Functional ParceNip GUI Manual

Functional ParceNip GUI created by Becky Jackson and Claude Bajada (Neuroscience and Aphasia Research Unit, University of Manchester).

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If you use this method please cite ***Jackson, Bajada et al. (2017). An emergent functional parcellation of the temporal cortex. NeuroImage.***

The Functional ParceNip GUI for use in Matlab allows you to parcellate an area of cortex based on the similarity between the timeseries of the constituent voxels in either resting-state or task data. For more details on the method see Jackson et al., 2017**.**

You will need to download the 'functional_parcenip_gui' folder and include this folder within your Matlab path. You will also need SPM (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) within your Matlab path.

Inputs

In order to perform the parcellation you will need -

- Pre-processed functional data normalised to a group space (e.g. MNI space). This should be in the format of one 4D nii file per participant or per run. If you have preprocessed your functional data in DPARSFA it will be in the right format. If you have multiple 3D hdr/img files per person (e.g. if you have preprocessed the data in SPM) you will need to convert to a 4D nii file. There are multiple ways to do this. Your options include the fslmerge function in FSL, the SPM utility (SPM-Util-3D to 4D file conversion) or MRICro (File- Convert 3D files to 4D). See the 'Folder Organisation' section for a discussion of how to deal with multiple runs of functional data per participant.
- A binary ROI file delimiting the area you wish to parcellate. This should be in Analyze format (hdr/img) and must be in the same space as the functional image files (e.g. MNI) and have the same dimensions. If it does not you can reslice it using SPM - Realign(Reslice).

Please note to get quality results from the parcellation it will likely be necessary to clean the functional data well. The effect of motion on resting-state connectivity is well documented and there are a number of approaches to deal with this (e.g. see Power et al. 2015 Neuroimage; Power et al. 2014 Neuroimage; Satterthwaite et al. 2013 Neuroimage; Van Dijk 2012 Neuroimage). Additionally, the use of the same basic functional connectivity measures with task data will be equally troubled by motion. Therefore, it may be pertinent to apply more advanced motion correction for functional parcellation using task data than is standard for univariate analyses of task data.

Folder Organisation

There should be a directory for the functional images which contains a separate folder for each participant. The participant folders must have names of equal length (e.g. _001, _002, _011 not _1, _2, _11). Within the participant folder there should be the functional data (the 4D nii file). No other folders may be present in this directory and no other files may be in these folders.

There should be a separate folder where you would like the results to be saved. A folder will be created within this to save the group results. The ROI file may be located anywhere outside of these two folders.

If you are using multiple runs of functional data per participant there are two possible approaches. Separate runs may be included in separate folders as if from separate participants. Provided that there are the same number of runs per participant this will result in the same effect as averaging within each participant and then across the group. However, if there are different numbers of runs for different participants this may skew the data, with different participants having more or less influence on the result. To avoid this you should create one 4D nii file per participant which includes all the data from that participant. This will concatenate all the participant's data so only one similarity matrix is created per participant. This can be done by converting all the 3D files for the participant in to one 4D file (using the fslmerge function in FSL or the SPM utility (SPM-Util-3D to 4D file conversion) or MRICro (File- Convert 3D files to 4D)). If you're starting with 4D files you can split them in to 3D files and then merge them all in to one 4D file.

Starting the GUI and Entering Inputs

Check your Matlab path includes the functional_parcenip_gui folder and type functional_parcenip_gui in to the command line. This will start the GUI (see Figure 1).

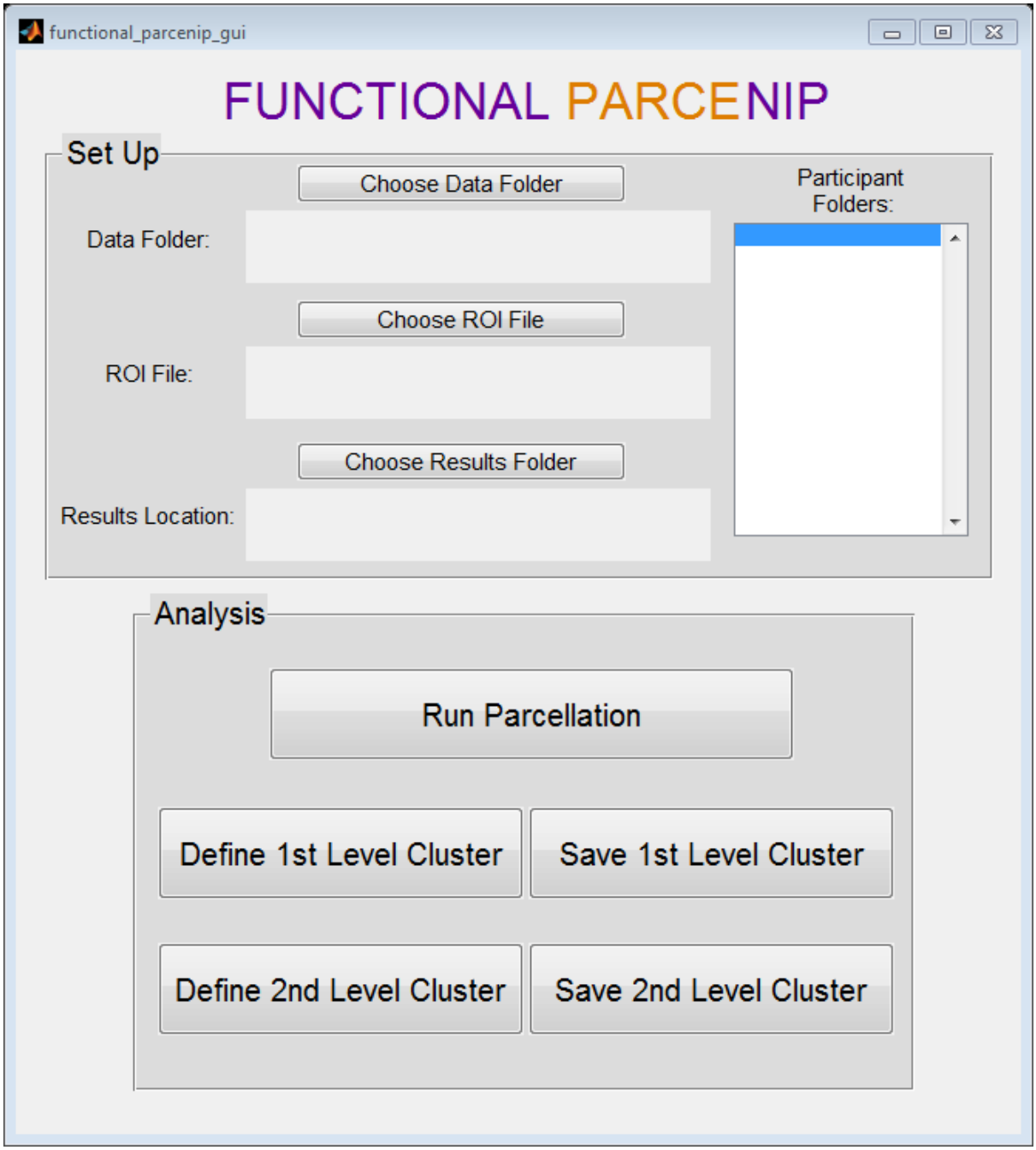

Figure 1. The Functional ParceNip GUI.

You will then need to tell the GUI where your functional data folder, ROI file and results output folder are.

Click 'Choose Data Folder' (see Fig.2.1) and select the functional image directory (i.e. the folder containing all the subject folders each containing the functional data, see Fig.2.2). Subject folders should appear in the list box on the right. Check they are correct. Participant folder names must all be the same length otherwise running the parcellation will lead to an error. No other folders may be present in this directory and no other files in the subject folders. If the folders are correct, select 'OK' (see Fig.2.3).

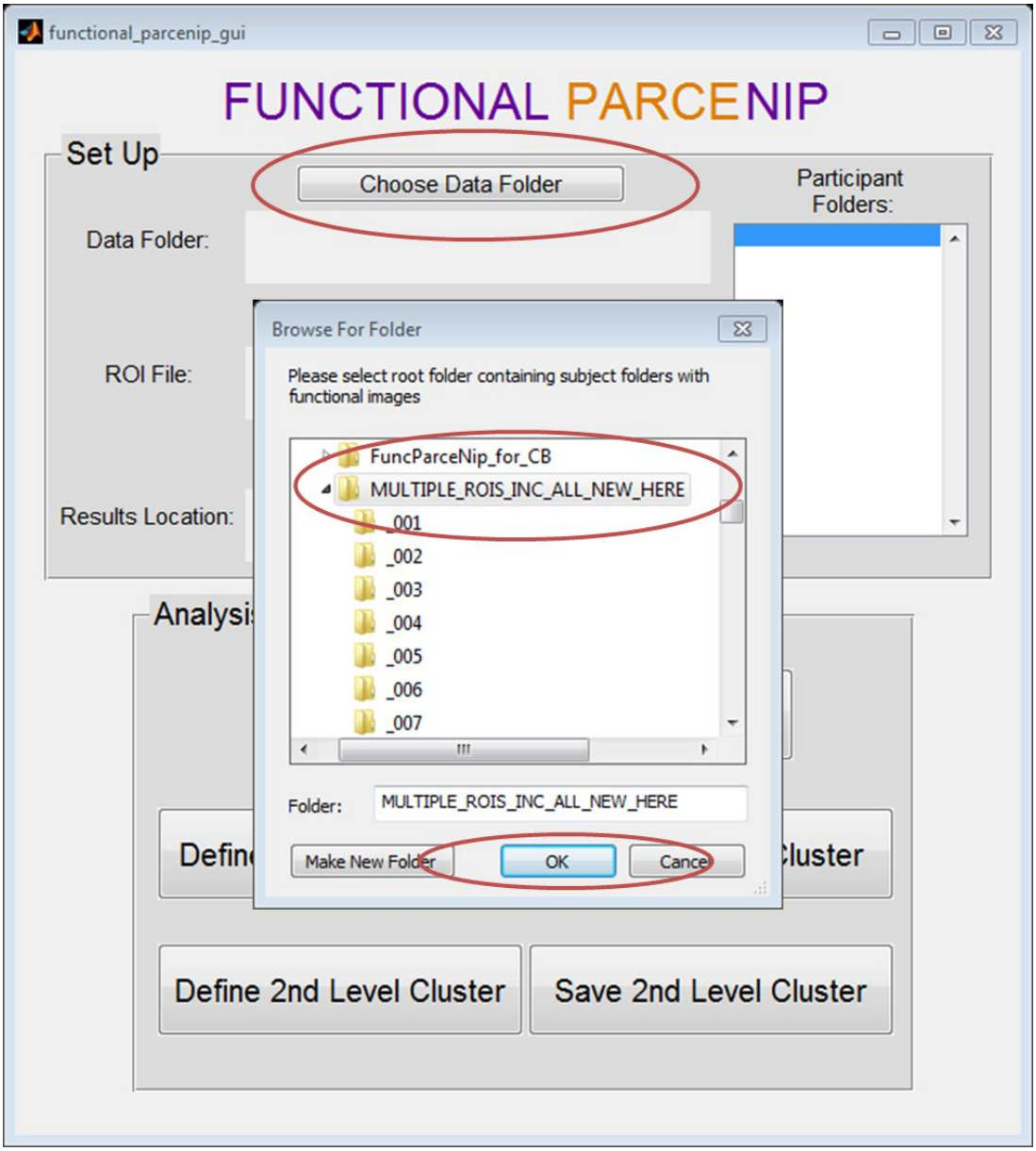

Figure 2. Choosing the directory containing the participant folders containing the functional images.

Click 'Choose ROI File' (see Fig.3.1) and select a binary ROI file in Analyze format in group space (see Fig.3.2). Select 'OK' (see Fig.3.3).

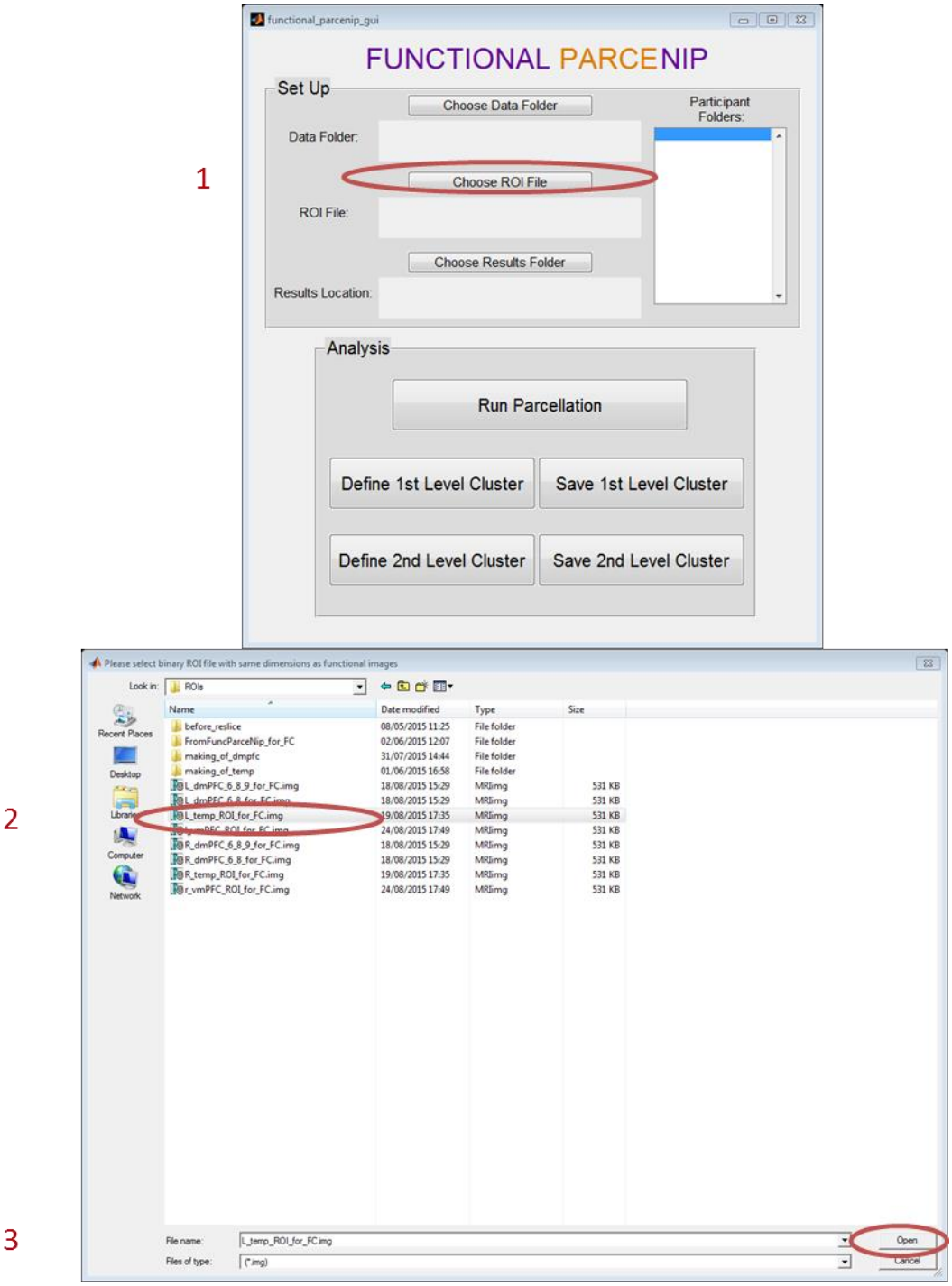

Figure 3. Choosing the ROI file.

Click 'Choose Results Folder' (see Fig.4.1) and select the directory where a new results folder will be created (see Fig.4.2). Select 'OK' (see Fig.4.3). The results folder will be named FuncPARCENIP_Results_[ROI filename]. The results files for the group level analysis will be saved here. An overwrite warning will appear when the analysis starts if this folder already exists, if you do not want to overwrite the existing results folder select 'No' and change the name of the existing folder.

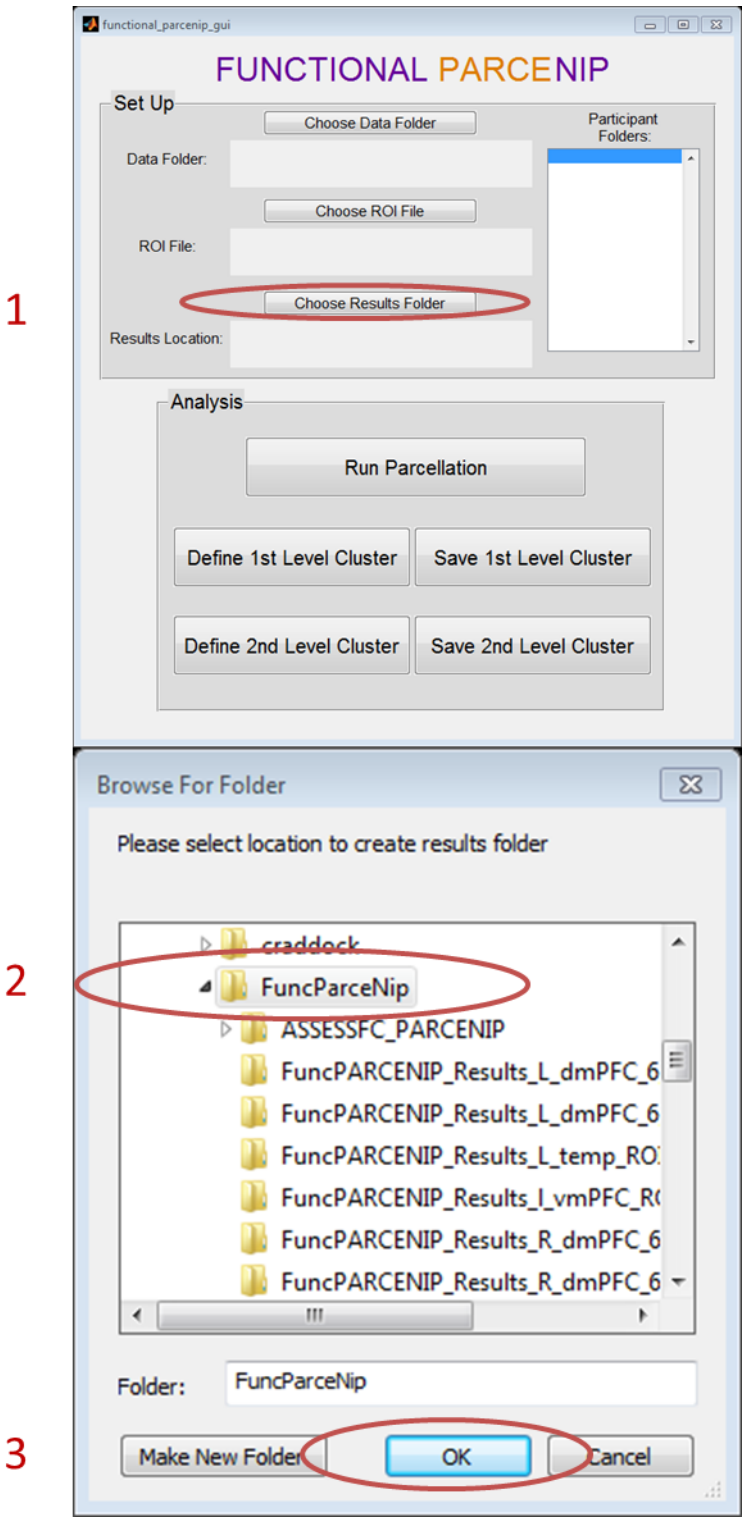

Figure 4. Choosing the location of the group results folder.

All three of these inputs must be entered whenever you open the GUI in order to perform any analysis or display steps.

Running the Main Analysis

You're now ready to run the main analysis. Pressing 'Run Parcellation' will perform the main 1st and 2nd level analysis (see Fig.5.1). First, the timeseries of the functional data is extracted. This is saved as a text file named my_timeseries_[ROI filename].txt in each participant's functional images folder. This is the timeseries for each voxel within the ROI in that participant's scan. During this process a wait bar will show your progress. When this has finished the wait bar will disappear and the first level analysis will begin (**do not shut the GUI at this point, the analysis is still running**).

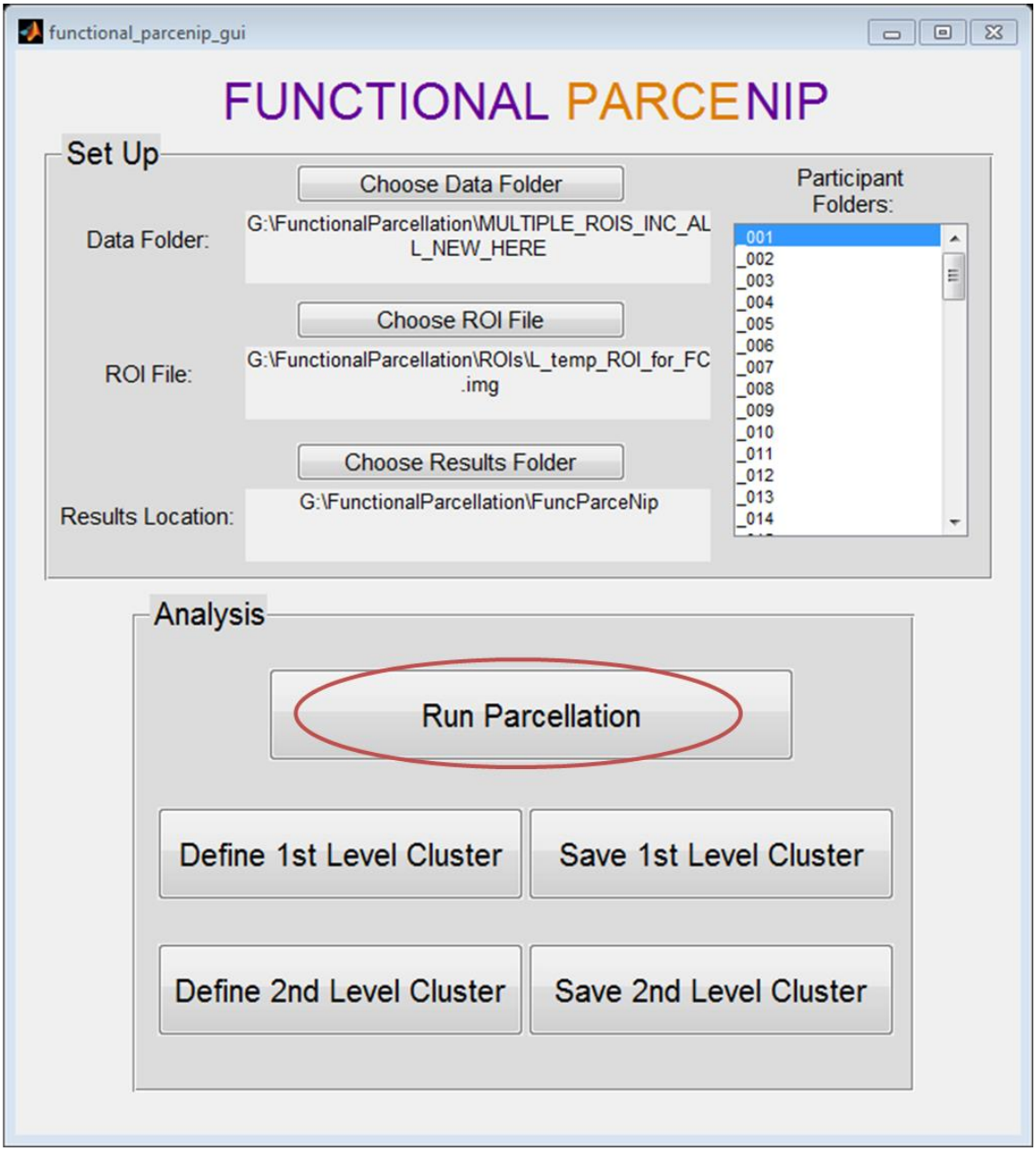

Figure 5. Running the main analysis.

In the first level analysis the pairwise similarity of the voxels timeseries is determined (i.e. the similarity of the timeseries of each voxel in the ROI to every other voxel in the ROI). The voxels are then ordered in terms of this similarity (see **[ref paper]** for more details). This results in a matrix showing the similarity of the timeseries of the voxels. The matrix is saved as a figure before and after the sorting process, resulting in _sortedsimilarity_matrix[ROI filename].tif and _unsortedsimilarity_matrix[ROI filename].tif files. Additionally, the graded connectivity pattern is projected on to the cortex and saved as a hdr/img file, named graded_similarity_map_[ROI filename].hdr/img. This can be opened in standard brain image viewers (e.g. MRICro) in order to see the graded change in connectivity for that participant. The individual participant's Matlab workspace, including the ordered matrices, is saved as sorted_seeds_L_temp_ROI_for_FC.mat. This can be accessed if you wish to perform further computation or view the ordered matrices within Matlab. All of the first level analysis results will be stored in the functional image subject folder. Analyses will not overwrite each other if the ROI filenames are different.

The second level analysis is then performed. The first level unordered matrices are averaged to make a group level matrix. This is reordered to create a graded similarity matrix for the group. The group similarity matrix is saved as _GROUP_sortedsimilarity_matrix_av_sim.tif. The projection of the similarity matrix on to the cortex is saved as graded_similarity_map_group.hdr/img. Again, this can be opened in standard brain image viewers (e.g. MRICro). The group Matlab workspace including the ordered matrices is saved as workspace_sorted_group_av_sim.mat. All the group results are stored in the folder created in the chosen Results Folder. You will know the analysis has finished running when all of these files are saved in the group results folder. Depending on the size of the ROI and the amount of functional data (as well as computer specifications) the main analysis is likely to take 1-3 hours.

Hard Parcellations – Finding Distinct Clusters

After assessing the structure identified across your ROI you may wish to define hard clusters of distinct subregions present within the ROI. To get a hard parcellation of the clusters formed in the matrix select 'Define 1st Level Cluster' (for one individual) or 'Define 2nd Level Cluster' (for the group, see Fig.6.1.). A pop-up window will display the sorted matrix. It may be useful to view the same image (_GROUP_sortedsimilarity_matrix_av_sim.tif) saved in the group results folder where it may be easier to see the structure.

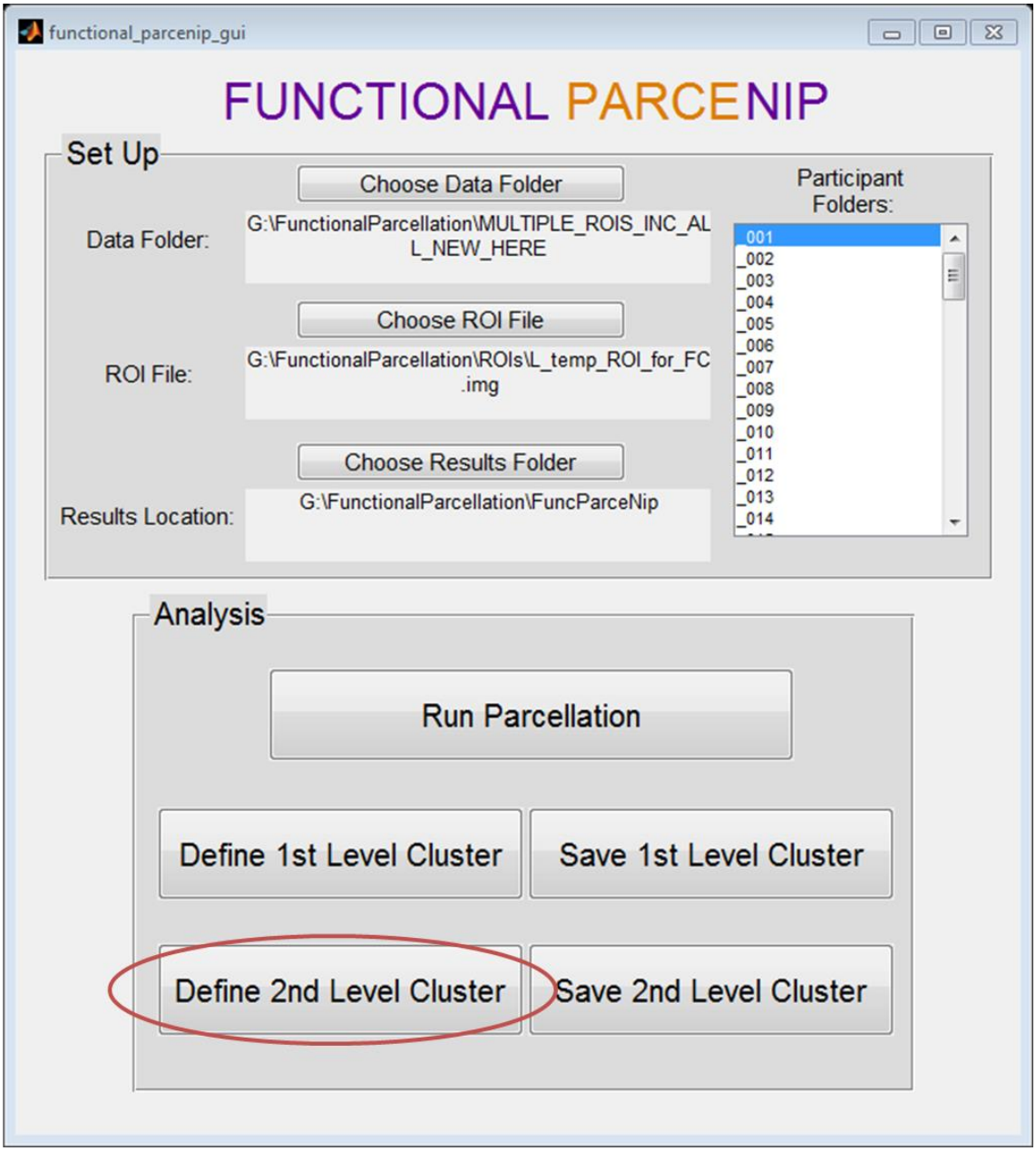

Figure 6. Defining a hard cluster at the group level.Pt.1.

To define the cluster press 'Start' (see Fig. 7.1). You will then be able to select points on the matrix with the mouse which will be displayed as a crosshair. Use this to select one edge of a cluster (along the diagonal) in the matrix (see Fig. 7.2). The number of the seed voxel selected will be displayed at the bottom of the window (see Fig. 7.2). You may wish to keep a record of this. The cross may be dragged to select a specific seed e.g. the first or last voxel before the voxel is selected. To confirm selection of the voxel as one edge of your cluster press 'Select Seed' (see Fig. 7.3).

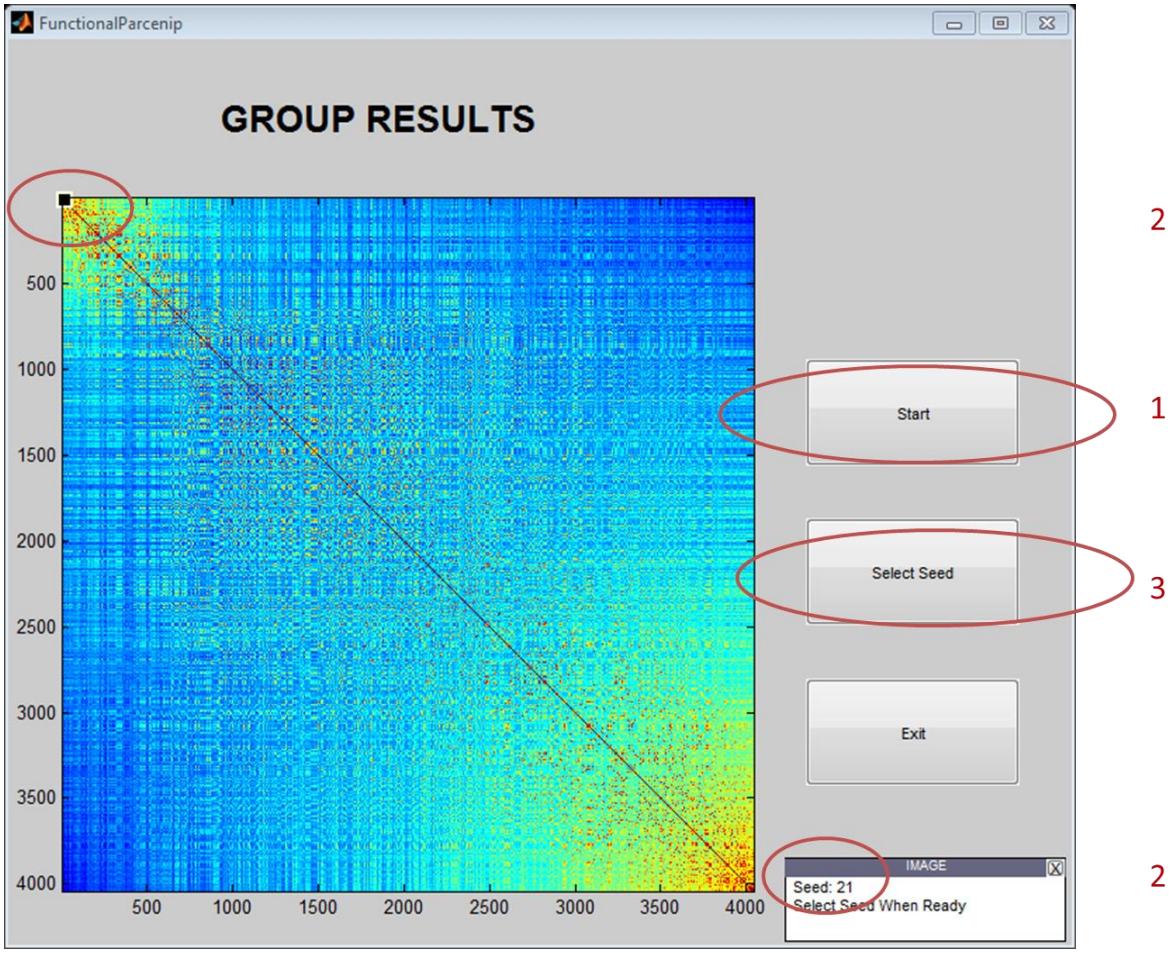

Figure 7. Defining a hard cluster at the group level.Pt.2.

You may now use the crosshair to choose the other edge of the cluster (see Fig.8.1) and check the selected seed number (see Fig.8.1). Press 'Select Seed' to confirm this edge (see Fig.8.2). Press 'Exit' to confirm this selection (see Fig.8.3) or 'Start' to restart. **In order to save this cluster as an image, select 'Save 1st Level Cluster' or 'Save 2nd Level Cluster'.** Choose an appropriate filename when prompted. This will output a binary mask of the ROI voxels within this cluster in Analyze format.

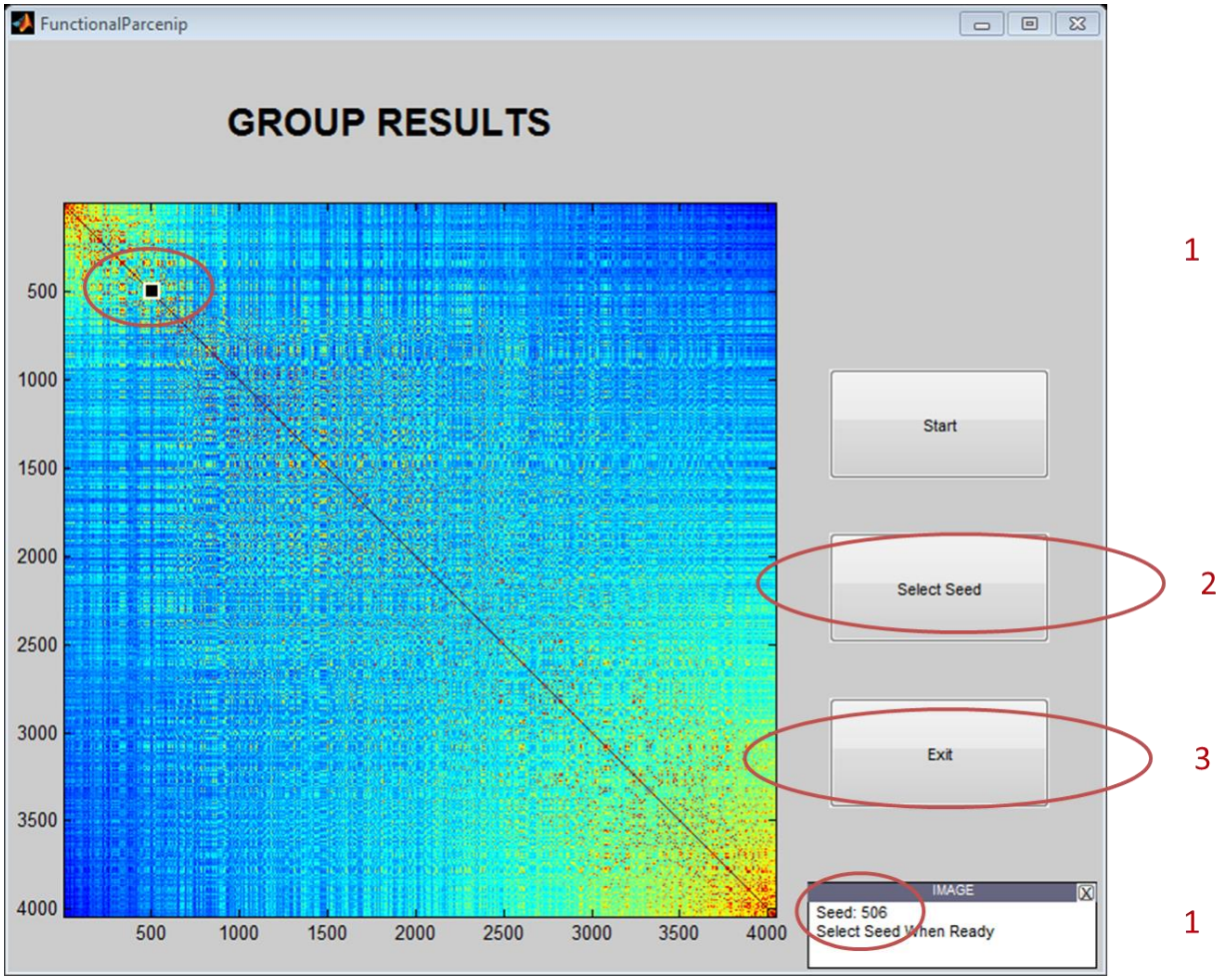

Figure 8. Defining a hard cluster at the group level.Pt.3.

The resulting output has been separated from other clusters within the ROI on the basis of its timeseries. In order to determine the functional connectivity of this cluster it can be used as an ROI within any standard functional connectivity assessment program, such as REST (http://www.restfmri.net/forum/REST_V1.8), DPARSFA (http://rfmri.org/DPARSF) or CONN (https://www.nitrc.org/projects/conn). The connectivity of distinct clusters may be compared. Within participant t-tests (such as those performed in SPM) allow an assessment of the connectivity of the cluster and between participant t-tests allow a comparison of the differences in functional connectivity between the different ROIs.

Note regarding task data: Please note, the use of simple correlations within task data is not an accurate measure of functional connectivity due to the problem of coactivation (two regions may be more active in a condition or timepoint in the task model causing them to appear more connected without any true change in connectivity). For this reason the output of the functional connectivity analyses for task data should be considered connectivity and /or coactivation. This does not affect the functional parcellation which is merely based on the pairwise similarity of the voxels timeseries

but care should be taken when interpreting the areas shown to be connected/coactivated (see **[ref paper]** for further details).

Key Outputs

_GROUP_sortedsimilarity_matrix_av_sim.tif – the similarity matrix showing the similarity between voxels, allowing assessments of whether there are hard clusters within the ROI

graded_similarity_map_group.hdr/img – the similarity of the voxels on the cortex, showing the changes in similarity across the ROI

Hard clusters with given filename – show areas where voxels show similar timecourses distinct from elsewhere, the limits of the clusters are user determined. Can use as connectivity ROI to assess the functional relevance of the subregions.

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