fMRI data analysis with ISC toolbox

Contents

1. Setting up the parameters

Before setting up the parameters, make sure that Harvard-Oxford atlas and m-files for reading nifti-files to Matlab are available. Set all paths in runInit.m. After this, go to ISCtoolbox directory and Launch parameter setup using the command:

```
>> runInit;ISCanalysis;
```

The GUI that appears is presented in Fig 1. By clicking File -> Open/Save you can load/save parameter files. After setting up the parameters, check that all parameters are valid by clicking the button *Validate parameters*. After validation, you can run analysis by using the *Run analysis* –button or by using runAnalysis.m function from the command line. Note that the analysis can be very time consuming especially when several time-frames and frequency subbands are selected.

The parameters in the four panels that must be filled before running the analysis are described next.

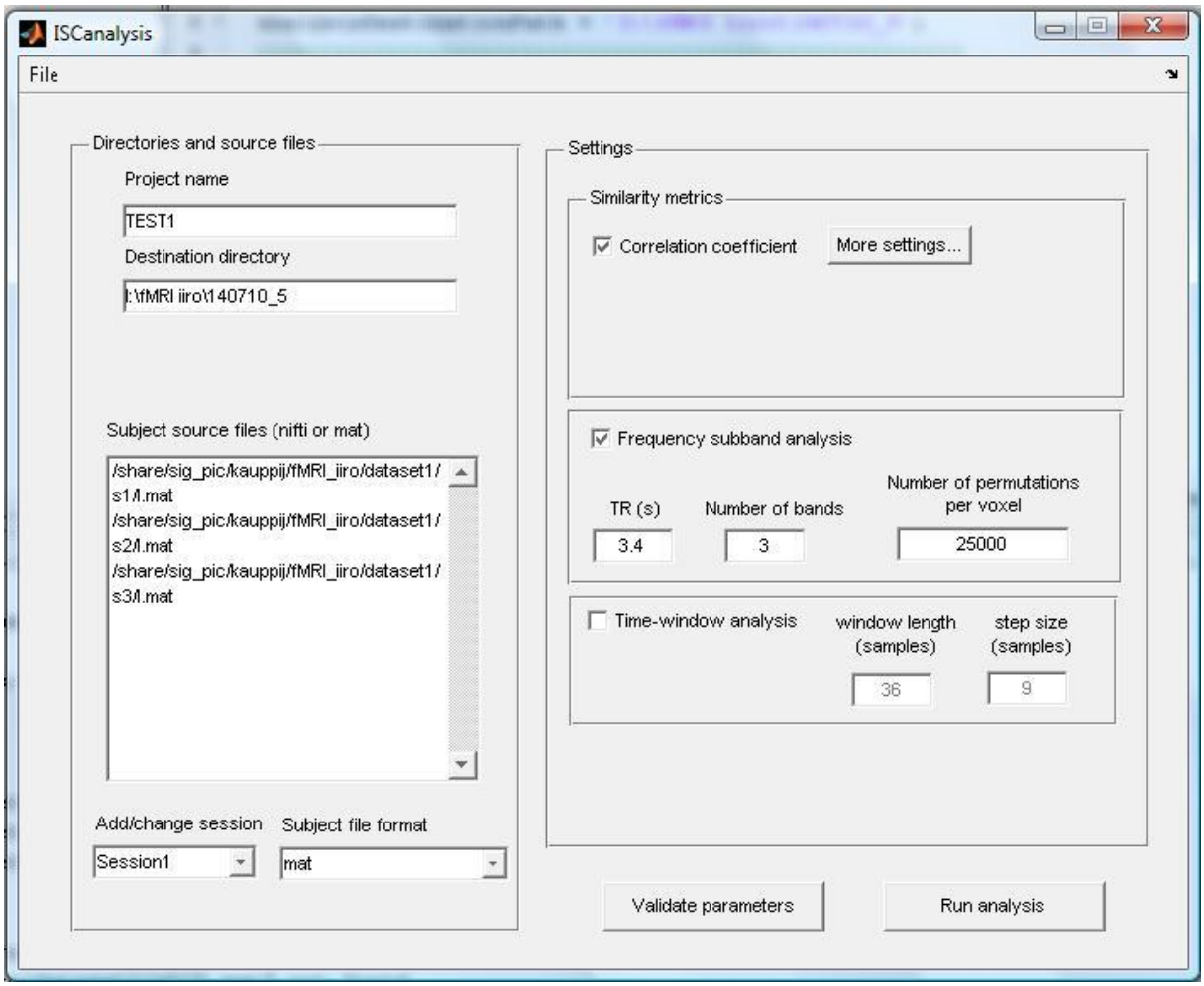

Figure 1. Parameter setup.

1.1 Directories and source files

Project name is the identifier of your project. After setting-up all the parameters and successfully validating them, file named "projectName.mat" will appear in your destination directory containing the parameters.

Destination directory must contain full path to the directory where you want to save the analysis results. If the given directory does not exist, it will be created automatically. Note: Given directory must end with "slash".

Subject source files –box must contain full path names of all subject source files. File extension must be either .mat or .nii. Make sure that nifti-files are uncompressed. Each file name should be separated with enter. Verify from command line display that all files are typed in correctly.

Add/change session allows you to add more than just one scanning session to the analysis. Currently correlation analysis across sessions is not supported but you can simply investigate ISCs independently within distinct sessions.

Subject file format must be set either to .mat or .nii depending on your source file format.

1.2 Similarity metrics

Select intersubject similarity measure. In current version, only correlation coefficient is supported which have some additional options (see Fig. 2). Default setting is to calculate average of the subject pairwise ISC maps. By checking *Calculate median, quartile, std and t-stats* also other type of maps can be computed. Under *permutation test*, set total number of samples in the permutation distribution to assess statistical significance. If you wish to compute samples using a grid, you can increase the number of batches.

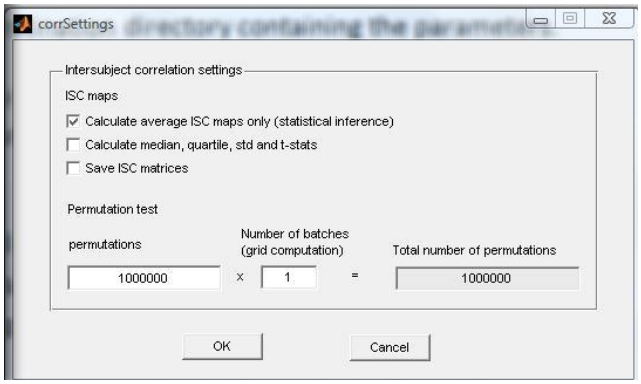

Figure 2. Correlation coefficient settings

1.3 Frequency subband analysis

If you wish to perform frequency subband analysis, set TR (in seconds) and the number of subbands. Length of your fMRI time-series determines the maximum

number of subbands that can be computed (see information in the command line when changing the number of bands). Set also the number of permutations for frequency band comparisons (the total number of permutations is the given value x the number of voxels).

1.4 Time-window analysis

If you wish to perform ISC analysis in time-frames, set window and step size in samples.

2. Analysis using the visualization GUI

2.1 Launching the GUI

After you have run the analysis successfully using runAnalysis.m –function, you can launch the visualization GUI from Matlab command line using one of the following commands:

```
>> ISCtool; % opens the GUI.

>> ISCtool('ProjectName.mat'); % opens the GUI and loads the data.
```

When the GUI opens successfully, no error messages appear in the command line. If you launched the GUI without project file name, select File -> Open and find the project file from the analysis destination directory.

Important! Before opening the GUI, be aware of two things:

- Every time you copy analysis results to another directory/computer, you must update data access pointers using the changeDirectory –function. Otherwise the GUI cannot access the data.
- Make sure that workspace is empty from data access pointers (memMaps -variable) when launching the GUI. Otherwise the GUI may become very slow.

Figure 1 presents the main window of the GUI, where most important settings are highlighted with red rectangles. These settings will be explained next.

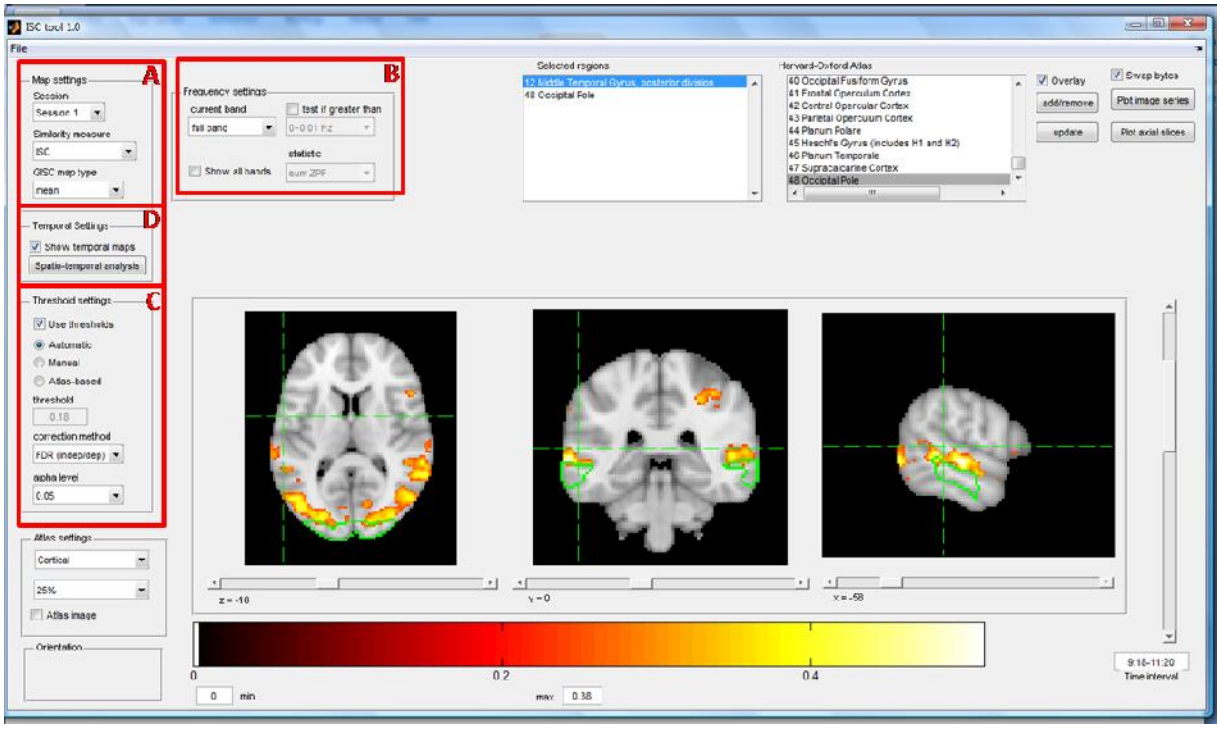

Figure 1. Visualization GUI main window.

2.2 Map settings

Map settings are marked in Fig. 1 by letter A. Map settings consist of three pop-up menus: 1) Session, 2) Similarity measure and 3) ISC map type.

2.2.1 Session

If you have included more than one scanning session in your analysis, you can switch from one session to another here.

2.2.2 Similarity measure

Change the similarity measure here. In current version, only intersubject correlation (ISC) is implemented:

ISC similarity measure is based on correlation coefficients calculated across each pair of subjects.

2.2.3 ISC map type

This menu is active when the ISC similarity measure is selected. Consider correlation coefficients calculated between the time-series of *N* subjects. When correlations between all possible subject pairs is calculated, this leads to *N*x*N* correlation matrix consisting of *N*(*N*-1)/2 interesting correlations. For example, when *N*=6 the correlation matrix can be represented as follows:

	subject 1	subject 2	subject 3	subject 4	subject 5	subject 6
subject 1	X	c1	c2	c3	c4	c5
subject 2	X	X	c6	c7	c8	c9
subject 3	X	X	X	c10	c11	c12
subject 4	X	X	X	X	c13	c14
subject 5	X	X	X	X	X	c15
subject 6	X	X	X	X	X	X

Because correlation matrix is symmetric and correlation between the subject itself is 1, there are altogether 6(6-1)/2 = 15 interesting correlations. Here, interested correlations are marked as c1, c2, ..., c15, and redundant/uninterested coefficients are marked as X and are discarded from the analysis. Based on interesting correlations, several statistics can be computed. Options implemented in the GUI are:

I) mean

Mean of the subject pairwise correlation coefficients is the default option. Nonparametric permutation test and false discovery rate (FDR) based multiple comparison correction is implemented to assess statistical significance of the mean ISC.

II) t stat

This map is obtained by first transforming correlation coefficients using the Fisher's z-transform, calculating the subject pairwise mean and dividing the result by the standard deviation. For more details, see:

Wilson, M. et al: "Beyond Superior Temporal Cortex: Intersubject Correlations in Narrative Speech Comprehension", Cerebral Cortex, 18:230-242, January 2008.

III) standard deviation

Standard deviation of the subject pairwise correlation coefficients.

IV) median

Median of the subject pairwise correlation coefficients.

V) upper quartile

Upper quartile (75%) of the subject pairwise correlation coefficients.

VI) lower quartile

Lower quartile (25%) of the subject pairwise correlation coefficients.

2.3 Frequency settings

Changing frequency settings (denoted by B in Fig. 1) allow investigation of frequency-specific inter-subject similarities. You have three options: 1) investigate ISC in individual frequency bands, 2) investigate multi-frequency ISC maps, and 3) compare ISCs between two frequency bands.

2.3.1 Investigation of individual frequency bands.

Change frequency band from “current band” –menu.

2.3.2 Investigation of multi-frequency map.

Check the box “show all bands”. Color code shows which frequency band shows highest ISC among all frequency bands. Note: Frequency band activated in “current band” determines the threshold for multi-frequency map.

2.3.3 Comparison of two frequency bands.

Two frequency bands can be directly compared when “test if greater than”-checkbox is activated. In this case, current band is compared against the frequency band shown under the check box. To activate the frequency-band comparison, mean ISC must be selected and multi-frequency option must be deactivated. You can use two statistics for frequency band comparison:

I) ZPF

This option uses Pearson-Filon test based on Fisher’s Z-transformation (ZPF) to assess statistical significance of the difference between correlation coefficients in two frequency bands. Test is calculated separately for each subject pair, and the final mapping presents the number of subject pairs which show statistically significant difference for a given voxel.

II) sum ZPF

This option uses group level statistic obtained by summing up ZPF values of each subject pair. Statistical significance of sum ZPF values are assessed using permutation test and multiple comparison correction based on maximal statistic.

2.4 Threshold settings

There are three possibilities to threshold the brain maps: 1) automatic, 2) manual and 3) atlas-based.

2.4.1 Automatic thresholding

When using automatic thresholding, you must specify two options: alpha level and the method for multiple comparison correction. The correction method can be one of the following:

- none = no multiple comparison correction
- FDR (no assump.) = False Discovery Rate without assumptions.
- FDR (indep/dep) = False Discovery Rate with independence or positive dependence assumption (recomm.)
- Bonferroni = Bonferroni correction.

Important:

- Automatic thresholding can be used only when mean ISC statistic or t statistic is selected. In these cases, separate permutation distributions are generated for each scanning session because the length of the scanning session affects the distribution. Permutation distributions are sampled across all frequency bands meaning that the distribution for every frequency band is the same. However, FDR multiple comparison correction gives individual threshold for each frequency band.
- If mean ISC together with frequency band comparison is selected, thresholding is based on different permutation test that uses maximal statistic to deal with multiple comparison correction. In this case, correction method is fixed to FWER (family wise error rate) and cannot be changed by the user.

2.4.2 Manual thresholding

To threshold maps manually, activate “manual”-radio button and type the desired threshold value to a “threshold”-text box.

2.4.3 Atlas-based thresholding

Atlas-based thresholding makes it possible to investigate effects in one brain region at a time. A brain region of interest can be selected by activating the region name in “Harvard-Oxford atlas”-list.

2.5 Temporal settings

Through temporal settings you can investigate how inter-subject similarity varies across time. By activating “show temporal maps”- check box, vertical slider together with the “time interval”-text box appears on the right side of the main window. By scrolling the slider, you can investigate ISC maps within the time-intervals shown in the text box.

Important:

- A special care must be taken when analyzing frequency subbands within time windows. If the number of samples captured by a time-window is *M* for the unfiltered data, the number of samples in successive subbands (from higher to lower) is *M*/2, *M*/4, *M*/8, *M*/16, .etc. due to time-frequency uncertainty principle. This means that there are only few samples to calculate correlation in the low-frequency band.
- time-window analysis does not work with frequency-band comparison analysis.