NIRS-KIT V3.0

User Manual

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Version updata

—— **NIRS-KIT V3.0** released in August 2023 added several features and made some improvements to the software. We hope these changes will result in a better experience for our users!

Updata 1: Marking and canceling exceptional channels in Data Viewer (See Sec 4.2.1).

Updata 2: Block/Event Averaging for task design experiments (See Sec 4.2.2 Block/Event Average).

Updata 3: Design information mat file maker for task design experiments (See Design information mat maker in Sec 4.4).

Updata 4: Integrating all subjects' individual-level index value to a single mat file, to facilitate the user to conduct customized group-level analysis or result display (See Integrating multi-subjects value to a single one in Sec 4.5).

1. Introduction

NIRS-KIT (Hou et al., 2021) is a MATLAB-based cross-platform toolbox for both task and resting-state fNIRS data analysis with a user-friendly GUI. This toolbox covers the entire data analysis pipeline with batch processing, including raw data format conversion, data preview and quality check, preprocessing, individual-level analysis, group-level statistics, and result visualization.

This toolbox has been successfully tested under a variety of operating systems with MATLAB installed, including Windows, Linux, and Mac OS.

Citation inf:
Hou, X., Zhang, Z., Zhao, C., Duan, L., Gong, Y., Li, Z., Zhu, C., 2021. NIRS-KIT: a MATLAB toolbox for both resting-state and task fNIRS data analysis. Neurophotonics 8. https://doi.org/10.1117/1.NPh.8.1.010802

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2. Install and start

2.1 Requirements

- **Matlab**: Matlab R2012a or later version
- **NIRS-KIT**: It can be downloaded from https://www.nitrc.org/projects/nirskit/.
- **SPM12**: if it is not at your Matlab search path (you can type 'which spm' in the Matlab command window to confirm it), please add. You can get it from https://www.fil.ion.ucl.ac.uk/spm/software/spm12/.

2.2 Installation:

- Unzip the NIRS-KIT folder, and add it to the Matlab search path. In Matlab's **File->Set Path->Add with Subfolders**.
- Then select the NIRS-KIT directory.
- Click **Save** and then **Close**.

- Same operations for Spm12, if it was not at your Matlab search path.

2.3 Start

To start the NIRS-KIT, at the Matlab command window, type (must in uppercase characters):

```
>> NIRS_KIT
```

Then the NIRS-KIT mine GUI (Fig. 2.1) should now appear on the screen.

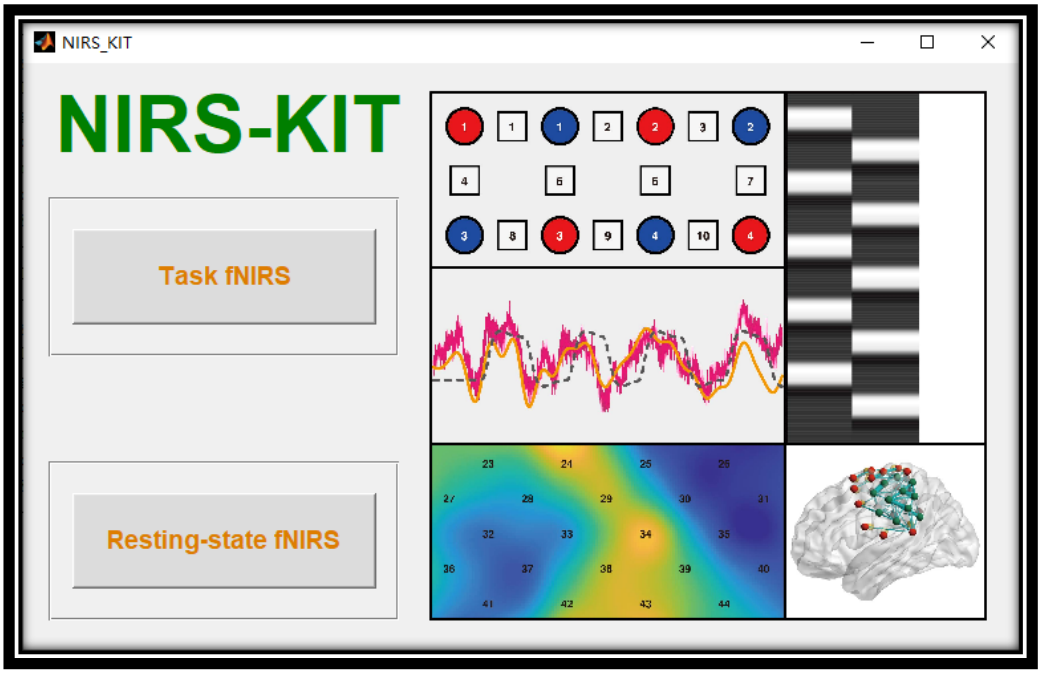


Fig. 2.1 The main interface of NIRS-KIT

3. General Overview of NIRS-KIT

NIRS-KIT has two main analysis modules: the resting-state fNIRS module and task fNIRS module (Fig. 2.1). The fNIRS data analysis pipeline implemented in NIRS-KIT is illustrated in Fig. 3.1. This pipeline consists of common and necessary processing steps for fNIRS data analysis, including data preparation, quality control, preprocessing, individual-level analysis, group-level statistics, and results in visualization.

For resting-state fNIRS individual-level analysis, FC analysis, ALFF, and fALFF, and graph theory-based network analysis to investigate complex topological properties of brain networks (such as local or global efficiency) are supported. In the individual-level analysis for task fNIRS, a general linear model (GLM) is used to detect task activation.

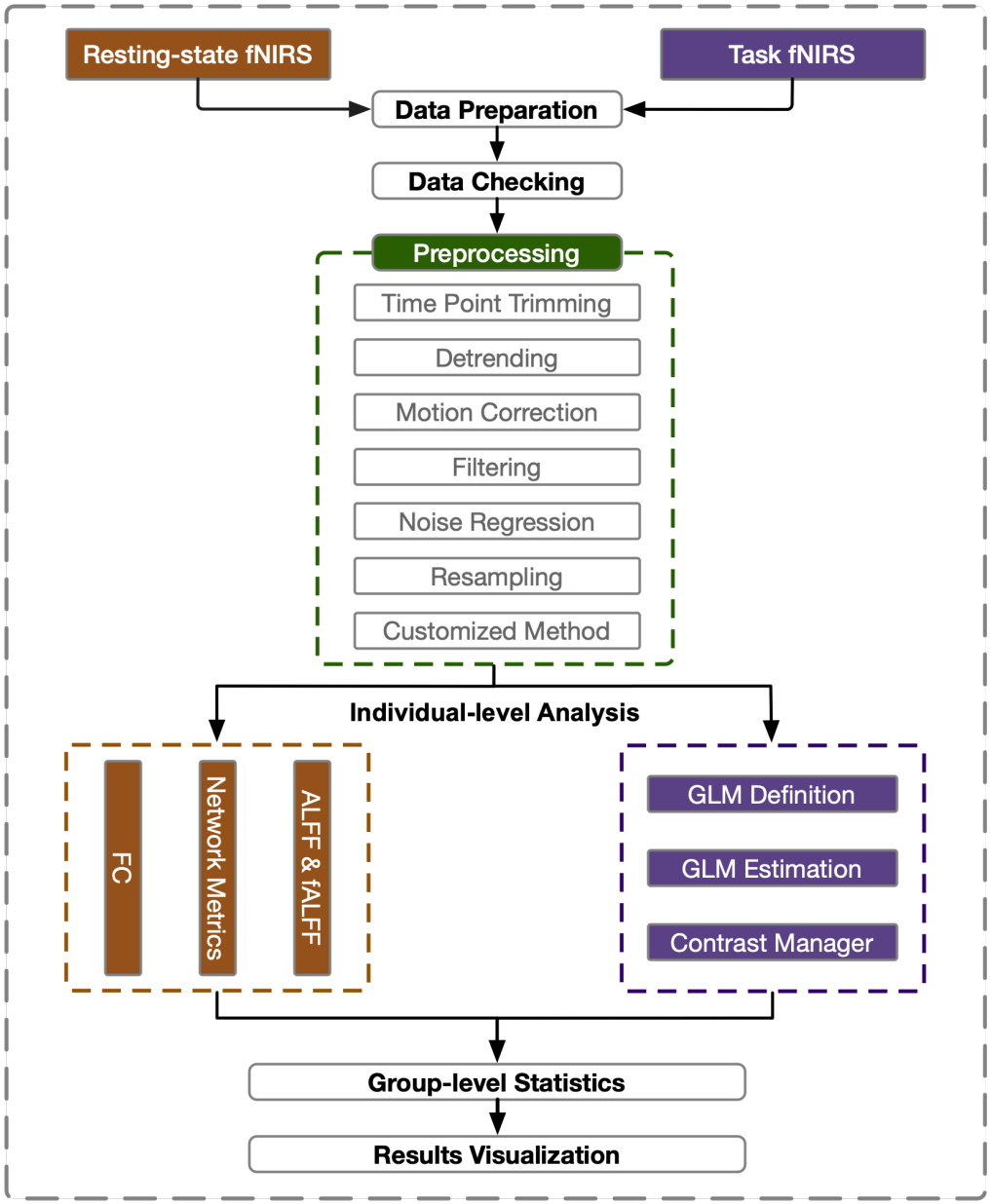


Fig. 3.1 Main processing pipeline in NIRS-KIT. FC, functional connectivity; GLM, general linear model; ALFF, amplitude of low-frequency fluctuation; and fALFF, fractional ALFF.

4. Task-design fNIRS data analysis

Task-design fNIRS module (Fig. 4.0) in NIRS-KIT provides the main functions of Data Preparation, Data Viewer, Preprocessing, Individual-level Activation Analysis, Group-level Statistics, and Result Visualization.

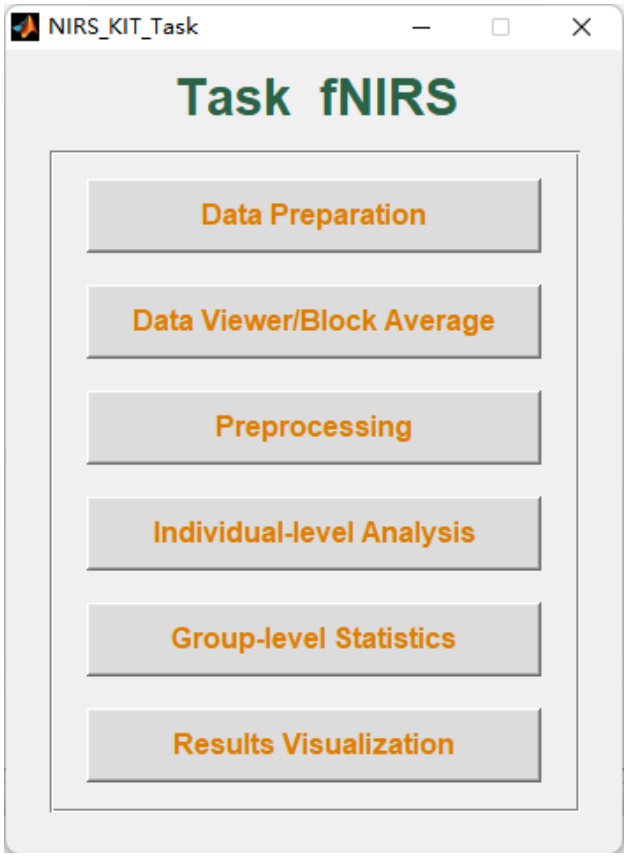


Fig. 4.0 The main interface of Task-design fNIRS module.

4.1 Data preparation

A variety of data are obtained in an fNIRS experiment, and data first need to be imported into the analysis toolkit. The most important data, the raw fNIRS signal time series, are the relative concentration changes of oxyhemoglobin (HbO), deoxyhemoglobin (HbR), and/or total hemoglobin (HbT). Besides, to signal data series, the fNIRS recording's spatial information, consisting mainly of probe and channel locations in two-dimensions (2D) or three-dimensions (3D), are also very important for some analyses, results visualization, data sharing, and publication. Thus, we designed a NIRS-KIT data format [stored in MATLAB .mat files using Data Preparation module (Fig. 4.1)], which includes not only the time-series signals, but also spatial information about probe set geometry and standard coordinates of source, detector, and channel positions in Montreal Neurological Institute (MNI) coordinates.

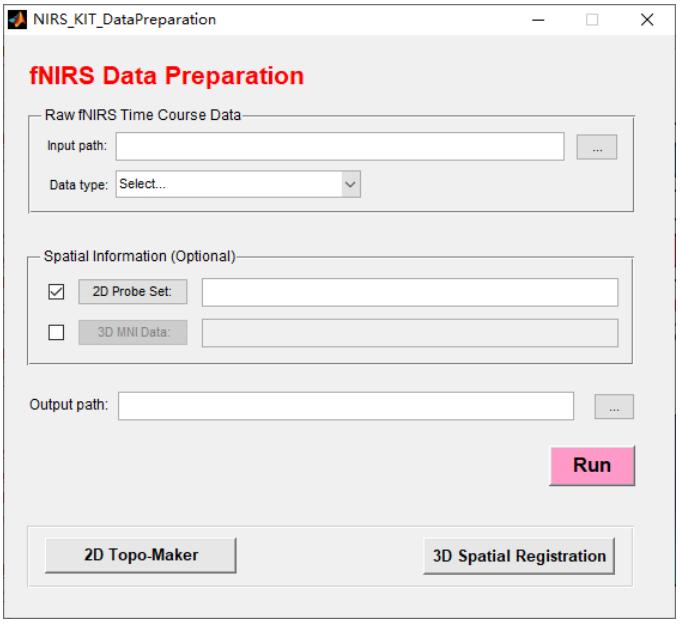


Fig. 4.1 The main interface of NIRS-KIT Data Preparation

4.1.1 Preparation of temporal signals (hemoglobin concentrations)

fNIRS time course data are obtained from diverse commercial devices in different types of formats. Some devices only output the raw optical intensity data (OD), whereas others support the calculation of the relative hemoglobin concentration change (HB) with in-house conversion functions. Different fNIRS recording systems use different output formats (such as .txt and .csv). These inconsistent data sources pose difficulty for following-up analysis, thus a unified format for hemoglobin concentration is needed.

NIRS-KIT provides time course preparation functionality for diverse fNIRS data sources (Fig. 4.1). If the raw optical intensity data are obtained, such as from Hitachi ETG4000/7000 (.csv), or NIRX (.wl1 and .wl2), they can first be converted into optical density data and then converted to concentration changes of HbO and HbR via the modified Beer-Lambert law (Cope and David T. Delpy, 1988). The converted hemoglobin concentration data will be saved in the NIRS-KIT data format. If the converted hemoglobin concentration change data are directly obtained, such as from Hitachi ETG4000/7000 (.csv) or Shimadzu LABNIRS (.txt), they will be reformed and saved in the NIRS-KIT supported format.

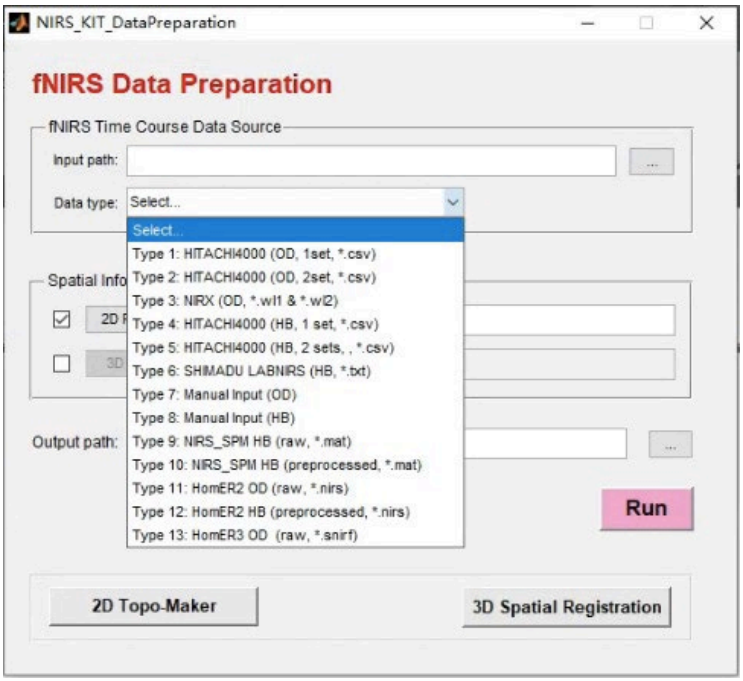


Fig. 4.1.1 The main interface of NIRS-KIT Data Preparation with supported data sources.

- Click '…' to select the *Input path* in the top panel 'Raw fNIRS Time Course Data' (see Fig. 4.1.1). [several raw sample data are provided in the NIRS-KIT package, see …\NIRS_KIT\Sample_Data\Temp_DataSource.]
  Note: All the raw fNIRS time course data files for every subject should be put in a single folder.
- Click the drop-down box to select your raw *Data Type* (see Fig. 4.1.1):
  OD: the raw optical intensity data;
  HB: the relative hemoglobin concentration change generated from fNIRS devices with in-house conversion functions.

  Note: Homer2 toolbox should be added to your Matlab search path when you want to use Type11 and Type12 to prepare your fNIRS data. And if so, you should also cite (Huppert et al., 2009) in your potential published academic paper.

- Click '…' to select the *Output path* to save the output data.
- Click '*Run*'. A few minutes later, the output files were generated into the output folder.

If the recording system is not one of the above, NIRS-KIT also provides a manual input function to generate the required data format. Here users just need to reorganize their raw data (optical density data or hemoglobin concentration data) into a specific format (.csv) according to the format in the sample file included in the toolbox (…\NIRS_KIT\Sample_Data\Temp_DataSource\Manual Input).

NIRS-KIT has good compatibility with two widely used fNIRS data analysis packages. Both raw and preprocessed fNIRS time course data from NIRS-SPM (.mat) or HomER2 (.nirs) can

be read and saved in the NIRS-KIT supported data format.

4.1.2 Preparation of topo-spatial information (probe setup)

The topographical geometry of the channels is very useful for checking time course signals for problems and result visualization, especially when adopting a complex or irregular probe design. NIRS-KIT provides several standard probe settings in the software package's sample folder (including standard 3 × 3, 3 × 5, 3 × 5 × 2, 3 × 11, and 4 × 4 probes).

[Optional but recommended]: If you want to integrate the probe set information with raw fNIRS time course data into a single NIRS-KIT format file (.mat), after selecting the raw fNIRS time course folder and corresponding data type, then please:

- Check '2D Probe Set' to add the probe set file (*.mat):
  The standard probe set files are provided in the sample folder (…\NIRS_KIT\Sample_Data\Temple_2D_Probeset);
- Click '*Run*' to execute fNIRS data integration.

If an appropriate probe setup file does not already exist, users can use the Topomaker Module to generate, in a simple and flexible way, a customized probe setup file with arbitrary arrangements of sources and detectors. Click the '*2D Topo-maker*' in the main interface of fNIRS Data Preparation (Fig. 4.1) to open it (see Fig. 4.1.2.1).

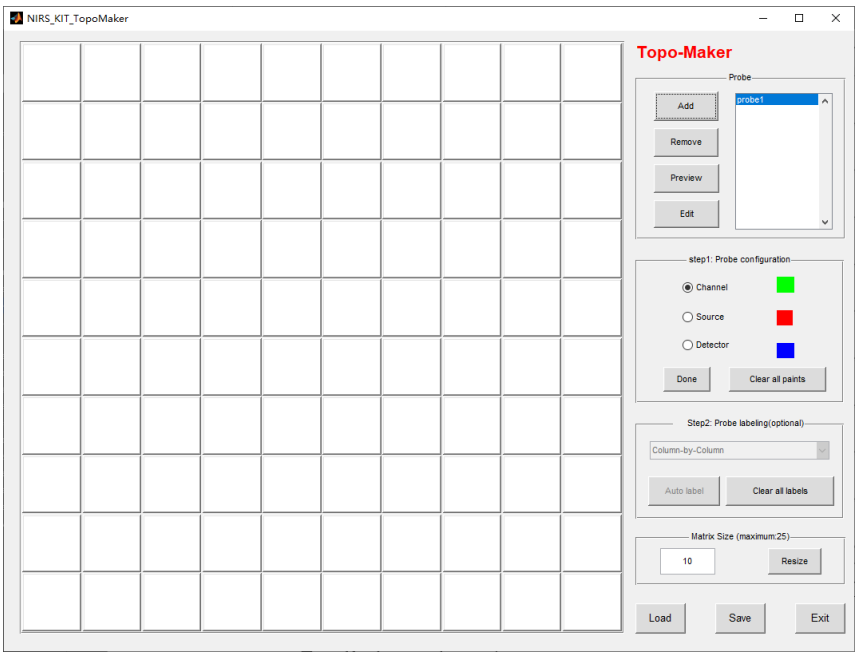


Fig. 4.1.2.1 The main interface of NIRS-KIT Topo-maker.

- Click '*Add*' to create a new probe set: (if multiple separate probes, please repeat this action to add them)
- Probe configuration:
  - Set the square matrix density of the blank canvas: Default (10x10). Resize the matrix if necessary;

- Switch the brush in ‘Probe configuration’, then click and color the corresponding squares (for example, see Fig. 4.1.2.2). red: source; blue: detector; green: channel;
- After that, click ‘*Done*’ to lock the Probe configuration panel, and active the Probe labeling panel. (You can unlock the Probe configuration by clicking ‘*Edit*’).

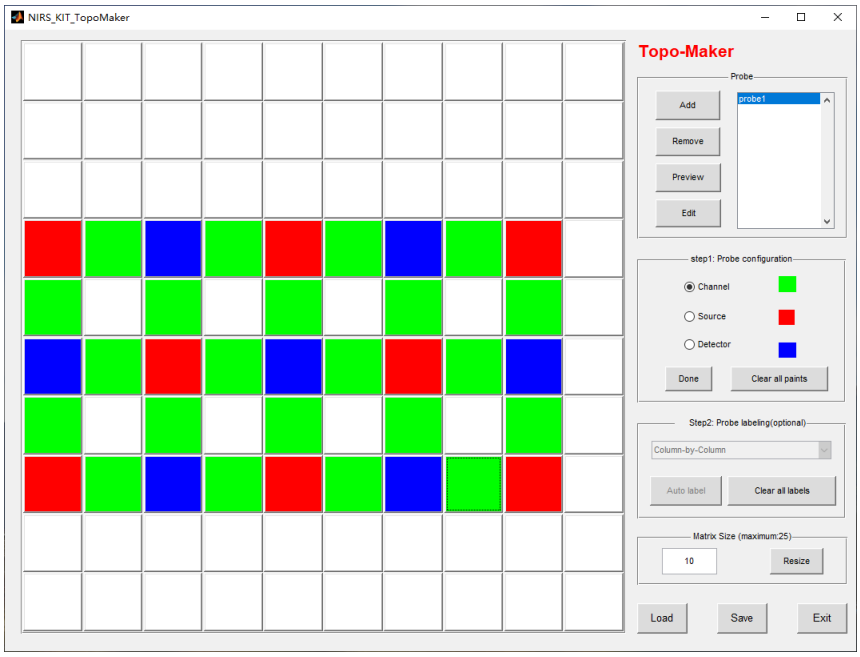


Fig. 4.1.2.2 The main interface of NIRS-KIT Topo-maker with Probe configuration.

- Probe Labeling:

  There are two ways to label the optodes and channels:
  - Auto label:
    - Select ‘Row-by-Row’ or ‘Column-by-Column’.
    - Click Auto label
  - Manual label:
    - Click the corresponding square, input, or modify the number at the pop-up window (see Fig. 4.1.2.3).
- After finishing all the above settings, click ‘Save’ to save the current probe set as a mat file.

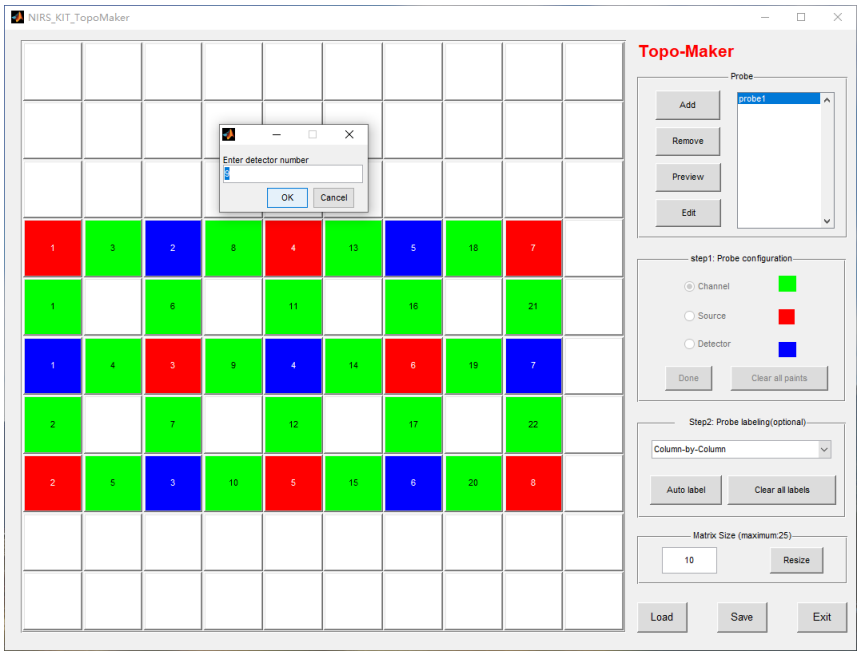


Fig. 4.1.2.3 NIRS-KIT Topo-maker Probe labeling.

Note:

1) If multiple separate probe sets are covering your regions of interest, edit all the probes and then save them to a single mat file (see Fig. 4.1.2.4);
2) During the editing, you can timely click 'Preview' to view them (see Fig. 4.1.2.5);
3) The already saved probe set file can be loaded by clicking 'Load', then re-edit by clicking 'Edit'.

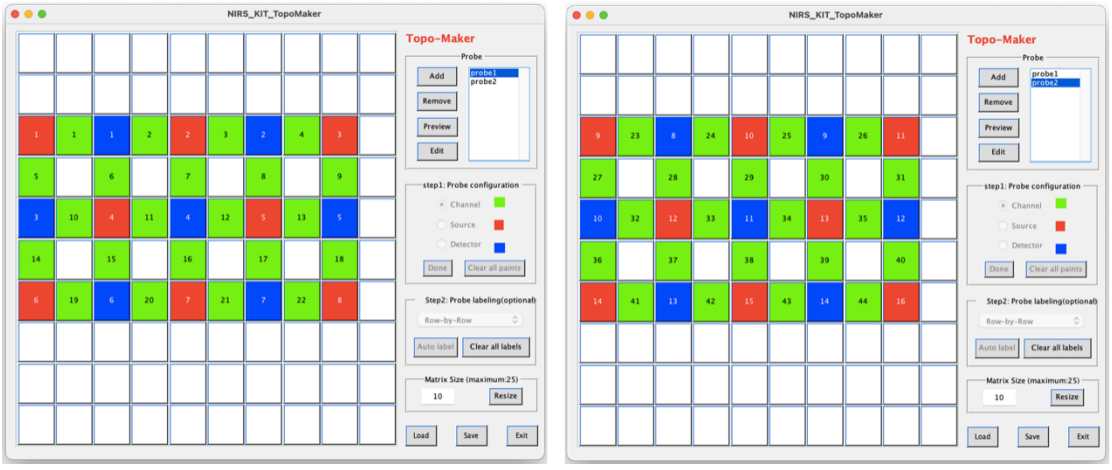


Fig. 4.1.2.4 Example for multiple probes. Setting to 3x5x2 probe sets were shown.

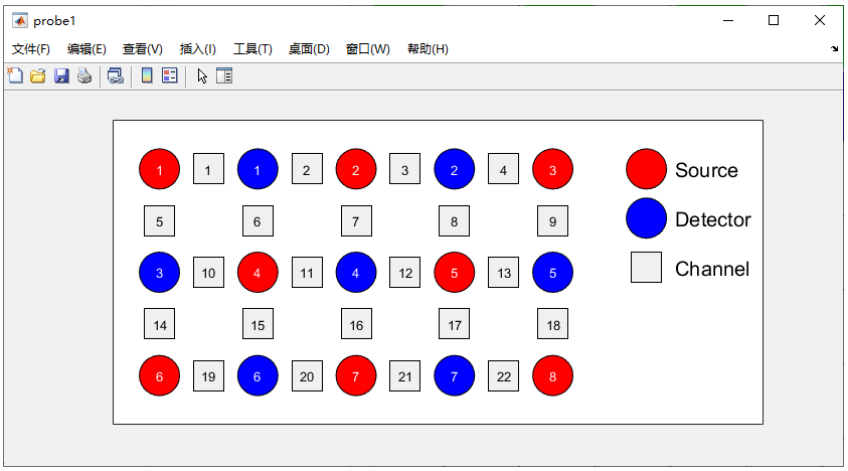


Fig. 4.1.2.5 The preview result of a 3x5 probe set in NIRS-KIT Topo-maker.

4.1.3 Preparation for spatial information in standard brain space

Three-dimensional information in standard brain space (MNI space), consisting of the spatial locations of optodes and channels, is important for result interpretation, visualization in brain space, and publication to enable replication and meta-analysis.

[Optional]: If the spatial coordinates in MNI space, and you want to integrate them into the NIRS-KIT format file. Firstly, the MNI coordinates for every subject should be put into the fixed-format .txt files (see the sample files in …\NIRS_KIT\Sample_Data\Temp_3D_Localization\Outp\MNI_Coordinates). After selecting the raw fNIRS time course folder and corresponding data type (with or without adding the probe set file), then please:

- Check '*3D MNI Data*' to add the folder containing the fixed-format MNI coordinate file. **Note:** If this file contains more than one MNI information mat file corresponding to each subject, make sure that the name of the MNI information file for each subject is the same as the name of the loaded time series data.
- Click '*Run*' to execute fNIRS data integration.

If the real coordinates information obtained from the 3-D digitizer was not available, the MNI coordinates can also be generated from the real coordinate through spatial registration by using the NFRI toolbox (Singh et al., 2005). Click 'Spatial Registration' in the main interface of the Data Preparation module to open the following GUI (see Fig. 4.1.3.1).

There are two kinds of spatial registration methods that can be used: 1): NFRI-based registration (Singh et al., 2005); 2): TBA-based registration (Xiao et al., 2018).

4.1.3.1 NFRI-based registration

If you want to use this method to produce your results, you are also required to cite the following paper.

For NFRI-based registration, two kinds of input files are necessary (see Fig. 4.1.3.1): 1) the '**origin**' file contains the in real coordinates for reference points; 2) the '**others**' file contains the in real coordinates for channel and optode positions. The sample files for NFRI-based registration are provided in …\NIRS_KIT\Sample_Data\Temp_3D_Localization\Raw_Localization_Data\NFRI_Based\;

- Click '*Add reference file path:*' to add the folder containing the 'origin' files, and click 'Add ch & opt file path:' to add the folder containing the 'others' files.
- Set the *Output path*:
- Select whether *Skip Reference Check* or not if method 1 (NFRI-based registration) selected;
- Click '*Run*' to execute spatial registration.

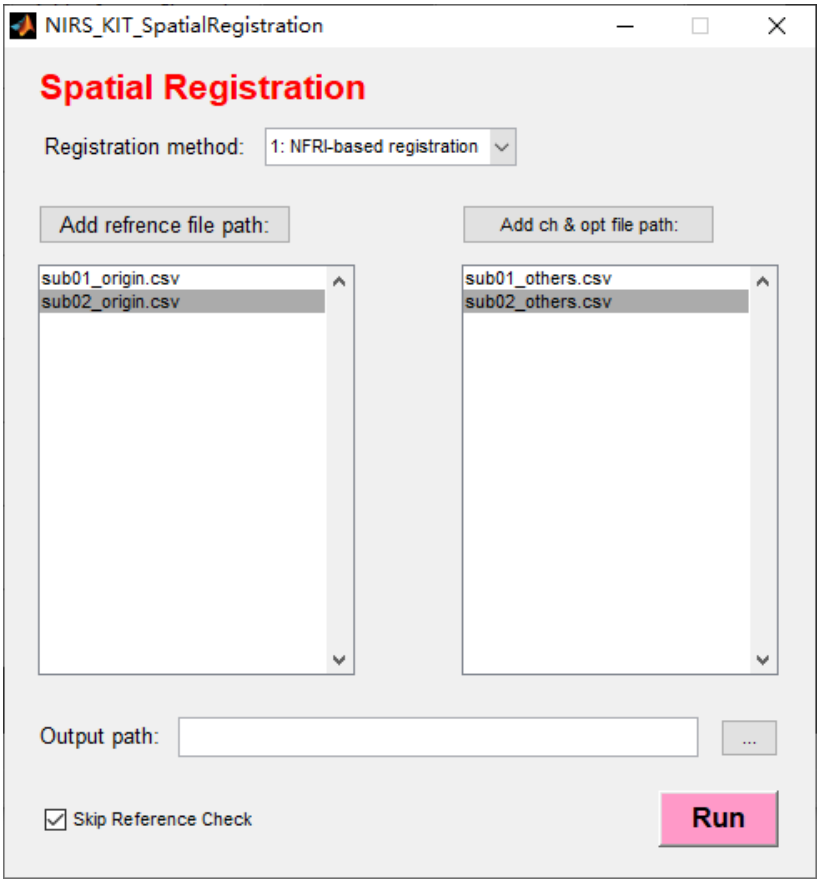


Fig. 4.1.3.1 NIRS-KIT Spatial Registration: NFRI-based registration

Note: For NFRI-based registration selected, in the origin .csv file, at least four reference points are given (Nz, Lz, AL, AR, if including Cz will be better).

4.1.3.2 TBA-based registration

If you want to use this method to produce your results, you are also required to cite the following paper.

Xiao, X., Yu, X., Zhang, Z., Zhao, Y., Jiang, Y., Li, Z., Yang, Y., Zhu, C., 2018. Transcranial brain atlas. Sci. Adv. 4, eaar6904. https://doi.org/10.1126/sciadv.aar6904

For TBA-based registration, two kinds of input files are necessary (see Fig. 4.1.3.2): 1) the '**landmark_sparse**' file contains the real coordinates for the landmark and sparse points that are necessary for scalp reconstruction and CPC system construction. Note: In this file, four landmaker points (Nz, Lz, AL, AR) and at least 21 sparse points (the name for each sparse point can be arbitrary or even empty) are necessary. Besides, please make sure that the sparse points are evenly and discretely distributed on the scalp when collecting these sparse points. 2) the '**opt_ch**' file contains real coordinates for measurement channel positions (with or without optode positions). The sample files for NFRI-based registration are provided in in …\NIRS_KIT\Sample_Data\Temp_3D_Localization\Raw_Localization_Data\TBA_Based\;

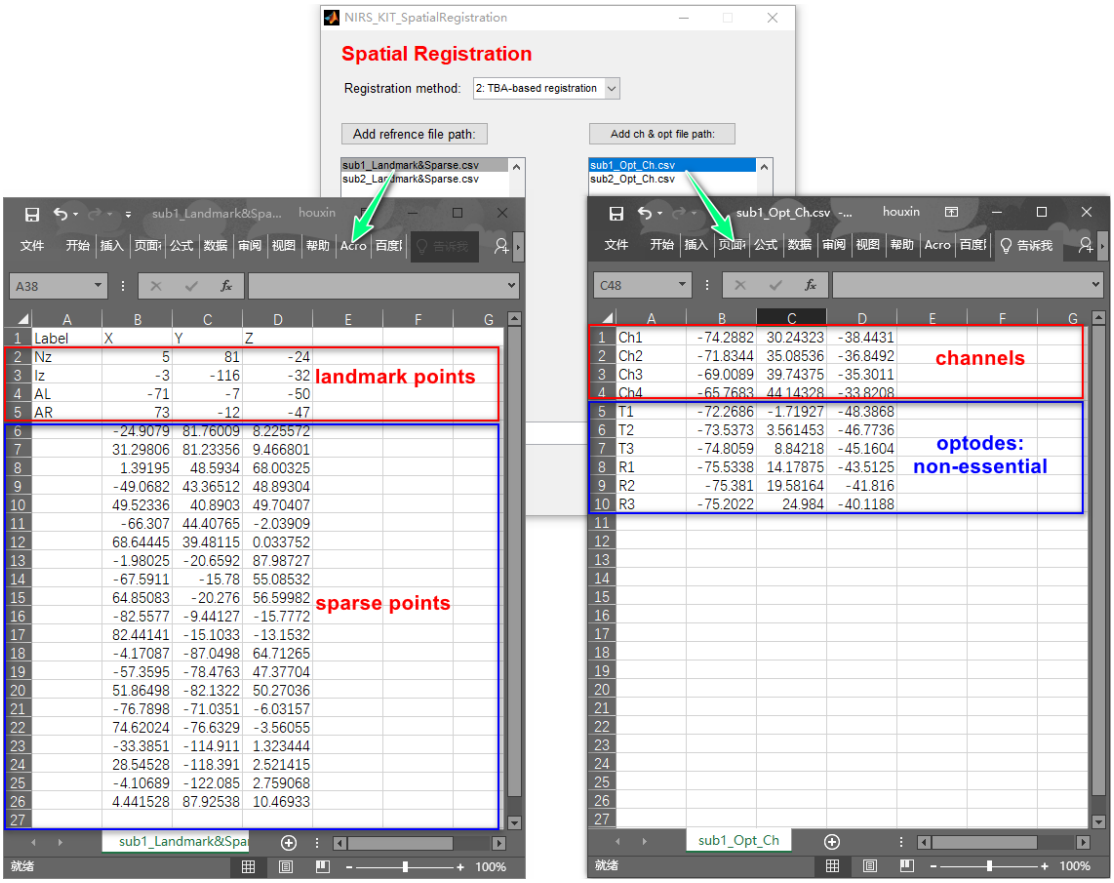


Fig. 4.1.3.2 NIRS-KIT Spatial Registration: TBA-based registration

- Click '*Add reference file path:*' to add the folder containing the 'origin' files, and click 'Add ch & opt file path:' to add the folder containing the 'others' files.
- Set the *Output path*:
- Click '*Run*' to execute spatial registration.

A few minutes later, and the output files (MNI_Coordinates && Anatomical_Label) were generated under the output folder.

- The MNI_Coordinates folder contains the output MNI coordinate files for each subject.

- The Anatomical_Label folder contains the anatomical labels and corresponding probabilities for each optode or channel (if TBA-based registration was applied, only the label with maximum probability for each optode or channel would be reported now). Three kinds of anatomical labeling were reported here.
  1) Anatomic anatomical labeling (AAL, Tzourio-Mazoyer et al., 2002);
  2) Brodmann area;
  3) LONI Probabilistic Brain Atlas (LPBA40, Shattuck et al., 2008).

4.1.4 Output NIRS-KIT data format and structure

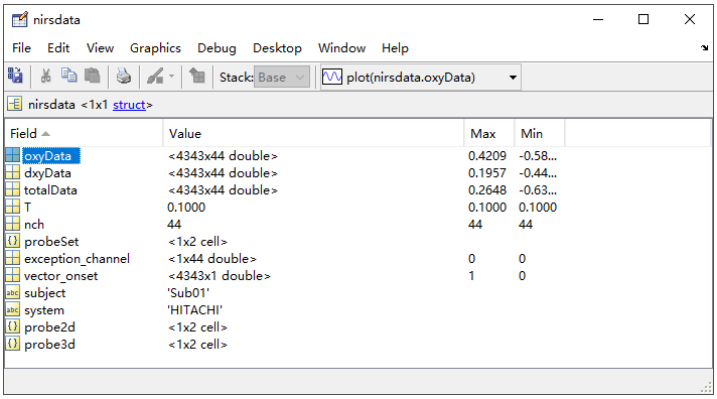


Fig. 4.1.4 The data structure of the output file after fNIRS data integration. 'oxyData','dxyData', and 'totalData': the time courses for three difference hemoglobin signals (time points × channels). T: the sampling period, and sampling frequency = 1/T; nch: total number of channels; In fNIRS data preparation, the '2D Probe Set' and '3D MNI data' are optional. If they were not been added, the probe2d and probe3d in the integrated data will be empty.

4.2 Data Viewer and Block/Event Average

4.2.1 Data Viewer

Before formal fNIRS data analysis, we need to preview our raw data to see the characters and check the quality of our data, such as check the physiological noise, head motion artifact from time-domain, frequency-domain, or spatial features. Data preview and check not only can help us to identify whether the fNIRS data of a certain subject is good or bad, but also can help to determine the parameters for following preprocessing. NIRS-KIT Task Data Viewer (Fig. 4.2.1.1) provides abundant data check functions for these aims, including presenting the time-domain information (time series), frequency-domain information (spectrum distribution), the comparison between raw data and preprocessed data. Especially, for task fNIRS data, we presented frequency spectrum information, preprocessing options, draw reference waves for task-design fNIRS data.

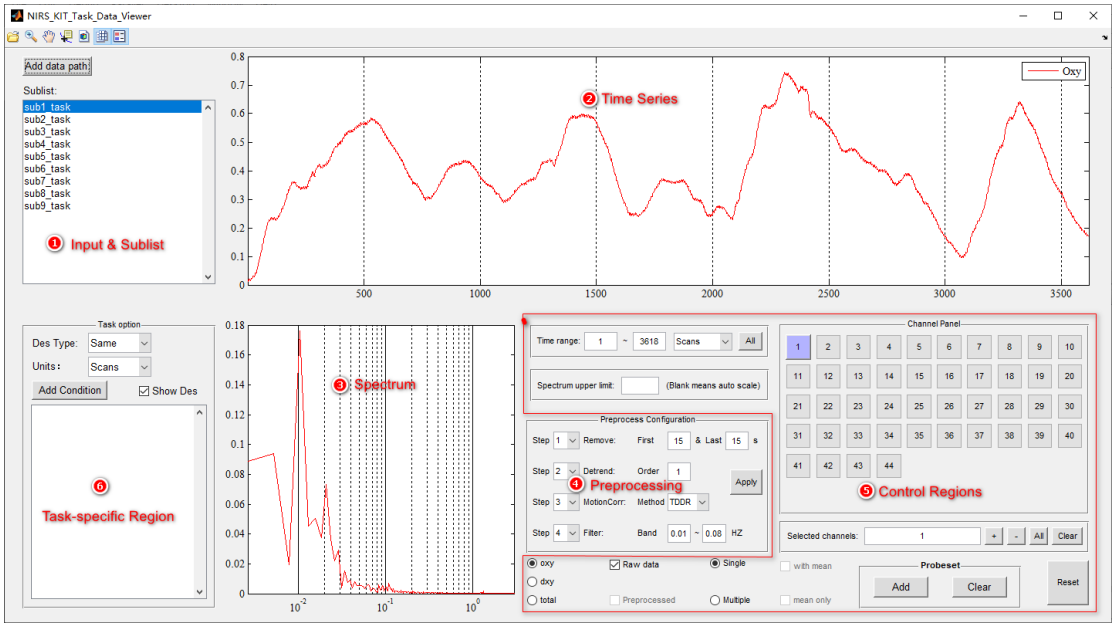


Fig. 4.2.1.1 The main interface of NIRS-KIT Task Data Viewer

To use this module:

- Click '*Add data path*': selected the data folder containing the data saved in NIRS-KIT format (.mat, see above Sec. 4.1), then the files within this folder will be present on the Sublist panel; Then, the first subjects' oxy data of channel_01 will be plotted (default);
  - In the time-series panel, the x-axis represents the time points (the units can be arbitrarily switched between seconds and scans), the left y-axis represents the hemoglobin concentration.
  - In the spectrum panel, the x-axis is the frequency component (Hz), the y axis represents the amplitude for each frequency component.
- You can set the preprocessing parameters and then click 'Apply' to preprocess the raw data. You can compare the raw data with the preprocessed data by presenting them simultaneously (see Fig. 4.2.1.2). And determine the final preprocessing parameters by adjusting them.
- Besides, you can freely change the display mode (refer to Table 4.2.1) according to your needs.

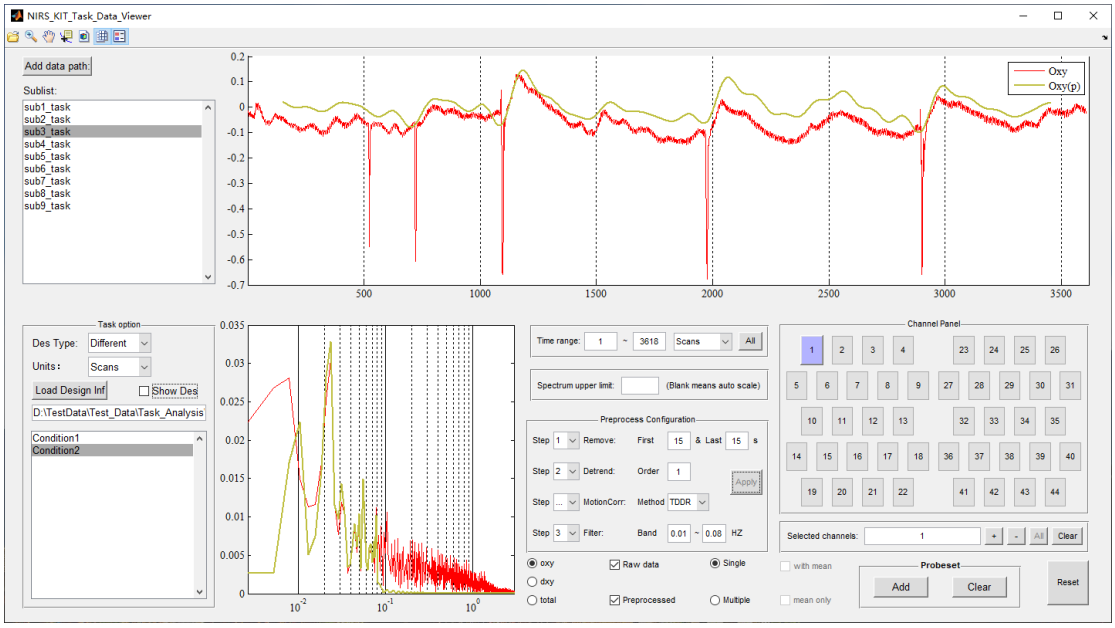
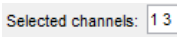
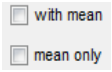


Fig. 4.2.1.2 NIRS-KIT Data Viewer: Comparation of the raw and preprocessed time series.

Table 4.2.1 Function descriptions of the control in Data Viewer interface

	Control Name	Appearance	Function
Toolbar	Zoom		Zoom in or out the time series or the frequency spectrum axes
	Move		Move the figure to the specified screen location
	Data cursor		Display data values at data cursor locations.
	Color schemes		Under multiple channel mode, you can click the color schemes switch button to change the colors of multiple time series lines or frequency spectrum lines.
	Gridline		Show or hide the grid lines
	Legend control		Show or hide the figure legends
Channel control options	Channel selection panel		Select or release the channel by clicking the corresponding button. Note: The channel arrangement mode will be shown as it is if the input data has the 2D probe set information. Otherwise, the channels will be arranged from small to large without showing the real relative spatial arrangement mode. (You can add the 2D probe set information by click the bottom 'ProbeSet-Add').
	Display & input the selected channels		(1) Display the selected channels; (2) Select a certain channel by input its corresponding number and then press 'Enter'.
	Increase/Reduce the selected channel number		Note: Only can be used in the single-channel mode.
	Select all channels		Select all channels to be presented. Note: Only can be used in the multiple channel mode.
	Clear selected channels		Clear all selected channels
	Mode switch options		Single: Only a single channel's time series and frequency spectrum can be presented; Multiple: Display the time series and frequency spectrum information of multiple channels simultaneously.
	Probe set		Add or clear the probe set for channel arrangement.
Signal	Single average		In multiple modes: With mean: select whether to show the mean single of the multiple selected channels. 选择是

			Mean only: Only present the average single.
	Single type selection	oxy dxy total	Select or switch the presented single type. In a single mode: multiple single types can be selected simultaneously; In multiple mode: only one single type can be selected.
	Data type options	Raw data Preprocessed	Select to present the raw data or the preprocessed data. In single mode: They can be chosen simultaneously; In multiple mode: Both cannot be chosen at the same time.
Range	Time series range	Time range: 2000 ~ 9000	Display the time series within the input time range.
	Spectrum range	Spectrum upper limit:	Set the upper limit of the spectrum.

If you want to show the task reference waves in this module. Add the task design information in task-specific panel (a .mat in fix-format, see the sample file: …\NIRS_KIT\Sample_Data\Temp_Design_Inf.mat, or refer to 4.4 task-design individual-level analysis to make it). Then you can selectively represent a certain task conditions reference wave and corresponding spectrum information (both in dashed line, see Fig. 4.2.1.3).

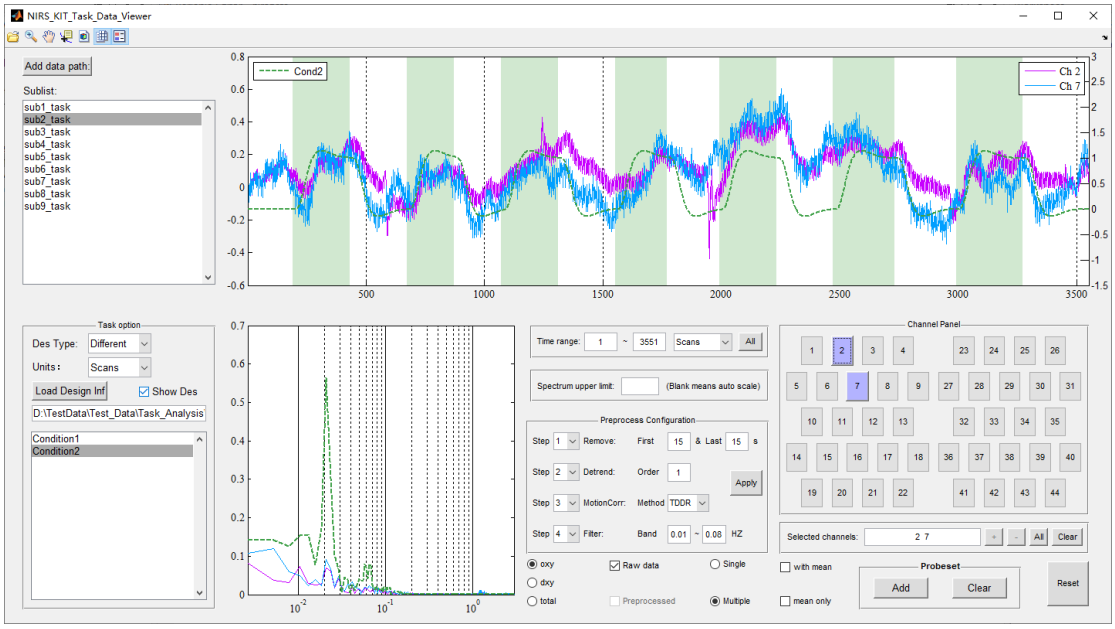


Fig. 4.2.1.3 NIRS-KIT Data Viewer: With task-specific reference wave.

Marking and canceling the exceptional channel

- In the Channel Control Regions panel, we have add a right click for marking and canceling the exceptional channel (V3.0 updated, see Fig. 4.2.1.4).
  - When there is exceptional (abnormal) channel information stored in the data you load into the Data Viewer (e.g. Hitachi NIRS devices allow users to mark abnormal channels), the foreground colour of these channels will be automatically set to grey.
  - When right-clicking on a normal channel, if the user selects "Yes", the foreground colour of this channel will turn grey and this marker information will be re-updated in the loading data for that subject. Similarly, when right-clicking on a channel that has been marked as abnormal, if the user selects "Yes", the foreground colour of this channel will be cancelled, and the new marking information will be stored in the loading data of the subject.
  - For group level statistics, this abnormal channels for this subject will be automatically excluded. For example, an experiment had 10 subjects and 2 channels (Ch1 and Ch2). And, Ch2 of two subjects was labelled as exceptional channel. In the group level analysis, 10 subjects were included in the analysis of Ch1 and 8 subjects were included in the analysis of Ch2. Of course, users need to be aware that when too many subjects are labelled as abnormal in a particular channel, that channel may not be very suitable for inclusion in group-level analyses.
  - Besides, In addition, if the user wants to cancel the existing exception markers of channels for all subjects, the user can run the *NK_clear_exceptional_lebels.m* script to perform the operation.

Fig. 4.2.1.4 NIRS-KIT Data Viewer: Marking and canceling exceptional channels

4.2.2 Block/Event Average

Block Average module (updated in NIRS-KIT V3.0) allows the user to perform the block (see Fig. 4.2.2.1) and event (see Fig. 4.2.2.2) average calculation and display. It retains most of the features of the NIRSk-KIT Task Data Viewer.

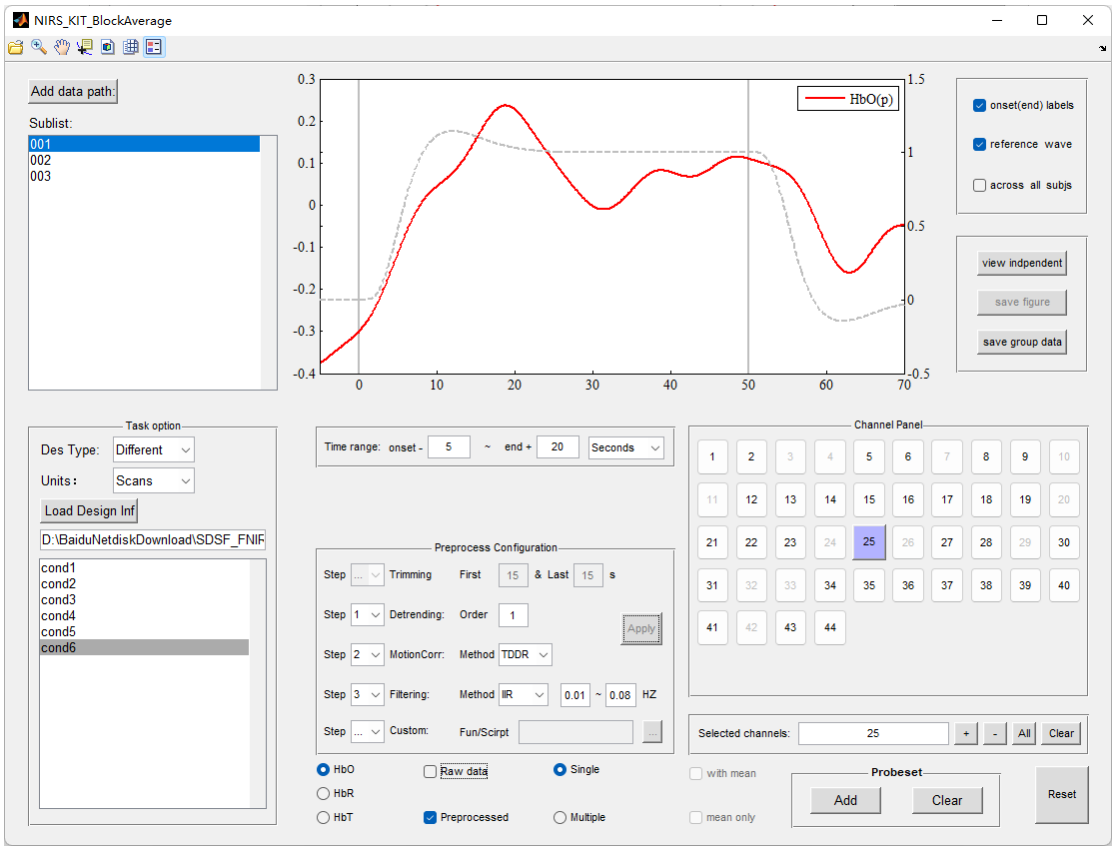


Fig. 4.2.2.1 NIRS-KIT Block/Event Average: block average with preprocessing

To use this module:

- Click '*Add data path*' to add the raw data or preproceseed data;
- Set your design information in Task option (manually input or add the design_inf.mat);
- Change the display mode according to your needs, such as display oneset (end) labels & reference wave or not;

- If you want to display the results at group level, select '*across all subjs*' button.
- If you want to store the currently displayed image, you can click '*view independent*' button (at this moment, you can adjust it to suit your needs), and then click the '*save figure*' button.
- Besides, you can click '*save group data*' to calculate and save the block average for conditions-subjects-channels, and You can use this data to draw the results you want with other tools, including adding confidence intervals and so on.

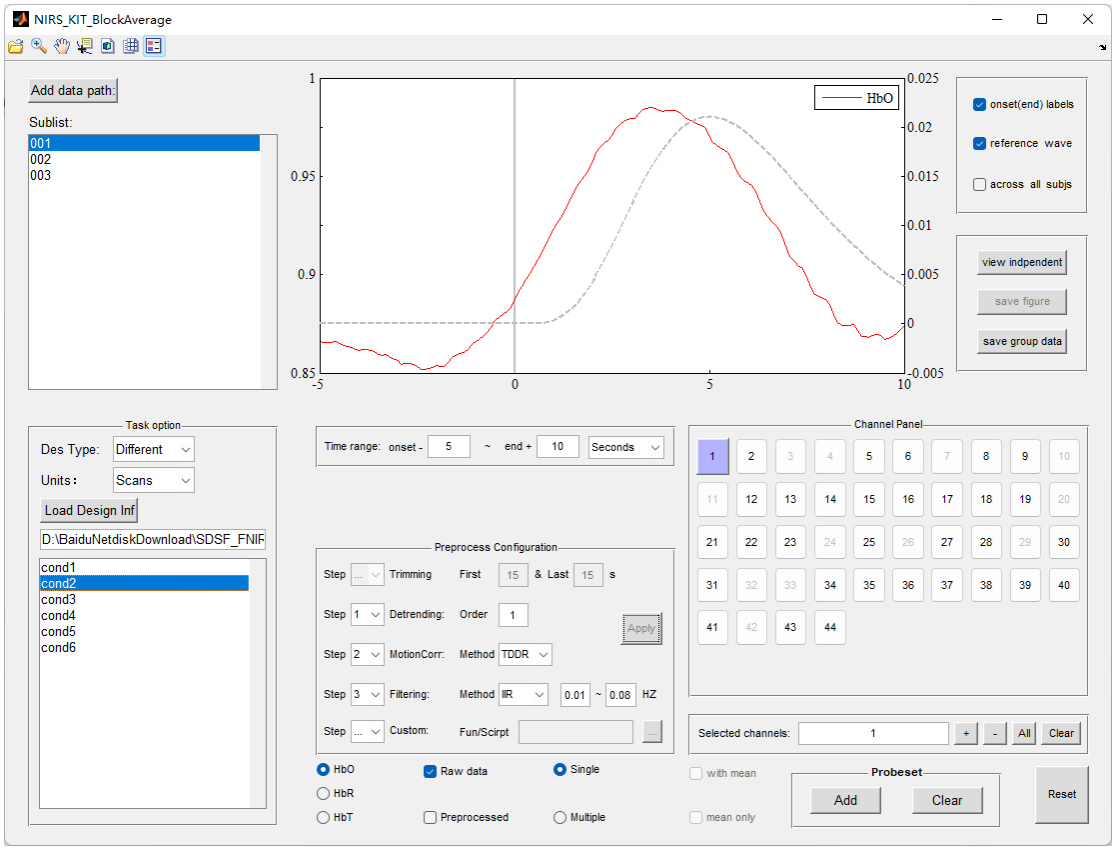


Fig. 4.2.2.2 NIRS-KIT Block/Event Average: event average (with duration = 0 for each event)

Note:

1) If a certain channel of a subject is labelled as abnormal (see Fig. 4.2.1.4), it will be excluded from the group-level block/event average calculation and display.

2) When we do group-level block average for block design, it is more appropriate when all blocks have the same durations. And when the duration of each block is not same, we take the maximum duration as the average duration, and the block average is calculated as shown in Fig. 4.2.2.3.

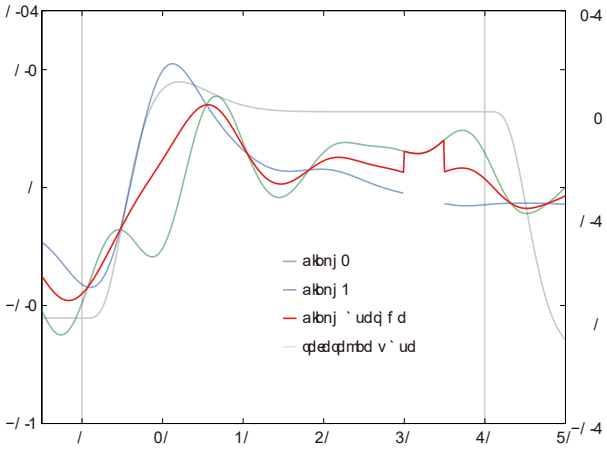


Fig. 4.2.2.3 Block average calculation for blocks with different durations

4.3 Preprocessing

Before statistical analysis, fNIRS data typically undergoes a series of preprocessing steps aimed at removing artifacts. The main goals are to minimize the influence of data acquisition and physiological artifacts. In this section, NIRS-KIT Preprocessing [Fig. 4.3(A)] provides several commonly preprocessing functions for fNIRS data. Researchers can arbitrarily designate the order of preprocessing steps.

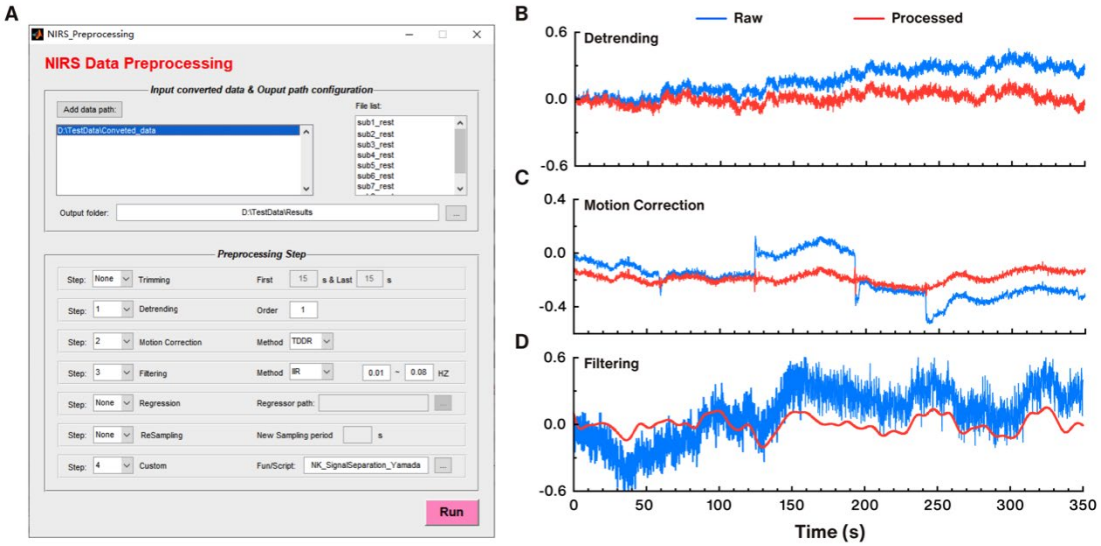


Fig. 4.3 The main interface of NIRS-KIT Preprocessing and demonstrations of processing methods on real data. (A) GUI of the data preprocessing module; (B) demonstration of the effect of detrending (linear detrending); (C) demonstration of motion correction using TDDR method; and (D) demonstration of filtering by a third-order IIR Butterworth bandpass filter (0.01 to 0.08 Hz).

- **Trimming**: Cut out the certain time series unwanted; If this option is checked, and you do not want to cut first or last time points, please enter '0'.
- **Detrending**: A polynomial regression model is used to estimate a linear or non-linear trend and then subtracts it from the raw hemoglobin concentration signal [as illustrated in Fig. 4.3(B)]. And order = 1 or 2 was recommended;
- **Motion Correction:** Two motion correction methods are provided. TDDR (Fishburn et al., 2019): Temporal Derivative Distribution Repair [as illustrated in Fig. 4.3(C)]; CBSI (Cui et al., 2010): correlation-based signal improvement.
- **Filtering**: NIRS-KIT provides three filtering models (high-pass, low-pass, and bandpass filtering) to remove irrelevant low-frequency and/or high-frequency components [as illustrated in Fig. 4.3(D)]. Three commonly used digital filter types are available: infinite impulse response (IIR) filter, finite impulse response (FIR) filter, and fast Fourier transform-based ideal filter (FFT-based filter). The IIR filter used in NIRS-KIT is a Butterworth filter (default third order). The FIR filter is a Hamming window filter (default 34th order). For the FFT-based filter, the time series of each channel is transformed into the frequency domain, the frequency-domain signals are filtered and then transformed back to the time domain. The default is bandpass filtering (0.01 to 0.08 Hz) using a third-order IIR filter. The filtering model, high- and/or low-frequency thresholds and filter type

can be specified by the user according to study objectives and noise characteristics. Note, for task analysis, the task-specific frequency should not be filtered.

- **Noise regression**: Undesirable systemic artifacts (especially scalp blood flow) usually contaminate fNIRS functional signals. A prominent approach is to use short-distance reference channels (that are sensitive only to signals in the superficial layers of the tissue outside the brain) to record superficial noise, then use regression to remove that noise from neural recordings. When short-distance channels are used, NIRS-KIT provides a noise regression functionality, in which the signals of short-distance channels can be used as the regressor and be removed. The regressors data should be saved in .txt files for each subject, see the example data in …\NIRS_KIT\Sample_Data\Regressor_Covariates.
- **Resampling**: Resample the original time series to a new fixed sampling rate.
- **Customized processing methods**: This module allows users to use the customized processing methods (as a MATLAB function) to process your fNIRS data [see Fig. 4.3(A)]. Users can refer to the sample customized processing method file in the package […\NIRS_KIT\Customizedfunctions\NK_SignalSperation_Yamada.m, a systemized noise remove method developed by Yamada et al. (2012) was implemented as an example] to make their customized function or script.

Please compare the preprocessed data and raw time series with Data Viewer, and then be careful to determine the preprocessing methods and step orders. Pay attention, if you performed trimming and resampling in preprocessing procedure, the raw time points and your mark points may be changed. You need to make sure the design information you entered in the next task fNIRS individual-level analysis (GLM build) is correct with your preprocessed data.

4.4 Individual-level analysis

After task fNIRS data preprocessing, individual-level statistical analysis is carried out to detect task-related neural activation based on the General Linear Model (GLM).

Individual-level statistical analysis of fNIRS data comprises the following steps (1) Specification of the GLM, which considers the observed hemodynamic signal (dependent variable) as a linear combination of regressors of interest (task variables), nuisance covariates (such as the superficial noise measured by short-distance channels), and an error term. For GLM specification, the canonical hemodynamic response function implemented in SPM is used to construct the reference time series representation from task variables; (2) Estimation of GLM parameters (3) Interrogation of results using contrast vectors.

Step1: Model Specification

- Add data path: The folder contains all subjects' preprocessed task fNIRS data
- Set the Output path;
- Model Specification:
  - Signal type: select one or more signal types (oxy, dxy or total) to be analyzed;
  - HRF: Canonical HRF is the default option.
  - Design Inf Type: Same or Different

- Units for Design: Seconds or Scans

If all subjects have the same design matrix, select the **Design Type** as **Same** (Fig. 4.4.1), click ***Add New Condition***, and the input window will be open. You need to input the condition name of each condition, the onset times, and corresponding durations of each trial or block. Specify a vector of onset times for this condition type.

Onset: Specify a vector of onset times for this condition type.
The onsets vector can be entered using the keyboard e.g. typing in "100 300 500" or "100:200:500", and then hitting return.
Duration: Specify the event durations.
Block and event-related responses are modeled in the same way but by specifying their different durations. Events are specified with a duration of 0. If you enter a single number for the duration, it will assume that all trials conform to this duration. If you have multiple different durations, then the number must match the number of onset times.

After Model Specification complete, the design matrix will be plotted on the right panel (see the following figure). The design matrix has one row for each scan and one column for each effect or explanatory variable.

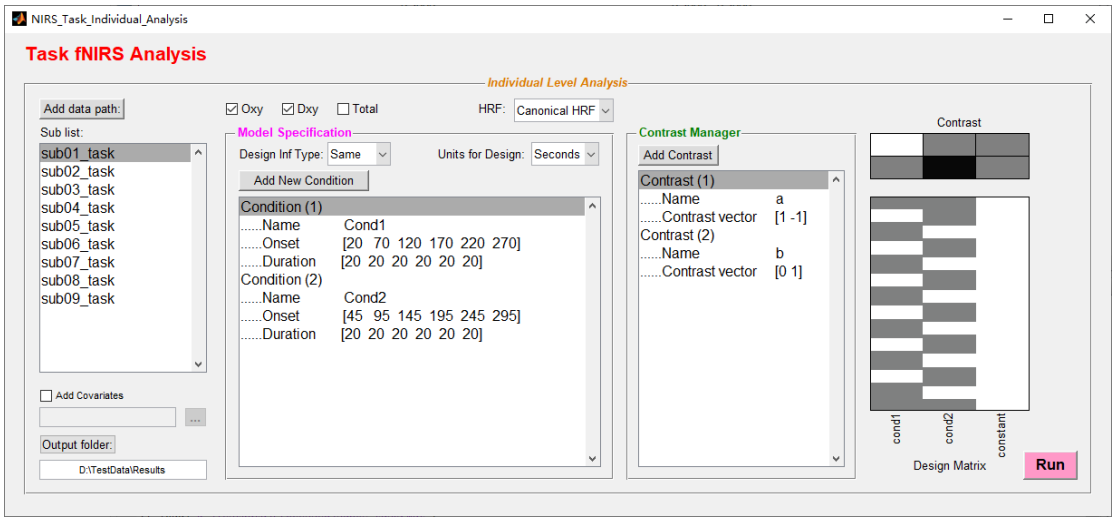


Fig. 4.4.1 Task analysis – Model Specification with same design information for everyone.

If every subject has its design matrix, select the **Design Inf Type** as **Different** (Fig. 4.4.2), and then click ***Load your Design Inf.mat***. The users can refer to the Temp_Design_Inf.mat (in …\NIRS_KIT\Sample_Data\ by clicking **Open the sample folder** to open) to make their own *.mat file contains all the subjects' design information.
Please make sure that sub names in the design information mat file are identical with the name in the input folder in the same order (see Fig. 4.4.2).
And then the design matrix of the first subject will be plotted (Fig. 4.4.3).

If the users want to add the nuisance covariates (such as the superficial noise measured by short-distance channels) to the GLM, the covariates should be put into a .txt file (see the sample file in …\NIRS_KIT\Sample_Data\Regressor_Covariates) for each subject.

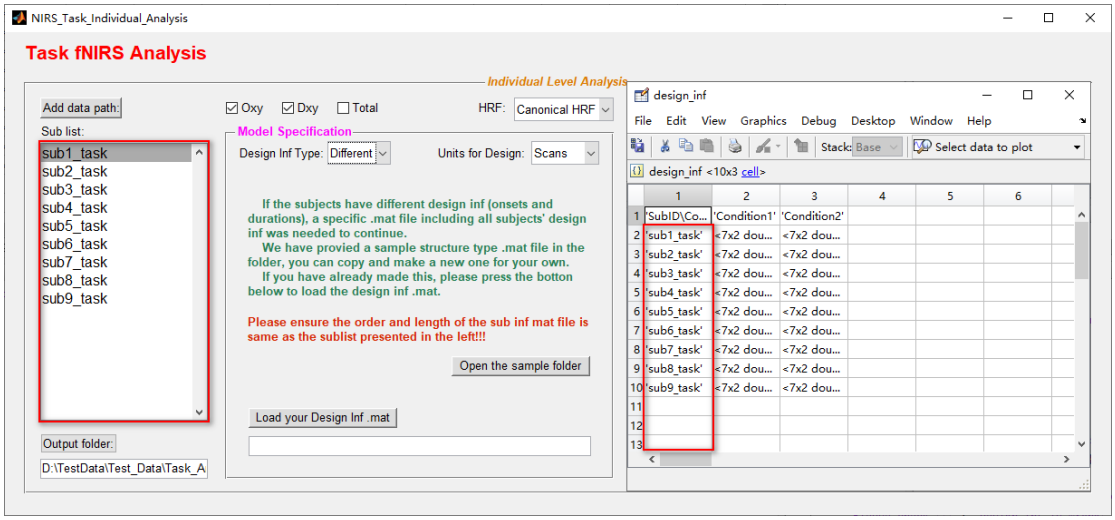


Fig. 4.4.2 Please make sure the design information file (*.mat) has identical names and the same order as the sub list.

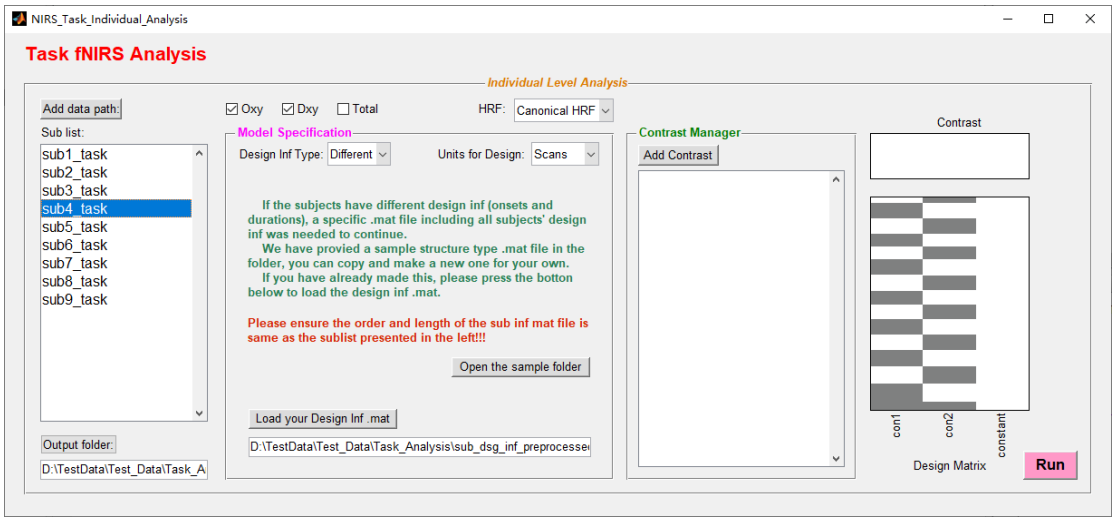


Fig. 4.4.3 Task analysis - Model Specification with different design information for everyone.

Step2: Model Estimation

After GLM has been specified, click ***Run*** on the low right corner. Then, after a few seconds or minutes, all the work will be done, and the output files were generated in the output folder, as follow:

…\output folder\GLM\
…\output folder\Oxy\beta_0
…\output folder\Oxy\beta_1
…\output folder\Oxy\beta_*

…\output folder\Dxy\beta_0

…\output folder\Dxy\beta_1
…\output folder\Dxy\beta_*

Note: beta_1 is corresponding the first column-condition_1 in the design matrix, beta_n is corresponding the n-st column-condition_n in the design matrix; while beta_0 is corresponding the last column - the constant in the design matrix.

Step3: Creating Contrasts

The contrast vector can be used to estimate signal magnitudes in response to a single condition, an average over multiple conditions, or the difference in magnitude between two conditions (Fig. 4.4.4).

- Add data path: The folder contains all subjects' GLM file generated after the last step Model Estimation;
- Output folder:
- Add Contrast:
  - Name: enter the contrast name
  - Contrast Vector: and enter the contrast vector e.g., "1 -1" or "0 1 0"

Note: 1. F contrast is not allowed here, you can do this in the group-level analysis.
2. The right zero(s) in your contrast vector can be omitted because that NIRS-KIT will automatically zero-pad this vector if you specify fewer contrast weights than the number of columns in your design matrix.

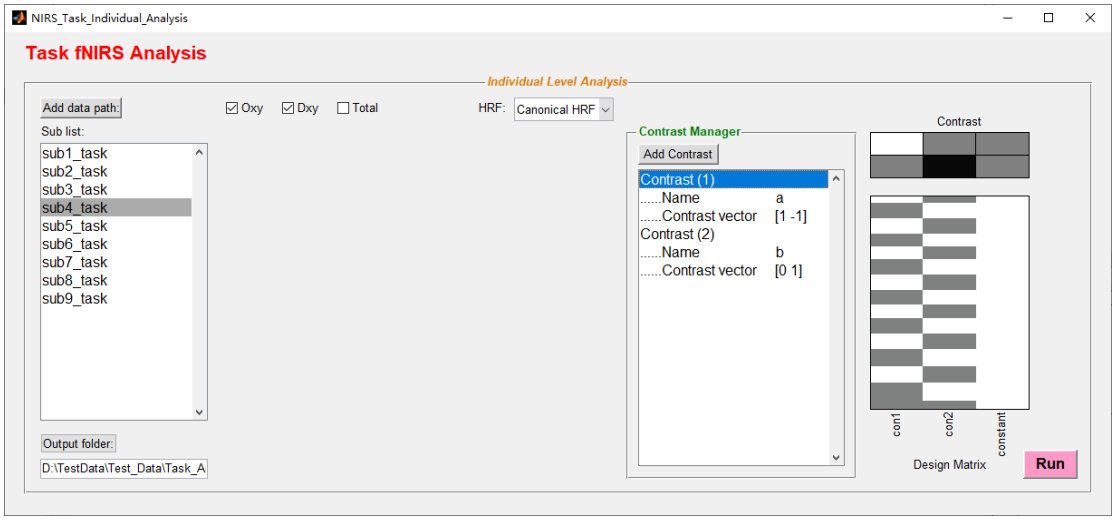


Fig. 4.4.4 Contrast manager of task-design fNIRS individual analysis.

After definition the contrast, click 'Run' on the low right corner. Then, after a few seconds or minutes, all the work will be done, and the corresponding output contrast files were generated in the output folder, as follow:
…\output folder\Oxy\con1
…\output folder\Oxy\con2

…\output folder\Oxy\con*

…\output folder\Dxy\con1
…\output folder\Dxy\con2
…\output folder\Dxy\con*

Or the user can define and run all the three steps (Model Specification, Model Estimation, and Contrast Manager) at once as follow:

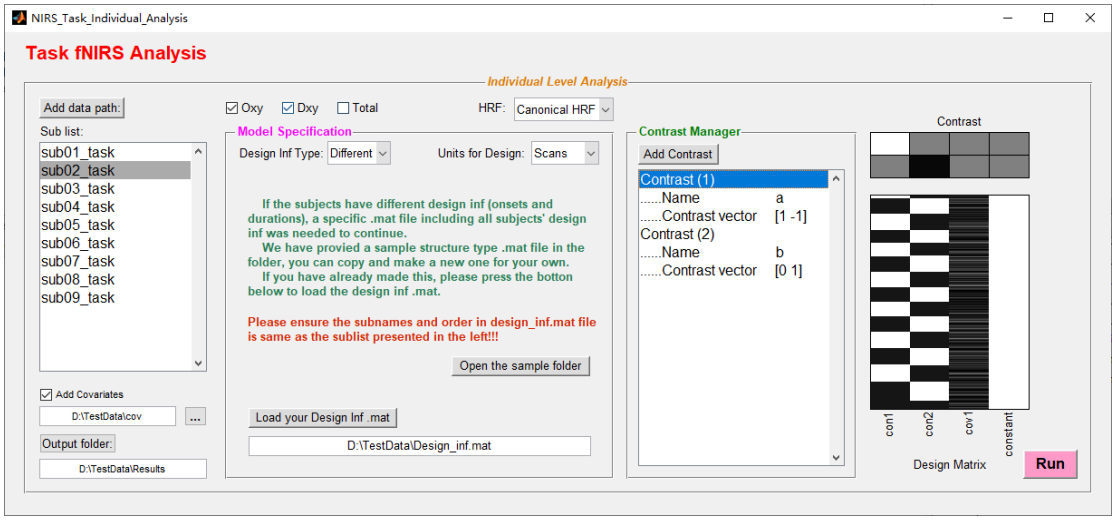


Fig. 4.4.5 One-stop setting of NIRS-KIT task-design fNIRS individual analysis. One column of randomly generated covariates is added into the GLM as an illustration.

Design information mat maker

When using the NIRS-KIT software for Task Individual Analysis, Data Viewer, or Block/Event Average, users will be required to provide design information for the subjects. When the design information for each subject is consistent, users can manually enter it through the interface. However, when the researcher balances or randomizes the presentation of conditions for each subject in the experiment, there may be significant differences in the onset of each condition block/event and the duration time of each block. In this case, users need to provide a design_inf.mat file containing information for each subject's settings. This may be difficult for users without coding experience. To facilitate the process of creating a design_inf.mat file, we have added a Design_Inf_Maker module to the Task Individual Analysis interface (updated in NIRS-KIT v3.0, as shown in the figure below).When researchers record mark information in the raw data while collecting data with the near-infrared device through the experimental procedure, they can use this module to quickly create the design_inf.mat file.

To use this module:

- Click '*Design Inf mat Maker*' when Design Inf Type is'Different' (see Fig. 4.4.6);
- Load your data (raw data or preprocesing data. After preprocessing data will be better, because if you do trimming or resampling in preprocessing, your mark information will be changed) that included the vector onset information (see Fig. 4.1.4 for the subjects' data structure);
- Then, the software will then automatically determine the number of marks in the data and generate a corresponding number of design information setting lines (see Fig. 4.4.7 left) .
- Users can click '*Mark Display*', select a representative data to display (see Fig. 4.4.7 right) that will help you to perform the next operations.
- Set change or import the settings according to your experimental design. Then click '*Run*' to save.

Note:

1) Design_Inf_Maker generates the output Design_Inf.mat in scans, so when it is used in Data Viewer/Block Average/Task Individual Analysis, the Units of Design should be set as 'Scans'.

2) Sometimes, due to issues with the experimental procedure, machine problems, or incorrect user settings, there may be some problems with the generated design_inf.mat file. Therefore, before using the file officially(such as using DataViewer or Block/Event Average loading for inspection) , users should carefully check it to ensure that there are no errors.

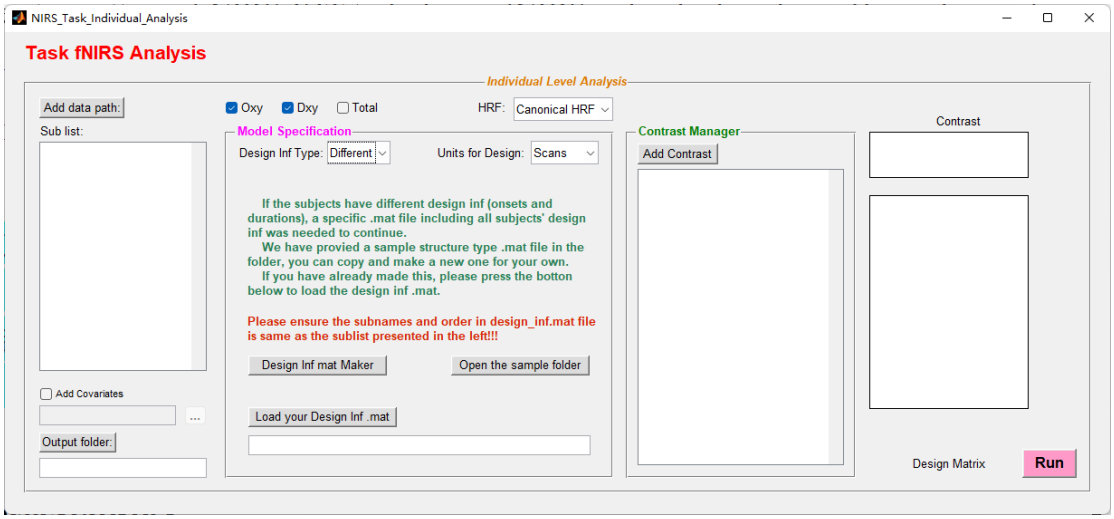


Fig. 4.4.6 Design_Inf_Maker interface in NIRS_Task_Individual_Analysis.

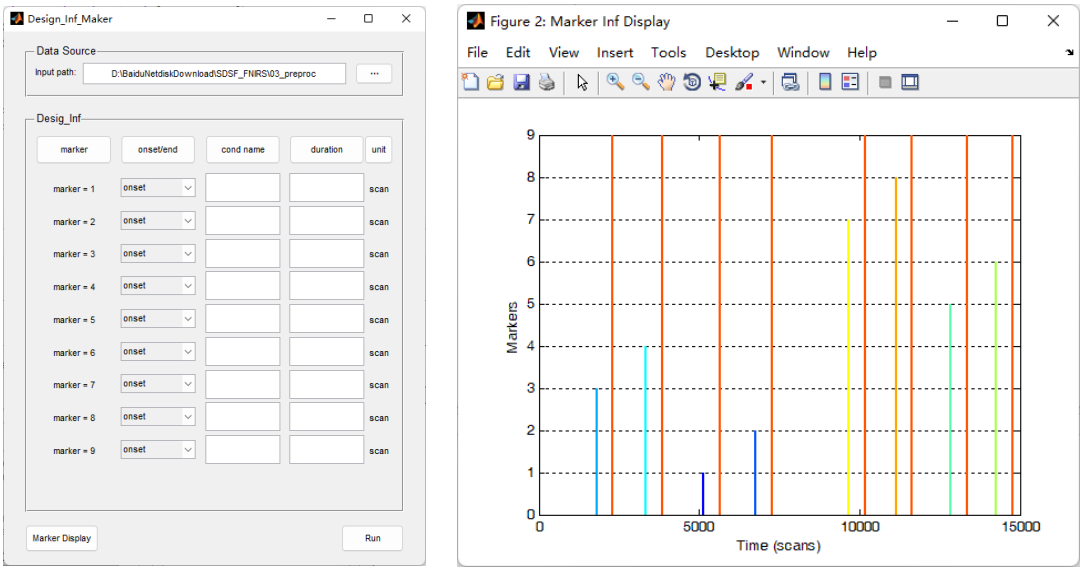
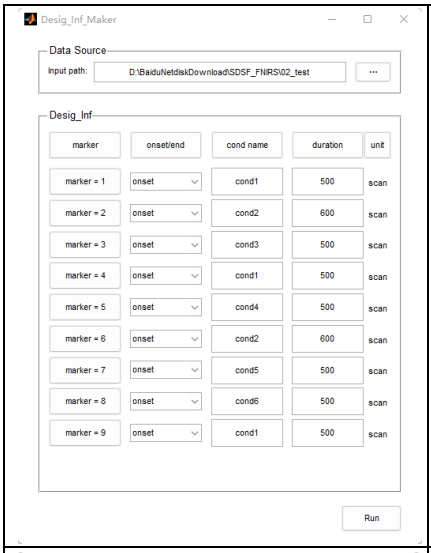
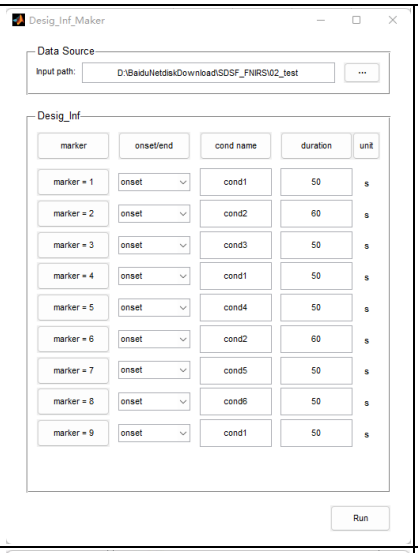
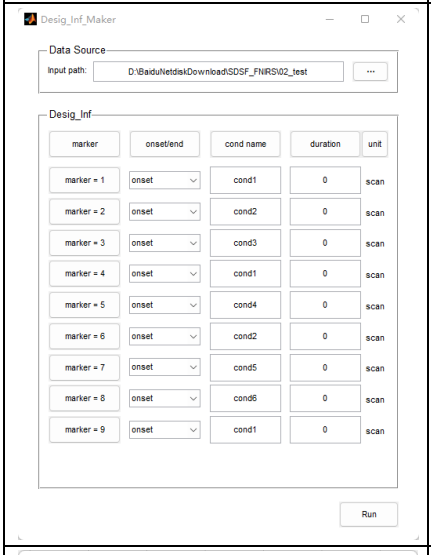
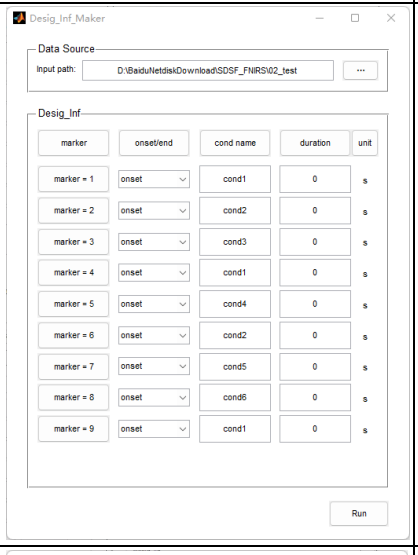
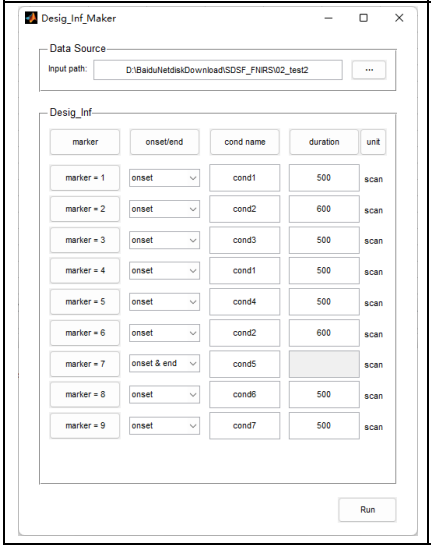
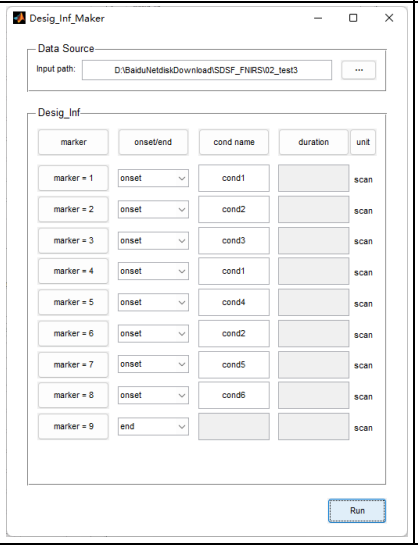


Fig. 4.4.7 Design_Inf_Maker interface (left) and Marker Inf Display (right).

Table 4.4 Several example settings for Design Information mat Maker

	**Setting1:** Cond1: marker=1&4&9,duration = 500 scans; Cond2: marker=2&6,duration = 600 scans; …… ……		**Setting2:** Exactly the same as Setting1, except that here the units are seconds.
	**Setting3:** Event design information setting with duration=0		**Setting4:** Event design information setting with duration=0
	**Setting5:** …… …… In this setting, Cond5 with both onset and end = mark7		**Setting5:** In this setting, Each condition has the same end mark=9

4.5 Group-level statistics

Comparing the activations or differences across multiple subjects and making population inference is called the second-level or group-level analysis (Fig. 4.5).

For group-level analysis, NIRS-KIT provides several popular parametric models, including the one-sample t-test, two-sample t-test, paired t-test, correlation analysis, ANOVA (independent and repeated measurement), and average the individual indexes. Besides, covariates of no interest (e.g., age, gender, and training time) can be added into these statistical models (except average). Besides, NIRS-KIT also provides multiple comparison correction approaches, including the false discovery rate (FDR) and Bonferroni correction. Finally, if multiple correlation approaches are applied, they can be only performed within a mask (contains your interest channels).

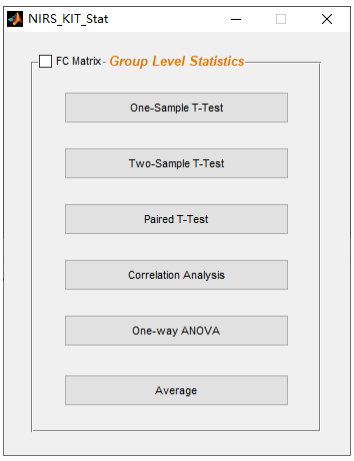


Fig. 4.5 The main interface of NIRS-KIT Group-Level Statistical.

4.5.1 One-Sample T-Test

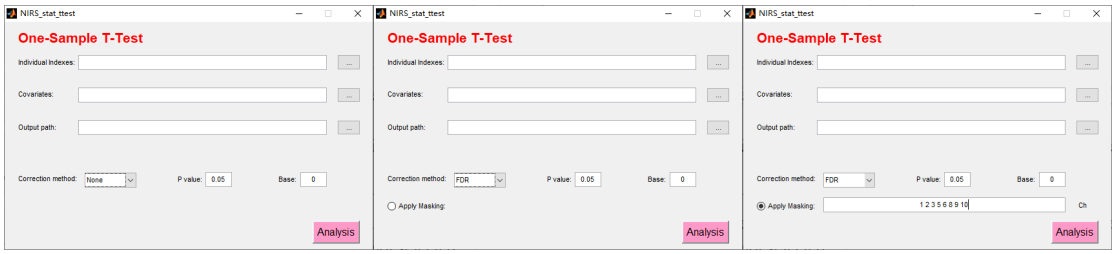


Fig. 4.5.1 One-sample t-test of group-level statistics.

One sample t-test (Fig. 4.5.1) can be used to test whether the group indexes are significantly different from a given value (e.g., 0).

- Click the button '…' to add the individual-level resulted folder contains the interested indexes (the beta values or contrast values);
- Add the covariates text file to input the covariates of no interests, if you have;
  Note: the covariates text file should be organized like this (Fig. 4.5.2): The rows represent subjects in the same order with your input Individual Indexes files. The columns represent the different covariates.

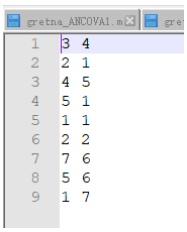


Fig. 4.5.2 A sample of a covariates text file.

- Set the output path;
- Select the correction method: None, FDR or Bonferroni

  If FDR or Bonferroni is selected, the Apply masking will be presented, then

  If Apply masking is not selected (middle panel), the multiple comparison correction will be done within all channels (Fig. 4.5.1 middle panel);

  If Apply masking is selected (Fig. 4.5.1 right panel), the multiple comparison correction will be only done within the mask channels;
- After setting all of the parameters, click Run, and the statistical result (a mat file) will be generated (Fig. 4.5.3).

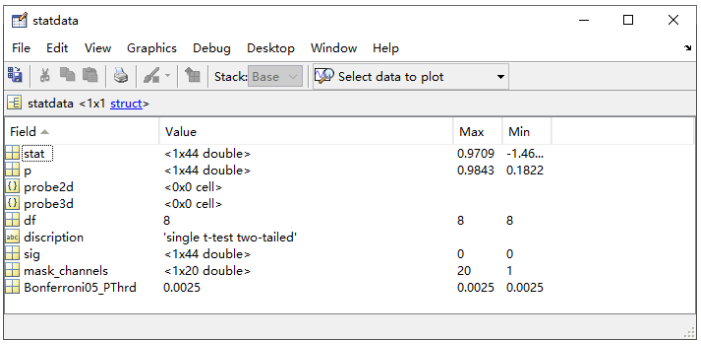


Fig. 4.5.3 A sample of the group-level statistical result.

Note:

In the result file, statdata.stat is the statistical value (here is the T value), statdata.p is the raw p-value corresponding to statdata.stat;

The statdata.sig marks each channels' significance with 0 (non-significant, with corresponding raw p-value > the statdata.*_PThrd) and 1(significant, the raw p value ≤ the statdata.*_PThrd);

If multiple comparison correction and the masking were applied, only the channels within the statdata.mask_channels with their raw p values ≤ statdata.*_PThrd will be set 1.

4.5.2 Two-Sample T-Test

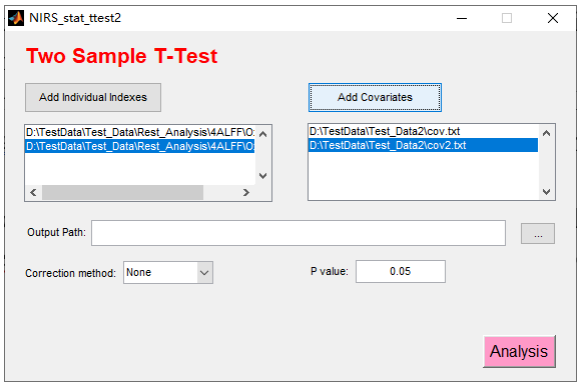


Fig. 4.5.2 Two-sample t-test of group-level statistics.

The two-sample t-test (Fig. 4.5.2) can be used to test whether the interested indexes in two independent groups are significantly different from each other.

- Click Add Individual Indexes to add the individual indexes folder for each independent group;
- If there are covariates, add two covariates text files for each group in the same order with the individual indexes folders in the left panel.
- Other parameters setting please refer to above one-sample t-test;

4.5.3 Paired T-Test

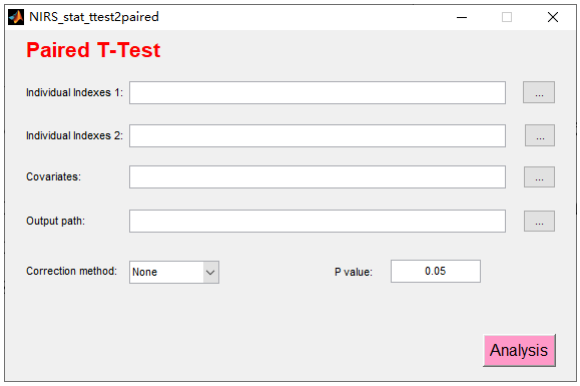


Fig. 4.5.3 Paired t-test of group-level statistics.

The paired t-test (Fig. 4.5.3) can be used to test whether the interested indexes in two related groups (such as pre-and post-tests) are significantly different from each other.

- Click the button '…' to add Individual Indexes 1 and paired Individual Indexes 2;
- If there are covariates, add a single one covariates text file for the group;
- Other parameters setting please refer to above one-sample t-test;

4.5.4 Correlation Analysis

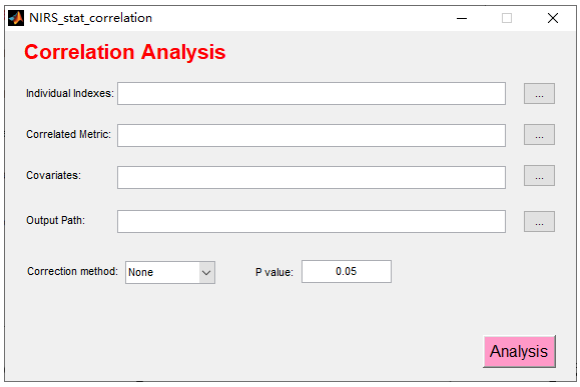


Fig. 4.5.4 Correlation analysis of group-level statistics.

Correlation analysis (Fig. 4.5.4) can be used to test whether the interested indexes are significantly correlated with the variable of interest (e.g., demographic, or cognitive, or clinical variables) across subjects while controlling the differences of the covariates of no interest.

- Click the button '…' to add the individual-level resulted folder contains your interesting indexes (the beta values or contrast values);
- Add the correlated metric text file contains your variable of interest.

  Note: the covariates text file should be organized like this: n (the number of subjects) rows represent subjects with the same order with your input Individual Indexes files. The single-column represents your variable of interest.
- Other parameters setting please refer to above one-sample t-test;

4.5.5 One-way ANOVA

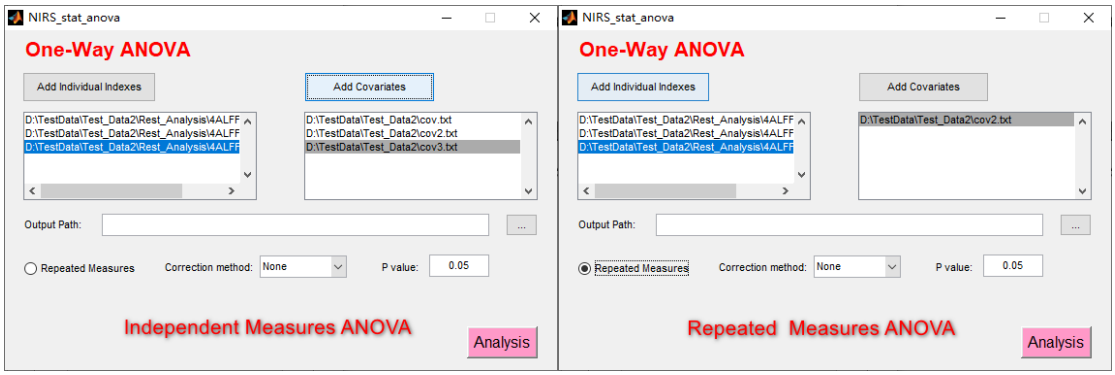


Fig. 4.5.5 One-way ANOVA (left: independent measures; right: repeated measures) analysis of group-level statistics.

One-way ANCOVA (Fig. 4.5.5) can be used to test whether the interested indexes are significantly different across categories/levels of an independent (a repeated) variable while controlling the differences of the covariates. Here, NIRS-KIT provides both independent and repeated measures one-way ANOVA.

- Click 'Add Individual Indexes' in the left column to input the interested indexes folders;
- Select Repeated Measures if you want to do repeated measures one-way ANOVA;
- Click 'Add Covariates' in the right column to input the covariates of no interest, if

necessary:

Note:

- If independent measures: add the covariates text files for each group in the same order with the individual indexes folders in the left panel (see above-left figure);
- If repeated measures: add a single covariates text file for the group (see above right figure)

- Other parameters setting please refer to above one-sample t-test;

4.5.6 Average

Fig. 4.5.6 Group average analysis.

Average functional (Fig. 4.5.6) can be used to calculate the mean indexes across subjects.

- Click the button '…' to add the individual-level resulted folder contains your interesting indexes (the beta values or contrast values);
- Click the button '…' to select the path for outputting results;
- Click 'Analysis' to run.

Integrating multi-subjects value to a single one

This feature supports the integration of individual-level analysis indicators for all subjects into a single file so that users can perform customized group-level statistical analysis or presentation of results based on this (updated in NIRS-KIT V3.0).

- To perform this function, you can run *NK_ind2grp.m* in the Matlab command window, select the folder to be integrated containing the individual level analysis metrics of all subjects. Then, a separate table format *. mat document will be generated (Fig. 4.5.7).

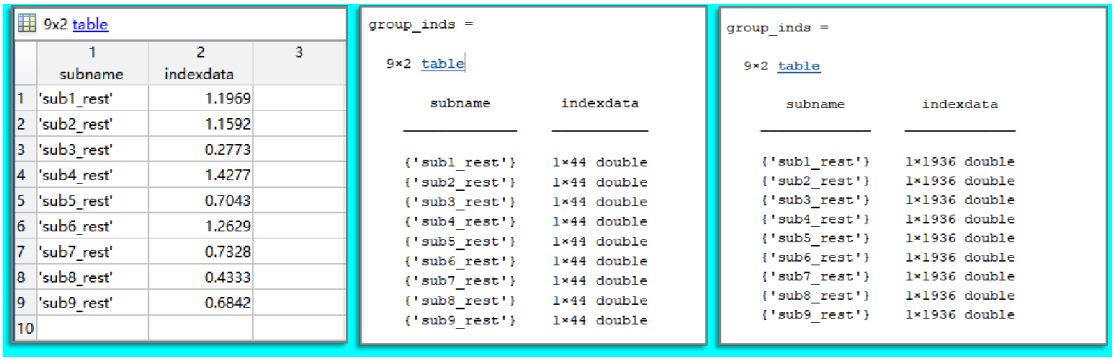


Fig. 4.5.7 Demonstration of integrating multi-subjects value to a single one.

4.6 Result visualization

NIRS-KIT provides both 2D and 3D visualization functions (Fig. 4.6) to visualize task-design fNIRS individual-level analysis indexes (beta value or contrast value) or group-level statistical results (e.g., t-test, correlation results) in an easy, flexible and quick manner.

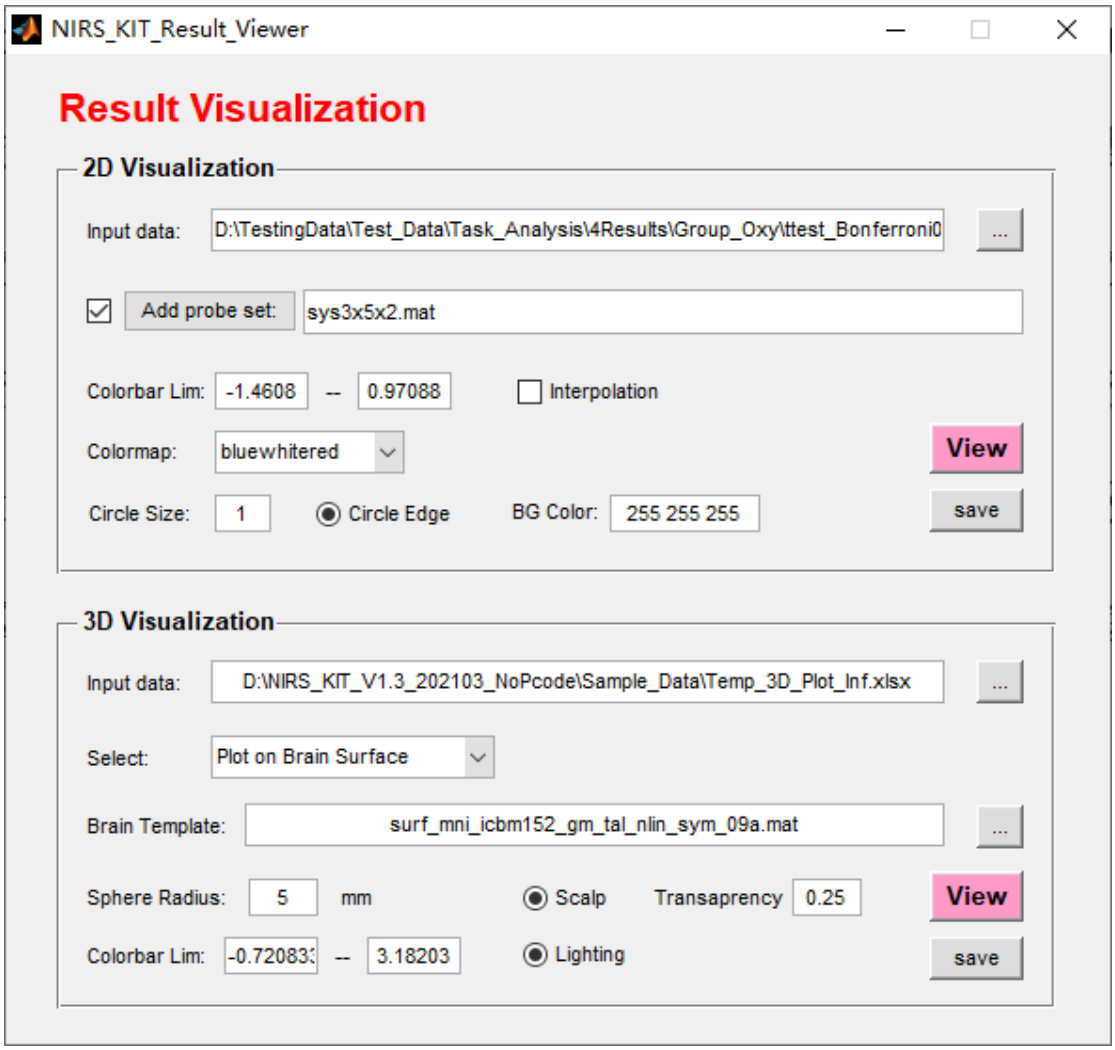


Fig. 4.6 The main interface of NIRS-KIT Result Visualization.

4.6.1 2D result visualization

Here, NIRS-KIT allows you to show your individual-level indexes or second-level statistical results in interpolation or non-interpolation 2D visualization mode (Fig. 4.6.1.1).

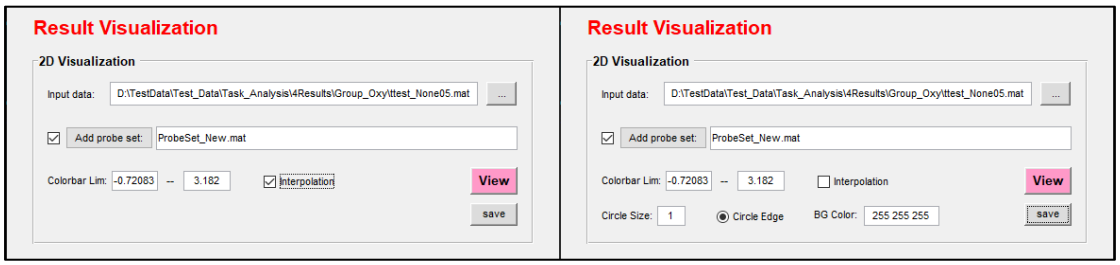


Fig. 4.6.1.1 NIRS-KIT 2D Result Visualization.

- Click the button '…' to add the individual-level analysis index or group-level statistical result;
- If there is no probe set data in your input data file, please pitch on the box in the left of 'Add probe set', and select the corresponding probe set file;
- Set the lower and upper limit if necessary to restrict the color scale of the data;
- Select the display type: Interpolation (Fig. 4.6.1.2) or not (Fig. 4.6.1.3):
  - If the interpolation was not been selected, set the following parameters: relative circle size (1 was default value), whether or not show the circle edge, the background color (BG Color).
- After setting all of the above parameters, click 'View' to plot and present the figure.
- Click 'save', set the output folder and output file name, and the output file format to save the present shown figure. Here, NIRS-KIT supports saving the figure as a bitmap format ('tif') file or vector diagram format ('pdf') format file. Here, the export_fig function was applied to enhance the visualization effects (https://github.com/altmany/export_fig).

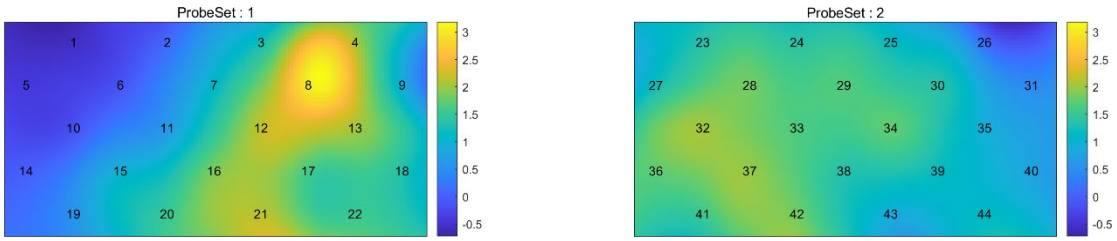


Fig. 4.6.1.2 The 2D interpolated visualization map.

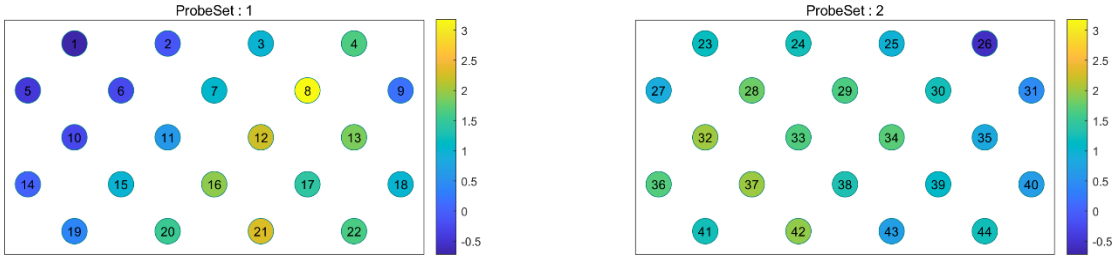


Fig. 4.6.1.3 The 2D non-interpolated visualization map.

4.6.2 3D results visualization

In NIRS-KIT, three non-interpolated and one interpolated channel-wise 3D visualizations are provided (Fig. 4.6.2.1).

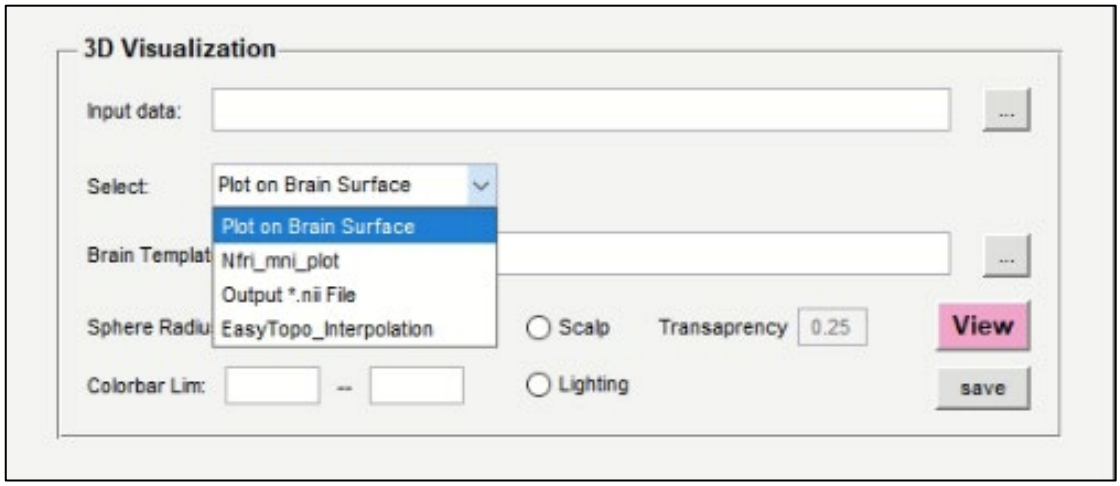
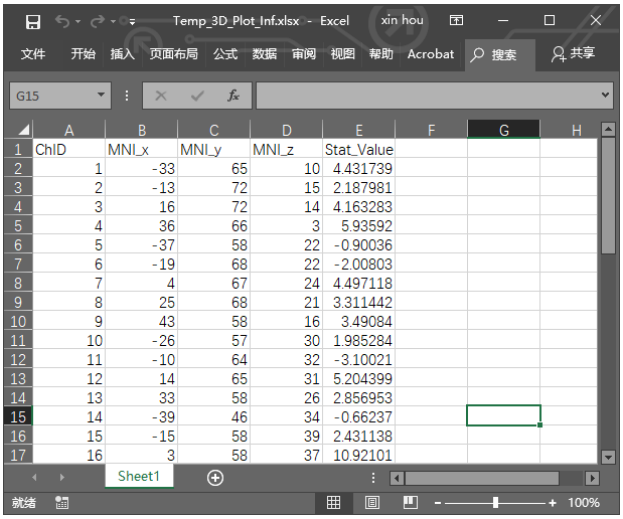


Fig. 4.6.2.1 NIRS-KIT 3D Result Visualization.

For non-interpolated 3D visualization, three models can be used**: The first** is to project the analysis result of each measurement channel directly onto a standard surface template (e.g., ICBM152), set the display parameters (such as the sphere size and color bar limits), and then output the resulting figure. **The second** is mapping the channel-wise statistical values onto the standard brain space using the nfri_mni_plot function in the NFRI toolbox [if this method was applied, please cite this paper (Singh et al., 2005)]. **The last**, more flexible approach, is to output a brain image in the widely used NIFTI format, that can be loaded and visualized by other external imaging visualization toolbox [such as BrainNet Viewer (Xia et al., 2013), MRIcroGL, Surfice, et al.].

For interpolated channel-wise 3D visualization, the EasyTopo toolbox provided by Tian et al. (2013) is used (if this method was applied, please cite this paper).

To present 3D visualization, you need to integrate the statistical values and the MNI coordinates of each channel into an excel file in the following specified format (see the template file Temp_3D_Plot_Inf.xlsx in the sample folder). In the 3D plot information xlsx (Fig. 4.6.2.2) the first column is the channel ID, the second to fourth columns are the corresponding MNI coordinates for each channel, the last column is the statistical value.



	A	B	C	D	E
1	ChID	MNI_x	MNI_y	MNI_z	Stat_Value
2	1	-33	65	10	4.431739
3	2	-13	72	15	2.187981
4	3	16	72	14	4.163283
5	4	36	66	3	5.93592
6	5	-37	58	22	-0.90036
7	6	-19	68	22	-2.00803
8	7	4	67	24	4.497118
9	8	25	68	21	3.311442
10	9	43	58	16	3.49084
11	10	-26	57	30	1.985284
12	11	-10	64	32	-3.10021
13	12	14	65	31	5.204399
14	13	33	58	26	2.856953
15	14	-39	46	34	-0.66237
16	15	-15	58	39	2.431138
17	16	3	58	37	10.92101

Fig. 4.6.2.2 The input file format for 3D result visualization.

- Click the button '…' to add the 3D plot information excel file contains the MNI coordinates and corresponding statistical values as the input data;
- Select the display type:
  - If Within Matlab on Surface was selected.
    - Select the standard surface brain template: click '…' to add surf_mni_icbm152_gm_tal_nlin_sym_09a.mat in NIRS-KIT template folder, the surface file extracted form ICBM152 nonlinear atlases (version 2009, https://www.mcgill.ca/bic/icbm152-152-nonlinear-atlases-version-2009);
  - If Output Volume (*.nii) File was selected.
    - Select the standard surface brain template: click '…' to add mni_icbm152_gm_tal_nlin_sym_09a_mask.nii in NIRS-KIT template folder (refer to ICBM152 nonlinear atlases (version 2009, https://www.mcgill.ca/bic/icbm152-152-nonlinear-atlases-version-2009);
- Set the sphere radius, set the Colorbar limitation (if necessary), with scalp or not (and the transparency for scalp), lighting or not;
- Click 'View', to plot and present the resulted brain map. Then rotate the brain map to an appropriate angle;
- Click 'save', set the output folder and output file name, and the output file format (*.tif or *.pdf) to save the brain map.

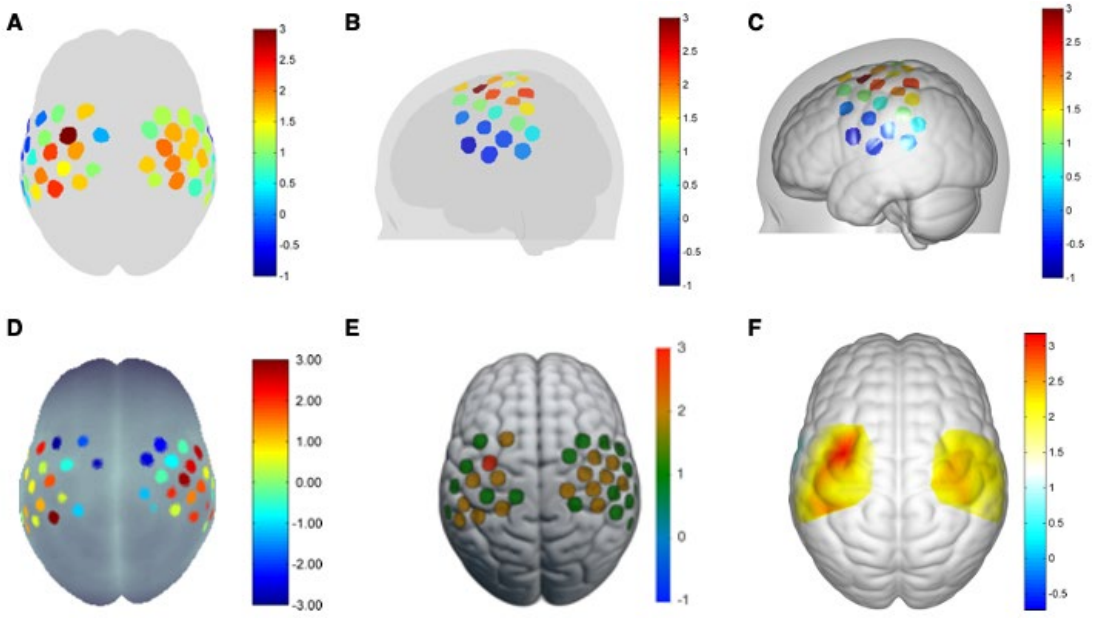


Fig. 4.6.2.3 The 3D visualization brain map. (A-C): No-interpolated 3D visualization using Within Matlab on Surface. (A) no scalp, no lighting; (B) with a scalp, no lighting; (C) with a scalp, with lighting. (D) Resulted brain map plotted using nfri_mni_plot function. (E) Show the fNIRS analysis result using MRIcroGL by loading the generated NFTI file. (F) 3D interpolated visualization.

5. Resting-state fNIRS data analysis

Resting-state fNIRS module (Fig. 5) in NIRS-KIT provides the main functions of Data preparation, Data Viewer, Preprocessing, Individual-level Analysis, Group-level Statistics, and Result Visualization.

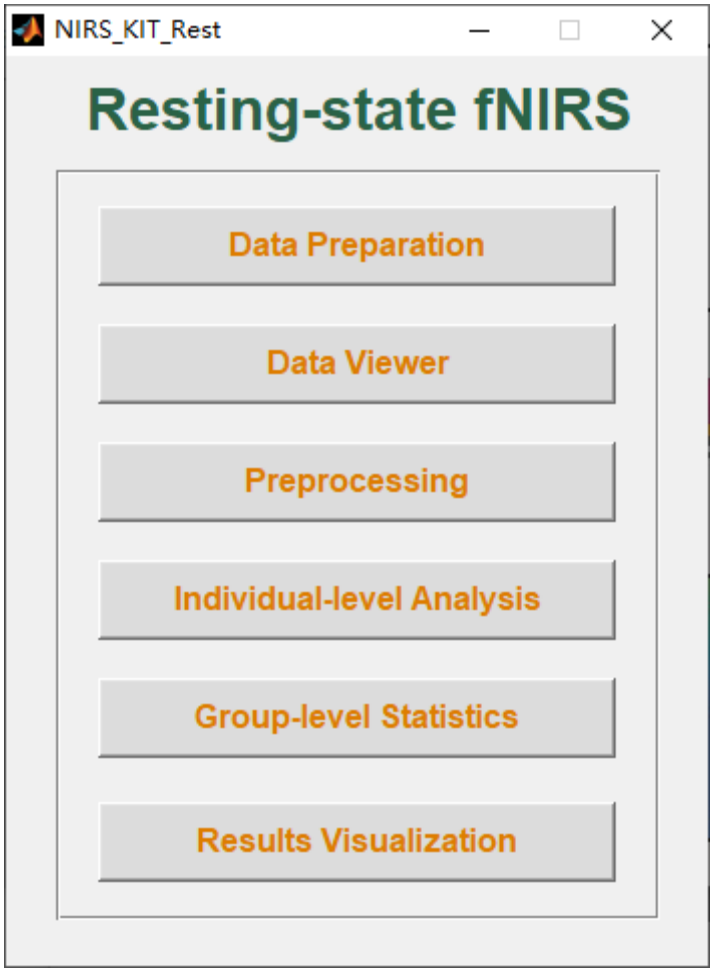


Fig. 5 The main interface of the Resting-state fNIRS module.

5.1 Data preparation

There is no difference between task fNIRS data preparation and resting-state fNIRS data preparation (see Sec. 4.3).

5.2 Data previewing and quality check

Data Viewer in Resting-state fNIRS module (Fig. 5.2) has the same functions as Data Viewer in task module, except without the task-specific panel for plotting task reference waves. Please refer to Sec. 4.2 Data Viewer to use it.

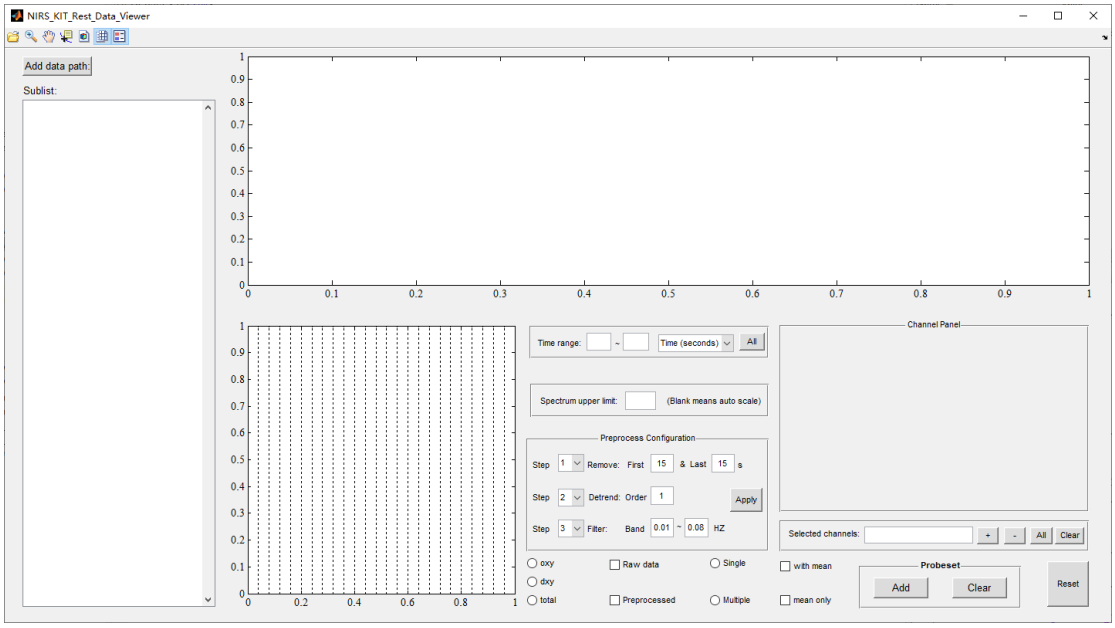


Fig. 5.2 The main interface of the Resting-state fNIRS module.

5.3 Preprocessing

The Preprocessing module was shared by NIRS-KIT task-design and resting-state analysis. Please refer to Sec. 4.3 Preprocessing to use it. For resting-state, low-frequency fluctuations (0.01 to 0.08 Hz) have been suggested to be of physiological importance and may reflect the spontaneous neural activity.

Note: When performing fALFF analysis, the bandpass filtering should be applied to reserve the entire frequency band of the neural signals, 48 and frequency range selection can refer to fALFF analysis in fMRI studies (usually 0 to 0.25 Hz).

5.4 Individual-level analysis

After resting-state fNIRS data preprocessing, individual-level statistical analysis is carried out to calculate the functional connectivity (FC), amplitude low-frequency fluctuation (ALFF, Yu-Feng et al., 2007), fractional ALFF (fALFF, Zou et al., 2008). Besides, NIRS-KIT can produce the functional connectivity matrix that can be used as the input for Gretna (Wang et al., 2015) to calculate the network metrics.

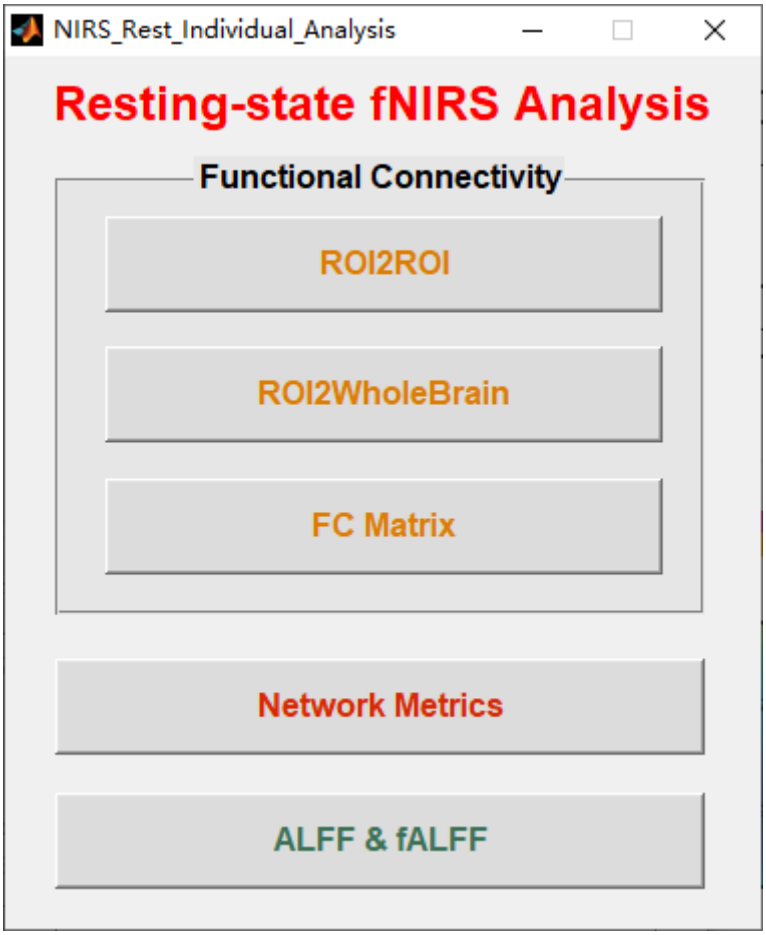


Fig. 5.4 The main interface of Resting-state fNIRS Individual Analysis.

5.4.1 Functional connectivity analysis

ROI2ROI FC, ROI2Wholebrain FC, and FC Matrix analysis can be performed by NIRS-KIT.

5.4.1.1 ROI2ROI FC analysis

Click the 'ROI2ROI' button showed in Fig. 5.4.1.1 to perform ROI2ROI functional connectivity analysis.

- Add data path: The folder contains all subjects' preprocessed resting-state fNIRS data, then the subjects in this folder will be presented in the right File list panel;

- Set the Output path;
- Select one or more signal types (oxy, dxy or total) to be analyzed;
- Define ROI1: One or more channel numbers can be typed in the right textbox of ROI1. When more than one interested channel was defined as ROI1 (for example channel 2 and channel 3 were defined as the ROI1, see Fig. 5.4.1.1), the averaged time series was generated firstly, and then functional connectivity was calculated between this averaged time series with ROI2.
- Define ROI2: One or more channel numbers can be typed in the right textbox of ROI2. When more than one interested channel was defined as ROI2, the averaged time series was generated firstly, and then functional connectivity was calculated between this averaged time series with ROI1.
- Select input the functional connectivity Fisher z-score or raw time-series correlation coefficient results.
- Click 'Run' to calculate the ROI2ROI functional connectivity.

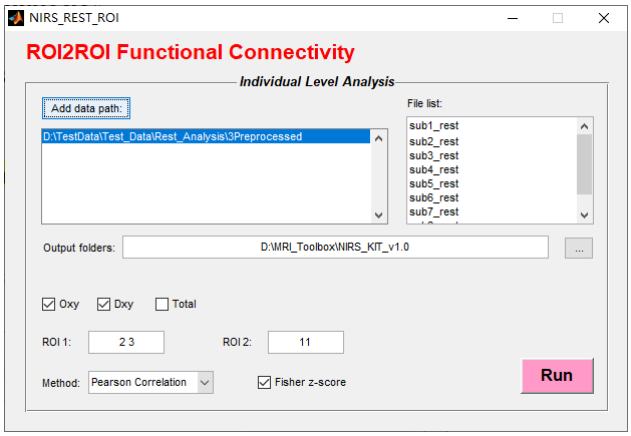


Fig. 5.4.1.1 Resting-state fNIRS ROI2ROI functional connectivity analysis.

5.4.1.2 ROI2Whole FC analysis

Click the 'ROI2Wholebrain' button showed in Fig. 5.4 to perform functional connectivity analysis between the ROI channel(s) with every channel in the fNIRS data (Fig. 5.4.1.2).

- Add data path: The folder contains all subjects' preprocessed resting-state fNIRS data, then the subjects in this folder will be presented in the right File list panel;
- Set the Output path;
- Select one or more signal types (oxy, dxy or total) to be analyzed;
- Define ROI: One or more channel numbers can be typed in the right textbox of ROI1. When more than one interested channel was defined as ROI, the averaged time series was generated firstly, and then functional connectivity was calculated between this averaged time series with every channel in the fNIRS data.
- Select input the functional connectivity Fisher z-score or raw time-series correlation coefficient results.
- Click 'Run' to calculate the ROI2Wholebrain functional connectivity.

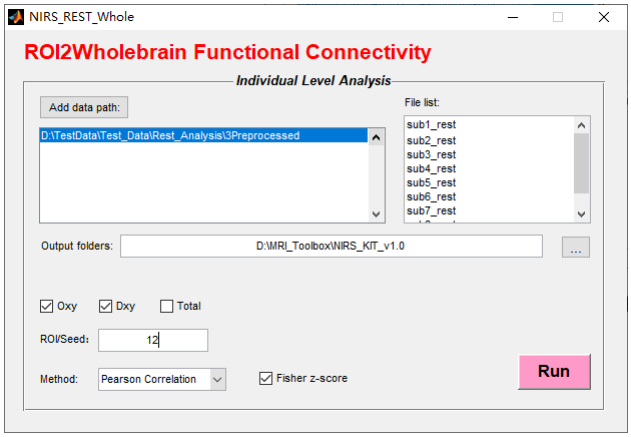


Fig. 5.4.1.2 Resting-state fNIRS ROI2Wholebrain functional connectivity analysis.

5.4.1.3 FC Matrix analysis

Click the 'FC' button showed in Fig. 5.4 to perform functional connectivity analysis of every paired-channels, and output the FC matrix (Fig. 5.4.1.3).

- Add data path: The folder contains all subjects' preprocessed resting-state fNIRS data, then the subjects in this folder will be presented in the right File list panel;
- Set the Output path;
- Select one or more signal types (oxy, dxy or total) to be analyzed;
- Select input the functional connectivity Fisher z-score or raw time-series correlation coefficient results.
- Click 'Run' to calculate the functional connectivity matrix.

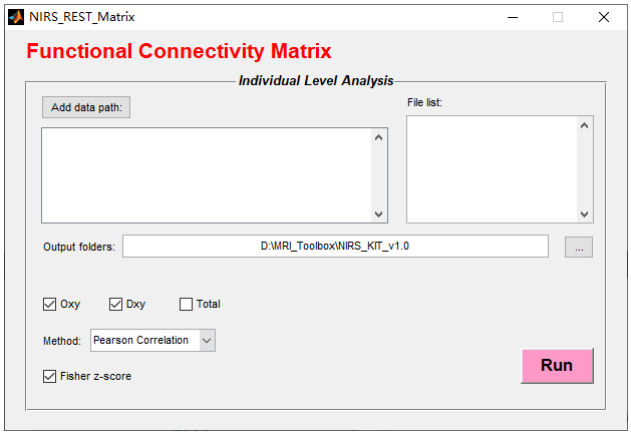


Fig. 5.4.1.3 Resting-state fNIRS FC matrix analysis.

5.4.2 Network metrics analysis

NIRS-KIT can produce the functional connectivity matrix that can be used as the input for Gretna (Wang et al., 2015) to calculate the network metrics, such as the global network metrics (small-world, efficiency, et al.), nodal and modular network metrics (clustering coefficient, degree centrality, et al.).

Gretna is needed for fNIRS Network Metrics analysis. Before performing fNIRS Network Metrics analysis, Gretna should be download (http://www.nitrc.org/projects/gretna) and added to the Matlab search path.

Step1: Generate the FC Matrix

- Add data path: The folder contains all subjects' preprocessed resting-state fNIRS data, then the subjects in this folder will be presented in the right File list panel;
- Set the Output path;
- Select one or more signal types (oxy, dxy or total) to be analyzed;
- Select input the functional connectivity Fisher z-score or raw time-series correlation coefficient results.
- Network Definition: Input the channel numbers within the interested mask (for example, see Fig. 5.4.2.1).
- Click 'Run' to calculate the functional connectivity matrix.

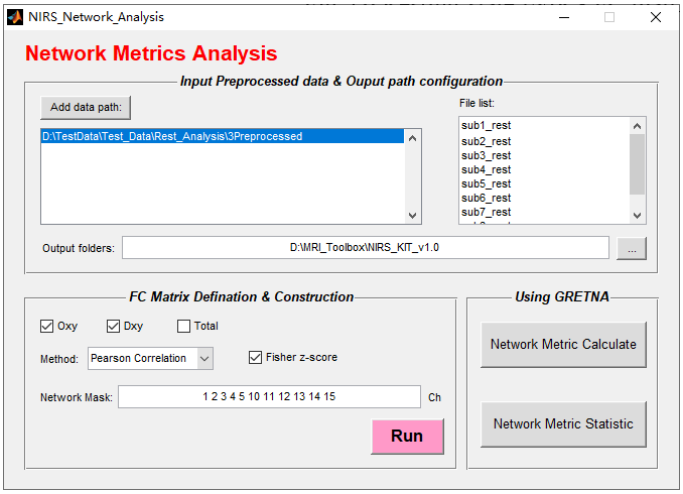


Fig. 5.4.2.1 Resting-state fNIRS network metrics analysis.

Step2: Network Metrics calculation and statistics.

Click 'Network Metric Calculate' in Fig. 5.4.2.1 to open the Gretna-Network Analysis module; Add the FC Matrix files generated from Step1 to the 'Brain Connectivity Matrix'; The set the parameters to perform network metrics analysis (Fig. 5.4.2.2 left panel).

Click 'Network Metric Statistic' in Fig. 5.4.2.1 to perform group-level network metric statistics (Fig. 5.4.2.2 right panel).

Detailed usage please refer to Gretna manual, and cite the following paper:

Wang, J., Wang, X., Xia, M., Liao, X., Evans, A., He, Y., 2015. GRETNA: a graph theoretical network analysis toolbox for imaging connectomics. Front. Hum. Neurosci. 9, 1–16. https://doi.org/10.3389/fnhum.2015.00386.

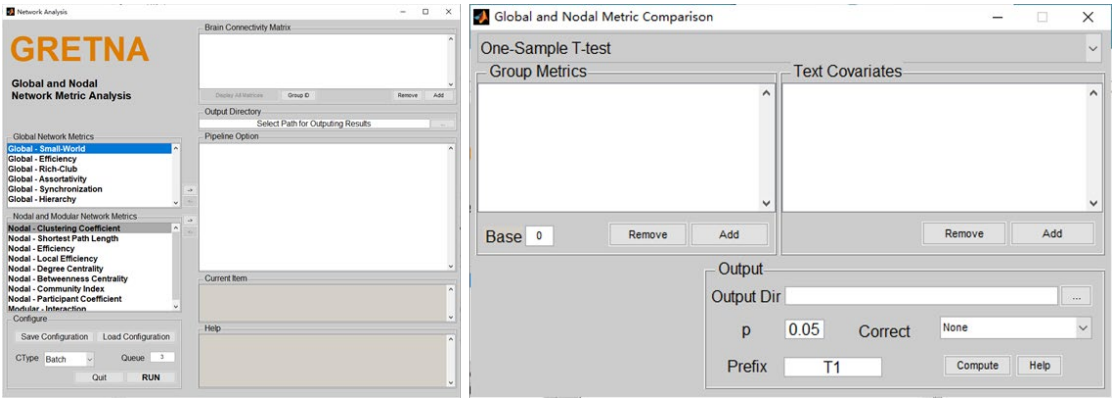


Fig. 5.4.2.2 Resting-state fNIRS network metrics analysis.

5.4.3 ALFF and fALFF analysis

Click the 'ALFF & fALFF' button showed in Fig. 5.3 to perform resting-state fNIRS ALFF or fALFF analysis (Fig. 5.4.3).

- Add data path: The folder contains all subjects' preprocessed resting-state fNIRS data, then the subjects in this folder will be presented in the right File list panel;
- Set the Output path;
- Select one or more signal types (oxy, dxy or total) to be analyzed;
- Set the Amplitude of Low-frequency Fluctuation Band;
- Select which types of indexes to be calculated;
- Click 'Run' to calculate the corresponding indexes.

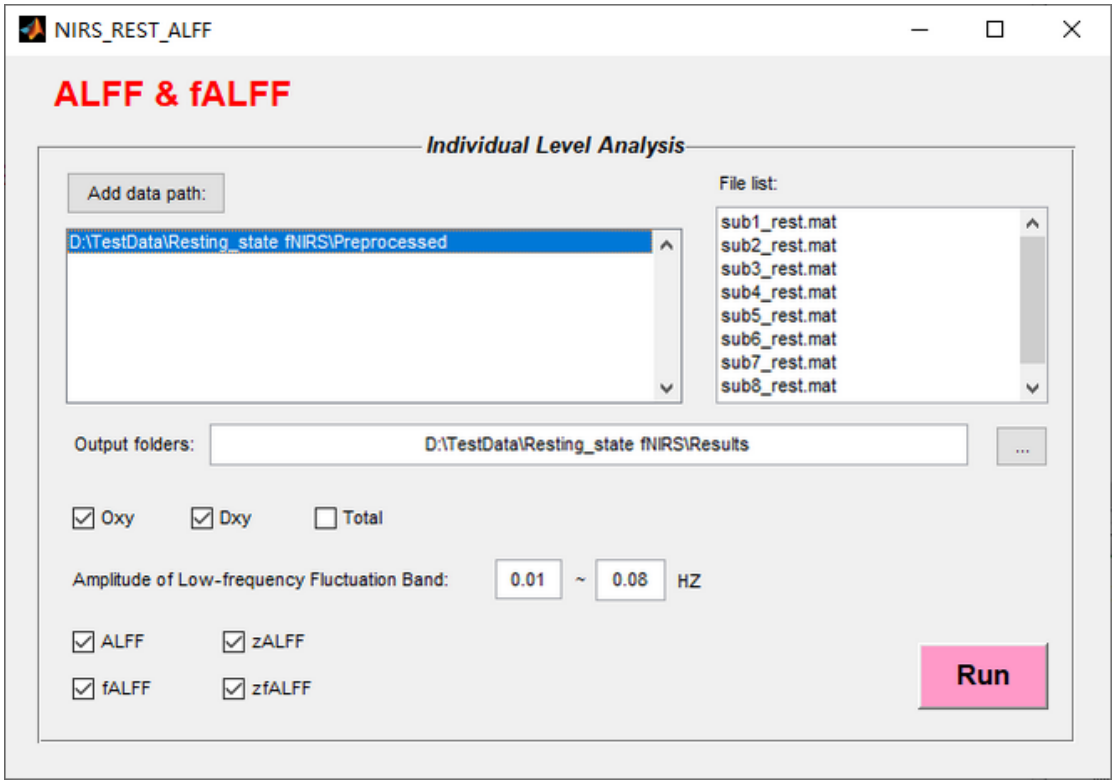


Fig. 5.4.3 Resting-state fNIRS ALFF & fALFF analysis.

5.5 Group-level statistics

Please refer to Sec. 4.5 to perform group-level statistics for resting-state fNIRS individual results.

Note: Because the output results format of the FC matrix is different from other individual resulted indexes, please choose the 'FC Matrix' checkbox, then select the corresponding statistical model to perform group-level FC matrix analysis (Fig. 5.5 right).

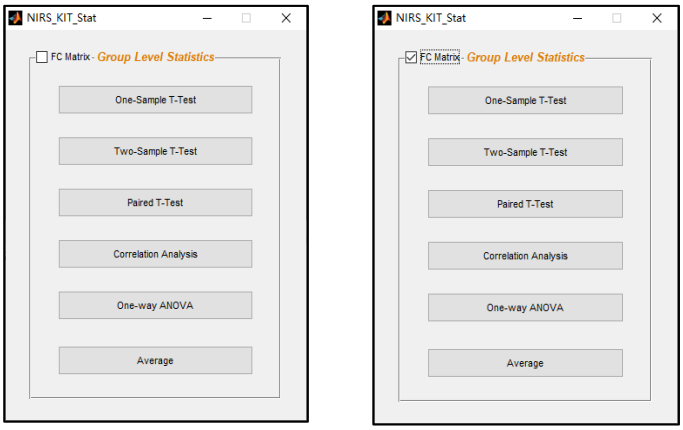


Fig. 5.5 Resting-state fNIRS Group-Level Statistics.

5.6 Results visualization

NIRS-KIT provides both 2D and 3D visualization functions to visualize resting-state fNIRS individual-level analysis indexes or group-level statistical results (channel-wise or FC matrix results, see Fig. 5.6).

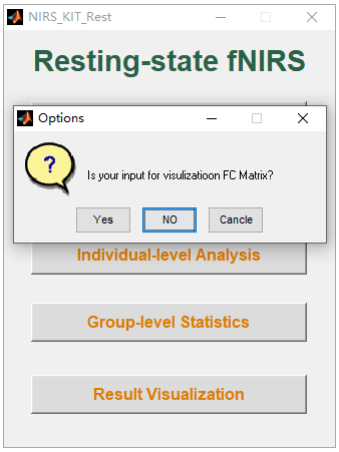


Fig. 5.6 Resting-state fNIRS Group-Level Statistics.

5.6.1 Channel-wise Visualization

Click the 'Result Visualization' button in the main interface of Resting-state fNIRS analysis, then an Options pop-up window appears (Fig. 5.6); Select 'No' to open an identical visualization interface (Fig. 5.6.0) with task-design fNIRS result visualization to visualize the

channel-wise resting-state fNIRS analysis results (such as ROI2WholeBrain and ALFF/fALFF results).

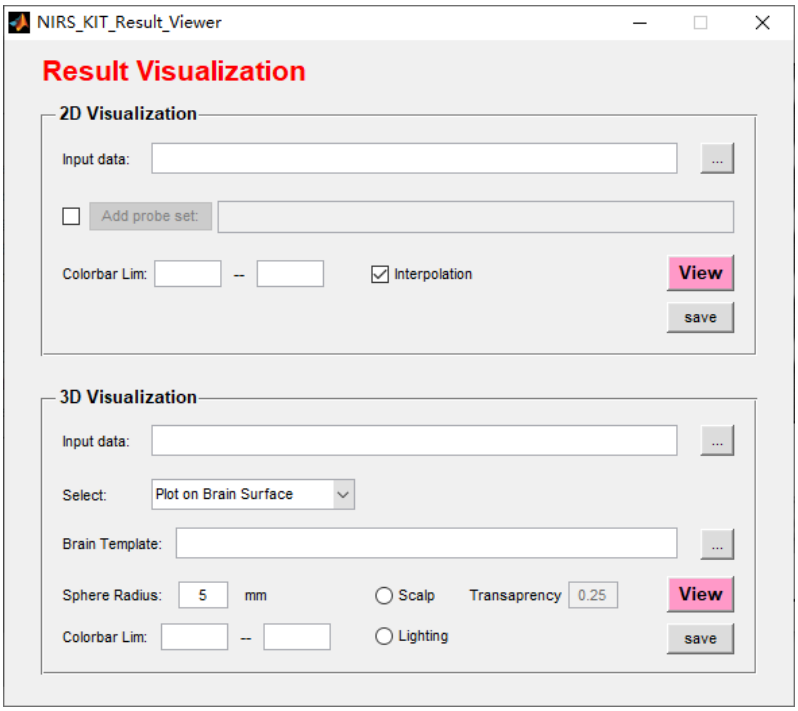


Fig. 5.6.0 The main interface of NIRS-KIT Result Visualization.

5.6.1.1 2D channel-wise visualization

1) Because there is only a single statistical value for the ROI2ROI functional connectivity result, it is not suitable for plotting 2D result mapping.
2) When performing ROI2Wholebrain functional result visualization, mapping the result without interpolation is suggested. And NIRS-KIT will automatically identify the ROI channel(s), and then circle them without filling color (for example, see Fig. 5.6.1.1).
3) ALFF/fALFF 2D result visualization please directly refer to Sec. 4.6.1.

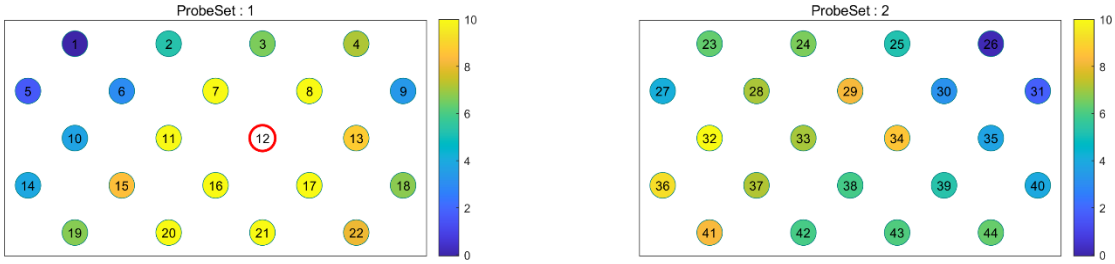


Fig. 5.6.1.1 The visualization of ROI2Wholebrain functional connectivity (ROI = channel 12).

5.6.1.2 3D channel-wise visualization

Please refer to Sec. 4.6.2.

5.6.2 FC Matrix Visualization

Click the 'Result Visualization' button in the main interface of Resting-state fNIRS analysis, then an Options pop-up window appears (Fig. 5.6); Select 'Yes' to open Fig. 5.6.2 to visualize the resting-state functional connectivity matrix results.

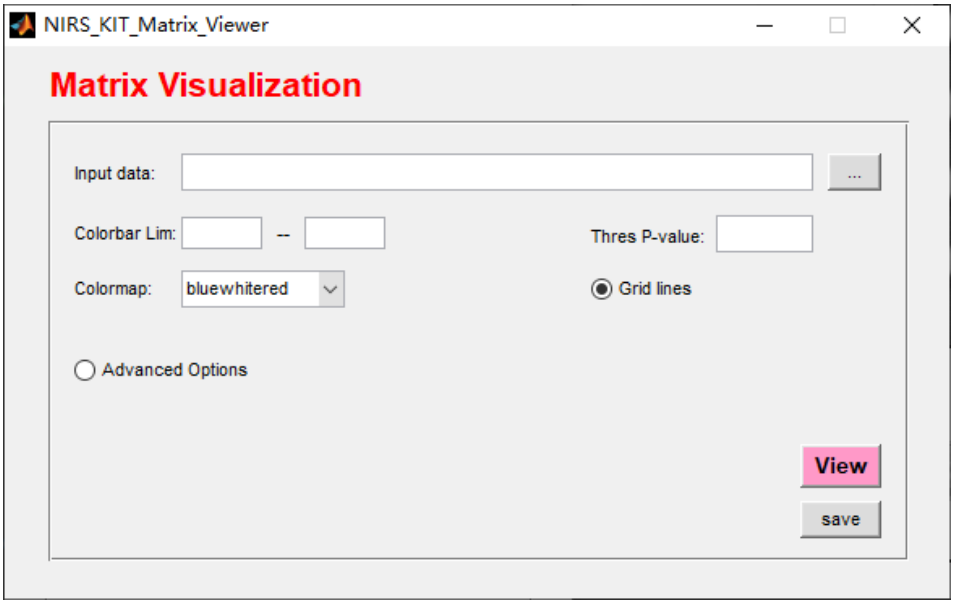


Fig. 5.6.2 The main interface of result visualization for functional connectivity matrix.

5.6.2.1 Basic Visualization for FC Matrix

- Click the button '…' to add the individual-level or group-level FC matrix result;
- Set the lower and upper limit if necessary to restrict the color scale of the data;
- Setup the threshold p-value (default: null), the statistic values with p > threshold p-value will be zeroed.
- Select *Colormap*.
- Select whether shown the grid lines or not.
- Click '*View*' to plot the FC matrix map, and then click '*Save*' to save the resulted map (Fig. 5.6.2.1).

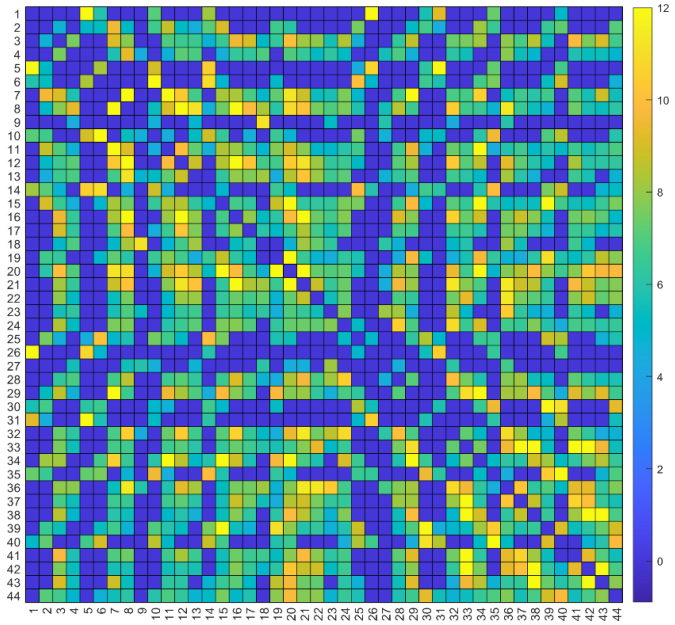


Fig. 5.6.2.1 The basic visualization of the functional connectivity matrix.

5.6.2.2 Advanced Visualization for FC Matrix

NIRS-KIT FC Matrix Visualization supports Advanced visualization functions for re-ordering or cutting the displayed matrix, plot sub-networks for FC matrix, and output the node and edge files for 3D visualization (with MNI information for each channel).

1) Re-order or cut the displayed matrix;
   - Select '*Advanced Options*' in Fig. 5.6.2, then 'Advanced Inf .xlsx:' appears (Fig. 5.6.2.2a);
   - Click the button '…' to add an Advanced Information file (*.xlsx, see Temp_FC_Matrix_Inf.xlsx in the sample folder, and Fig. 5.6.2.2b) with a new displayed channel ID in a single column. The channels not included in the xlsx-file will be cut.
   - Click 'View' to plot the new FC matrix map, and then click 'Save' to save the resulted map.

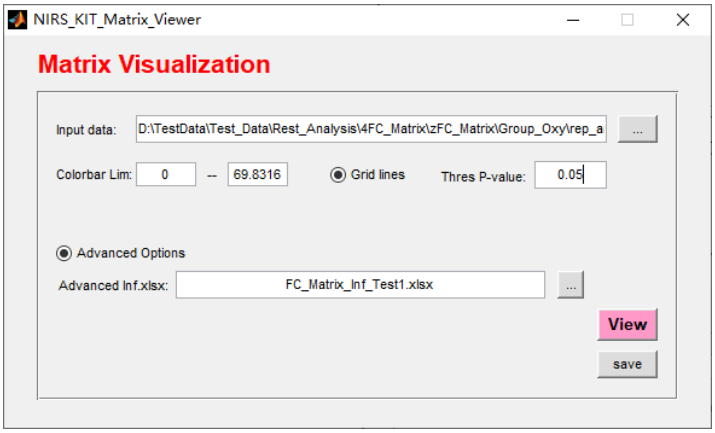


Fig. 5.6.2.2a The main interface of result visualization for FC matrix with re-order the displayed matrix channels.

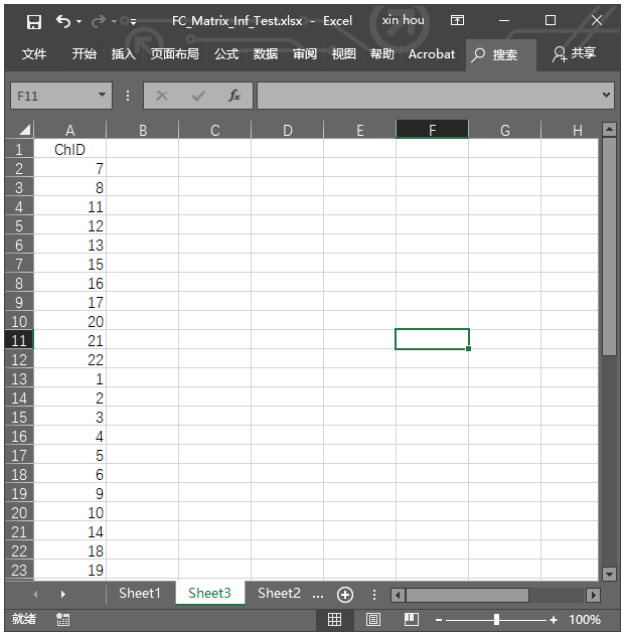


Fig. 5.6.2.2b The example excel for re-ordering or cutting the FC matrix.

2) Plot sub-networks for FC matrix;

- Click the button '…' in Fig. 5.6.2.2a to add an Advanced Information file (*.xlsx, see Fig. 5.6.2.2c) for plotting the FC matrix with sub networks. Six columns are needed in the inputted xlsx: Column1 = Channel ID, Column2 = Sub network ID, Column3 = Sub network name, Column4-6 = RGB color values. Then 'SubNet View Type' option appears (see Fig. 5.6.2.2d);
- Click 'View' to plot the new FC matrix map with sub-networks, and then click 'Save' to save the resulted map (see Fig. 5.6.2.2e).

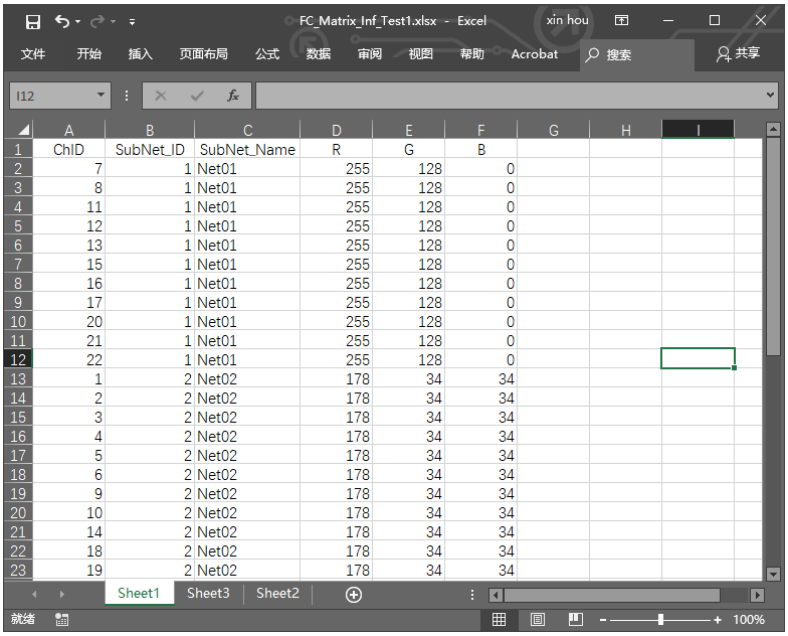


Fig. 5.6.2.2c The example excel for plotting the subnetworks for FC matrix.

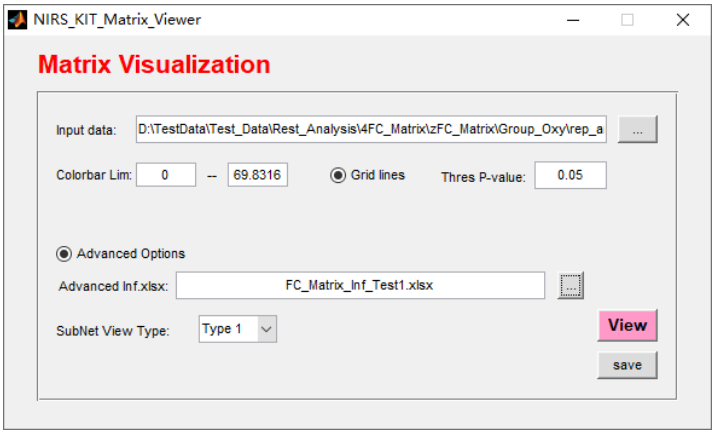


Fig. 5.6.2.2d The main interface of result visualization for FC matrix with re-order the displayed matrix channels.

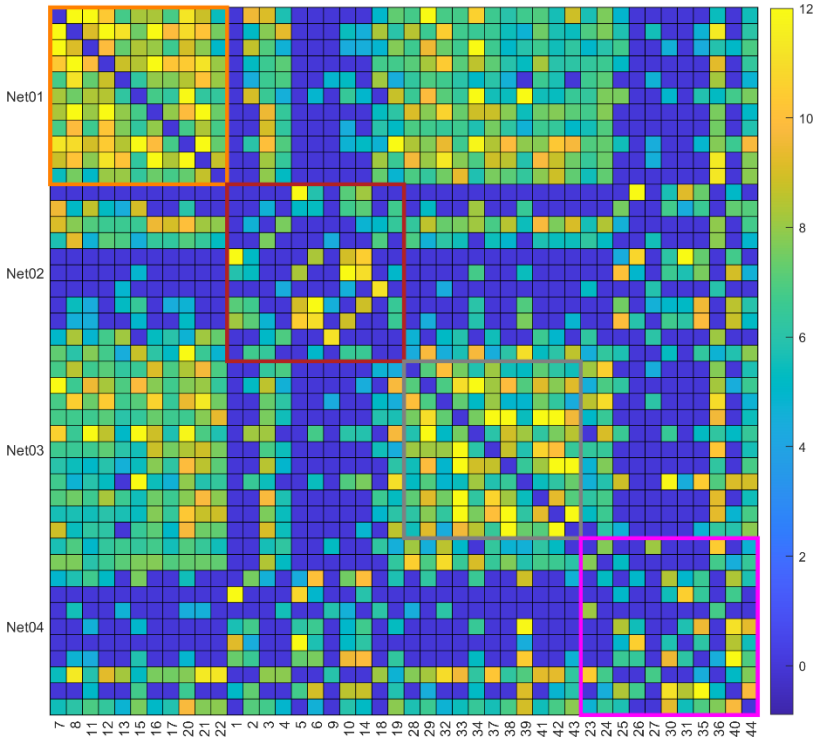
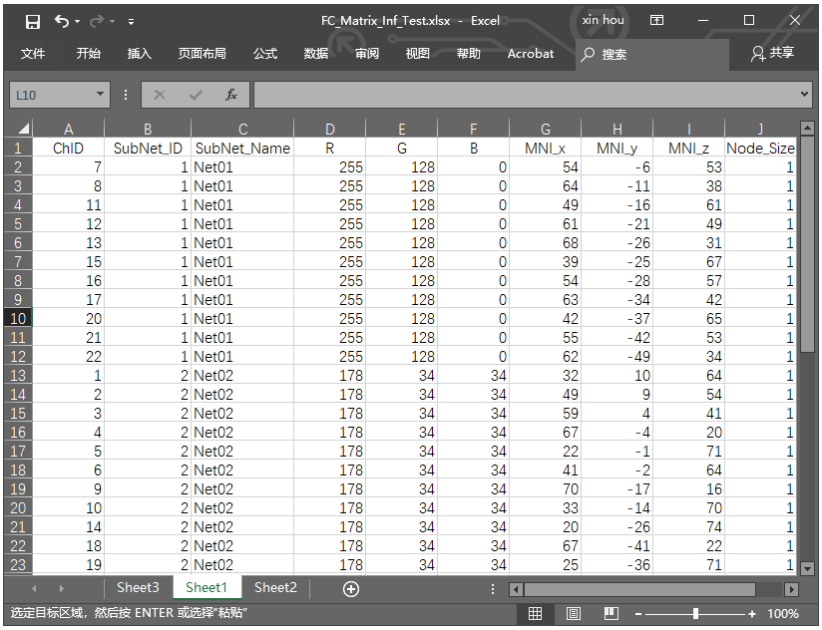


Fig. 5.6.2.2e The example excel for plotting the subnetworks for FC matrix.

3) Generate the network node and edge files

- Click the button '…' in Fig. 5.16 to add an Advanced Information file (*.xlsx, see Fig. 5.6.2.2f) with each channel's MNI coordinates for generating the network node and edge files. Columns 1 && 7-10 are necessary in the inputted xlsx: Column7-9 = MNI coordinates (X, Y, Z); Column10 = Node size.
- Then 'Generate Node & Edge files for 3D visualization' checkbox appears, check on (Fig. 5.6.2.2g);
- Click 'View' to plot the new FC matrix map, and then click 'Save' to save the resulted map and the node and edge files.
- Then load the node and edge files using an external brain network visualization

toolbox (such as BrainNet Viewer, Xia et al., 2013) to make the 3D brain connectome map (Fig. 5.6.2.2h).



FC_Matrix_Inf_Test.xlsx - Excel

ChID	SubNet_ID	SubNet_Name	R	G	B	MNI_x	MNI_y	MNI_z	Node_Size
7	1	Net01	255	128	0	54	-6	53	1
8	1	Net01	255	128	0	64	-11	38	1
11	1	Net01	255	128	0	49	-16	61	1
12	1	Net01	255	128	0	61	-21	49	1
13	1	Net01	255	128	0	68	-26	31	1
15	1	Net01	255	128	0	39	-25	67	1
16	1	Net01	255	128	0	54	-28	57	1
17	1	Net01	255	128	0	63	-34	42	1
20	1	Net01	255	128	0	42	-37	65	1
21	1	Net01	255	128	0	55	-42	53	1
22	1	Net01	255	128	0	62	-49	34	1
1	2	Net02	178	34	34	32	10	64	1
2	2	Net02	178	34	34	49	9	54	1
3	2	Net02	178	34	34	59	4	41	1
4	2	Net02	178	34	34	67	-4	20	1
5	2	Net02	178	34	34	22	-1	71	1
6	2	Net02	178	34	34	41	-2	64	1
9	2	Net02	178	34	34	70	-17	16	1
10	2	Net02	178	34	34	33	-14	70	1
14	2	Net02	178	34	34	20	-26	74	1
18	2	Net02	178	34	34	67	-41	22	1
19	2	Net02	178	34	34	25	-36	71	1

Fig. 5.6.2.2f The example excel for plotting the subnetworks for the FC matrix.

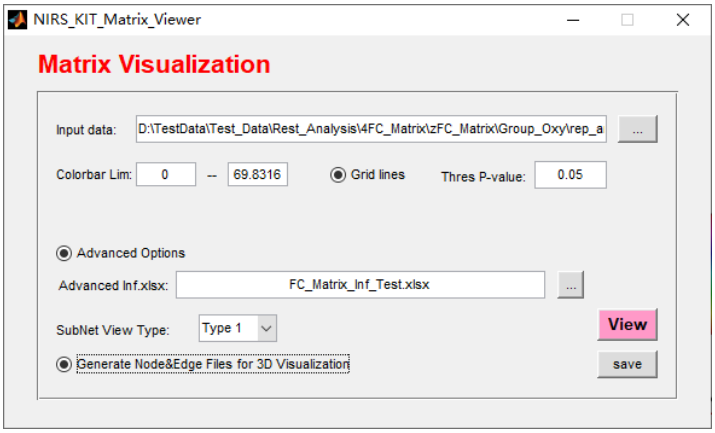


Fig. 5.6.2.2g The example excel for plotting the subnetworks for the FC matrix.

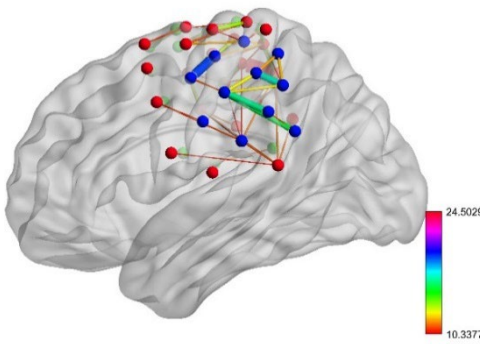


Fig. 5.6.2.2h The 3D visualization of FC Matric with BrainNet Viewer.

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