RESTing-state fMRI data analysis toolkit (REST) Manual
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Thank Xiangyu Long very much for this English manual!
-- Xiaowei Song
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Some abbreviations

AAL: Anatomical Automatic Labeling template (Tzourio-Mazoyer et al., 2002)

ALFF: amplitude of low frequency fluctuation

AFNI: Free software, website: http://afni.nimh.nih.gov/afni

fMRI: functional magnetic resonance imaging

GUI: Graphical User Interface

MRIcon/Mricon: Free software, website:

   www.sph.sc.edu/comd/rorden/mricron/

ReHo: regional homogeneity analysis

REST: RESTing-state fMRI data analysis toolkit

ROI: region-of-interest

SPM: Statistical parametric maps (Friston et al., 1995), a free software.

1. What can you do by REST

1. Batch processing of the fMRI data analysis;

2. Perform ReHo analysis, ALFF analysis and linear correlation analysis;

3. Band-pass filter, remove linear trend of the fMRI data

4. Viewing the time series and power spectrum of the fMRI data

5. Define the ROI, extract the mean time series within the ROI;

6. Viewing the MRI image, display the fMRI result on a anatomical image;
7. Extract the specific cluster from the AAL template, Brodmann template or statistic map, and create the mask image.

2. Notice before using REST

1. Image file format must be ANALYZE 7.5, and MRIcron or AFNI (Cox, 1996) can be used to transform the format;

2. When processing the fMRI data by REST, fMRI data of each session (or subject) must be stored in one folder volume by volume, i.e., each pair of files (hdr/img) is one time point. All volumes must be named in the order of the scanning sequence, e.g., the fMRI data of one subject was stored in the independent folder, it means that there were exactly all volumes in this folder and the files in this folder must like this: a001.hdr/img, a002.hdr/img, etc. Remember that, the unwanted image file must be moved out of the folder.

3. When using REST to define the seed voxel, if the coordinates of seed was input manually, the original and the input coordinates of all images must be corrected, and the position of ROI can be viewed after clicking the “View ROI” button.

3. Start to use REST toolbox

3.1 Installation

a) MATLAB 2006b for Windows XP was recommended.

b) Unzip the package, then start MATLAB, choose the option in toolbar as following: File -> Set Path... -> Add Folder... (Here select the REST folder) -> Save -> Close.

3.2 Remove the REST

Start MATLAB, and choose the option in toolbar as following: File -> Set Path... (Here select the REST folder) -> Remove -> Save -> Close, then delete the REST folder on the disk.

3.3 Start REST

1. Start MATLAB.

2. Enter “rest” in the command line (Figure 1).

Figure 1, Enter ‘rest’ to start REST toolbox GUI
3. After typing “rest” in the MATLAB command line, the REST GUI interface will popup (Fig. 2).

![REST GUI](image)

Figure 2. REST main interface.

### 3.4 Regional homogeneity analysis

#### 3.4.1 What is regional homogeneity analysis

Kendall coefficient of concordance (KCC) (Kendall and Gibbons, 1990) (Fig. 4) was used for measuring the similarity of the time series within a functional cluster based on the regional homogeneity hypothesis (Zang et al., 2004). 27, 19 or 7 nearest neighboring voxels (Fig. 3) were defined as a cluster and a KCC value (range 0–1) was given to the voxel at the center of this cluster (Zang et al., 2004). Recently, this method was widely used in the resting-state study (Cao et al., 2006; He et al., 2004,
Liu et al., 2006; Wu et al., 2007; Zhu et al., 2005; Long X-Y et al., 2008).

![Figure 3](image)

Figure 3. Nearest 7 voxels (yellow voxels and its up, down, left and right voxels), 19 voxels (except the 8 white voxels) and 27 voxels constitute a cluster.

![Figure 4](image)

Figure 4. KCC’s formula. Where $W$ is the KCC among given voxels, ranged from 0 to 1; $R_i$ is the sum rank of the $i$th time point; where $\bar{R} = ((n+1)K)/2$ is the mean of the $R_i$’s; $K$ is the number of time series within a measured cluster (here, $K$ could be 7, 19, and 27, respectively; one given voxel plus the number of its neighbors); $n$ is the number of ranks (here $n$ is the number of volume).

### 3.4.2 ReHo analysis

The GUI for performing regional homogeneity analysis will popup (Fig 5) after clicking the ‘ReHo’ button at the REST main interface (Fig. 2).
3.4.2.1 Remove linear trend (optional)

Before calculating ReHo, there are some optional processes. First is the option for removing signal linear trend.

In Fig. 6, if the “detrend” option was selected, the fMRI signal will be...
removed linear trend before the following process. After selecting the “detrend” option, the “Detrend” button at right side can be used. If this button was clicked, the process of linear trend removal will be performed immediately for all of data which were selected in the input window.

The output data will be saved in a new folder. This folder will be created in the same directory with the original directory, and with a suffix “_detrend”. For example, the input data was saved in the “c:\data\OneCleanDir” directory, and the output data will be saved in the “c:\data\OneCleanDir_detrend” directory.

**Extremely Caution:** If the “c:\data\OneCleanDir_detrend” directory already existed, the REST will first delete the “c:\data\OneCleanDir_detrend” directory and then recreate the “c:\data\OneCleanDir_detrend” directory and saved the data in this directory.

### 3.4.2.2 Temporal filter (optional)

There is another optional process: temporal filter.

![Figure 7. Interface of temporal filter.](image)

In Fig 7, if the “Band (Hz)” was selected, temporal filtering will be performed for the fMRI data before the following process. Moreover, if the “detrend” option was selected, the temporal filtering will be
performed after the linear trend removal procedure.

After selecting the “Band (Hz)” option, the “Filter” button at right side can be used. If this button was clicked, the process of temporal filtering will be performed immediately for all of data which were selected in the input window.

The output data will be saved in a new folder. This folder will be created in the same directory with the original directory, and with a suffix “_filtered”. For example, the input data was saved in the “c:\data\OneCleanDir” directory, and the output data will be saved in the “c:\data\OneCleanDir_filtered” directory.

**Extremely Caution:** If the “c:\data\OneCleanDir_filtered” directory already existed, the REST will first delete the “c:\data\OneCleanDir_filtered” directory and then recreate the “c:\data\OneCleanDir_filtered” directory and saved the data in this directory.

The cut-off frequency value and TR can be defined in the three separated blank boxes. The first is the lower limit value and the second is the upper limit value. The third is TR, the unit is second. For the Band-pass filter, both boxes must be filled and the second value must higher than the first value; for the high-pass filter, the second value must be zero, and the first value must higher that zero (Fig. 8); for the low-pass filter, the first value must be zero, and the second value must higher that zero (Fig. 9).
3.4.2.3 Setting the input data

In this stage, the input data will be defined by the directory (Fig. 10). The input data must be stored as mentioned above (please see details in section 2. Notice before using REST). The input directory can be added or removed in this interface (Fig 11). Multi subjects can be selected and batch process can be implemented by this setting.

One directory can be added by clicking the button “…”. Moreover, in fig 11, a menu popup after right mouse click in the blank area. The directory of input data can be added or removed in this way. If a directory was selected, all of the subfolders in this directory can be added by choosing “Add recursively all sub-folders of a directory”. The “Delete” and “Backspace” button of the keyboard of a computer are available in this interface.
3.4.2.4 Define the mask option

In this interface, a brain tissue mask can be selected during the calculating (Fig. 12). There are three options: default mask (provided by REST, REST can automatically select a mask which match the size of the input data), no mask (do not use mask during calculating) and user’s defined mask (user can selected a self-defined mask). A mask can make the calculating faster.

Figure 12. Mask definition interface. In this figure, a “User’s defined mask” was selected, and the mask file was “D:\Temp\mask_MNI_61_73_61.img”.

The “Default mask” options provide the following types of masks, the masks were created based on the SPM MNI template. Voxels within the brain can be selected by these mask. Their voxel size and dimension are:

- Voxel Size=3 mm x 3 mm x 3 mm, Dimension = 61 x 73 x 61;
- Voxel Size=3 mm x 3 mm x 3 mm, Dimension= 53 x 63 x 46;
- Voxel Size=2 mm x 2 mm x 2 mm, Dimension=79 x 95 x 69;
Voxel Size=2 mm x 2 mm x 2 mm, Dimension=91 x 109 x 91;
The mask can also be self-defined. The mask file could be ANALYZE 7.5 format (img/hdr) or mat file (variable name must be “mask”). The directory of the mask can be entered into the blank or selected by clicking the “…” button.

3.4.2.5 Define the cluster size
As mentioned above (please see details in section 3.4.1), the cluster size can be defined (Fig. 13). “27 voxels” is the most frequently used option.

3.4.2.6 Configuring the output directory
The output setting in ReHo analysis included prefix, directory for saving the results and whether divide the global mean (Fig. 14).

About “Prefix:” REST will add a prefix and a underline with the name of the input folder as the name of the output result. For example, if the input directory was “c:\data\OneCleanDir” and the prefix name if “ReHoMap”, the name of the result will be “ReHoMap_OneCleanDir.img/hdr”. All
results will be saved in the directory which can be defined in “Directory” section or selected by clicking the “…” button.

If the “Divide ReHo brain by the mean within the mask(mPrefix_DirectoryName.{hdr/img})” was selected, a normalized ReHo map which divide the global mean by using a brain tissue mask will be generated, and a prefix “m” will be added to the result map. For example, if the ReHo map was “ReHoMap_OneCleanDir.img/hdr”, the normalized ReHo map is “m ReHoMap_OneCleanDir.img/hdr”. These two results will be saved in the same directory.
3.4.2.7 Calculating the ReHo map

![Image of ReHo software interface]

Figure 15. The demo for calculating ReHo maps

After clicking “Do all” (Fig. 16), the fMRI data will be processed as following: i) remove linear trend; ii) band-pass (0.01~0.08 Hz) filtering; iii) Setting 27 voxels as a cluster, calculating the ReHo map voxel by voxel: iv) ReHo map will be normalized by dividing the whole brain mean by using brain tissue mask “mask_MNI_61_73_61.img” (Fig. 15).
Prefix of the output file is “ReHoMap” and “mReHo”. “ReHoMap” indicated that the spatial map is a KCC map. “mReHoMap” indicated that the spatial map is normalized. Generally, the “mReHoMap” will be entered into the second-stastic analysis. All of the result maps (result maps in Fig. 14 are RehoMap_test_Control-1.img/hdr, RehoMap_test_ADHD-1.img/hdr, mRehoMap_test_Control-1.img/hdr, mRehoMap_test_ADHD-1.img/hdr) will be saved in the “D:\MATLAB\work\GUI_Reho\Reho_V1\SampleData\out” directory.

Figure 16. ReHo map will be calculated after clicking “Do all".
3.5 ALFF analysis

3.5.1 What is the ALFF analysis

ALFF is the abbreviation of amplitude of low-frequency fluctuation. In this analysis, the fMRI time series was transformed to a frequency domain with a fast Fourier transform (FFT) (parameters: taper percent = 0, FFT length = shortest) and the power spectrum was then obtained. Since the power of a given frequency is proportional to the square of the amplitude of this frequency component of the original time series in the time domain, the square root was calculated at each frequency of the power spectrum and the averaged square root was obtained across a frequency band at each voxel. Generally, the frequency band of the low-frequency fluctuation is 0.01 Hz to 0.08 Hz (Biswal et al., 1995; ). This averaged square root was taken as the ALFF value (Fig. 17). This method was used in the recent resting-state study (Yang et al., 2007; Zang et al., 2007).
Figure 17. Schematic illustration of the current ALFF analysis. The signal intensity is measured in arbitrary units. (A) The fMRI time course. (B) Power spectrum using fast Fourier transform. (C) Square root of the power spectrum between 0.01 and 0.08 Hz, i.e., ALFF.

3.5.2 Start ALFF analysis

Click the “ALFF” button at the REST main interface (Fig. 2), the GUI for performing ALFF analysis will popup (Fig 18). The usage of some options is same with which also in the ReHo GUI.
3.5.2.1 Setting the ALFF parameters

Before performing ALFF analysis, the frequency band (e.g., 0.01 Hz and 0.08 Hz) and TR (e.g., 2 s) need to be defined (Fig. 19). The button “Band Hint” can provide the hint about the frequency band.
3.5.2.2 Output setting of ALFF

This option is similar with the same section in ReHo GUI (see 3.4.2.6 Configuring the output directory).

3.5.3 Calculating the ALFF map

After clicking “Do all” (Fig. 21), the ALFF map for each fMRI data will be calculated. The frequency band was 0.01 Hz to 0.08 Hz in this example. ALFF map will be normalized by dividing the whole brain mean by using a default brain tissue mask (Fig. 21, also see 3.4.2.4 Define a mask). Prefix of the output file is “ALFFMap” and “mALFFMap”. “ALFFMap” indicated that the spatial map is a ALFF map. “mALFFMap” indicated that the spatial map is normalized. Generally, the “mALFFMap” will be entered into the second-stastic analysis. All of the result maps (result maps in Fig. 21 are ALFFMap_test_Control-1.img/hdr, ALFFMap_test_ADHD-1.img/hdr, mALFFMap_test_Control-1.img/hdr, mALFFMap_test_ADHD-1.img/hdr) will be saved in the “D:\MATLAB\work\GUI_Reho\Reho_V1\SampleData\out” directory.
3.6 Functional connectivity analysis

Click the “Functional Connectivity” button at the REST main interface (Fig. 2), the GUI for performing functional connectivity analysis will popup (Fig. 22). The usage of some options is same with which also in the
ReHo and ALFF GUI.

![Functional Connectivity GUI](image)

**Figure 22.** the functional connectivity GUI

### 3.6.1 Setting the parameters of functional connectivity analysis

Before performing the functional connectivity analysis, the ROI and covariate (if exist) need to be defined. REST provide two ways for calculating the functional connectivity: Voxel wise (functional
connectivity between ROI and each voxel within the brain) and ROI wise (functional connectivity between two or more ROIs). The result of voxel wise analysis is a spatial map. For the ROI wise analysis, a text file will be created including the correlation coefficient matrix.

3.6.1.1 Define ROI

After clicking the “Voxel wise” button of the functional connectivity GUI or “Add ROI” button of the ROI Definition List GUI (this GUI will popup after clicking the “ROI wise” button of the functional connectivity GUI, see Fig. 23), the ROI (Region of interest Definition interface will popup (Fig. 24).

There are three kinds of ROI can be defined in REST:

(1) Seed ROI, i.e., a spherical ROI area defined by a center voxel’s coordinates and the radius (unit: mm);

(2) ROI mask, here included four situations:

   i) Extracted a cluster from a statistic map (after cut the threshold);

   ii) Extracted a brain area from AAL template (MNI template, provided by MRICro (Smith SM, 2002));

   iii) Extracted a brain area from Brodmann template (MNI template, provided by MRICro);

   iv) Self-defined mask file.

(3) A specific time series. The time series must be stored in a text file (*.txt) as one column, and the length of this time series must be the
same with the input fMRI signal.

First choose one kind as mentioned above, then click “Next” and set the ROI. Click “Done” after finishing the ROI definition.

**Extremely Caution:** i) The ROI position must be inspected by visualization after defining. This procedure can be done by clicking the “View ROI” button; ii) In the seed ROI section, the view of the image in REST is as following: positive value of the x coordinate located in the left of the image, and positive value of the y coordinate located in the anterior part of the image, and positive value of the z coordinate located in the superior part of the image. The original coordinate of the image must be checked, because REST will use the original coordinate to locate the position of the seed.

![ROI Definition List](image)

Figure 23 ROI Definition List. Here could add more than one ROI to perform the ROI wise analysis. The mean time series can be extracted by choosing the related option in Misc list in this interface.
3.6.1.2 Add the covariates

The covariate’s directory can be defined in the “Covariables” section. The covariates file could be text file (*.txt) or 1D file (extracted time series by AFNI, *.1D), and each column of the input file is considered as a covariate. If the covariate of each data was different, the covariate file must be changed for each data when calculating. If the covariates of each data were the same or no covariates were used, functional connectivity analysis can be performed for the multi-data at one time.

**Extremely Caution:** If the covariate was used, REST will add the linear trend to the covariates before calculating.

3.6.1.3 Fisher’s z transform
In the “Output Parameters” section, there is an option about Fisher’s $z$ transform (Fig. 25). The correlation coefficient need to be transformed to the normal distribution by Fisher’s $z$ transform before performing the random effect t-tests. If selected, REST will calculate the Fisher’s $z$ map using the following formula:

$$z = 0.5 \log \frac{1 + r}{1 - r}$$

The result will be saved in the output directory with a prefix “$z$”. For example, if the correlation coefficient map was “FCMap_OneCleanDir.img/hdr”, then the $z$ map will be “$z$ FCMap_OneCleanDir.img/hdr”. It is also available for the text file of the result of the ROI wise analysis.

![Output Parameters](image)

**Figure 25.** Output setting of functional connectivity analysis.

### 3.6.2 Calculating the functional connectivity map

After clicking “Do all” (Fig. 26), the fMRI data will be processed as following: i) remove linear trend; ii) band-pass (0.01~0.08 Hz) filtering; iii) A spherical region of interest (ROI) (radius = 10 mm) was centered at the given coordinates ($-2$, $-51$, $27$), and the mean time series within this ROI was considered the reference time course. Then a seed correlation analysis (SCA) will be performed in a voxel-wise way. Prefix of the
output file is “FCMap” and “zFCMap”. “FCMap” indicated the spatial map is the correlation coefficient map, and “zFCMap” indicated the spatial map is the Fisher’s z map. “zFCMap” can be used for the t-test. All of the result maps (result maps in Fig. 26 are FCMap_test_Control-1.img/hdr, FCMap_test_ADHD-1.img/hdr, zFCMap_test_Control-1.img/hdr, zFCMap_test_ADHD-1.img/hdr) will be saved in the “D:\Temp” directory.
Figure 26. The demo of functional connectivity analysis. In this example, the seed ROI was setting through clicking “Voxel wise” button and then enter the center (-2, -51, 27) and radius (10 mm) of ROI. “Covariables” was empty, so there was no covariate used in this process.

3.7 Other tools

REST also provides series of tools for manual operations (Fig. 27, Fig. 28).
Figure 27. The manual options of ReHo and ALFF GUI.

Figure 28. The manual options of functional connectivity analysis GUI.

- Help: Get the help document.

- Divide mean: In this option, the spatial map can be divided by that map’s average value within a selected mask. After clicking it, a 3D image can be selected and calculated. The result image will be saved in the directory of selected image with a prefix “m”.

- Fisher Z: In this option, the spatial map can be calculated by using the Fisher’s z transform (see details in 3.6.1.3. Fisher’s z transform). After clicking it, a 3D image can be selected and calculated. The result image will be saved in the directory of selected image with a prefix “z”.

- Slice Viewer: Similar with the MRIcro (Fig. 29). This tool can view the image in orthogonal and multi-slice manner (Montage is also available). It can also merge a low resolution image on a high resolution image and select these two images from the same subject is recommended. Slice viewer can view ROI, cut a threshold of overlay, extract a cluster which included the selected voxel (left click the mouse) in the overlay as a mask file, and draw the time series and its
power spectrum of the selected voxel (left click the mouse) (Fig. 30).

**Extremely caution when using slice viewer:**

a) In REST coordinates, left is positive value and right is negative value in the axis view; anterior of the image is positive value and posterior of the image is negative value in the coronal view; superior of the image is positive value and inferior of the image is negative value in the sagittal view.

b) Slice viewer do not reverse the original image.

c) The entered coordinates must in term with the input image. If the image was in the MNI standard space, the MNI coordinates should be used during the analysis by REST. If the image was in the Talairach view, the Talairach coordinates should be used during the analysis by REST.

d) The coordinates showed in the slice viewer can be edited. The slice viewer round the coordinates to show the voxel’s position. This coordinates (X, Y, Z) is underlay’s coordinates. The coordinates of overlay can be viewed by clicking the “Click to Toggle Hdr info”.
Power spectrum: draw the time series and its power spectrum of the selected voxel (left click the mouse) and viewed by slice viewer. If this button was clicked, one volume which stored in the selected folder need to be selected as the underlay and this tool will read all volumes of the selected folder, and then show the figures (Fig. 30). Voxel was selected by left mouse click.
Figure 30. Time series and its power spectrum of one voxel viewed by REST.

4. Websites


a) Download:

http://sourceforge.net/project/platformdownload.php?group_id=19736

3)

b) Main page: http://resting-fmri.sourceforge.net/

c) Project page: http://sourceforge.net/projects/resting-fmri

d) Code and the program:

http://resting-fmri.sourceforge.net/index_RESTCodes.html

5. Reference list


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