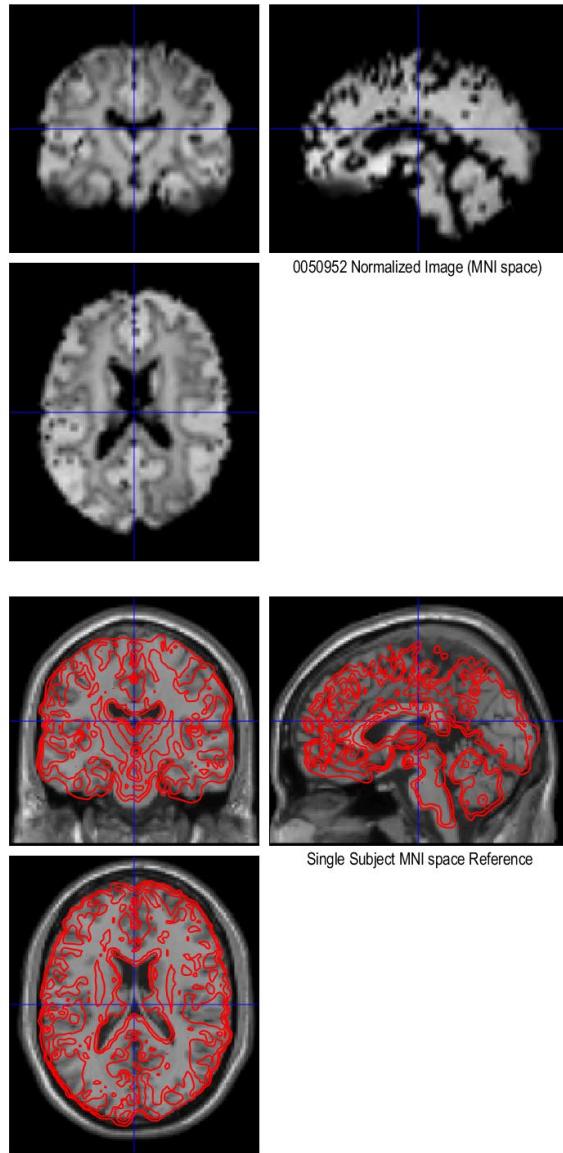


Figure 5-8: Normalization QC plot. The Normalized functional image in the MNI space is plotted with its contour overlapped on the standard MNI template.

5.9 Time-Series Check

The user can observe the associations between the global and noisy signals at this step. *WhiFuN* plots the raw unprocessed global signal, the six rigid motion parameters computed during realignment, the CSF signals, the global mean of the preprocessed image, and the correlation of all the above-mentioned signals. *WhiFuN* also plots pairwise variance between consecutive volumes (similar to DVARS (Power et al., 2014)) for the unprocessed image, the framewise displacement, the derivate of CSF signals, the pairwise variance between consecutive volumes for the preprocessed image and the correlation of these signals. The user must check the correlation plots and observe if the preprocessed global signal is correlated to the noisy signals. If the correlation of the

global signal corresponding to the final preprocessed signal with any of the noisy signals is significant, the user may exclude the participant.

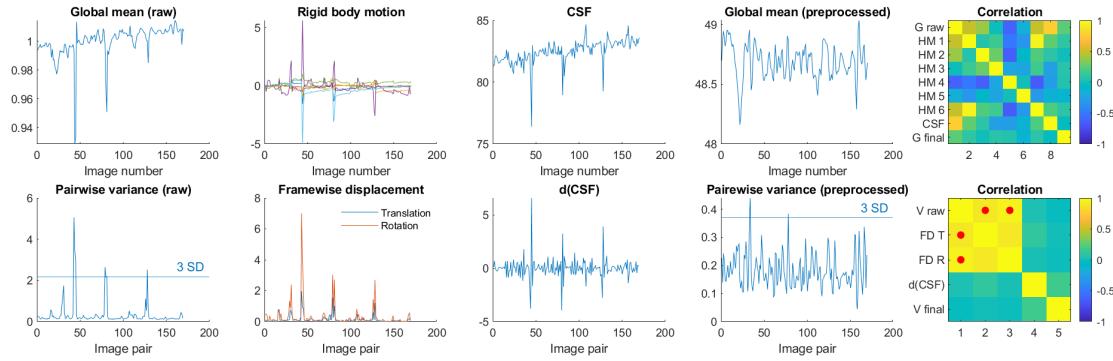


Figure 5-9: Timeseries plot showing the global time series before preprocessing, the 6 motion parameters, the mean CSF time series, the global mean signal after preprocessing and a correlation matrix correlating these signals. The same is repeated for pairwise variance across consecutive scans instead of the global mean in the 2nd row.

5.10 Error Handling

If *WhiFuN* encounters any unexpected error while processing a particular participant at a particular preprocessing step, it will store the error message in a txt file named <Participant ID>_error_info.txt in the Error_info folder located in the Quality Control folder.

It is recommended that the user examines all QC plots of all the preprocessing steps to remove outlier participants from further analysis.

6. Tools for Preprocessing

After checking the Quality Control plots, the user can use the following tools to improve or redo the preprocessing if there were some errors.

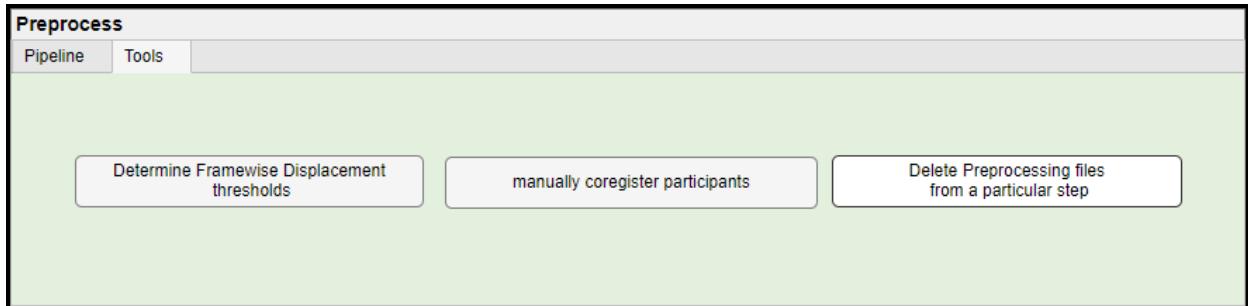


Figure 6-1: Preprocessing tools

6.1 Determine Framewise Displacement thresholds

After preprocessing, based on the motion thresholds, WhiFuN skips preprocessing steps for the participants that had excessive motion. Thus, it may be possible that not all the participants were preprocessed. However, the Realignment step will be done for all the participants that were selected and the rp_<functional_image_name>.txt file will be generated for every participant in the functional folder. The user can see how many participants were rejected and the reason for the rejection by using the Determine Framewise Displacement thresholds button. Once the button is clicked, a GUI shown in *Figure 6-2* opens. This shows the Maximum FD, the Mean FD, the percentage of FD greater than 0.2 for every participant and the corresponding histograms along with the thresholds used. Using the Drop down.

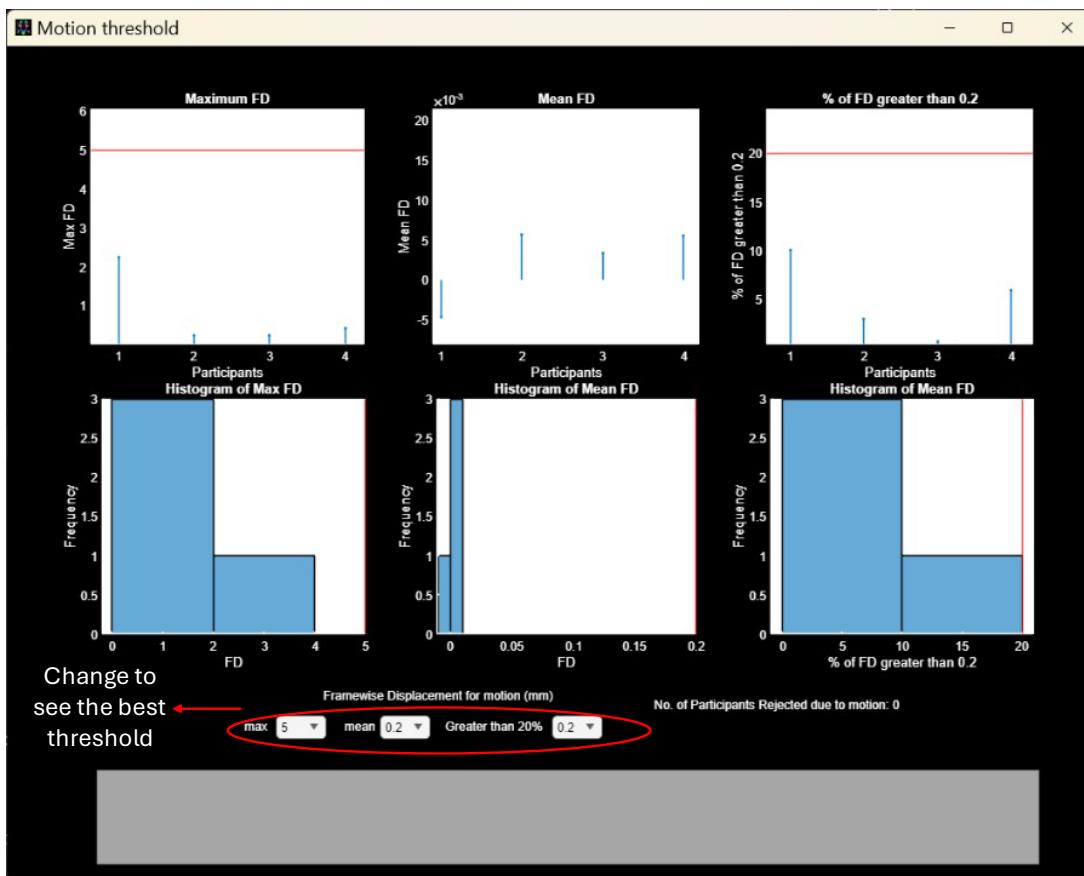


Figure 6-2: Determine the Framewise Displacement thresholds.

If a Participant is rejected as it did not pass the threshold then the participant ID and the reason why it would be rejected will be shown in the gray text box (See *Figure 6-4*).

Based on this the user can decide what threshold to keep such that optimal number of participants can be rejected. Once the optimal thresholds are decided then the user must use these new thresholds and run the preprocessing again. The user should make sure that the Overwrite existing files checkbox is not checked (See *Figure 6-3*). WhiFuN will run the

preprocessing with the new thresholds, if the preprocessing was already completed for participants, WhiFuN will skip the preprocessing corresponding to those participants.



Figure 6-3: Ensure the Overwrite existing files is unchecked.

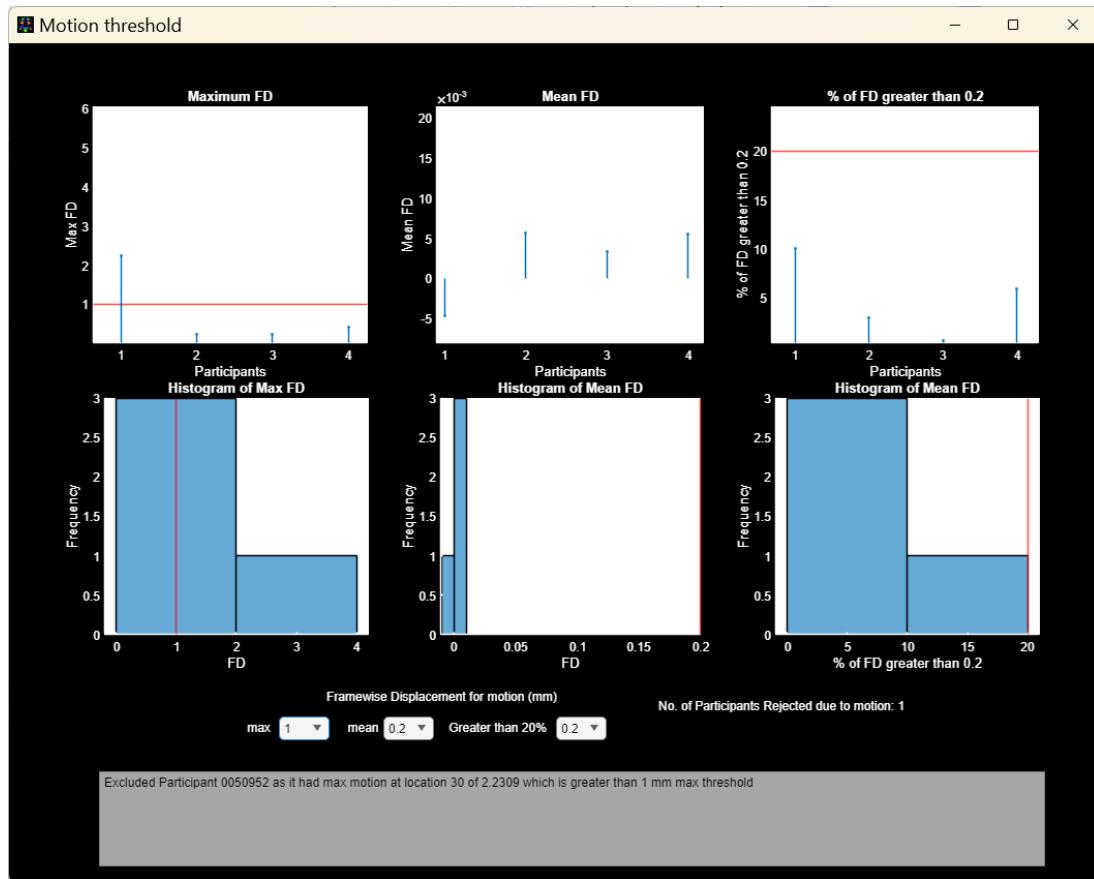


Figure 6-4: Participant Rejected as it exceeded the Max FD threshold.

6.2 Manually Coregister Participants

If the User observes that the coregistration corresponding to few participants is not proper (See Section 5.4) then those can be manually coregistered using this module. Once the user clicks the manually coregister participants button, a file explorer will pop asking the user to select the participant folder corresponding to the participant that has bad coregistration (*Figure 6-5*)

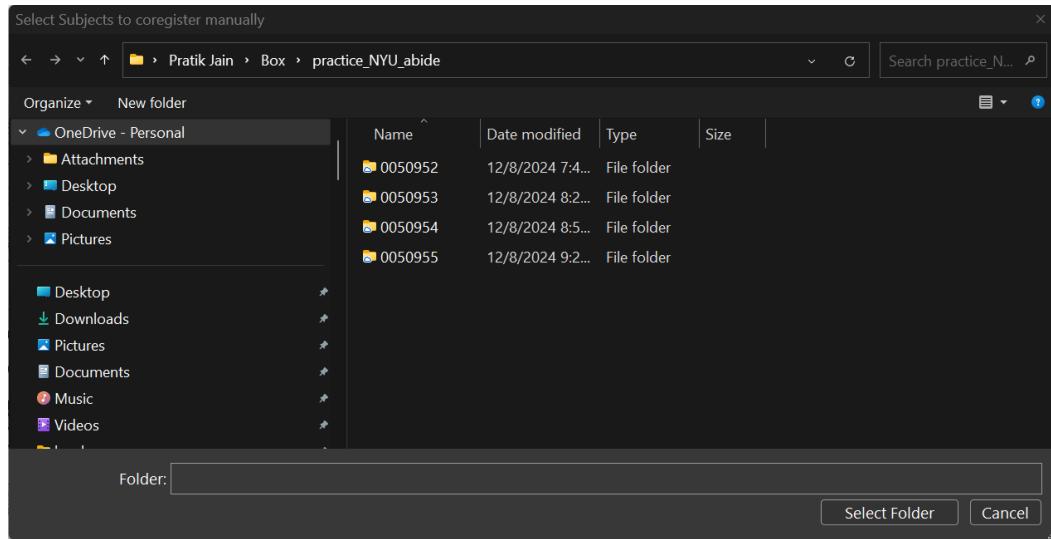


Figure 6-5: Select Participant folder that has bad coregistration.

Once the participant folder is selected, WhiFuN will prompt that the preprocessing files created after realignment steps (if exist) will be deleted. Since the Coregistration have to be performed, the preprocessing has to be redone. Once the user clicks the Yes button, WhiFuN will delete all the preprocessing that are not required and open the SPM manual coregistration module.

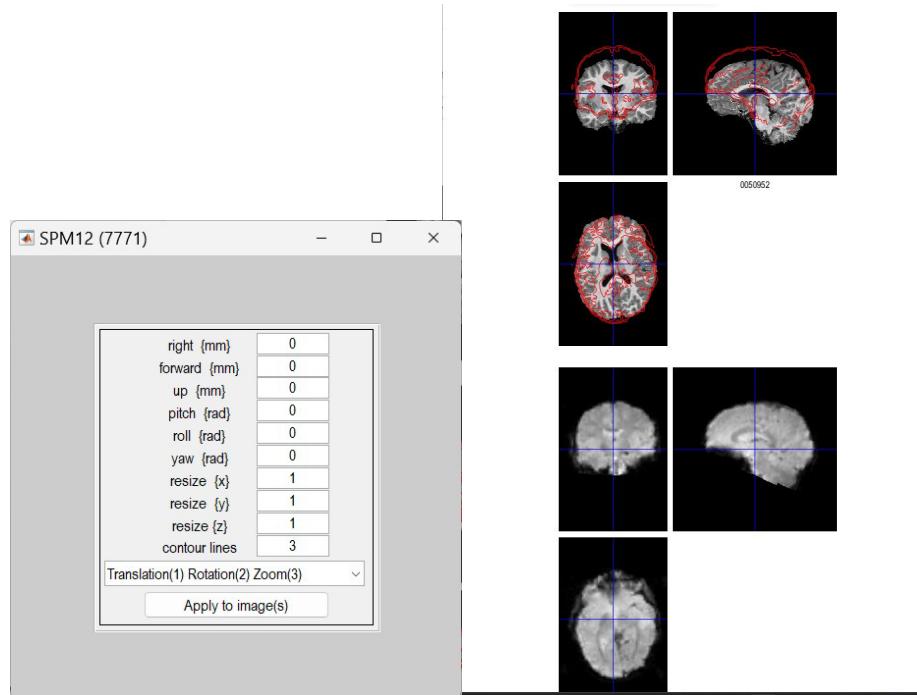


Figure 6-6: The SPM coregistration module pops up when the Participants are selected to manually coregister.

The user can manually enter the amount of translation or rotation required so that the contour of the functional image aligns with the anatomical image.

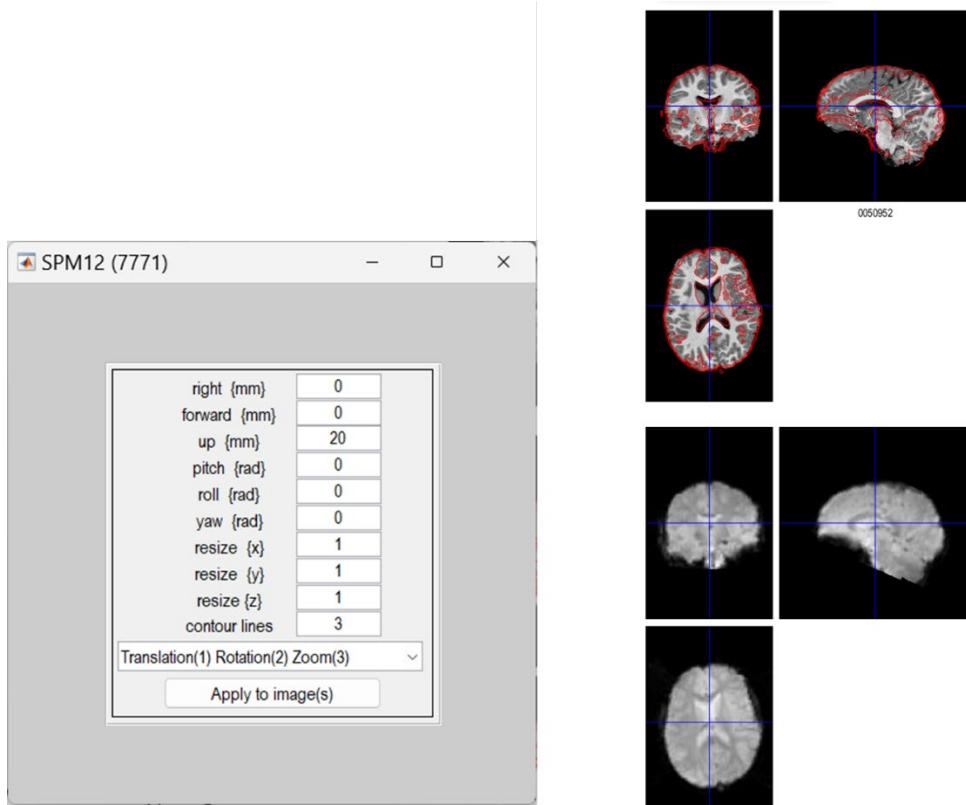


Figure 6-7: Users can manually enter the amount of translation or rotation required to align the contour of the functional image to the anatomical image.

Once the contours are aligned, the user should click the Apply to image button, which will open an SPM pop up (See *Figure 6-8*).

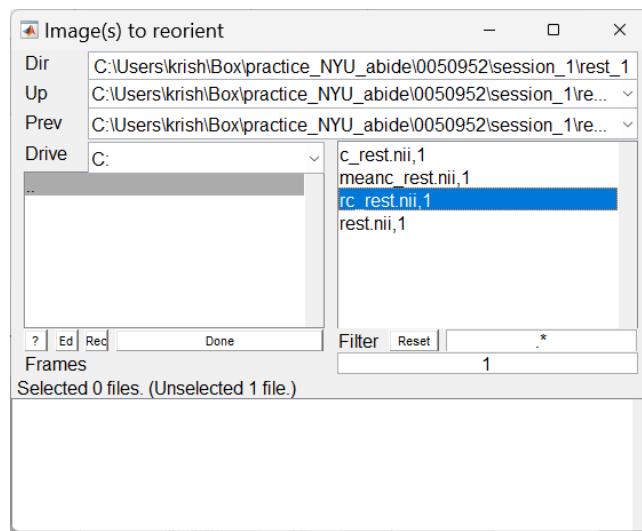


Figure 6-8: SPM pop up to apply the manual coregistration to the images

The user must select the realigned images that start with prefix ‘r’ if the user has also discarded some initial volumes the file name will be rc_<functional_image_name>.nii, else it will be r<functional_image_name>.nii. (in this example, <functional_image_name> = rest). Based on the preprocessing steps the prefix can be given to the filter as follows

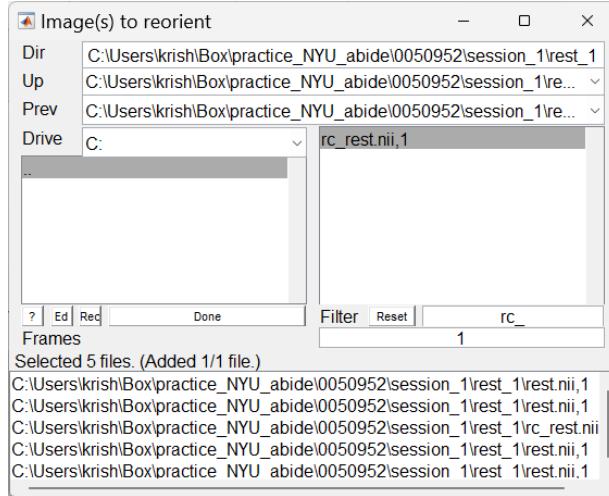


Figure 6-9: Enter the prefix of the Realigned file as filter to just select the Realigned files. Prefix = r, if no initial volumes were discarded, elseif initial volumes were discarded Prefix = rc_ (default and also shown here)

It is essential to select all the functional volumes/time points, for that enter the array of volumes that has to be coregistered. In our example dataset, there are 170 time points or volumes so the array will be 1:170, In general if there are nt time points the array will be 1:nt (See *Figure 6-10*).

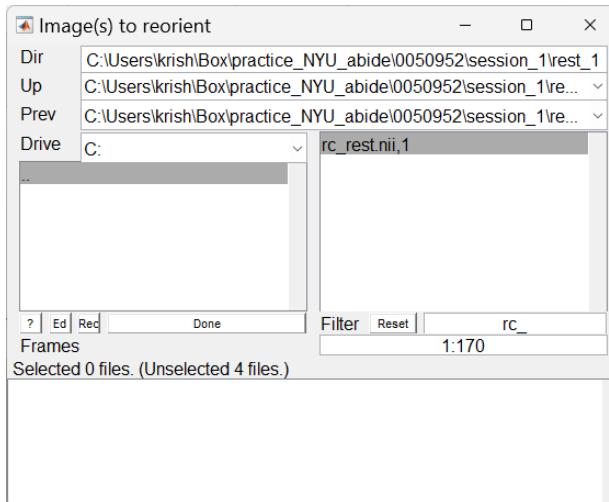


Figure 6-10: Selecting all the time points/volumes.

Then the user can use the right mouse click and select all the time points/volumes.

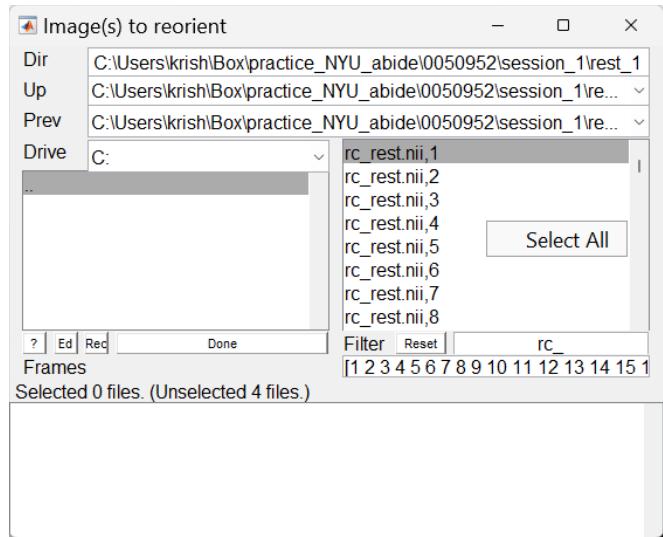


Figure 6-11: Select all time points/volumes.

Once all the files are selected, click on done and SPM will ask to save the transformation matrix (reorientation matrix), it is always advisable to save this matrix. Click yes and save the matrix in the functional folder of that participant and SPM will coregister the files.

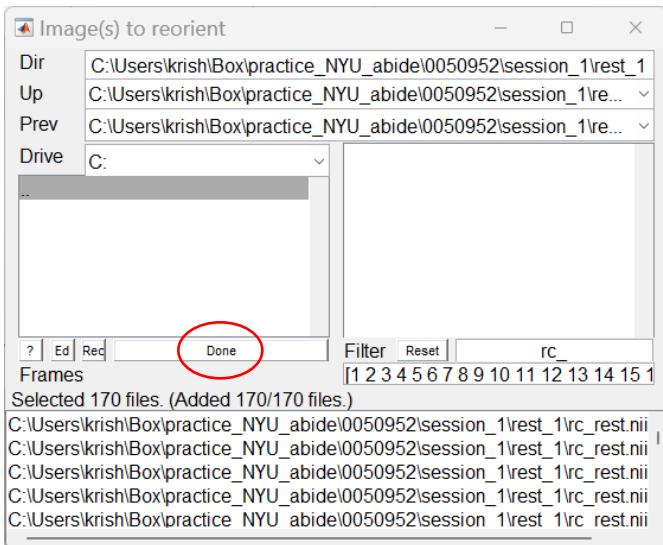


Figure 6-12: Finally Click on Done to coregister the images.

The user must ensure that the overwrite existing files check box is not checked and run the preprocessing again after this.

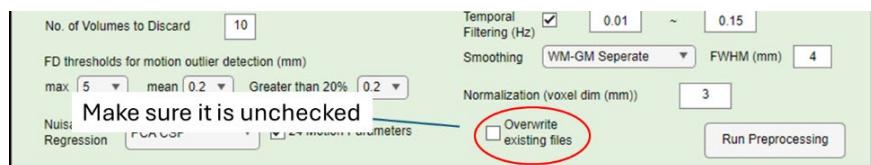


Figure 6-13: Ensure the Overwrite existing files checkbox is unchecked.

6.3 Delete Preprocessing Files from a Particular Step

If the user finds that the preprocessing was not done properly for a particular step and wishes to redo the preprocessing from that step, this module can be used. When the user clicks on Delete Preprocessing Files from a Particular Step, A GUI pops up (See *Figure 6-14*).

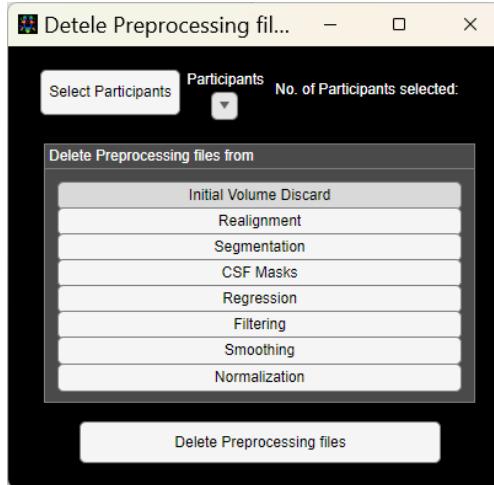


Figure 6-14: Delete Preprocessing files from a Particular step GUI.

The user will first select the participants for which the files have to be deleted and then the step after which the files must be deleted. For instance, the following *Figure 6-15* shows how all preprocessing files after CSF Masks (Including CSF Masks) are deleted for two participants.

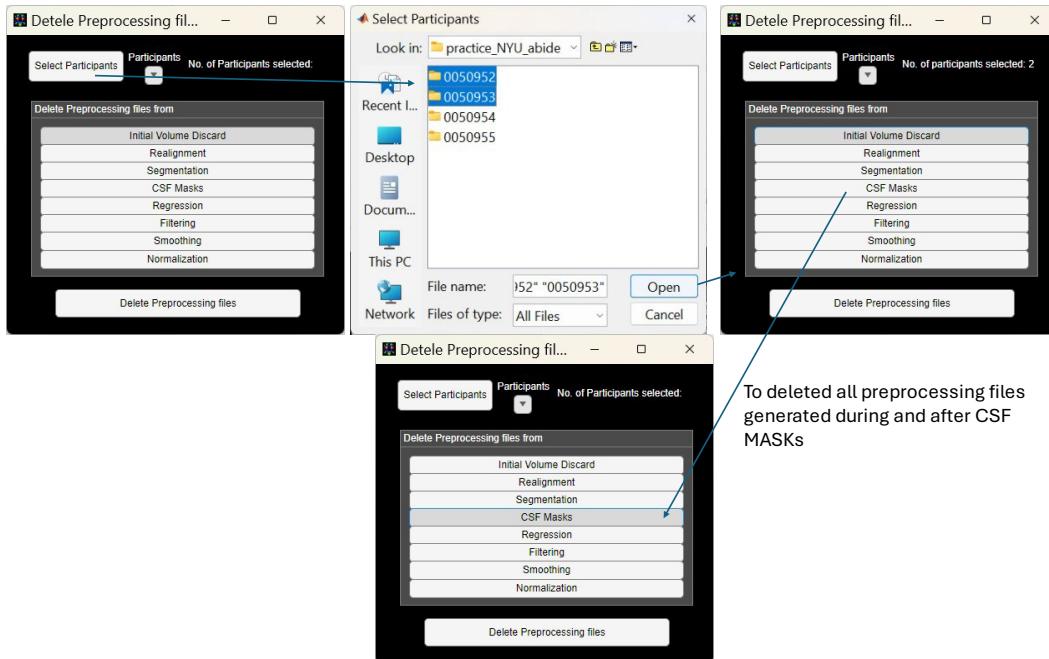


Figure 6-15: Delete Preprocessing files from a Particular step. Figure shows how preprocessing files generated during and after CSF Masks corresponding to two participants can be deleted

7. Construct Functional Networks and Functional Connectivity

This section discusses how the Functional Networks can be extracted using the K-Means algorithm and Functional Connectivity maps can be generated by storing the average timeseries corresponding to every region in a predefined atlas supplied by the user.

7.1 Excluding the participants with bad quality

After Preprocessing, the User must go through all the Quality controls plots described in *Section 5* and reject outlier participants. If the user detects outlier participants, the user can discard them by clicking on Manually exclude participants check box (See *Figure 7-1*).

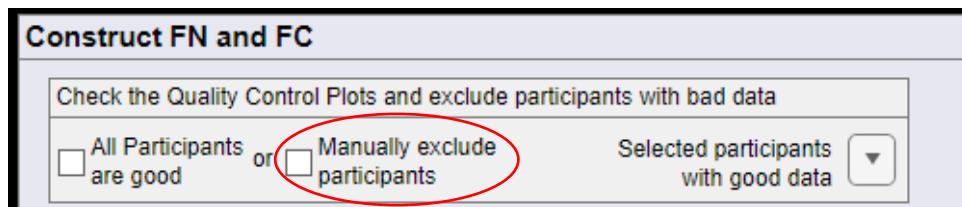


Figure 7-1: Reject participants that have bad Quality Control plots.

Once the user checks the Manually exclude participants check box, WhiFuN opens the participant data folder that has all the participant folders (See *Figure 7-2*). The

User can select the participant folders corresponding to the participants that need to be excluded due to bad quality. User can use the Ctrl button to select multiple participants.

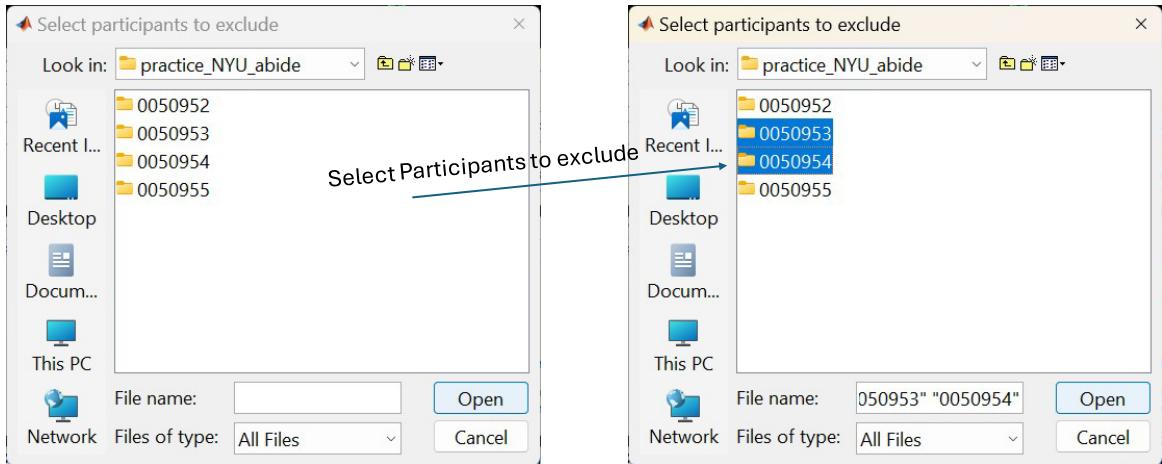


Figure 7-2: Select Participants to exclude.

Else, if all the participants are good, click on All participants are good checkbox (See *Figure 7-3*).

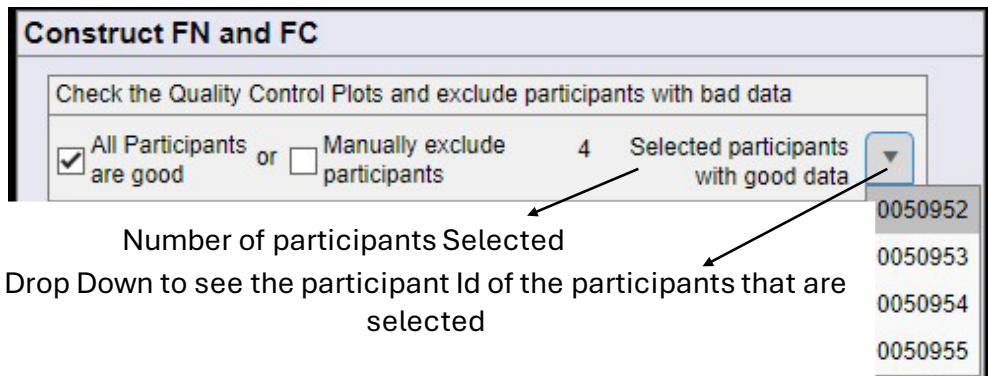


Figure 7-3: Select All participants and other details.

7.2 White matter and Gray Matter Functional Networks

This module discusses how the WM and GM Functional Networks (FNs) can be created using WhiFuN. This module is applied to the fully preprocessed rsfMRI images. Group masks are created first to identify the WM or GM voxels across the participants. The K-means algorithm clusters the voxels in WM and GM to create the WM and GM-FNs based on voxel-based FC (See *Figure 7-4*).

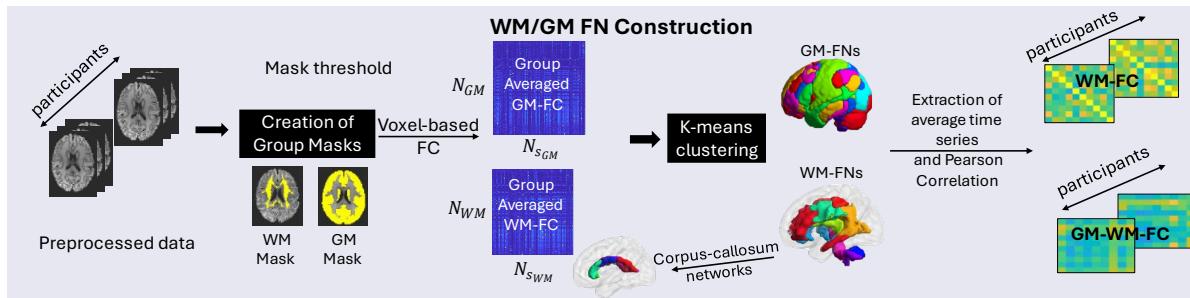
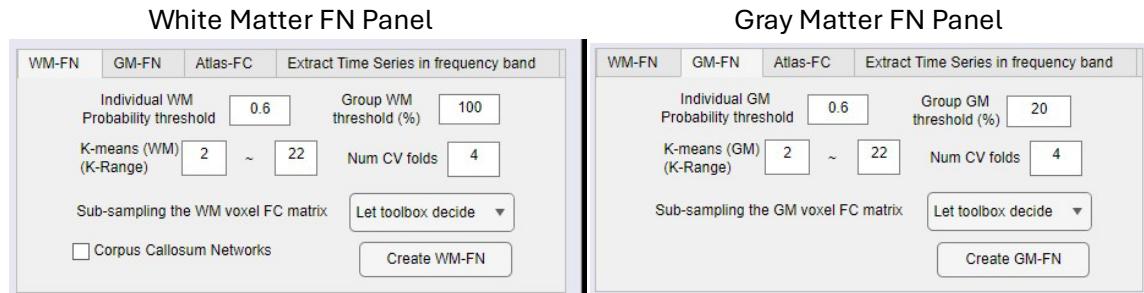


Figure 7-4: Flow Chart of WM/GM FN Construction

Once the FNs are constructed, the averaged time series corresponding to every FN is extracted and stored as .mat files.



7.2.1 Group Masks thresholds

The Individual WM/GM probability Threshold and the Group WM/GM threshold (%) are parameters to create the Group WM/GM Masks. These thresholds decide which voxels to consider in the Group WM/GM Masks.

All voxels for which at least Group WM/GM threshold (default 100% for WM and 20% for GM) of participants have WM/GM probability greater than an Individual WM/GM probability Threshold (default 0.6) are included in the group GM/WM mask.

The highest value that the Individual WM/GM probability can take is 1. Higher values will lead to a smaller WM/GM Mask.

The highest value that the Group WM/GM threshold (%) can take is 100. Higher values will lead to a smaller WM/GM Mask.

7.2.2 K-means parameters

WhiFuN requires the user to specify a K-value range (default 2 to 22). For every K-value in the range, the average dice coefficient and the distortion value are computed to help the user find the optimal K-value.

If the user inputs the same value for the lower and the higher range (Eg. 6 ~ 6) then WhiFuN will skip the cross validation and directly create clusters with the value mentioned (Eg. 6).

A cross-validation approach is applied to determine the optimal number of clusters (K). The user can specify the number of cross validation (Num CV folds (default 4)).

Based on the memory available in RAM, WhiFuN can decide whether to use the complete voxel FC matrix or use a subsampled version of the matrix proposed by (Peer et al., 2017).

7.2.3 Corpus Callosum Networks

Under the WM-FN tab, there is a checkbox for Corpus Callosum networks. If this is checked, WhiFuN will divide the corpus callosum into sub regions that are maximally correlated to the created WM-FNs. The method proposed by (Wang et al., 2020) is used to create the Corpus callosum networks.

7.2.4 Create WM/GM FN

Once all the parameters are set, users can click on the Create WM-FN or GM-FN button.

- 1) WhiFuN will first create the Group WM/GM mask. The Group Masks will be stored in the <output_folder_path>/Analysis/Group_Masks as WMmask_allsubjs.nii and Gmmask_allsubjs.nii for Wm and GM respectively.
- 2) Once the Masks are created, WhiFuN will compute the average voxel-level FC using the data from all the participants that were selected with good data. Thereafter WhiFuN uses the cross validation approach proposed by (Peer et al., 2017) to find the optimal value of K for the K-Means clustering (this step will be skipped if the user inputs the same value for the lower and the higher range of K values).
- 3) After the cross-validation Grid search is complete for all the values of K, WhiFuN will plot a graph of Dice coefficients and Distortion values vs the K values.
- 4) WhiFuN will have a pop up asking the user what value of K should be chosen See *Figure 7-5*.
- 5) An optimal K-value has a high average dice coefficient and low distortion value. As one can observe from *Figure 7-5*, there are local peaks of Average Dice Coefficients. The K values corresponding to these peaks can be chosen as the optimal K value. These plots are saved as .png files in

<output folder>/Analysis/WM_FN/K_grid_search_dice_coefficient_WM.png
<output folder>/Analysis/GM_FN/K_grid_search_dice_coefficient_GM.png

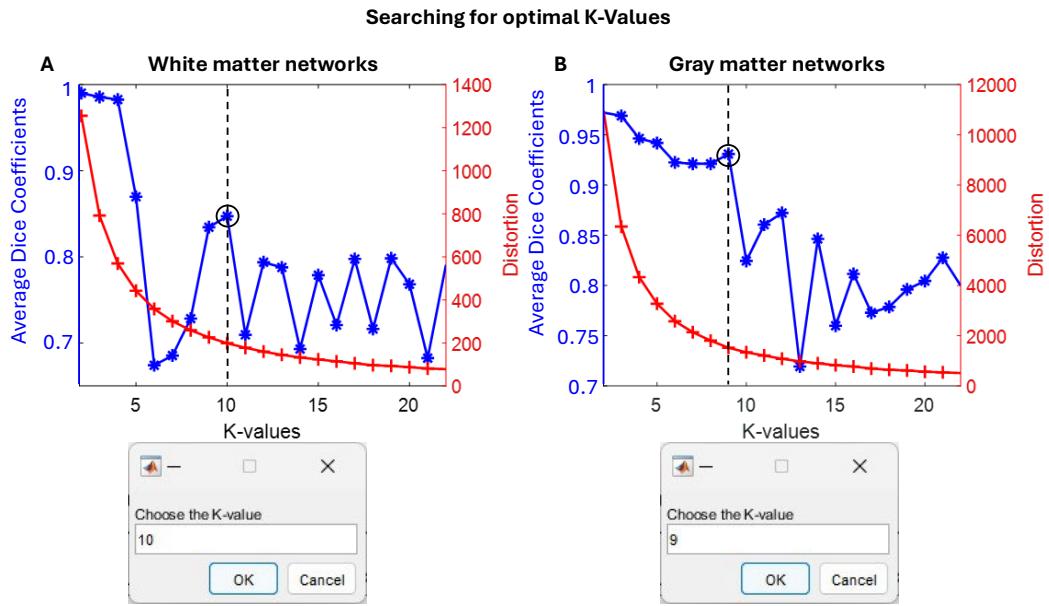


Figure 7-5: Finding the optimal number of FNs (K value). A: for WM-FNs and B: for GM-FNs. For this example K= 10 and 9 can be chosen as optimal K values for WM-FNs and GM-FNs respectively

- 6) Once the optimal K-value is selected, WhiFuN will compute the FNs and store the FN as a .nii file with name WM_clustering_K<value of K used>.nii for WM-FNs and GM_clustering_K<value of K used>.nii for GM-FNs.
- 7) The average BOLD time series of every WM and GM network for every participant are stored as a network_avgts_wm_K<value of K used>.mat and network_avgts_gm_K<value of K used>.mat file in
 - <output folder>/Analysis/WM_FN for WM
 - <output folder>/Analysis/GM_FN for GM
- 8) WhiFuN also stores 6 views of the WM-FNs in a folder WM_clustering_K<value of K used>_BrainNet_images in the WM_FN folder.
And the same for GM -FNs in the folder GM_clustering_K<value of K used>_BrainNet_images in GM_FN folder.
- 9) If the Corpus Callosum (CC) networks checkbox was checked, the CC-FNs will be stored in
 - <output folder>/Analysis/Corpus_Callosome_FN/CC_network_from_WM_K<value of K used for WM-FNs>.nii

The Mask used to identify the Corpus Callosum voxels is taken from Mori atlas (Mori et al., 2008) and is stored in the Corpus_Callosome_FN folder as

Corpus_callosum_atlas_MNI_<voxel dimension of MNI space>mm.nii

- 10) The average time series from every CC-FN is stored in the Corpus_Callosum_FN folder as network_avgts_cc_K<value of K used for WM-FNs>.mat
- 11) WhiFuN also stores the time series corresponding to every voxel in CC as cc_signals.nii.

7.3 Atlas based Functional Connectivity

If the user has a predefined atlas and they wish to compute the FC matrix using the atlas, WhiFuN's Atlas-FC module can be used.

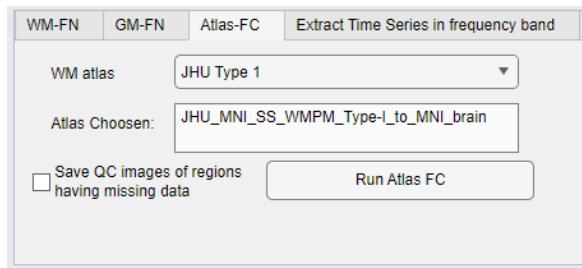


Figure 7-6: WhiFuN's Atlas FC module.

Here the user can choose the atlas. WhiFuN has already included JHU type 1, type 2 and type 3 atlases, but the user can also specify any other atlas (.nii file).

7.3.1 Quality Control for Atlas Based FC

Once the atlas is specified, the user can opt to save QC images for the participants that have some NAN or missing values in regions of the atlas. An example of the QC image is shown in *Figure 7-7*. It shows the functional image for a particular participant (in this case Participant 0050952) and Atlas region 19 from the JHU type 2 atlas, with the contour of the atlas region overlapped on the Participant's functional image. It can be observed that this participant does not have data for few voxels that are in the region. It is important to know that.

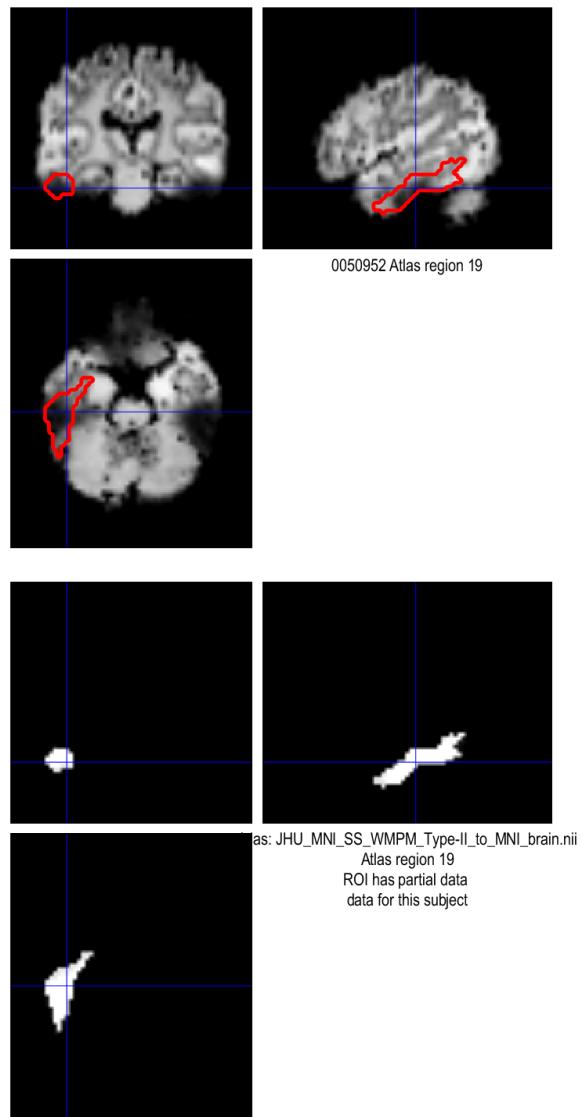


Figure 7-7: Quality Control for Atlas-FC

7.3.2 Run Atlas FC

Once the user Clicks on the Run Atlas FC button, WhiFuN will compute the average time series corresponding to every region in the atlas for every participant which can be used to compute the FC matrix using Pearson Correlation. (See *Figure 7-8*)

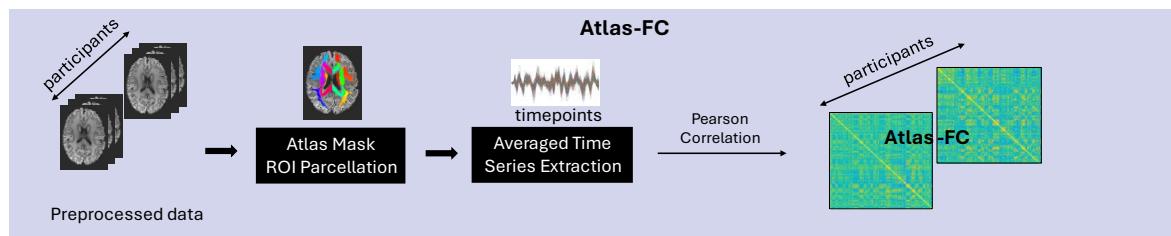


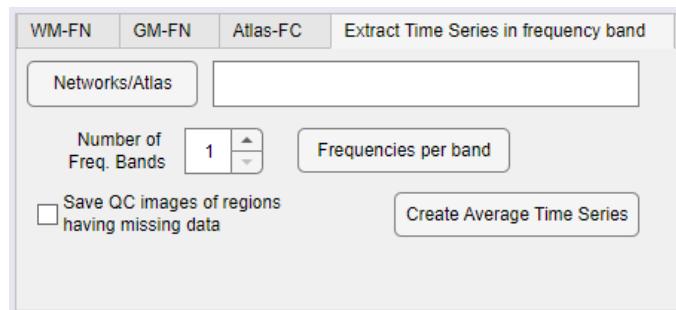
Figure 7-8: Atlas-FC computation

The average time series is stored as a .mat file with name <atlas_name>_reg_ts.mat in <output_folder>/Analysis/Atlas_FC folder.

If the dimensions of the specified Atlas is different from that of the functional images, WhiFuN will reslice the atlas and then use the resliced version of the atlas to compute the average timeseries. The atlas that is used to compute the average time series will also be stored in the Atlas_FC folder.

7.4 Extract Time series in frequency band

Users can extract the average time series in a particular frequency band, to study the frequency specific characteristics.



Users can specify the atlas and the number of frequency bands. Then by clicking on the Frequencies per band button a new window pops up where the user can specify the different frequency bands and the names corresponding to these frequency bands and click submit. (See *Figure 7-9*)

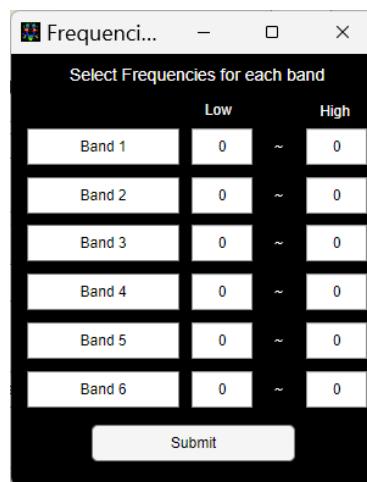


Figure 7-9: Frequencies for each band GUI

QC plots as mentioned in Section 7.3.1 can also be created here by checking the Save QC images of regions having missing data checkbox.

7.4.1 Create Average time series

Once the user clicks the Create Average time series button

- 1) WhiFuN creates the frequency specific time series in the following Atlas folder
`<output_folder>/Analysis/Extract_timeseries/<atlas_name>`
- 2) The frequency specific time series are stored as
`<atlas_name>_<band_name>_reg_ts.mat.`
- 3) Additionally, the magnitude and phase response of the filter used is also stored in
`<output_folder>/Analysis/Extract_timeseries/<atlas_name>/Filters`
With name `<band_name>_filter_freq_response.png`.
- 4) The WM/GM and CC FNs created by WhiFuN (see Section 7.2) can also be used here to extract the frequency specific time series.

8. Analyze Functional Networks and Functional Connectivity

This module analyses the FNs and FC created during the Construct FNs and FC module by finding significant connections using statistical tests in FCs, checking how symmetric the FNs are across the two hemispheres or comparing the FNs computed by WhiFuN with any other predefined atlas to understand the network labels.

8.1 Compare FNs

The compare FNs module can be used to compare two sets of FNs. This function compares the FNs based on the Dice coefficient for two sets user specified FNs. This feature is helpful if the user wants to label the FNs by comparing them to a predefined atlas. We explain the working of this module by demonstrating how the WM-FNs generated by WhiFuN can be compared to the JHU-DTI81 atlas (Mori et al., 2008).

When the user clicks the Compare FN button, WhiFuN asks the user to select the first network. Users are expected to select the nifti file corresponding to the first network (See *Figure 8-1*). For instance, we select the WM-FN created by WhiFuN (See Section 7.2 for details) as the first network.

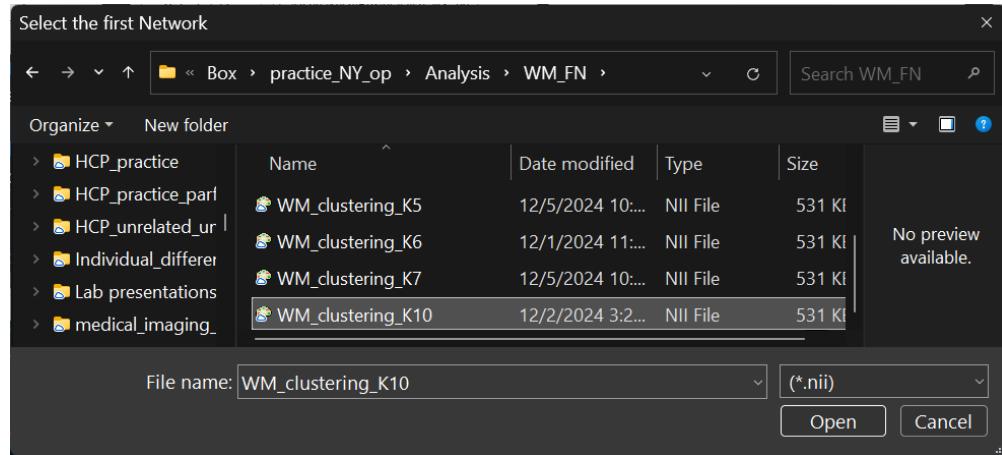


Figure 8-1: Select the First Network

Once the user clicks open, WhiFuN asks the user to select the second Network. We select the JHU-DTI-81 atlas (Mori et al., 2008).

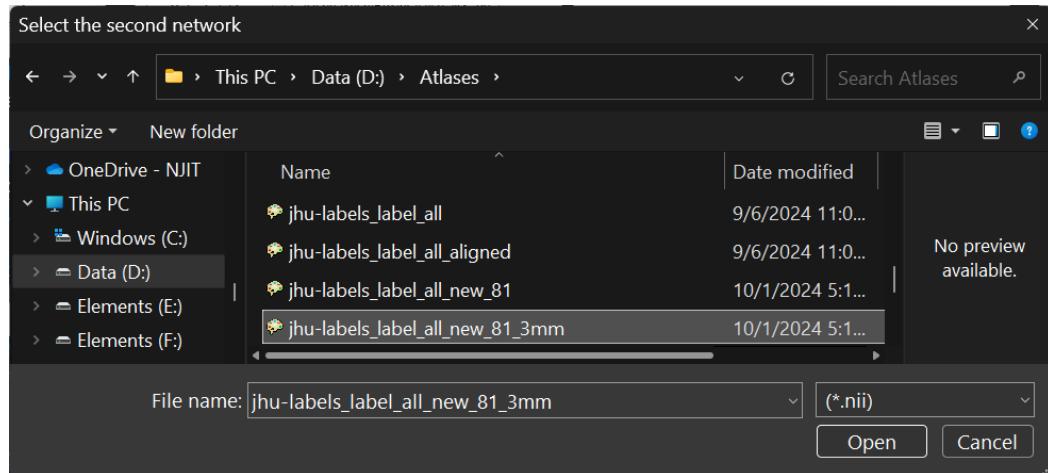


Figure 8-2: Select the Second Network

Once the user selects open, WhiFuN plots an $n_1 \times n_2$ matrix consisting of the Dice coefficient values calculated between every FN from the first set with that of the second set, where n_1 is the number of FNs in the first set and n_2 is the number of FNs in the second set.

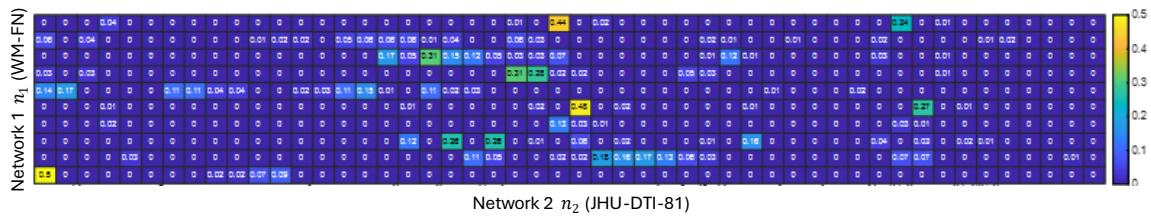


Figure 8-3: Result of Compare FN. Dice coefficients computed between every FN from the first set with that of the second set, where n_1 is the number of FNs in the first set and n_2 is the number of FNs in the second set

8.2 Check FN Symmetry

Using the Check FN Symmetry module, WhiFuN can calculate how symmetric the FNs are between the two hemispheres.

Once the user clicks the Check FN symmetry button, WhiFuN asks the user to select the network whose symmetry is to be computed (.nii file) (See *Figure 8-4*). For instance, we select the WM-FN created by WhiFuN (See Section 7.2 for details)

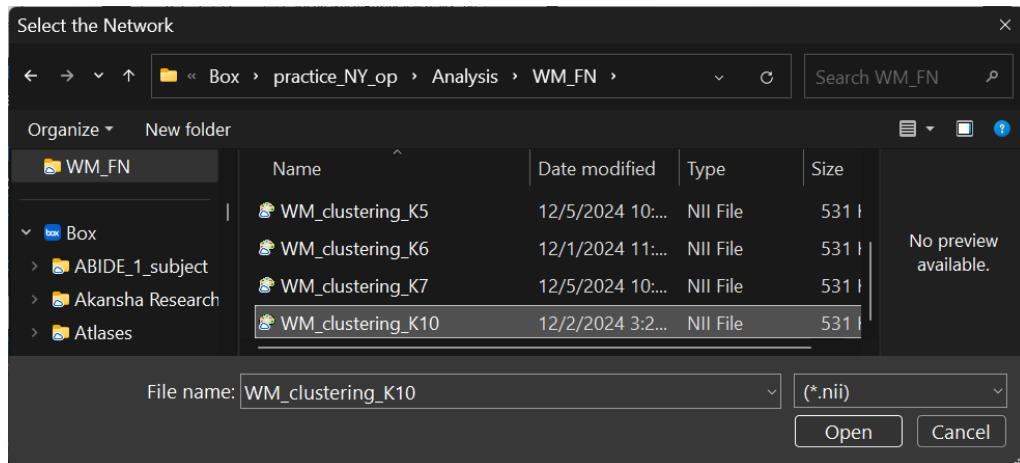


Figure 8-4: Select the network whose symmetry is to be computed.

To compute the symmetry, WhiFuN cuts the given network into two hemispheres (Left and Right).

Once the user selects the network in WhiFuN, the toolbox plots an $n_1 \times n_1$ matrix consisting of the Dice coefficient values calculated between every network in the left hemisphere with that on the right. Where n_1 is the number of networks.

WhiFuN also calculates the total symmetry of the entire .nii file (Using the method proposed by (Peer et al., 2017) and puts it in the title of the resultant plot (See *Figure 8-5*)

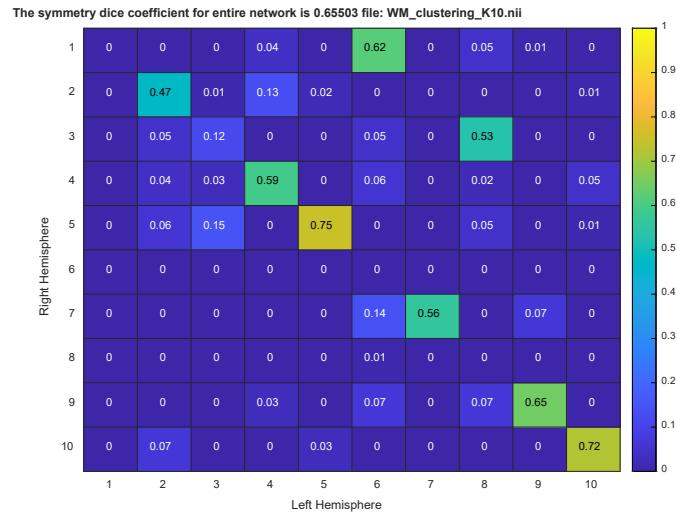


Figure 8-5: Result of Check FN symmetry. Dice coefficients computed between every network in the left hemisphere with that on the right

8.3 Statistics

WhiFuN can perform statistical tests (using MATLAB's `regstats` function) to find significant differences between groups of participants or how well a particular FC can explain a behavioral score. To avoid false positives due to multiple comparisons, false rate discovery (FDR) is computed using `mafdr` (the Benjamin and Hochberg method) or Bonferroni correction computed by correcting the alpha value based on the number of tests that are performed are included in the module.

8.3.1 Upload CSV file

After the WM, GM or CC FNs, or Atlas-FC (See Section 7 for details) is created, users can click on the statistics button to open the WhiFuN statistics module (See *Figure 8-6*).

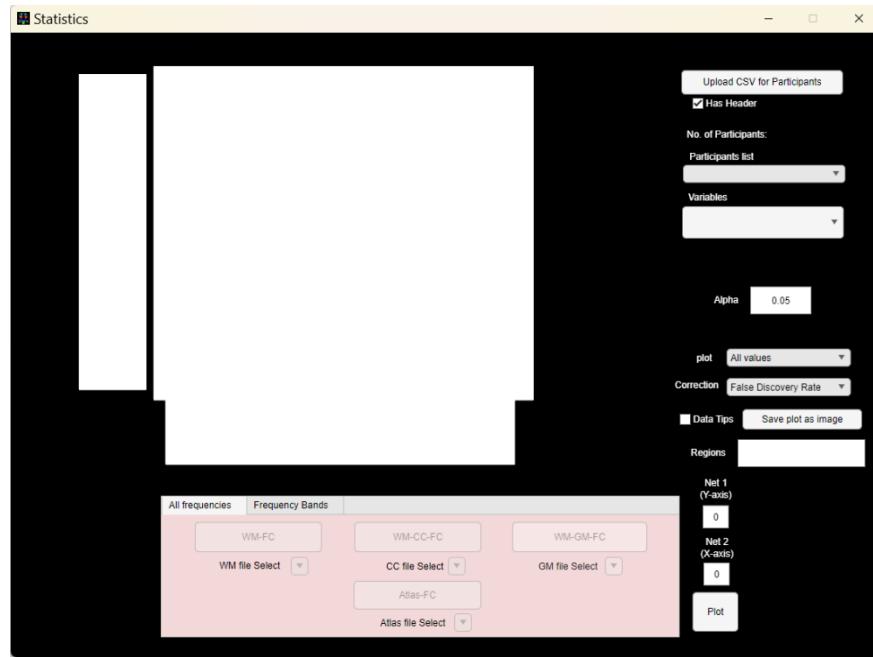


Figure 8-6: WhiFuN Statistics GUI

- 1) The user will be required to upload a CSV file. The first column of the CSV file should be Participant ID. The Participant IDs in the CSV should be the same as that of the Participant folder names.
- 2) The other columns of the CSV file can be any other metadata such as age, sex, or any other behavioral score. Ensure that except the Participant ID, all other columns should have numerical values. (For instance, for sex, Male can be represented by 0 and Female by 1).
- 3) Below, we show an example of the CSV file for the practice dataset that has been used here. (Note that these are random sex and age values and not the actual).

Participant List	sex	age
0050952	0	22
0050953	1	25
0050954	0	26
0050955	1	28

- 4) Users can click on the Upload CSV for Participants and choose the CSF file (See *Figure 8-7*).

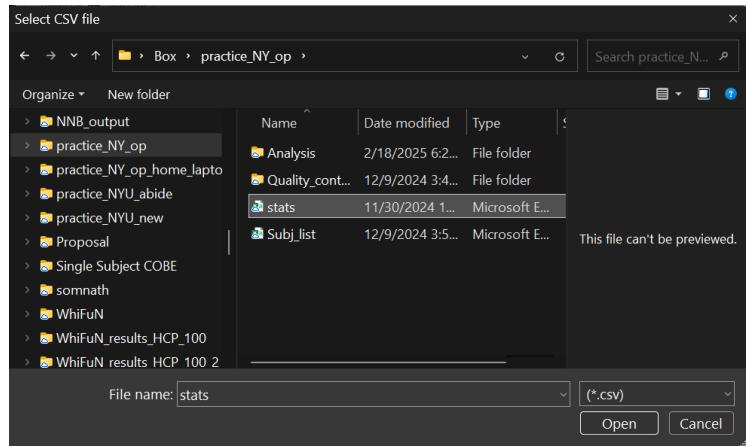


Figure 8-7: Select CSV file.

8.3.2 Statistics Module Features

Once the CSV file is uploaded the FC buttons (at the bottom of the GUI) corresponding to which the time series were stored will be enabled. If any of the buttons are not enabled that means WhiFuN did not find the time series corresponding to that particular FC creation.

- 1) Participant list drop down: This drop down shows the Participant IDs of the participant whose data is used in computation of the statistics.
- 2) Variables drop down: This drop down shows the variable that is being used to compute the statistical difference (T values) scores. The variables correspond to the columns of the CSV file that was uploaded except the 1st column (Participant ID).
- 3) Alpha: This is the significance alpha threshold. Default is 0.05.
- 4) Plot drop down: The user can choose to see the Statistics scores for every connection (All values) or only those that are significant (p-values less than Alpha).
- 5) Correction drop down: The users can choose the multiple comparisons corrections, the False rate discovery (FDR) (computed using MATLAB function mafdr (the Benjamin and Hochberg method) or Bonferroni correction (computing a new Alpha, by dividing it by the number of comparisons done).
The number of comparisons is computed by giving the c different p values obtained from every FC element. For WM-FC, if there are n_{WM} WM networks, $c = \frac{n_{wm} \times (n_{wm}-1)}{2}$, for WM-CC-FC $c = n_{wm}^2$ and for WM-GM-FC $c = n_{wm} \times n_{gm}$, where n_{gm} is number of GM networks. For atlas-FC, with atlas having n_{ROI} number of ROIs, $c = \frac{n_{ROI}(n_{ROI}-1)}{2}$.
- 6) Data Tips: When this is checked, the user can click on an element in the plot and see the corresponding T-value and p-Value.
- 7) Save plot as image: using this the user can save the plot as .png file for publications or posters.
- 8) Plot button: Users can see how different the FN connections are across the two groups (in this case sex). Since there are two groups, we see two bar plots, for n ($n < 5$) groups there will be n bar plots. If it is a continuous variable or if $n \geq 5$, it will show a scatter plot. User has to mention the network number from Y and X axis to get this plot.

9) Frequency Bands: Here the output from the Extract timeseries in specific frequency bands (see section 7.4) can be analyzed.

See *Figure 8-8* to get a summary of all the features stated above. (We use the HCP 100 unrelated participants' dataset to demonstrate the working of statistics module below)

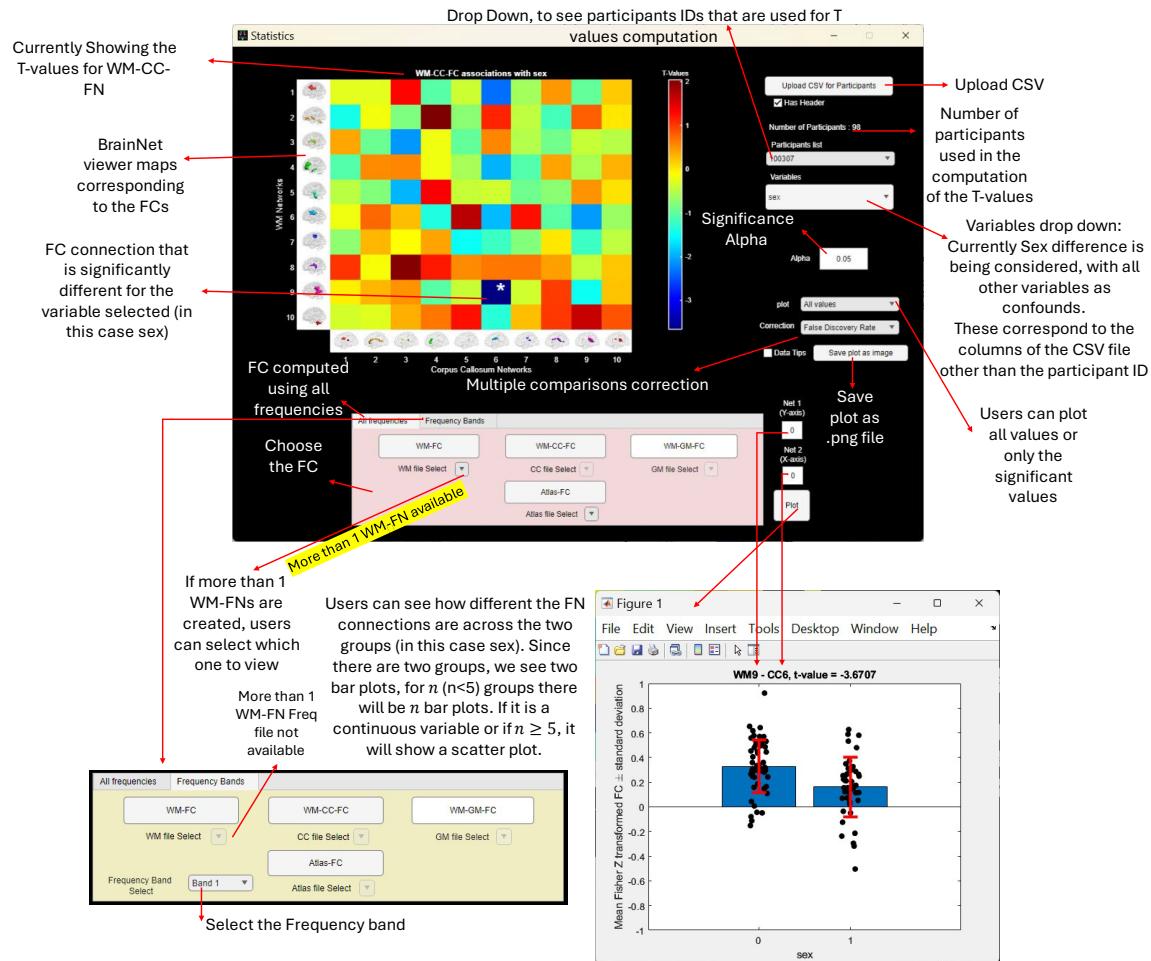


Figure 8-8: WhiFuN Statistics module Features

9. Visualizations

WM/GM FNs created can be visualized using the *Display FN* module, and the FC matrix can be visualized using the *Display FC* module.



Figure 9-1: WhiFuN Visualize module

9.1 Display FN

Once the functional networks are created using the details discussed in section 7.2, the networks can be viewed using the Display FN module.

As soon as the user clicks on the Display FN module, WhiFuN asks the user to select the clustering file (.nii file saved after WM/GM FNs are created). If the output folder is mentioned in the Setup, WhiFuN will directly open the WhiFuN Analysis folder. As an example, we show results from the HCP 100 unrelated participants' dataset.

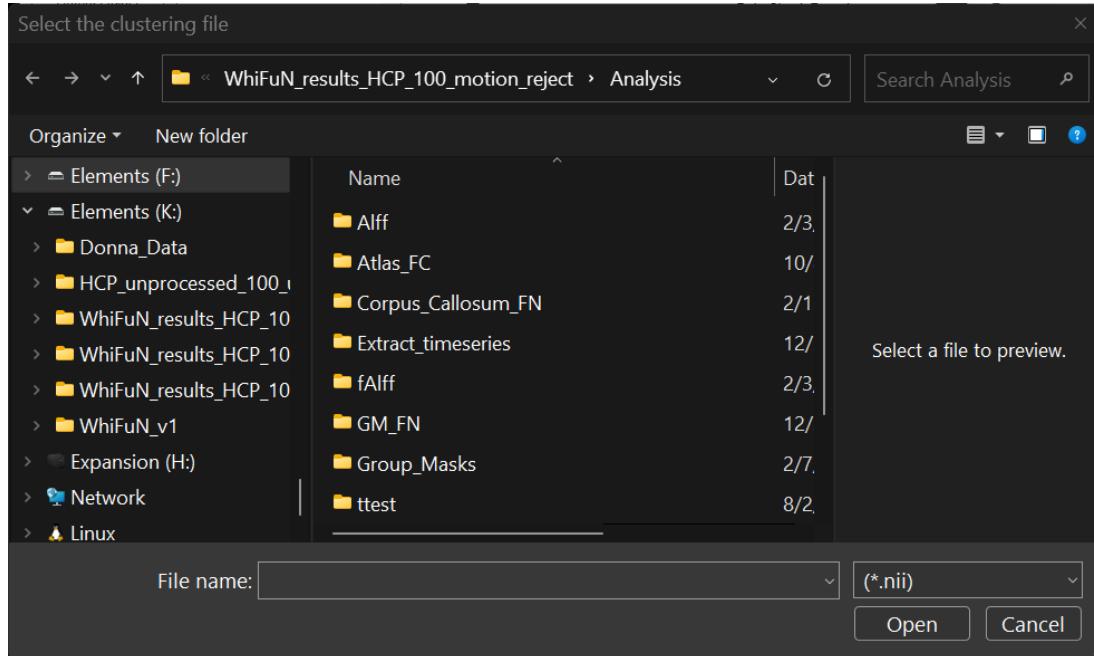


Figure 9-2: Select the FN file to visualize.

User can choose the desired FN file to visualize and click open.

WhiFuN can visualize the FN using the SPM display module or BrainNet viewer. WhiFuN will ask which toolbox the user would want to use to display the FN (see *Figure 9-3*).

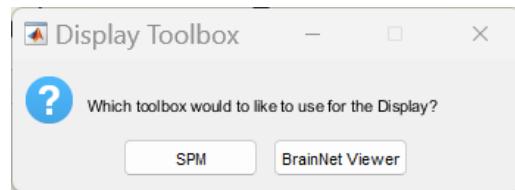


Figure 9-3: Which toolbox to use for Display FN.

9.1.1 SPM Display FN

If the user chooses SPM, A plot similar to *Figure 9-4* will be shown for the FN file selected. Users can move the crosshair and use any other SPM display feature here.

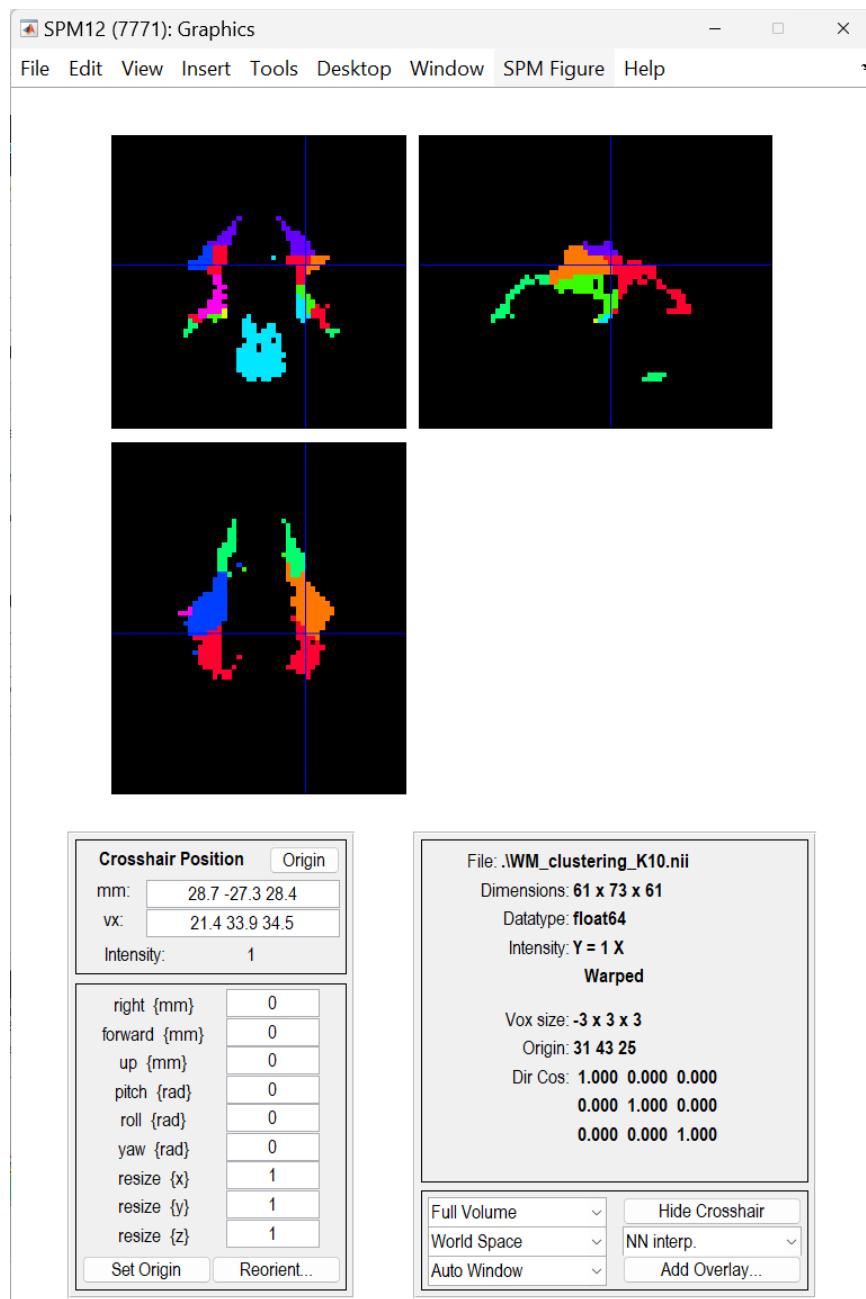


Figure 9-4: SPM display FN

9.1.2 BrainNet Viewer Display FN

If the user chooses BrainNet viewer, A plot similar to *Figure 9-5* will be shown for the FN file selected. Users can use any other features of the BrainNet viewer toolbox to see the plot as desired (For instance See *Figure 9-6*).

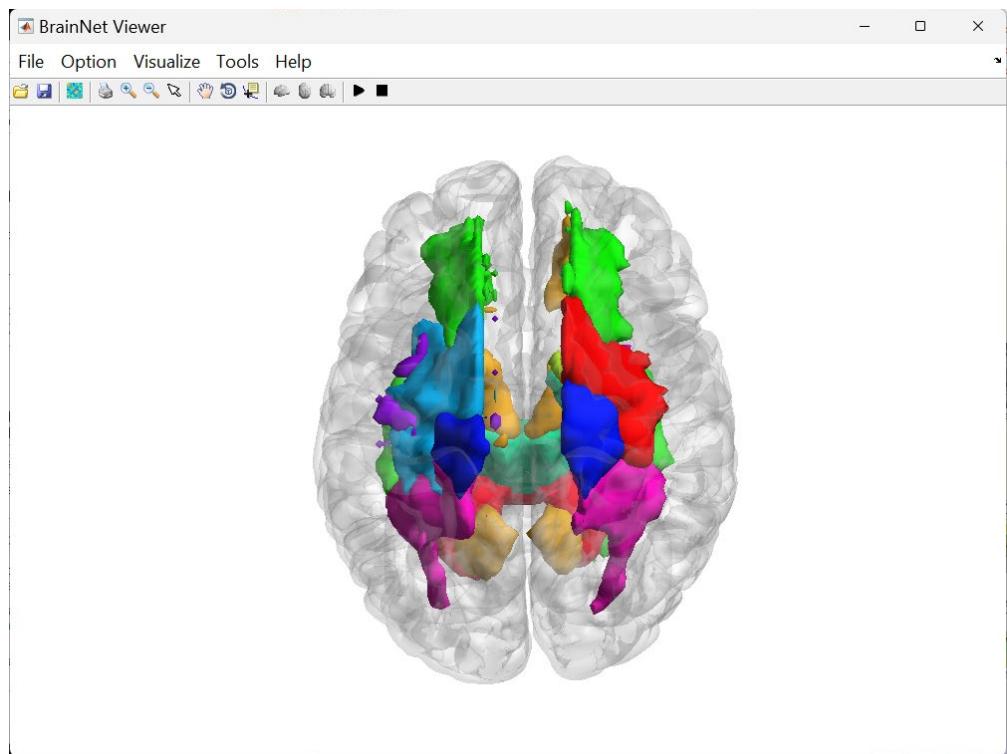


Figure 9-5: BrainNet viewer Display FN

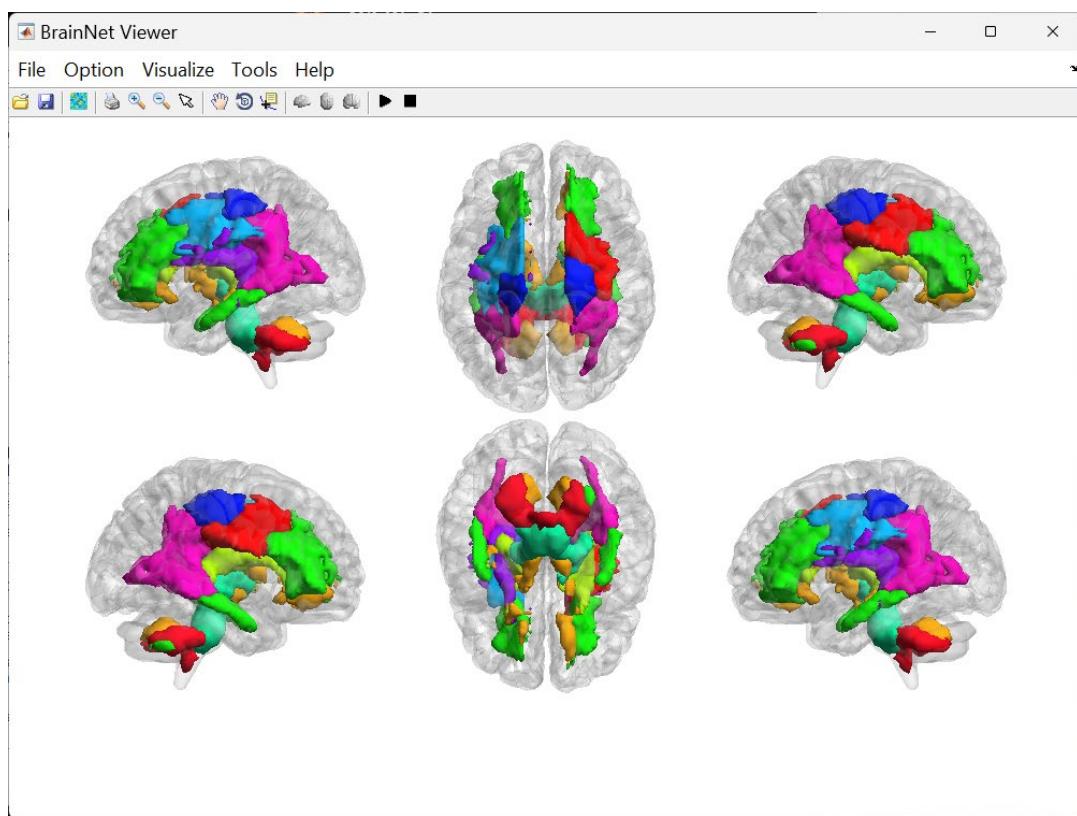


Figure 9-6: Click on option → option → Layout → Full view to see the above-mentioned plot.

9.1.2 Display FC

To visualize the FC matrices, the Display FC module can be used. Once the user clicks the Display FC button the Display FC GUI will open.

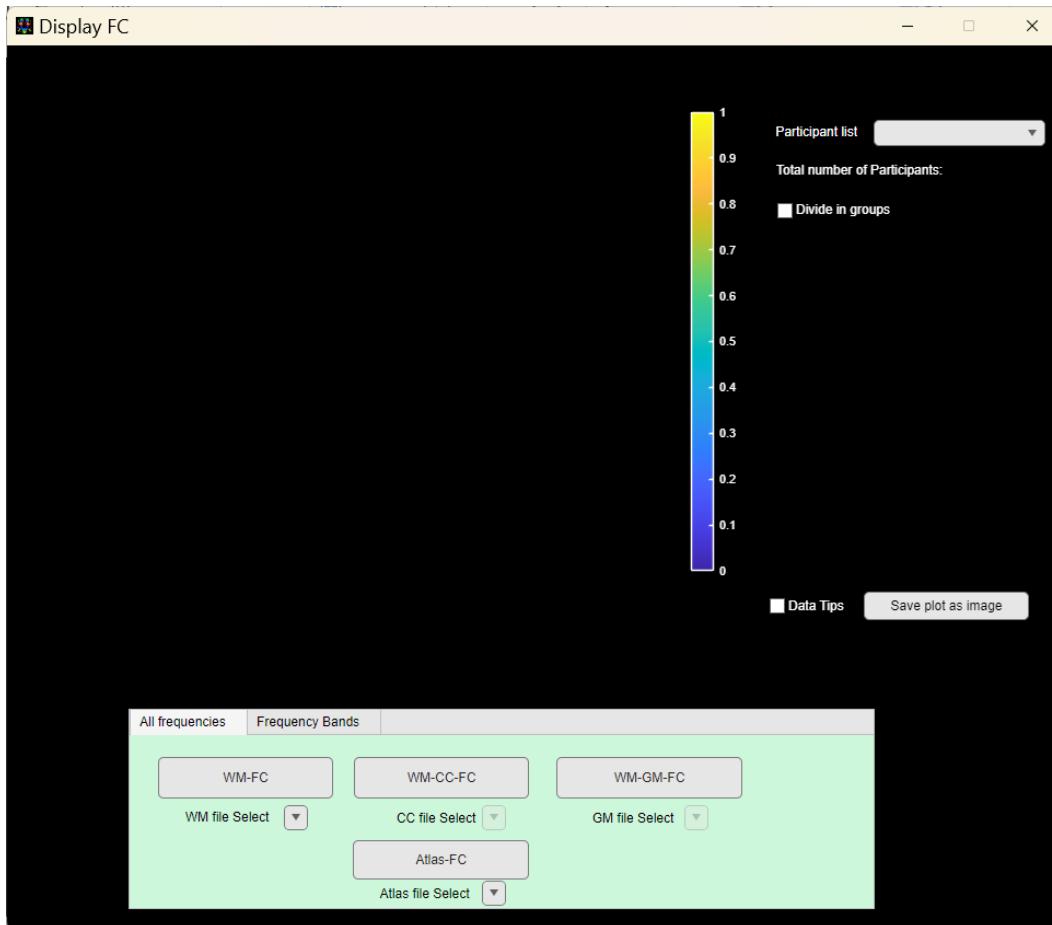


Figure 9-7: Display FC module.

Based on the average time series generated (see details in Section 7) the corresponding FC buttons (at the bottom of the GUI) will be enabled. If any of the buttons are not enabled that means WhiFuN did not find the time series corresponding to that particular FC.

Display FC Module Features

- 1) Participant List drop down: Users can use this drop down to choose to see the Mean, the Standard Deviation of the FC or the FC corresponding to individual Participants.
- 2) Divide in Groups: Users can divide the participants into two groups and then visualize the mean/STD of the FC from participants in the group by checking this checkbox.
If Divide in Groups Checkbox is checked
 - i) Upload CSV for Participant Groups: Upload a CSV file with two columns. The First column should be the participant ID (same as that in the Participant Folder), and the second column should have the groups. Only two groups

- are allowed. So, the unique values that the 2nd column can have should be two. (For instance sex, can be M or F).
- ii) Name of group 1 or group 2: Users can put any name here, corresponding to Group 1 or Group 2 participants.
 - iii) Group 1 or Group 2 participants select: Alternatively the user can manually select participants for group 1 and group 2.
- 3) Data Tips: When this is checked, the user can click on an element in the plot and see the corresponding FC value.
- 4) Save plot as image: using this the user can save the plot as .png file for publications or posters.
- 5) Frequency Bands: Here the output from the Extract timeseries in specific frequency bands (see section 7.4) can be visualized.

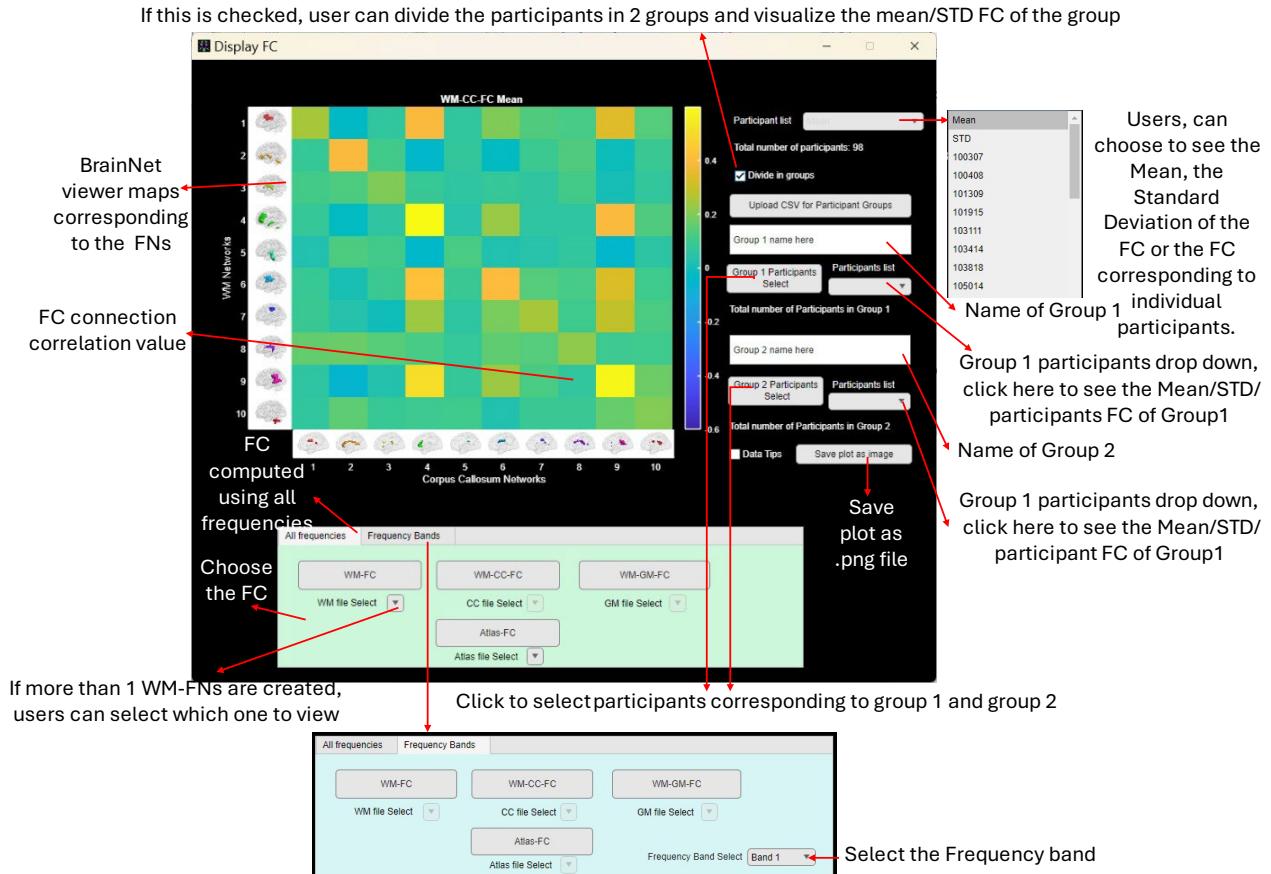


Figure 9-8: FC Display Module Features

10. Save/Load Parameters

Once WhiFuN is closed and a new session is open at a later date, the Load Parameters option can be used to fill all fields as it was when the first session was open.

After the user does the Run Data Check, a parameters.mat file is saved in the output folder. This file contains all the parameters used for preprocessing and setting up the data paths. Users can load this parameter and get all the fields populated.

Alternatively, users can also save the parameters at a different place or overwrite any other parameter.mat file using the Save parameters button.



Figure 10-1: Save/Load Parameters

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