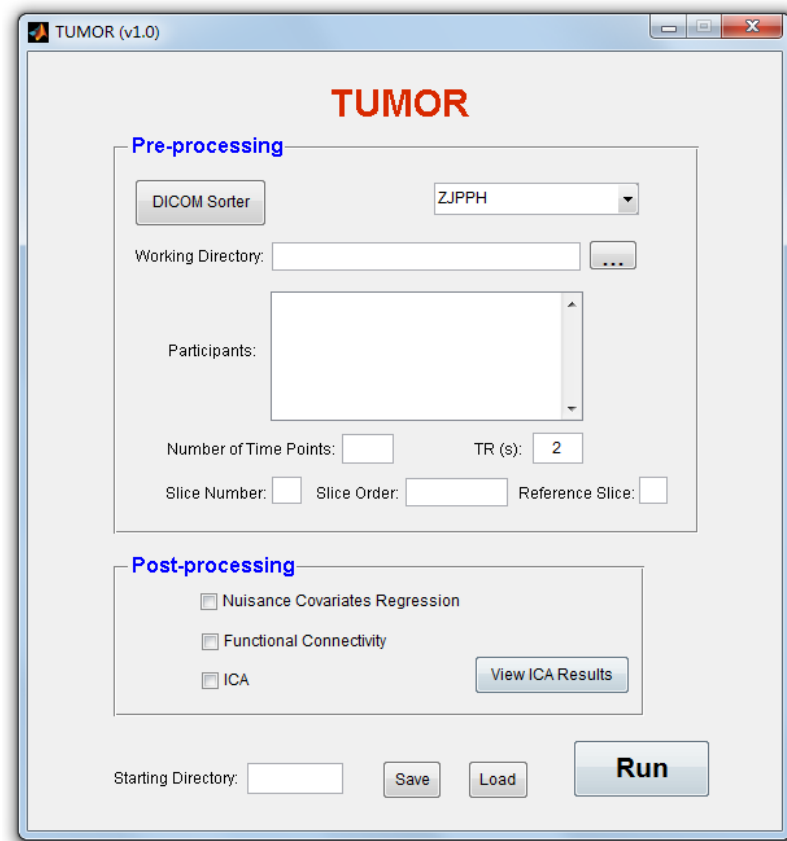


Toolbox for **f**unctional **M**apping pre**O**peratively
based on **R**esting-state fMRI (**TUMOR**)

Manual

(Version: 1.0)



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1. Set up

1.1 Running environment

Hardware Environment:

CPU: Intel 2.0 GHz or above

Memory: 2 G or above

Hard Disk: 20 G or above

Software Environment:

Support of Win7, Windows XP, Linux/Unix system

Support of Matlab version 2009 or later

1.2 Necessary software

Need to set path the software listed below in Matlab:

SPM8

REST_V1.8_121225

MICA_beta1.22

1.3 Install TUMOR

Download and unzip TUMOR:

Download the software from <http://restfmri.net/forum/node/2032>,

Unzip TUMOR.zip and add path in Matlab (Figure 1-2).

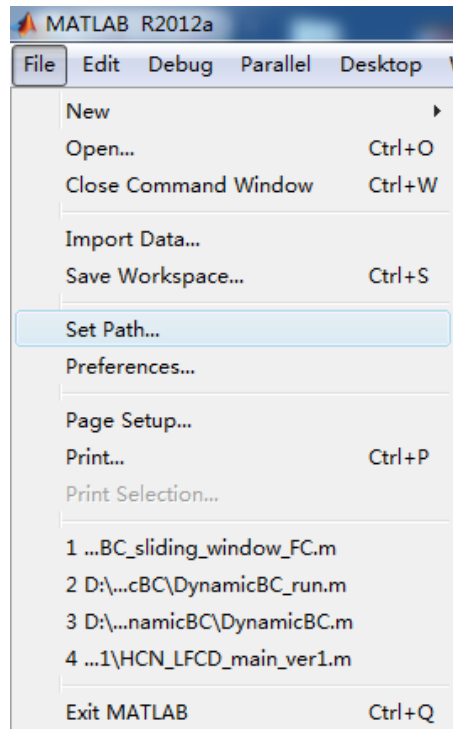


Figure 1

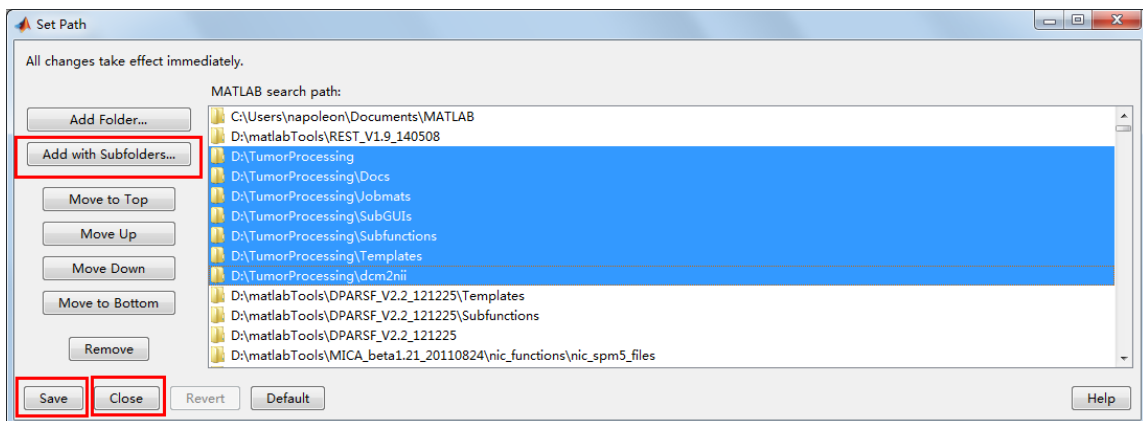


Figure 2

Matlab: 'File' -> 'Set Path' -> 'Add with subfolders' -> select the folder 'TUMOR'

-> 'save' -> 'close'

Then the TUMOR toolbox has been set up (Figure 2).

The same installing method for REST, SPM and MICA.

Open the TUMOR:

Open the Matlab;

Type 'TUMOR' in command Window and press enter (Figure 3):

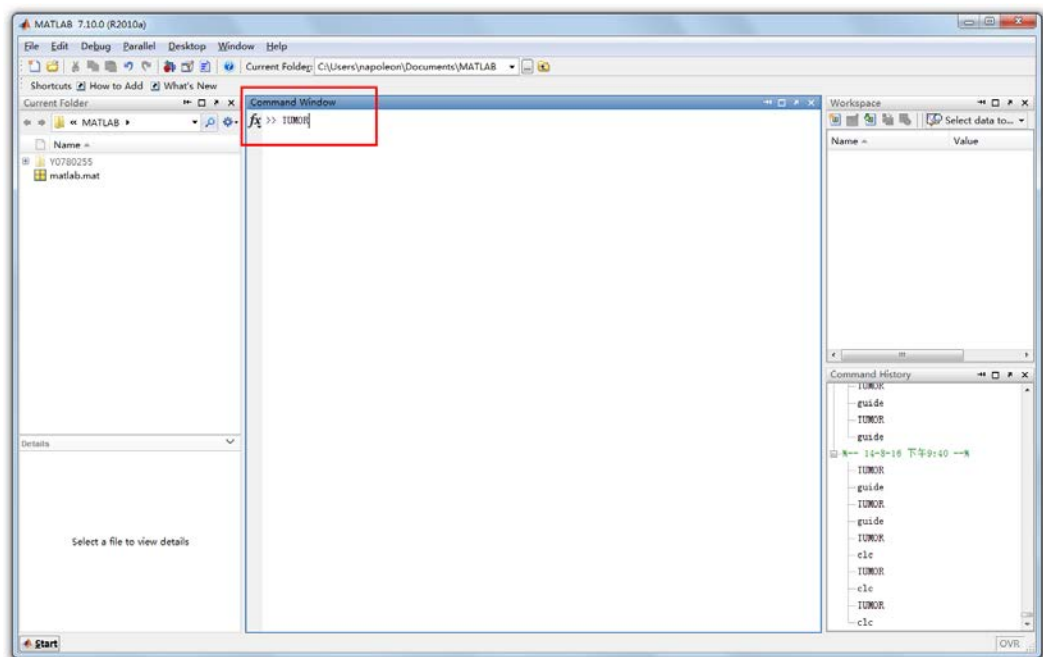


Figure 3

Then the Main Window of the toolbox will appear (Figure 4).

Introduction for the Main Window of the toolbox:

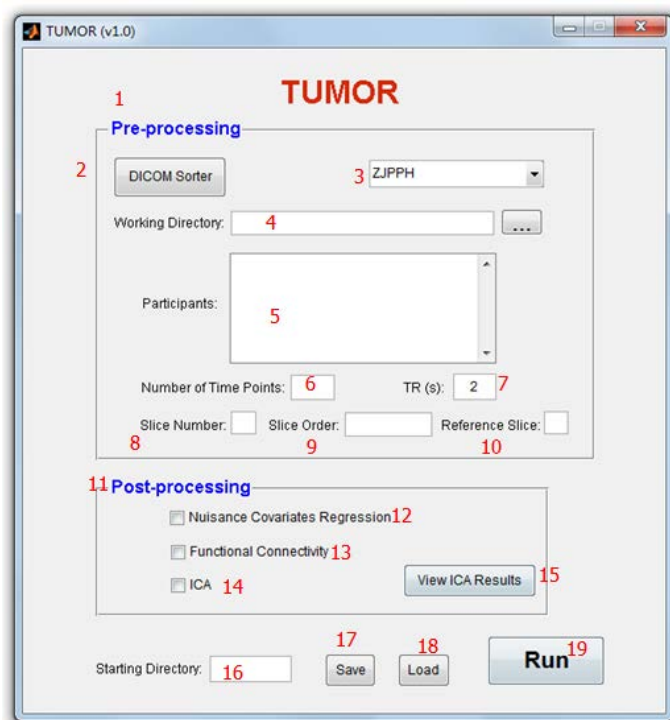


Figure 4

1. Pre-processing module, 2. DICOM Sorter, 3. Parameter templates, 4. Working directory selecting, 5. Display data from every subject, 6. The number of time points (volumes), 7. Temporal resolution (TR), 8. Slice number, 9. Scanning slice order, 10. Reference slice, 11. The post-processing module, 12. Nuisance covariate regression and de-noising, 13. Seed-based functional connectivity analysis, 14. Independent component analysis, 15. View the result of ICA, 16. Starting directory setting, 17. Button for saving the parameters, 18. Button for loading the parameters, 19. Run the program after finishing setting up the parameters.

2. Software Manual

2.1 DICOM Sorter

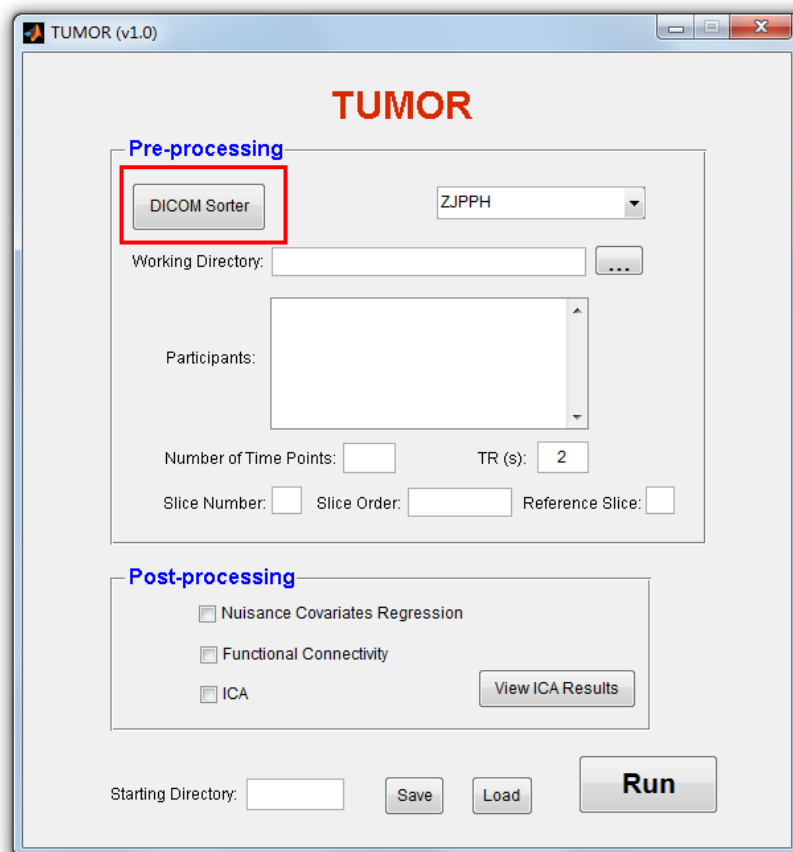


Figure 5

Since the original DICOM data from the hospital is directly exported from the MRI scanner, the data organization structure is often in chaos, with the images of different modal

may mix together, or there is no clear classification of data directories, see Figure 6.

名称	修改日期	类型	大小
17581403	2014/8/7 4:19	文件	349 KB
17581421	2014/8/7 4:19	文件	349 KB
17581439	2014/8/7 4:19	文件	349 KB
17581457	2014/8/7 4:19	文件	349 KB
17581475	2014/8/7 4:19	文件	349 KB
17581493	2014/8/7 4:19	文件	349 KB
17581511	2014/8/7 4:19	文件	349 KB
17581529	2014/8/7 4:19	文件	349 KB
17581547	2014/8/7 4:19	文件	349 KB
17581565	2014/8/7 4:19	文件	349 KB
17581583	2014/8/7 4:19	文件	349 KB
17581601	2014/8/7 4:19	文件	349 KB
17581619	2014/8/7 4:19	文件	349 KB
17581637	2014/8/7 4:19	文件	349 KB
17581655	2014/8/7 4:19	文件	349 KB
17581673	2014/8/7 4:19	文件	349 KB
17581691	2014/8/7 4:19	文件	349 KB
17581709	2014/8/7 4:19	文件	349 KB
17581727	2014/8/7 4:19	文件	349 KB
17581745	2014/8/7 4:19	文件	349 KB
17581763	2014/8/7 4:19	文件	349 KB
17581781	2014/8/7 4:19	文件	349 KB
17581799	2014/8/7 4:19	文件	349 KB
17581817	2014/8/7 4:19	文件	349 KB

Figure 6

Thus, it is necessary to sort the DICOM data according to its modality and scanning order:

Choose the button '**DICOM Sorter**' on the left corner of the interface (Figure 5).

You will see the following figure (Figure 7):

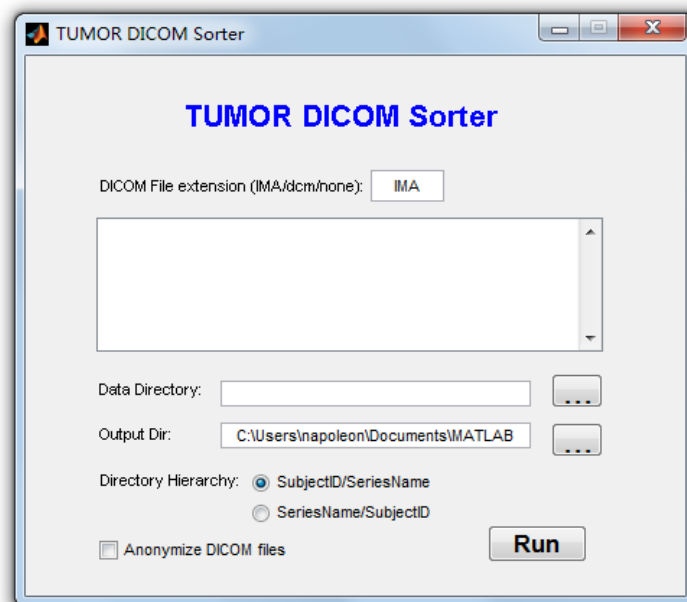


Figure 7

- **DICOM File extension (IMA/dcm/none):**

Fill in the format of the raw data (e.g. the suffix of your DICOM files). What is shown

in the brackets represents several common suffix of the DICOM files. As the data shown in the Figure 6, you should type 'none' because there is no suffix.

- **Right-click the mouse in the list box (the blank below where you input the DICOM extension), you will get the pop-up menu as shown in Figure 8:**

There are four choices:

1. **Add a directory:** Choose the folder where you store the raw data;
2. **Remove selected directory:** Remove the selected directory from the list box;
3. **Add recursively all sub-folders of a directory:** Add recursively all sub-folders in the root directory (**recommended**). Once you choose the root directory, all sub-folders and files in the root directory will be included;
4. **Remove all data directories:** Remove all data directories in the list box.

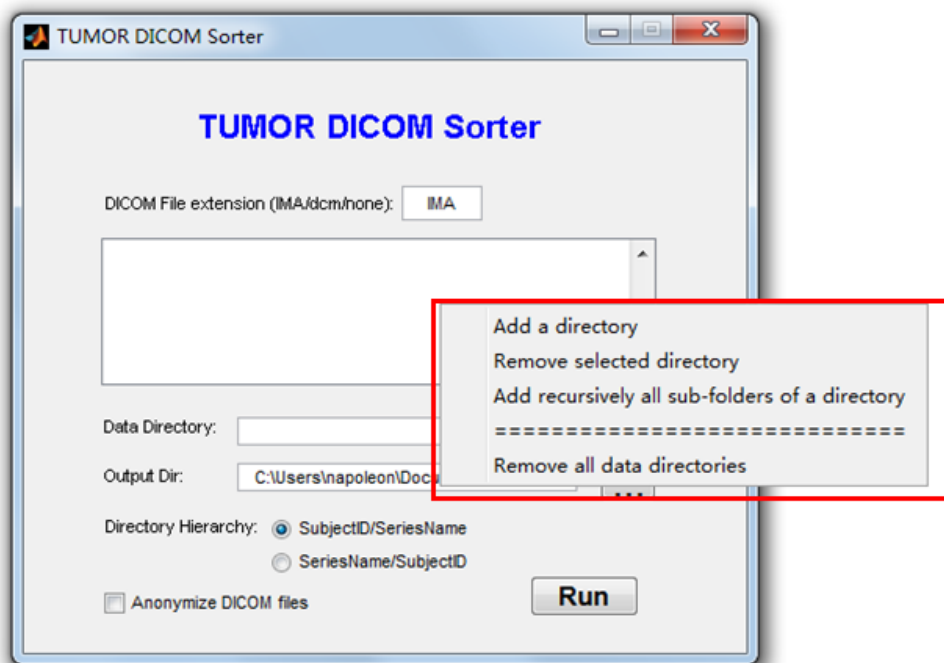


Figure 8

- **Data Directory:** The other way to choose a file folder where you store the raw data, but you can choose only one folder rather than add recursively all sub-folders;
- **Output Dir:** The output directory for newly sorted DICOM files;
- **Directory Hierarchy:** The new files after DICOM sorting can be stored in two directory naming conventions:
 1. SubjectID/SeriesName (in each "SubjectID" folder, there are several subfolders for each imaging modality with the names of "SeriesName");
 2. SeriesName/SubjectID (in each "SeriesName" folder, there are subject folders named as "SubjectID", **you should select this naming convention!**)
- **Anonymize DICOM files:** Anonymizing DICOM files, in order to protect privacy

information of subjects.

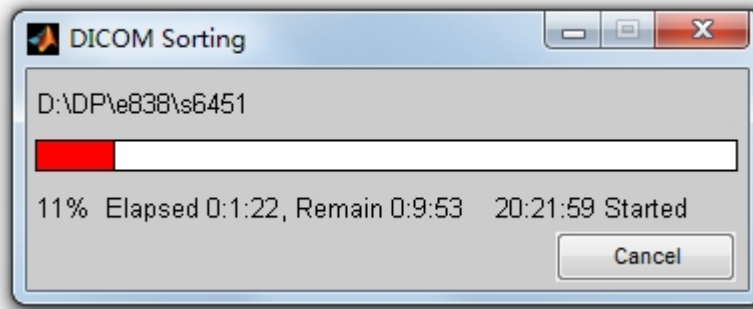


Figure 9

Finally, click button '**Run**' to begin the data sorting and a progress bar will appear (Figure 9). After the progress bar disappearing (please be patient), the data sorting has finished. A new folder will be generated in the output directory which you have set before, including various subfolders which has been well classified, as the figure shown below:

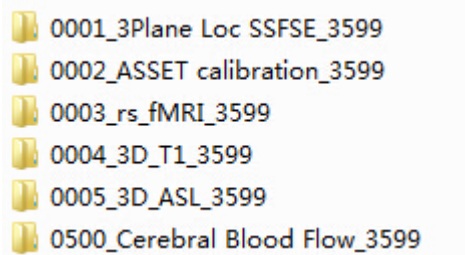


Figure 10

For the resultant folder with resting-state fMR images (such as, the '**003_rs_fMRI_3599**' folder in Figure 10 from our example), re-name it as '**FunRaw**' (Figure 11). Then re-name the subfolder in this Funraw folder, e.g., re-named as '**Li_YF**' (Figure 12).

For the T1 images (generally there dimensional high resolution anatomical images, such as, the '**004_3D_T1_3599**' folder in Figure 10 from our example), re-name it as '**T1Raw**' (Figure 11). Then rename the subfolder in this T1Raw folder, e.g., re-named as '**Li_YF**' (Figure 12). **Note that, the subfolder name in the T1Raw must be the same as that in the FunRaw.**

Put the two folders '**FunRaw**' and '**T1Raw**' into a root directory (e.g., 'LYF3' in Figure 11), which will later be the working directory of the whole data processing, see the figure below:



Figure 11

After the above steps, the organization of the data folder should be similar to Figure 12 below. The folder names '**FunRaw**' and '**T1Raw**' **cannot** be changed. The other folder names can be decided by you (LYF3, Li_YF, etc.), but please note that, without any spaces and non-English characters (but the underscore is allowed to use). Moreover, it is necessary to ensure that the subfolder name under '**FunRaw**' and '**T1Raw**' is the same, so that **TUMOR** can identify them and the program can run successfully.

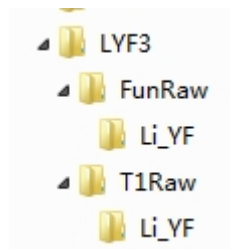


Figure 12

2.2 Parameter Templates

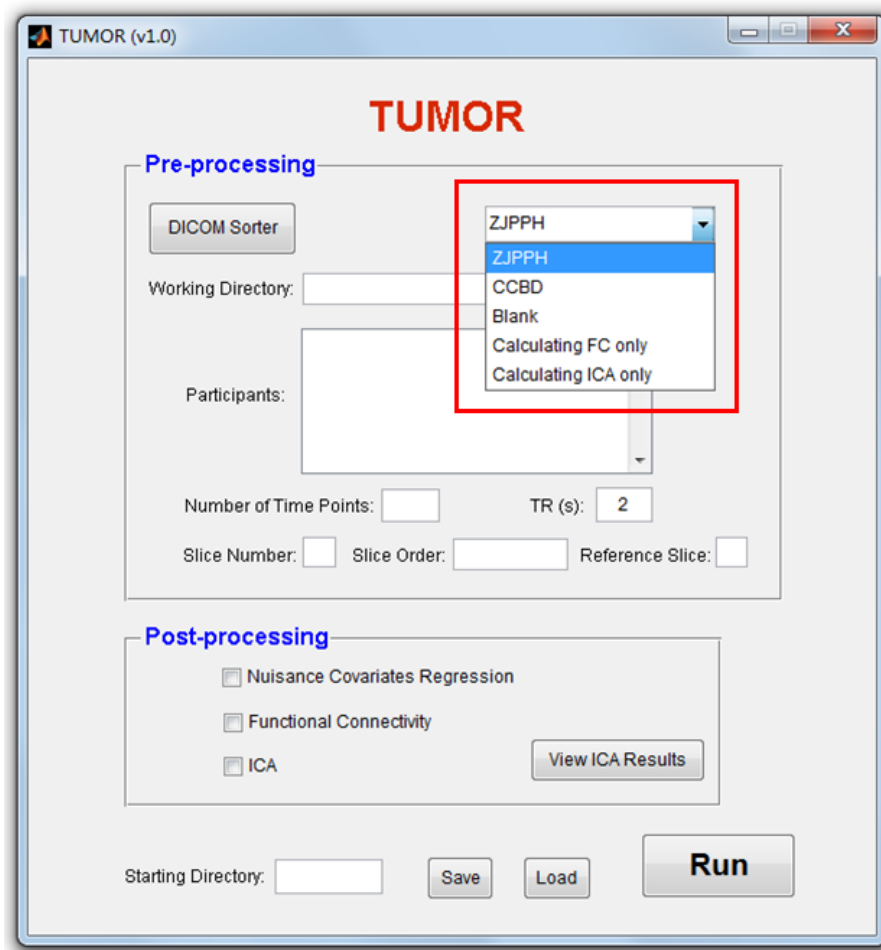


Figure 13

Click the popup menu on the top right corner of Figure 13, the parameter templates are displayed. The parameter template offers various scanning parameters, pre-processing parameters from different collaboration hospitals; it also offers different templates for different data processing procedures.

Parameters templates for Zhejiang Province People's Hospital (**ZJPPH**) and for Center for Cognitive and Brain Disorders (**CCBD**) are added into **TUMOR v1.0**. Provided your data comes from any of these two centers, you do not need to manually input these parameters one by one. What you need to do is simply select the corresponding parameter template in the popup menu.

For example, after selection of '**ZJPPH**', the parameters for processing the data from Zhejiang Province People's Hospital will be automatically loaded. These parameters include the number of time points, TR, Slice Number, Slice Order, and Reference Slice (Figure 14).

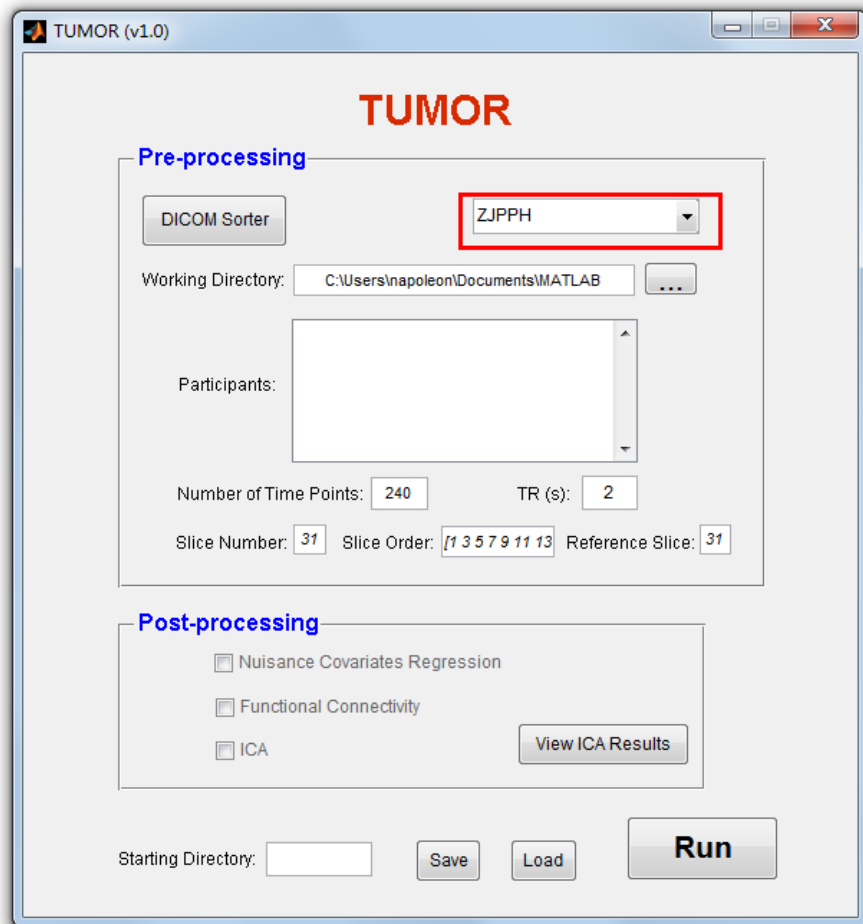


Figure 14

The parameters for processing the data acquired from the CCBd (from a 3T GE Discovery MR750 scanner) are shown in Figure 15.

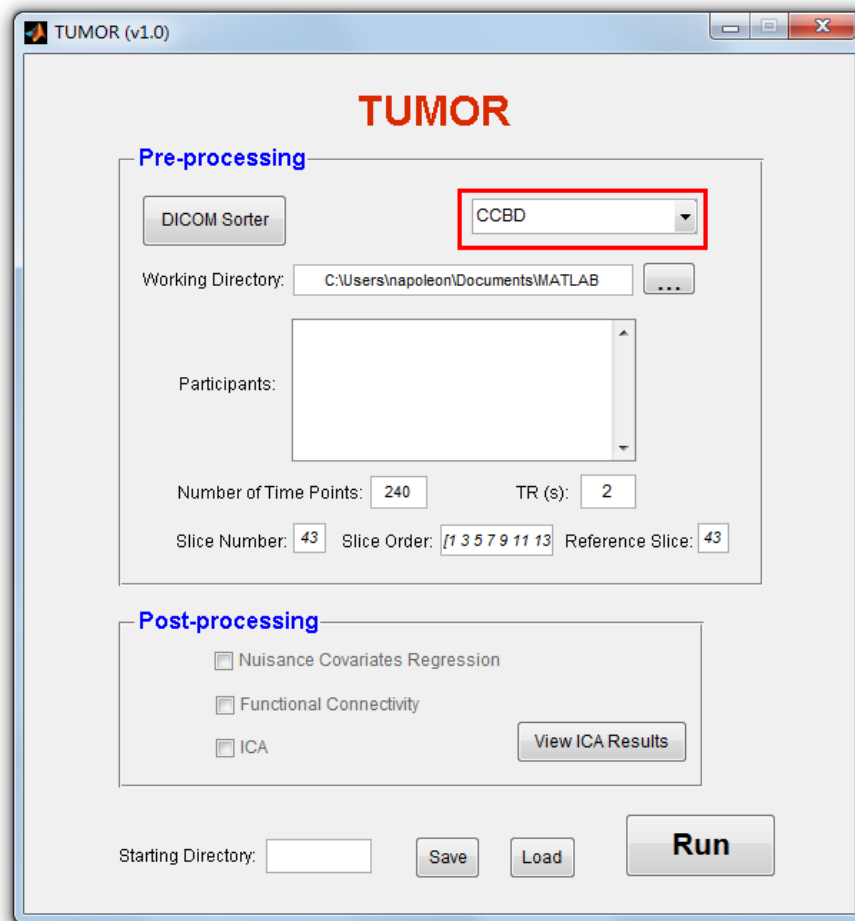


Figure 15

If your data has been acquired from other MRI scanner or other hospitals/institutes/ imaging centers, the parameter template is not included in the popup menu, you may need to select the '**Blank**' option; then, every parameter can be cleared (Figure 16). You can manually enter the value of each parameter.

Welcome to provide us with data from your own hospital, we can add it as a parameter template in the later version of **TUMOR**. We will also continue to add more parameter templates from different institutions, making the process of pre-processing more automatically, thereby saving the time of data processing.

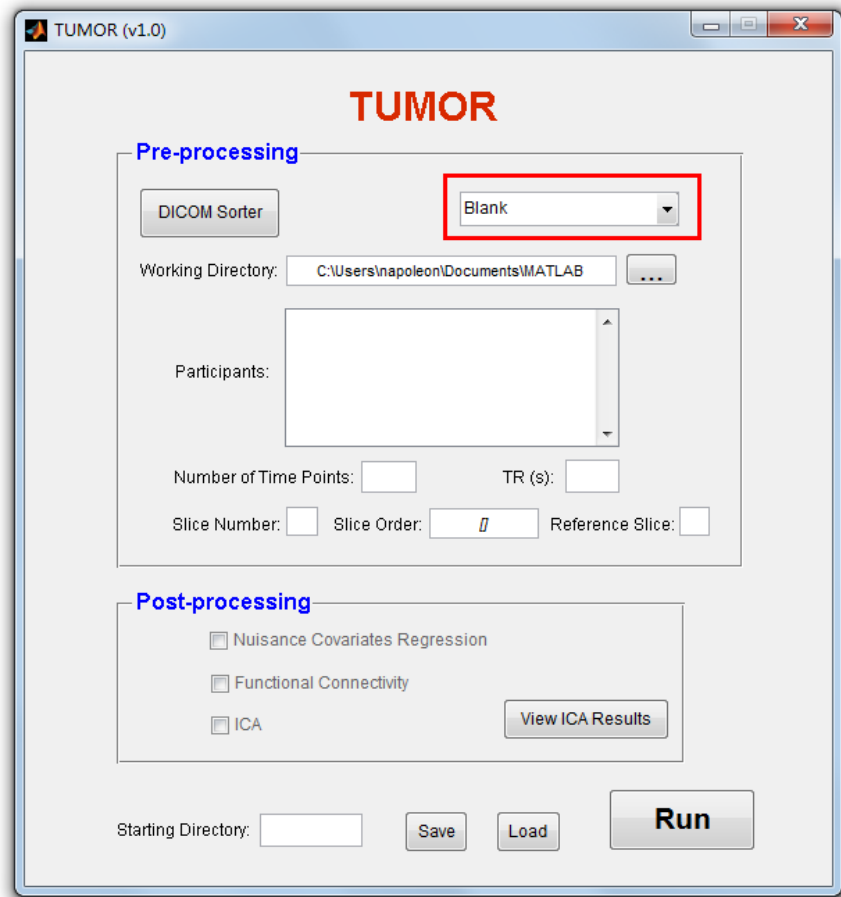


Figure 16

The third option of the popup menu, '**Calculating FC only**', and the fourth option, '**Calculating ICA only**', represent “performing seed-based functional connectivity only” and “performing ICA only”, respectively. Once one of the two options has been selected, the font color of the corresponding option will change into blue (Figure 17). You can choose either of them, or choose both.

Generally, seed-based functional connectivity is more recommended; and ICA serves as a supplementary method to the seed-based functional connectivity. So we recommend you always choose seed-based functional connectivity, and choose ICA if you need. In some case, where the seed region is difficult to define, ICA comes out as a better choice.

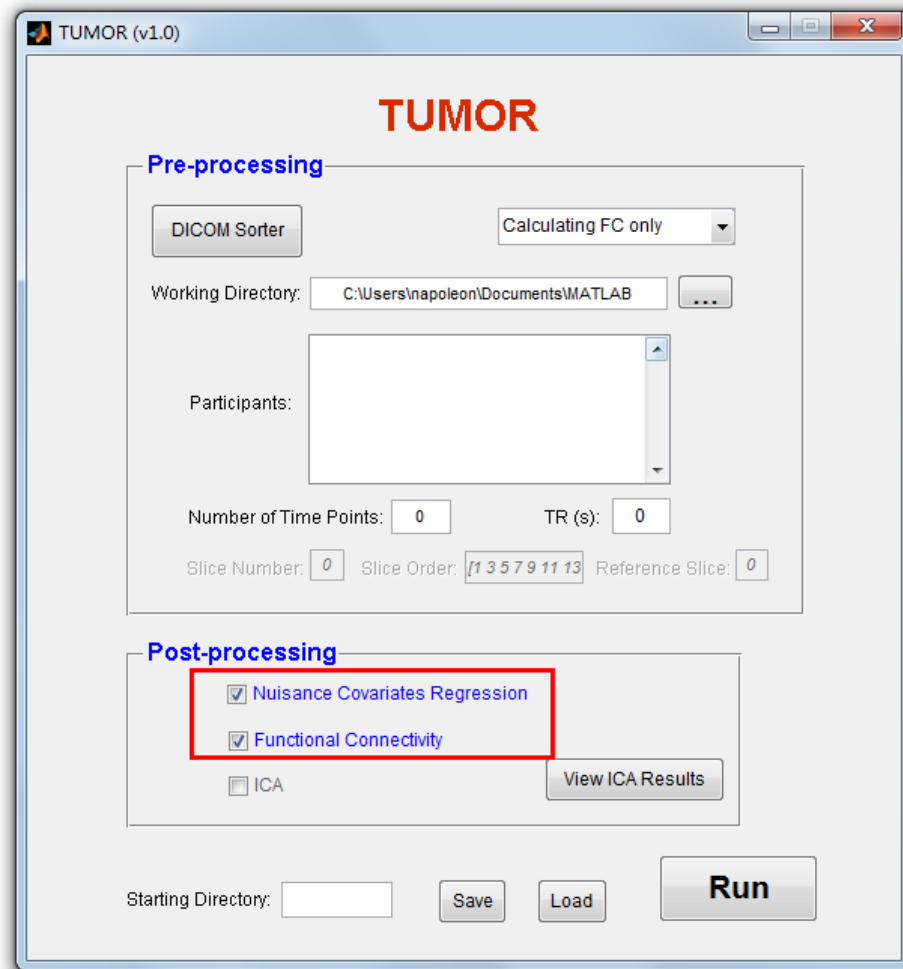


Figure 17

2.3 Working Directory

Click the button ‘...’ on the right of the edit box ‘**Working Directory**’, a file selecting dialog box will pop up (Figure 18). Select the root folder where you store ‘**FunRaw**’ and ‘**T1Raw**’ (in the example, this is ‘LYF3’), as the working directory for the whole data processing. All the results will be generated in this working directory.

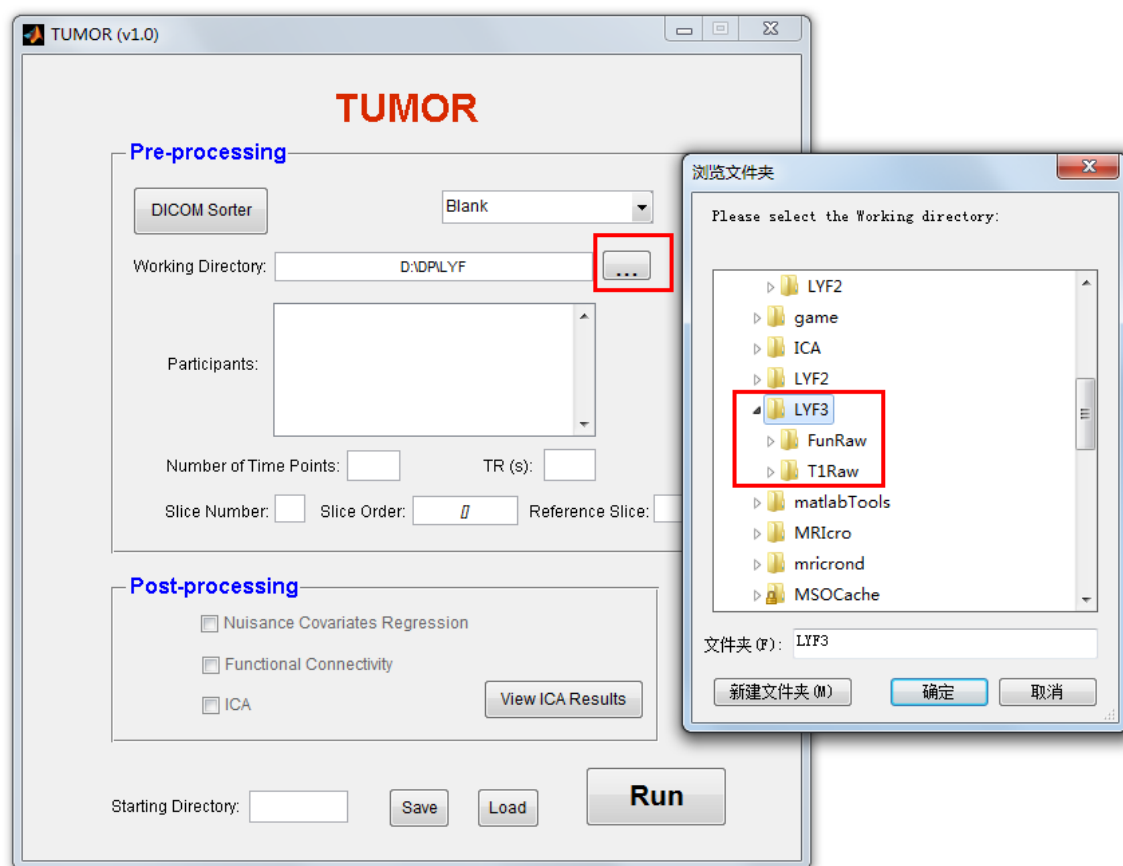
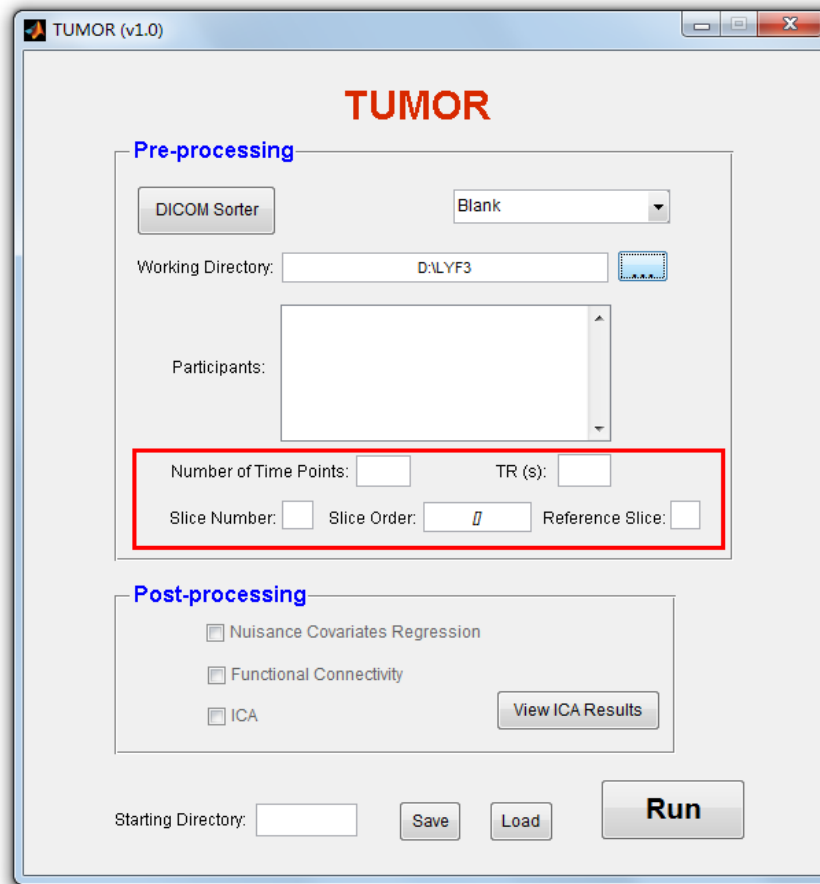


Figure 18

2.4 Parameter for pre-processing



The screenshot shows the TUMOR (v1.0) software window. The title bar reads 'TUMOR (v1.0)'. The main window has a red 'TUMOR' logo at the top. Below the logo, there are two sections: 'Pre-processing' and 'Post-processing'. The 'Pre-processing' section contains a 'DICOM Sorter' button, a 'Blank' dropdown menu, a 'Working Directory' text box with 'D:\LYF3', a 'Participants' list box, and a red-bordered box containing 'Number of Time Points', 'TR (s)', 'Slice Number', 'Slice Order', and 'Reference Slice' text boxes. The 'Post-processing' section contains three checkboxes: 'Nuisance Covariates Regression', 'Functional Connectivity', and 'ICA', along with a 'View ICA Results' button. At the bottom, there is a 'Starting Directory' text box, 'Save' and 'Load' buttons, and a large 'Run' button.

Figure 19

If your data is acquire from other hospital or other institute which is not included in the parameter templates, in this part should you set your own parameters. The pre-processing steps for preoperatively functional mapping generally include:

- Data format conversion: from DICOM to NIFTI
- Removal of the first 4 time points
- Slice timing correction
- Realignment
- T1 Image coregistering to mean EPI Image
- Smoothing
- Detrending (required for functional connectivity)

- Band-pass (0.01-0.08 Hz) filtering (required for functional connectivity)
- Nuisance Covariates Regression (required for functional connectivity)

Because several parameters for the above data processing are the same among different hospitals and institutions, for example, DICOM to NIFTI, removal of the first 4 time points, realignment, T1 Image coregistering to mean EPI Image, Smoothing, Detrending, and Filtering. Thus, these parameters are not displayed in the GUI, all these steps will be conducted automatically using the predefined parameters which you cannot see or change.

But for parameters such as '**Number of Time Points**', '**TR**', '**Slice Number**', '**Slice Order**', '**Reference Slice**', different scanners correspond to different settings. So these parameters are required to be set by users according to their data.

There are two ways to set these parameters:

1. Set by choosing one pre-exist parameter setting from 'Parameter Templates', see (2.2 Parameter Templates);
2. Set them manually by first selecting the 'Blank' option in the 'Parameter Templates' popup menu (Figure 16), and then manually input the values of these parameters in the blanks within the red box in Figure 19.

2.5 Functional Connectivity Analysis

Performing functional connectivity analysis via checking the check box on the left side of the '**Nuisance Covariate Regression**' and '**Functional Connectivity**' or, in another way, by selecting the option '**Calculating FC only**' in the '**Parameters Template**' popup menu (Figure 20).

The '**Calculating FC only**' means only performing functional connectivity analysis but not performing other steps such as preprocessing or ICA. Thus, if you choose this option, make sure that you have already preprocessed the data and had FunImgARSDF folder in the working directory. Once you select '**Calculating FC only**', check boxes '**Nuisance Covariate Regression**' and '**Functional Connectivity**' will be automatically selected and the corresponding color of the fonts will turn into blue (Figure 20).

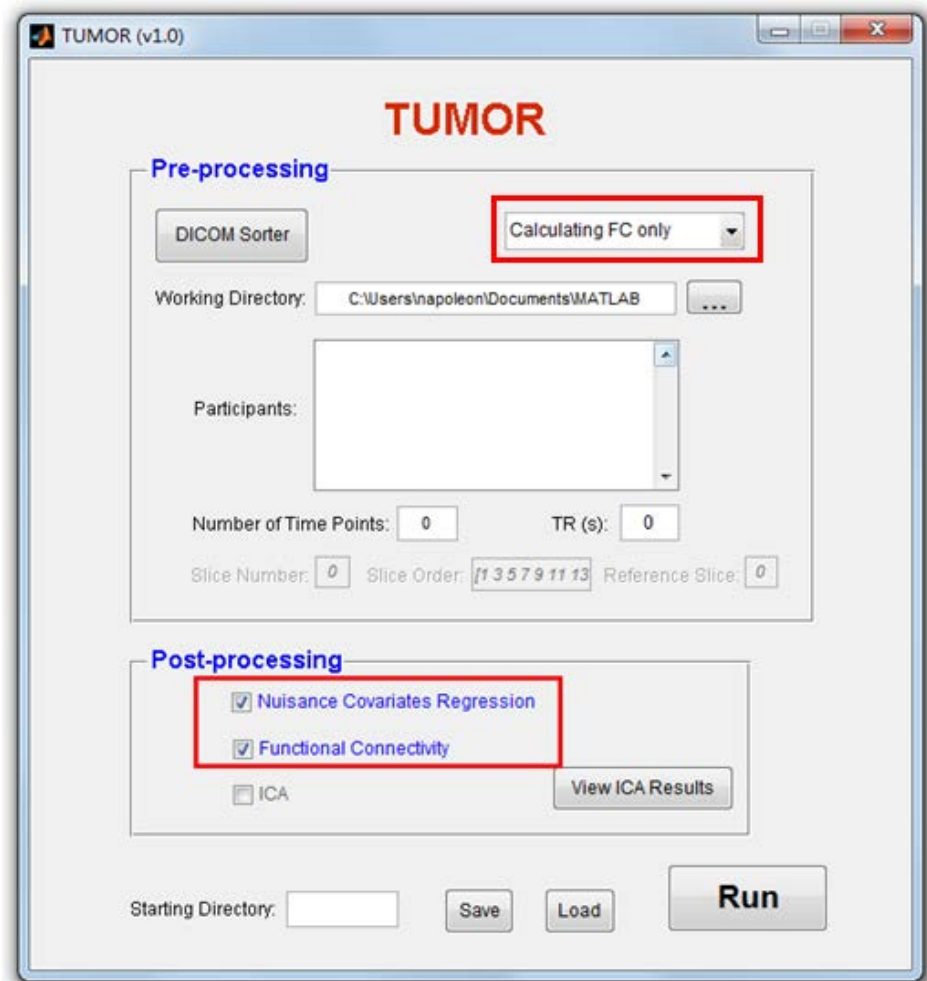


Figure 20

Functional connectivity analysis including two steps: nuisance covariate regression, and seed-based functional connectivity analysis. If check box '**Nuisance Covariate Regression**' is selected, when TUMOR toolbox is running to this step, the automatic processing will be held on. This pause is necessary for you to let you manually select the position of nuisance covariates (ROI). Since the data from different subjects will be in their own specific individual space, definition of the nuisance covariates ROI should be made individually. To do this, a GUI will pop up for you to let you select the position of the nuisance covariates ROI interactively (Figure 21).

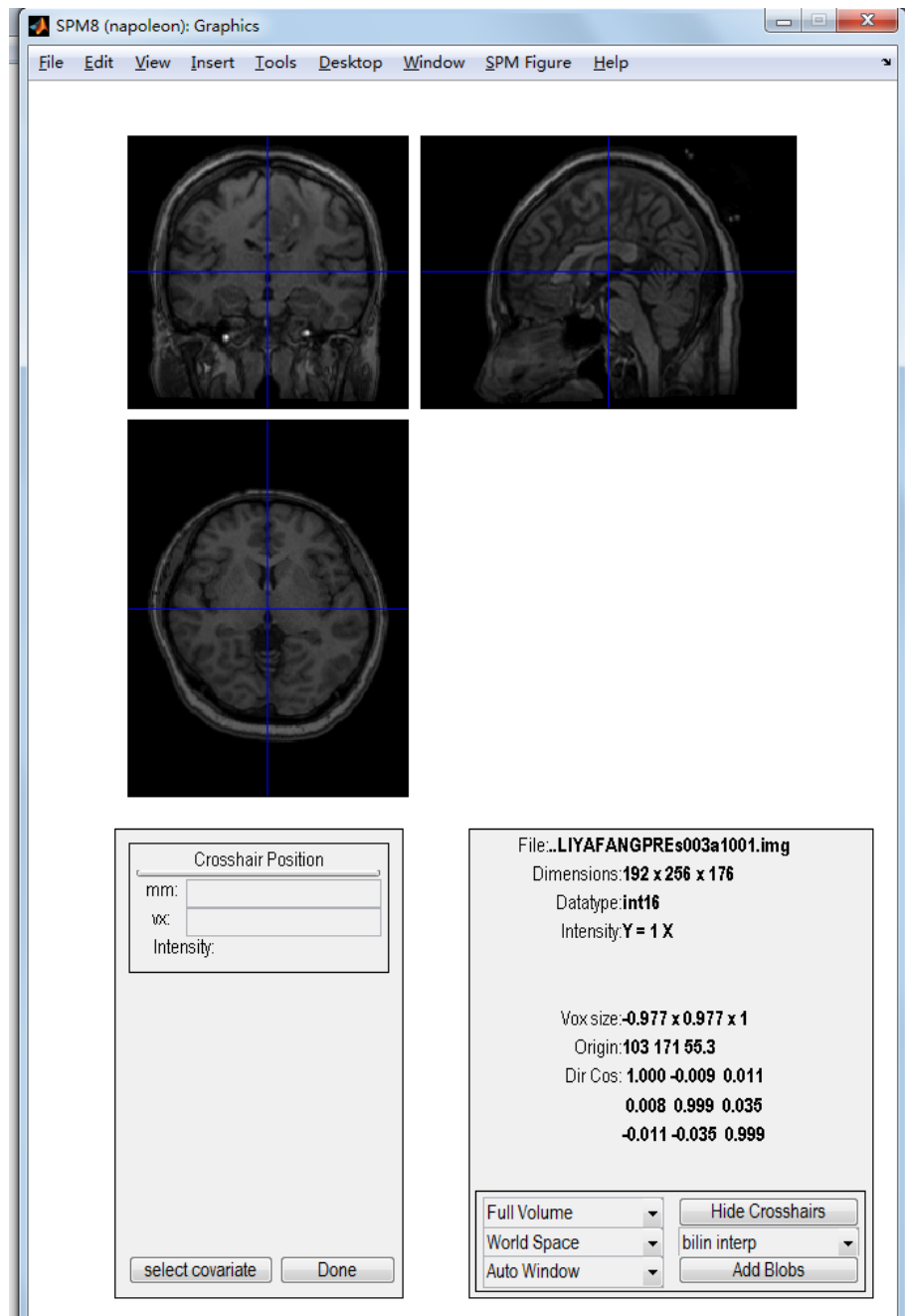


Figure 21

Figure 21 is the GUI interface for selecting nuisance covariates ROI. The anatomical image of the subject, which is the T1 image after coregistering to mean the subject's EPI image, is displayed and you can select the position where the nuisance covariates signal can be generated. To select, you just need to move the cursor (blue cross) to the position you want, and press 'select covariate' button in the left bottom corner (Figure 21).

The blue crosshair in Figure 21 is to indicate the position of the nuisance covariates ROI. Once you have chosen a specific position to be the nuisance covariates ROI, the GUI will generate the corresponding coordinates of this ROI and display it in the text box

'mm' (millimeter) of 'Crosshair Position' and in the text box 'vx' with voxel coordinates. Note that the two coordinates are all in individual space.

Once you have chosen a specific position with the crosshair, you can click the button '**select covariate**' to save it. Then, you will notice that another GUI, the same as the previous one, will pop up to let you select other nuisance covariates ROI. You can define any number of covariates as you like and push the button '**Done**' to exit the GUI for selecting covariates.

During functional connectivity analysis, signals from the white matter (Figure 22) and the cerebrospinal fluid (CSF, e.g., ROI in ventricles) are usually suggested to be regressed out. This step is recommended because it will make the functional connectivity result more accurate, and less affected by the physiological noise such as heart pulsation and respiration. You can select several ROIs in the white matter and CSF. The principle of choosing nuisance covariates ROI is:

- Don't select any location near the gray matter;

- Don't select any location near the functional area;

- Don't select any location outside the functional image's Field-Of-View (FOV).

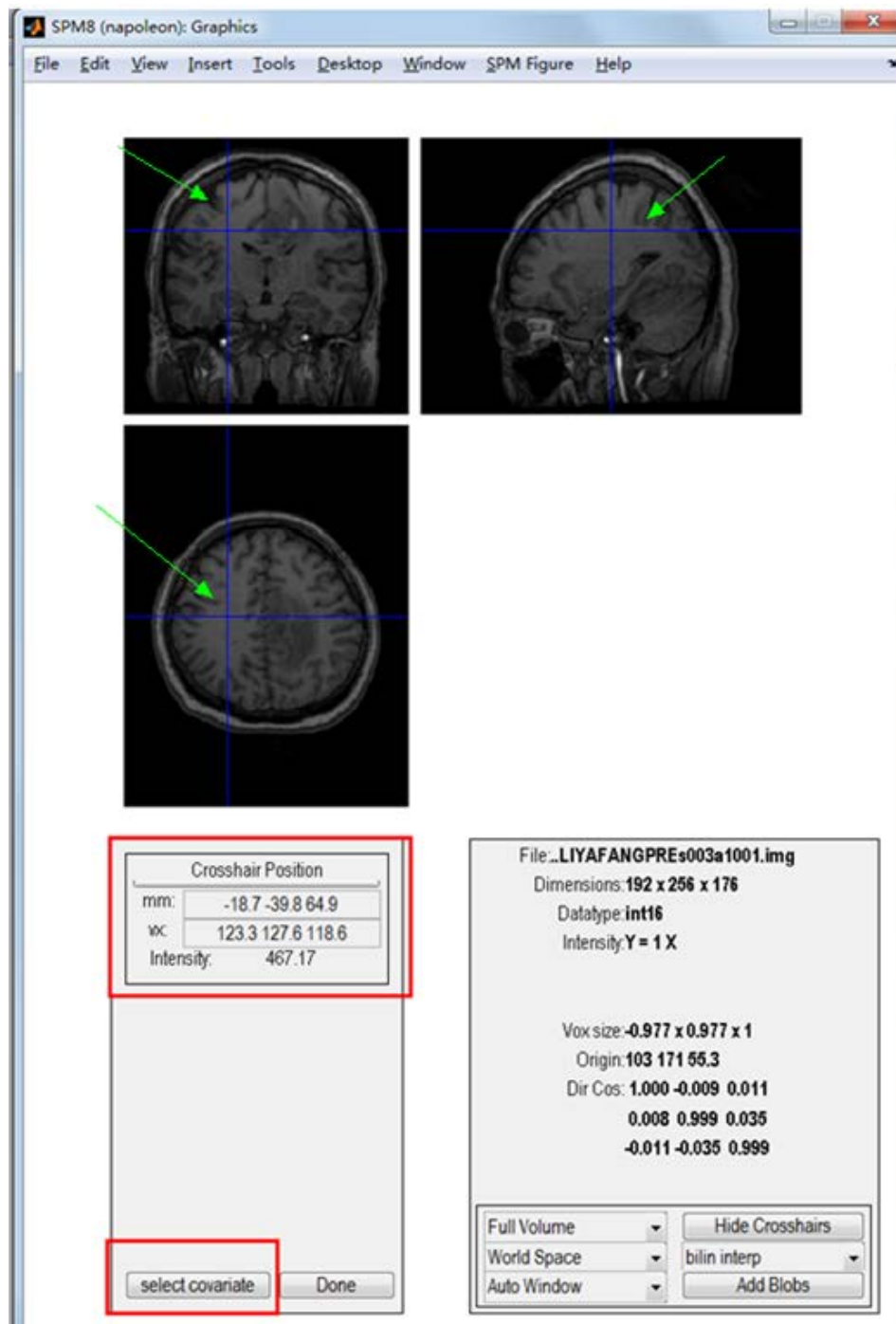


Figure 22

After nuisance covariate ROI definition, during seed-based functional connectivity analysis, a similar GUI will pop up for selecting seed ROI (Figure 24, in this example, we select the motor area as the seed). Similar to choosing a nuisance covariate ROI, choosing a seeds ROI(s) is by moving the blue crosshair to the location of the seed region, and click the left-bottom button '**define ROI**'. You can repeat this step to select multiple seed ROIs. When you don't want to define seed ROI any more, press button '**Done**' to exit the ROI

definition GUI.

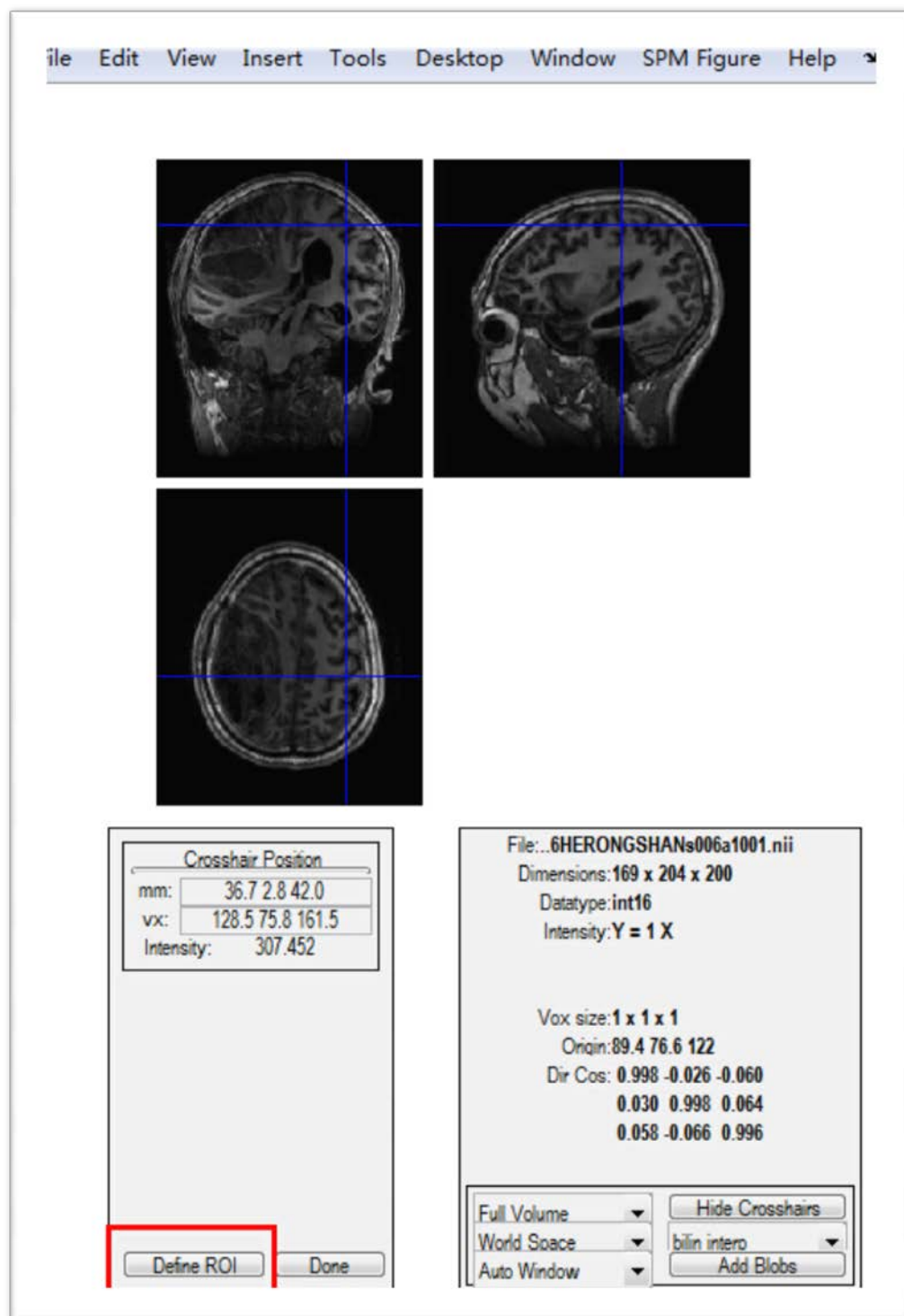


Figure 24

2.6 Independent Component Analysis (ICA)

Independent Component Analysis (ICA) is part of '**Post-processing**' in this software. As a popular method of functional connectivity analysis, ICA has wide applications. In particular, in the preoperative eloquent area localization for brain surgery, after performing ICA on resting-state fMRI data, the main functional networks of the patient can be preliminarily obtained, without the definition of seed regions or nuisance covariates ROIs.

Only a single check box '**ICA**' is designed for this module in the toolbox. Once you have chosen this check box, the program will run the ICA automatically without the need of manually inputting additional parameters. It realizes '**one-click-for-all**' function and greatly save the time of data processing. The ICA utilized by this toolbox will be run multiple times, each time with different initial value for starting the ICA estimation. Then, components from all ICA runs are integrated to produce the final result. This result has been demonstrated to be reliable and accurate than that derived from single ICA run.

There are two ways for specific ICA operation:

- Tick the check box '**ICA**' (processing will be automatically switched to ICA calculation);
- Select the fourth option '**Calculating ICA only**' in the popup menu '**Parameter Template**' (Figure 25). This will perform ICA only. Note that in this case, you should make sure the 'Working Directory' to be the data's root folder, and the 'Starting Directory' to be 'FunImgARS' (the folder where smoothed data is stored), and the 'Number of Time Points' to be *Total number of time points of the raw data - 2* (in the example, $240 - 2 = 236$), as well as the 'TR' to be set correctly.

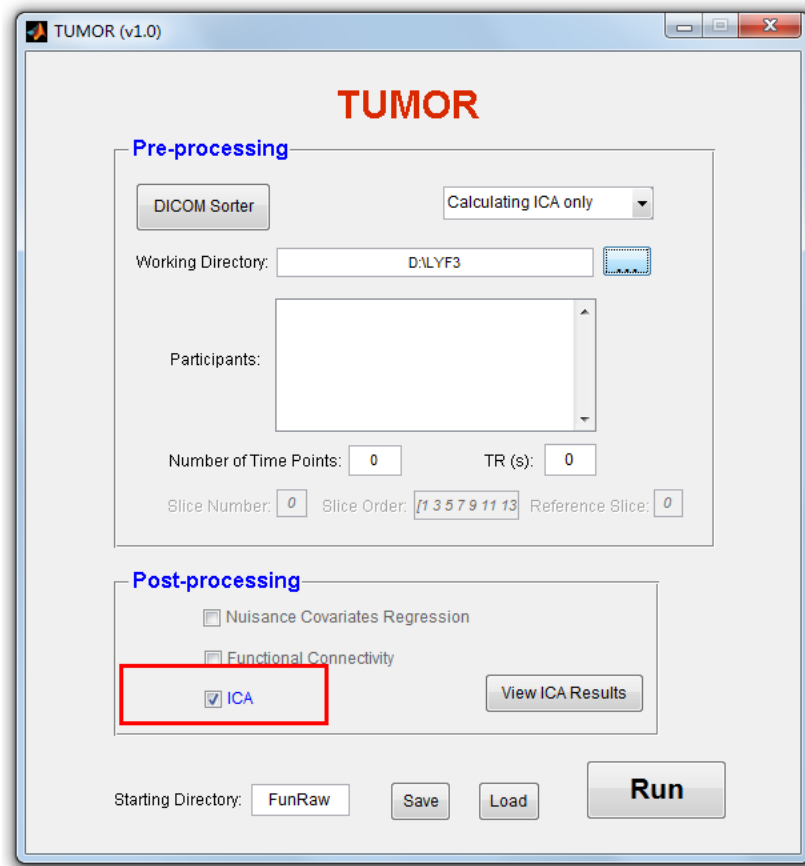


Figure 25

Click on the button 'Run', ICA will be started. A total progress bar and a single-run progress bar will appear as Figure 26 shown. Please wait patiently.

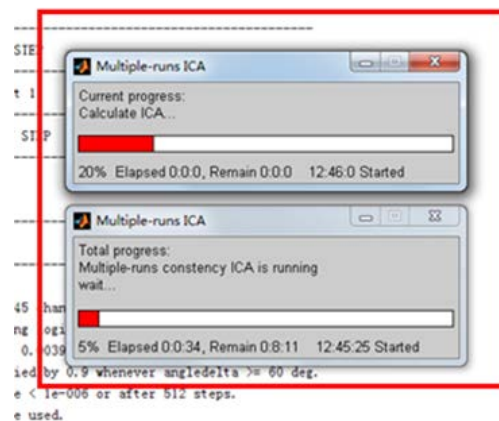


Figure 26

When finished ICA processing, a GUI for viewing the ICA result (Visualization tools) will show (Figure 27). A folder named '**ICA**' will be generated to save the result of ICA.

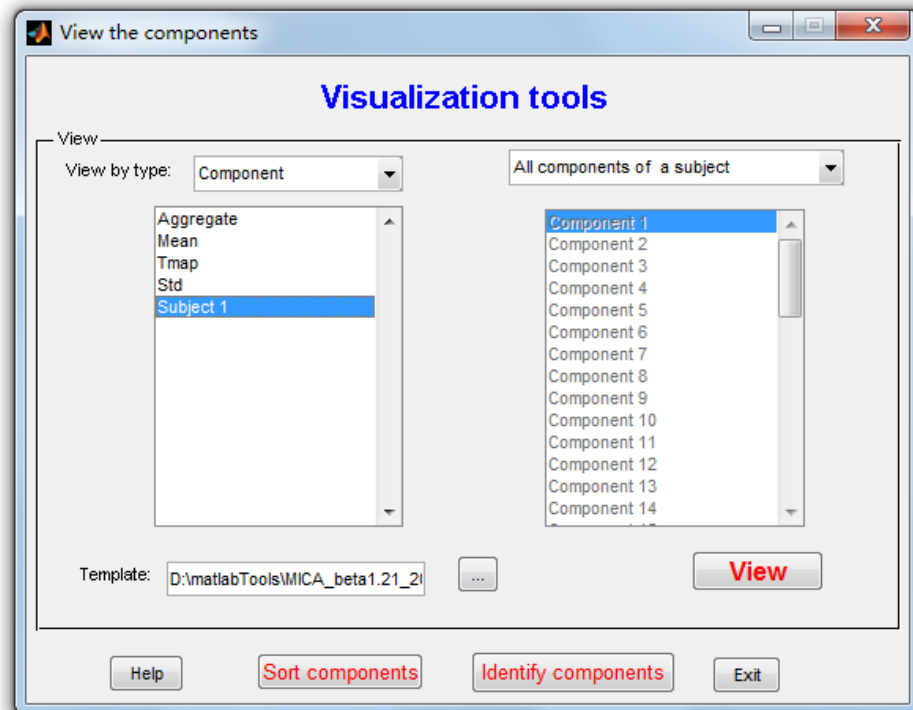


Figure 27

The result of '**ICA**' includes the following files (Figure 28):

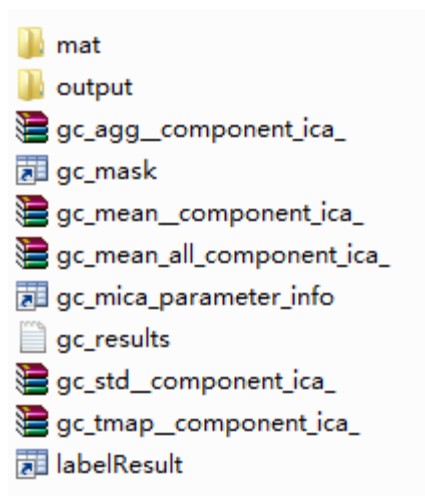


Figure 28

Among them, the result of ICA of specific subject, including the spatial maps and their associated time series for all components, is stored in folder ‘**output**’. The parameters of ICA are saved as a ‘.mat’ file named ‘**gc_mica_parameter_info**’.

If you happened to shut down the visualization GUI window in Figure 27, you can also view the result of ICA by pressing the button ‘**View ICA result**’ as Figure 29 shown.

Specific operations are as follows:

1. Press the button ‘**View ICA Result**’ (Figure 29, left). Then, press the button ‘Load’ for parameter file selection in the popup window (Figure 29, right);

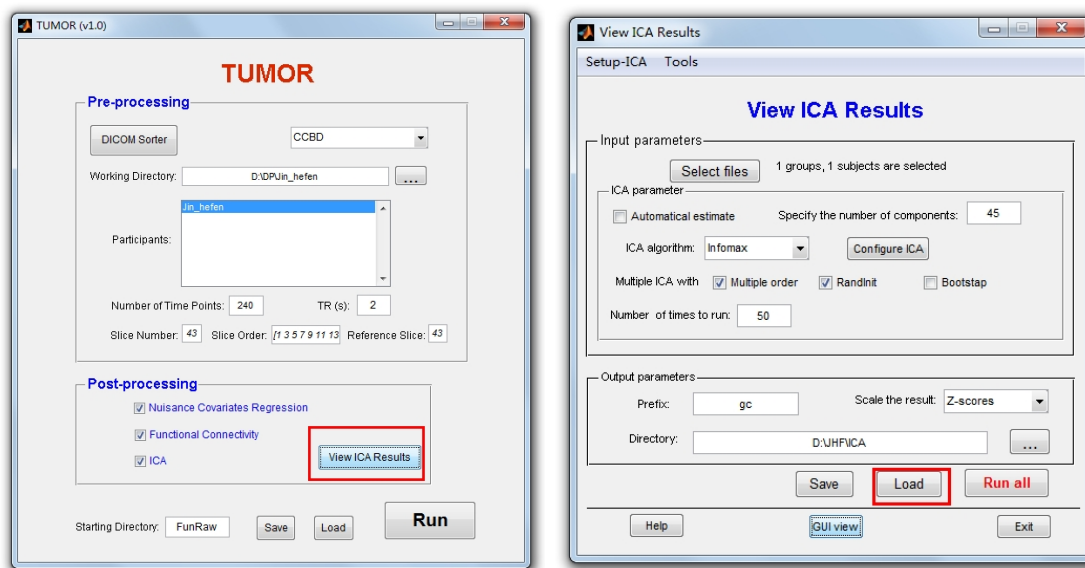


Figure 29

2. Select the ‘**gc_mica_parameter_info.mat**’ in the result folder ‘**ICA**’ (Figure 30), then press ‘Open’;

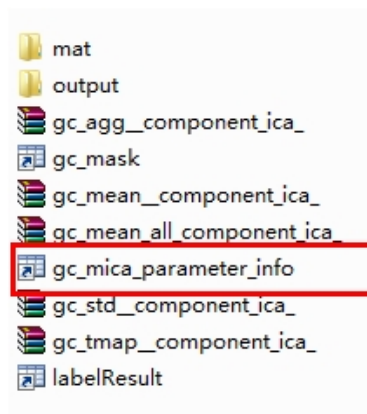


Figure 30

3. Set the parameters as Figure 31 (left) shown: View by type = Component, select 'Subject 1', select 'All components of a subject'. Then, click the button '...' besides '**Template**' to select the underlay anatomical image for this subject (Figure 31, right).

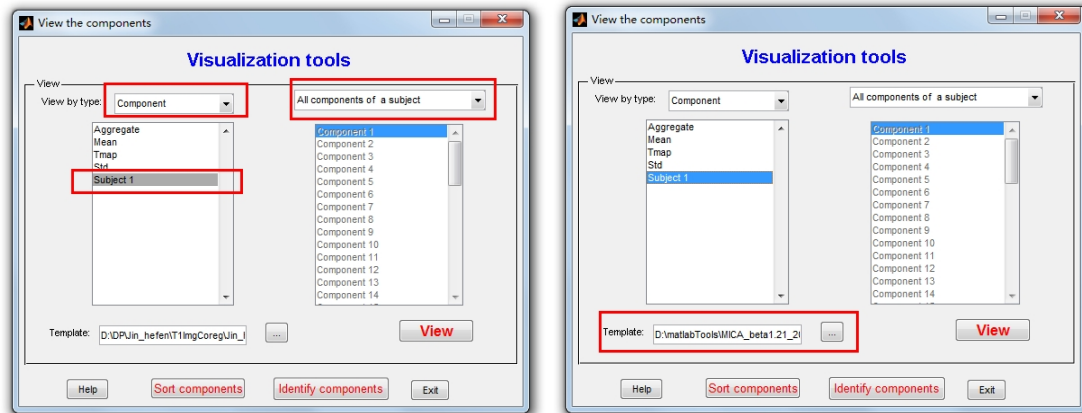


Figure 31

4. Select the T1 anatomy image in the folder of '**T1ImgCoreg**' in the pop up window (Figure 32), then, click 'open' to load the image.

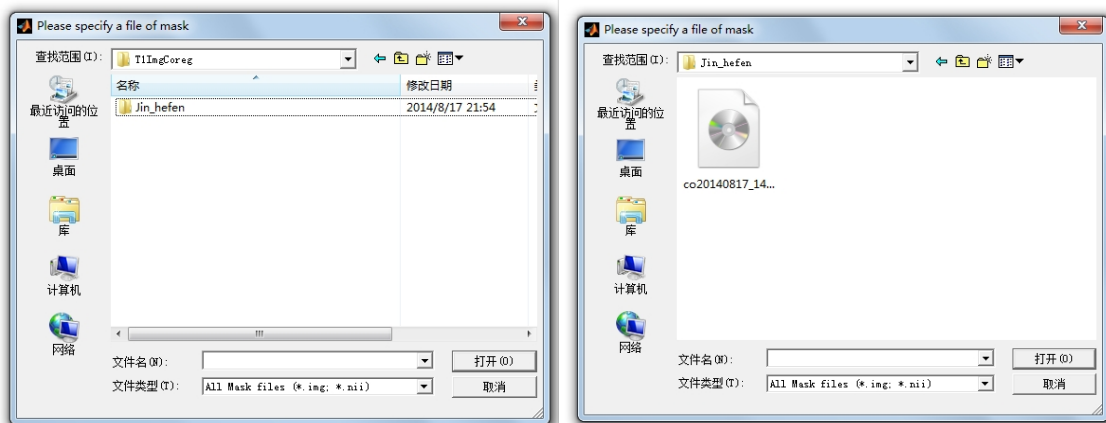


Figure 32

5. Click the button '**View**' (Figure 33, left), another window will pop up as Figure 33 (right) shown. Set the appropriate parameters as Figure 33 (right) shown. Then, click button 'OK'. We suggest you choose Images per figure = 1, and leave Slice position settings as default (-50:5:85). If the functional imaging FOV is far beyond this default setting, please change the slice position accordingly.

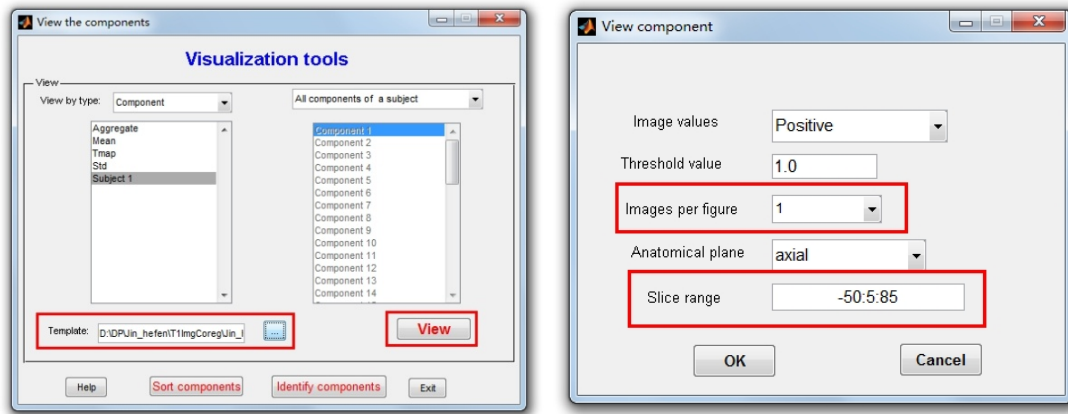


Figure 33

6. The program will show multiple components' spatial maps and associated time courses (Figure 34 shows them for a component). Press button '←' and '→' to view every component carefully to select components of interest. The label of the component is a green Arabic number shown on the top of the Figure 34. The time series of the component is displayed below the component label. Since resting-state networks are generally thought to be low frequency fluctuations dominant, we can take advantage of this feature to select some components with low frequency fluctuations as candidate components of interest. The noise-related components tend to have high frequency dominant time series. The spatial map of the component is located on the bottom of Figure 34. You can pick out some components with biological meaning according to its spatial pattern.

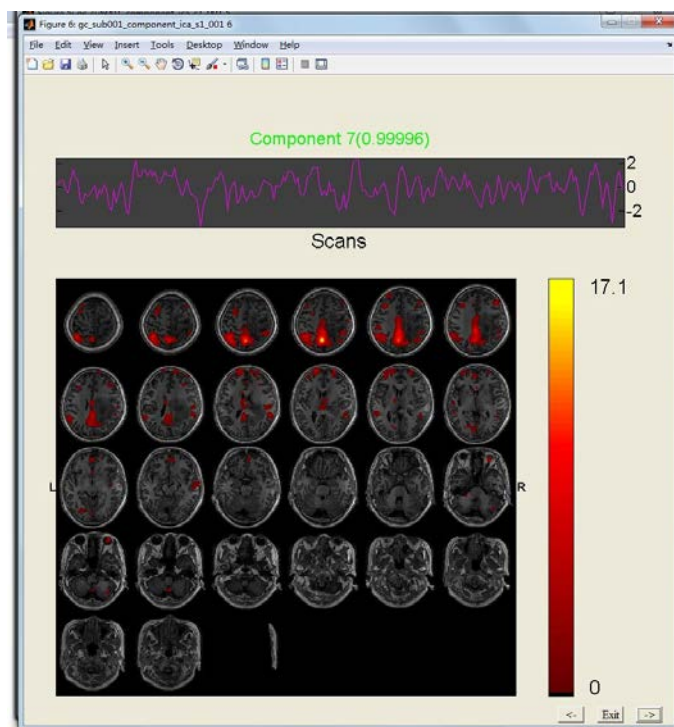


Figure 34

3. An example for the whole data analysis

3.1 An example for setting parameter

We take the data from a patient, whose tumor is near the motion area, as an example. The aim is to illustrate how to conduct data analyses using TUMOR for preoperative functional mapping.

1. Set the parameters as the figure shown below.

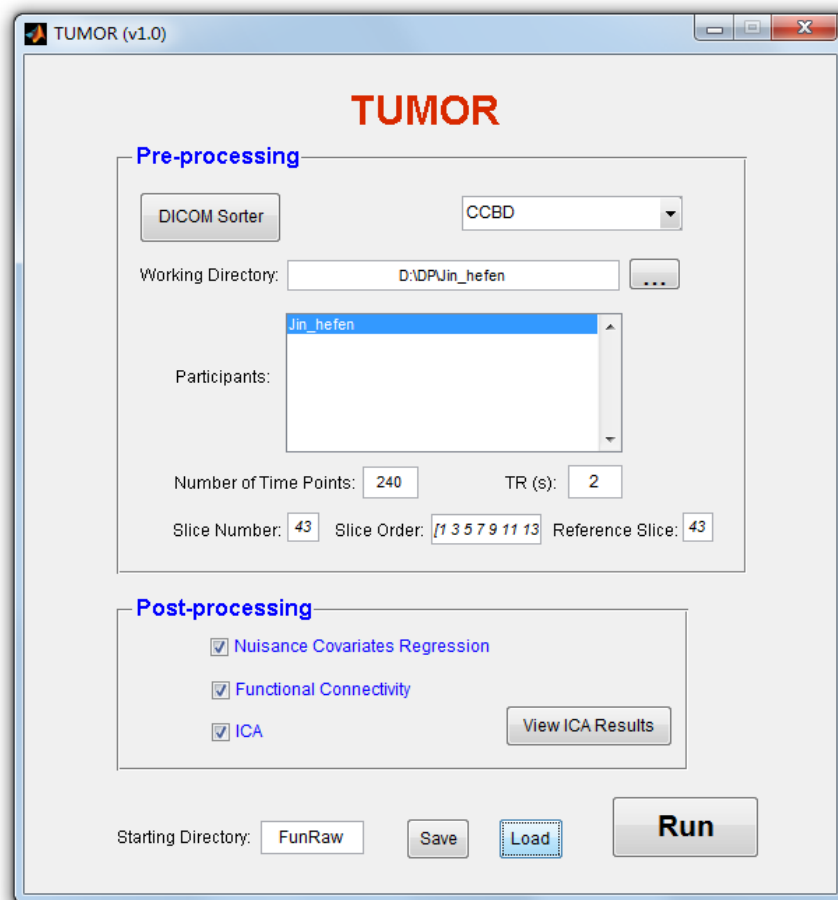


Figure 35

2. Run TUMOR.
3. When finished running, all the files and folders generated are shown in Figure 36.

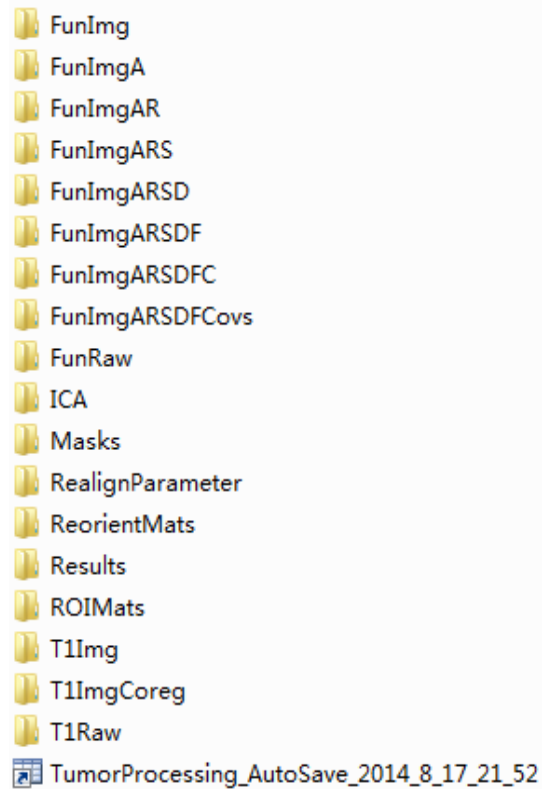


Figure 36

3.2 Result of functional connectivity

We selected 5 ROIs for nuisance covariates (See Figures 37-41). Figures 37-39 show the position of white matter ROIs, Figure 40 shows the position of CSF ROI and Figure 41 shows an ROI which is located in the bottom of the brain.

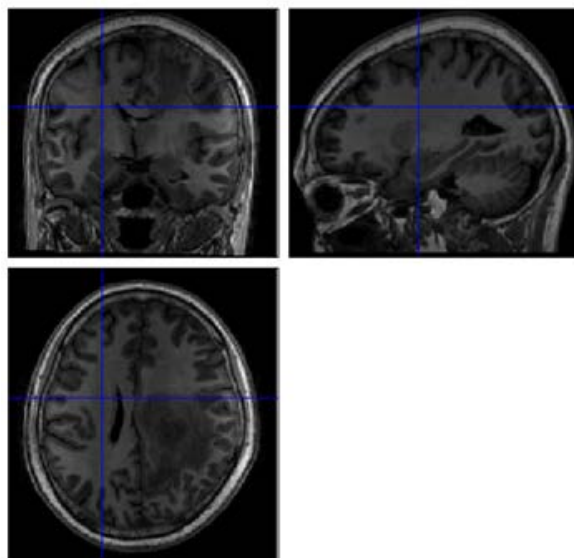


Figure 37

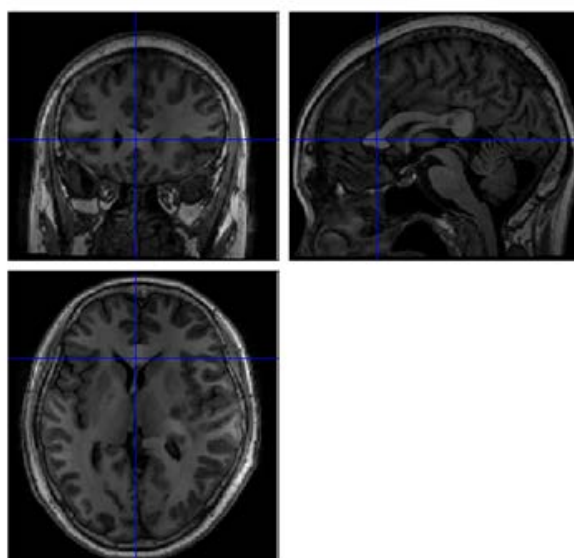


Figure 38

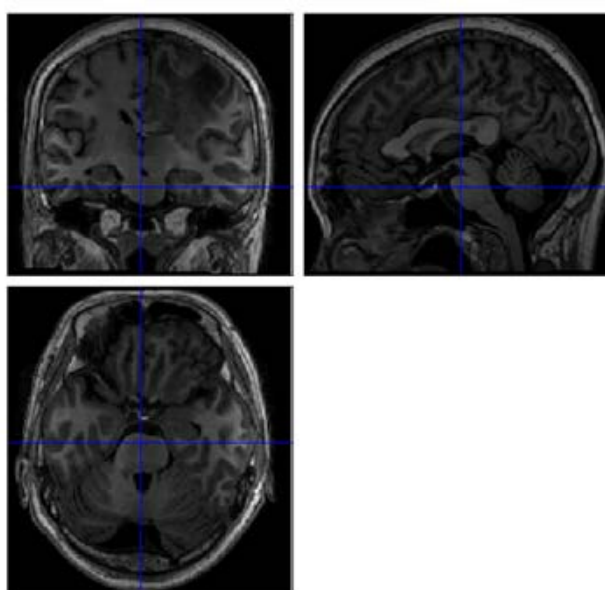


Figure 39

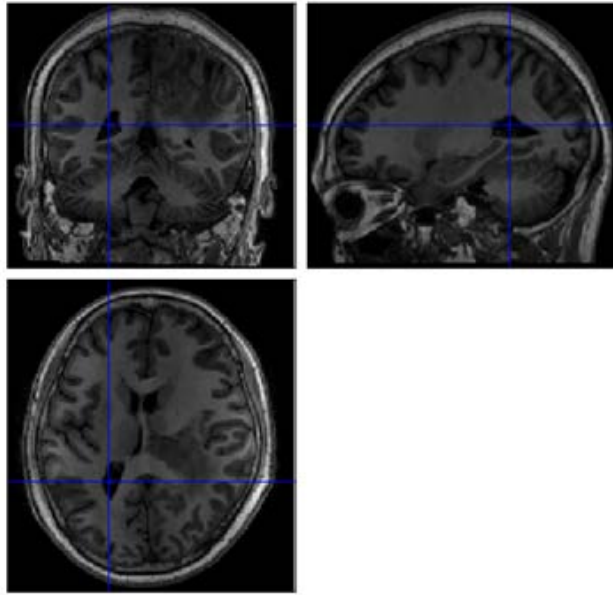


Figure 40

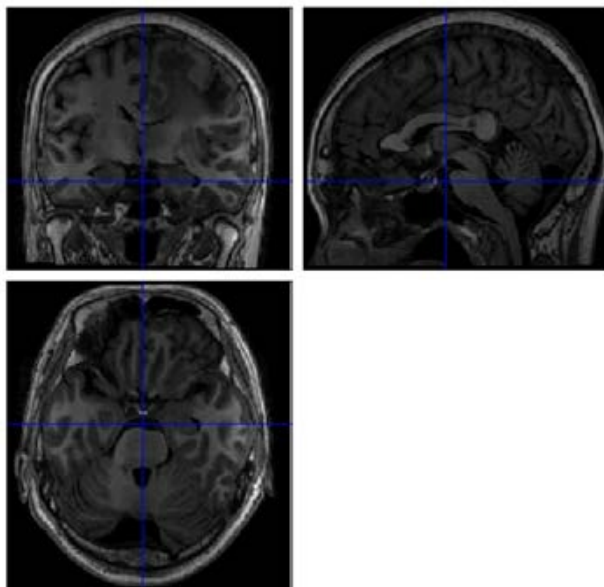


Figure 41

Figures 42-43 show the position of the two seed ROIs for functional connectivity analysis. Both of them are located in the left hand motion area.

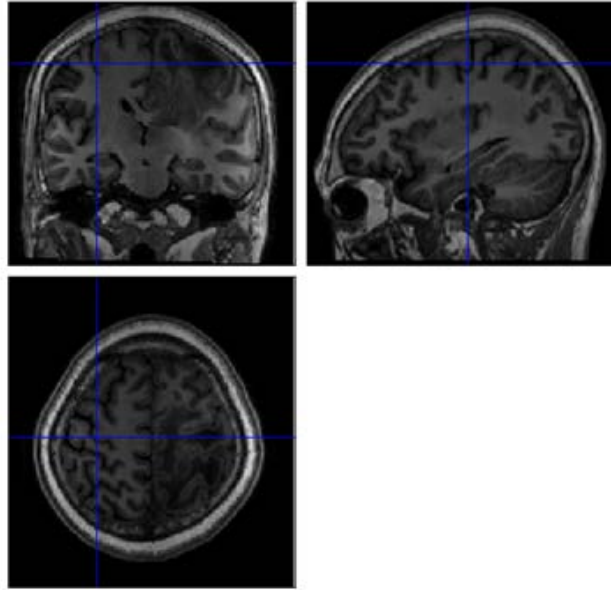


Figure 42

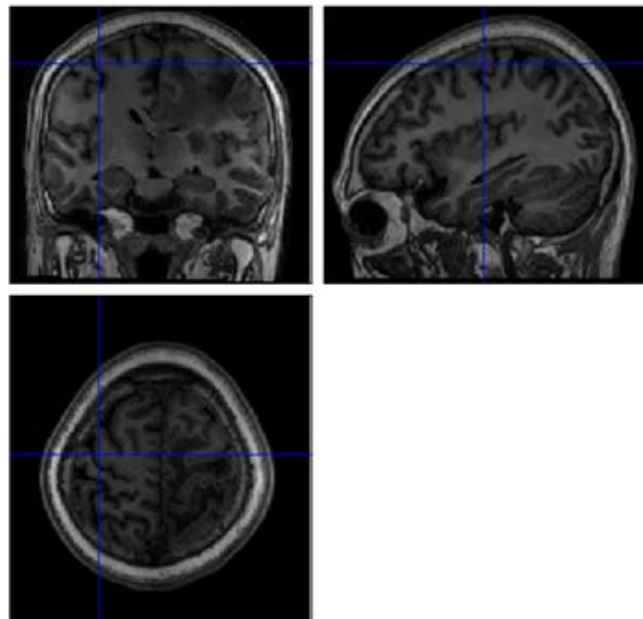


Figure 43

The result of the seed-based functional connectivity is present using software MRICron. The functional connectivity with the first seed ROI is displayed on the top of the Figure 44; and that with the second seed ROI is displayed in the bottom of the Figure 44.

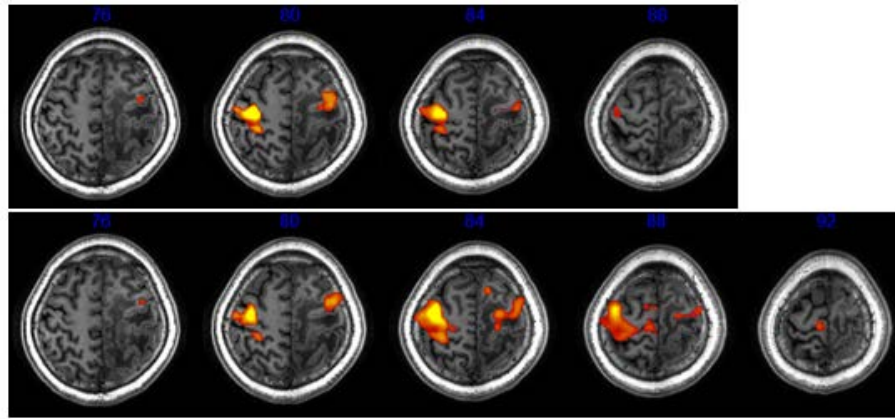


Figure 44

3.3 Result of ICA

ICA separated two sensorimotor network-related components, which were displayed in warm and cold colors, respectively, in Figure 45.

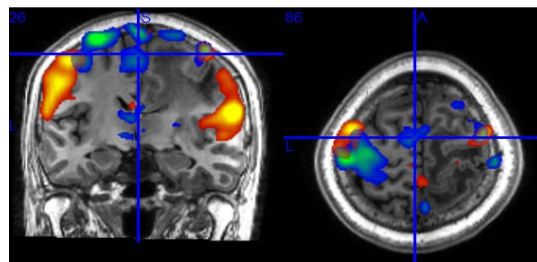


Figure 45

Using MRICroN, the result of these two components is further displayed in Figure 46.

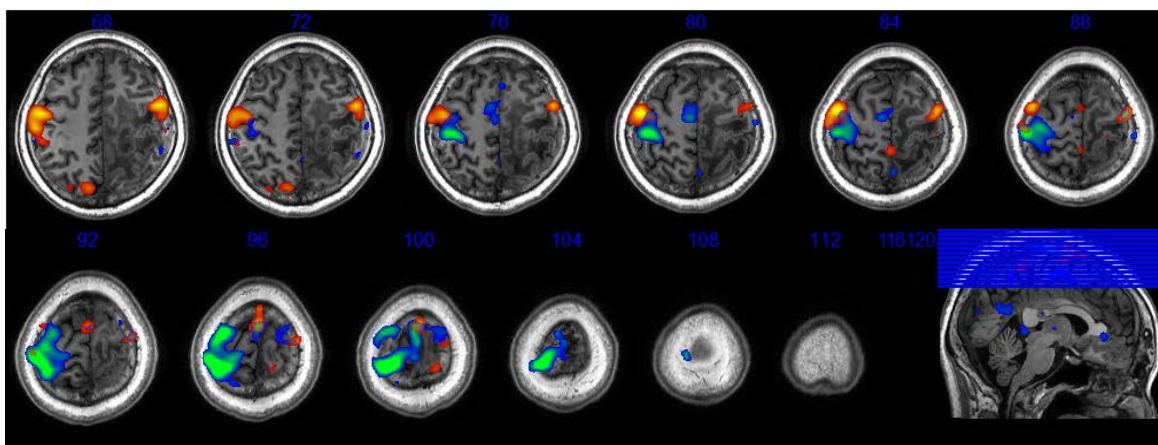


Figure 46

5. Appendix

5.1 Coder

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5.2 References

[1] Zhang H., Zuo X.N., Ma S.Y., Zang Y.F., Milham M.P. and Zhu C.Z., Subject order-independent group ICA (SOI-GICA) for functional MRI data analysis. Neuroimage, 2010, 51 (4): 1414-1424.

[2] Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, He Y, Yan CG, Zang YF*. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS ONE. 2011, 6(9): e25031.

[3] Yan C, Zang YF. DPARSF: a MATLAB toolbox for "pipeline" data analysis of resting-state fMRI. Front. Syst. Neurosci. 2010, 4:13.

5.3 Supportive software

SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>)

DPARSFA (<http://rfmri.org/DPARSF>, http://d.rnet.co/DPARSF_V2.3_130615.zip)

REST (<http://www.restfmri.net>, http://pub.restfmri.net/Anonymous/REST_V1.8_130615.zip)

MICA (<http://www.nitrc.org/projects/cogicat>)