

CONN

Functional Connectivity SPM Toolbox 2017



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Overview

CONN is a Matlab-based cross-platform software for the computation, display, and analysis of functional connectivity in fMRI (fcMRI). Connectivity measures include seed-to-voxel connectivity maps, ROI-to-ROI connectivity matrices, graph properties of connectivity networks, generalized psychophysiological interaction models (gPPI), intrinsic connectivity, local correlation and other voxel-to-voxel measures, independent component analyses (ICA), and dynamic component analyses (dyn-ICA).

CONN is available for resting state data (rsfMRI) as well as task-related designs. It covers the entire pipeline from raw fMRI data to hypothesis testing, including spatial coregistration, ART-based scrubbing, aCompCor strategy for control of physiological and movement confounds, first-level connectivity estimation, and second-level random-effect analyses and hypothesis testing.

Installing the toolbox:

download and unzip `conn*.zip`, and add the resulting `./conn/` directory to the matlab path (in Matlab's *File-Set path*)

Requirements:

SPM8 or above

Matlab R2008b or above (no additional toolboxes required)

To start the toolbox:

On the Matlab command window, type : `conn`

(make sure your matlab path includes the path to the connectivity toolbox)

Updating to latest version:

On the CONN gui click on *Help->Update*

Note: a standalone version of CONN for linux64 systems (precompiled including both SPM and CONN) that does not require Matlab installed or a Matlab license can be downloaded from www.nitrc.org/projects/conn.

OS-specific notes: On Mac OS/X use “ctrl-click” instead of “right-click” to bring contextual menus in the CONN gui; If the default GUI display fonts are too small click on *Tools->GUI settings* (top-right corner) to change the GUI font size (your choice of font sizes will be kept across CONN sessions and across toolbox updates); When using VNC to connect remotely to Linux machines type in Matlab's command window *opengl software* right after starting Matlab if you experience VNC crashes when displaying 3d renderings or when printing;

General

In order to perform connectivity analyses using this toolbox you will need:

Functional data. Either resting-state or task designs can be analyzed.

Structural data. At least one anatomical volume for each subject (this is used mostly for plotting purposes but also to derive the gray/white/CSF masks used in the aCompCor confound removal method)

ROI definitions. A series of files defining seeds of interest. ROIs can be defined from mask images, text files defining a list of MNI positions, or multiple-label images. The toolbox also provides a series of default pre-defined regions of interest that will be loaded automatically. These include a series of seed areas useful for investigating resting state connectivity –regions characterizing DMN, dorsal attention network, executive control network, etc.-, as well as a complete brain parcellation including 91 cortical areas and 15 subcortical areas from the FSL Harvard-Oxford Atlas as well as 26 cerebellar areas from the AAL atlas. See the `conn/utils/otherrois/` folder for additional/optional ROI files, including Brodmann areas, large-voxel parcellations, etc.

tip: To try out the toolbox in the absence of any data, select *Help. Sample Data* in the CONN gui in order to automatically download and process the NYU test-retest dataset

The toolbox operation is divided in four sequential steps:

Setup: Defines basic experiment information, data locations, regions of interest (seeds), temporal covariates, and second-level models. Optionally, perform functional and anatomical preprocessing steps if necessary, including realignment, slice-timing correction, coregistration/normalization, segmentation, outlier identification, and smoothing.

Denoising: Define, explore, and remove possible confounds in the BOLD signal, including motion, physiological and other noise sources.

Analyses: Perform first-level analyses. Define the seeds of interest and explore the functional connectivity of different sources separately for each subject. Define ICA, voxel-to-voxel analyses, dynamic analyses, etc.

Results: Perform second-level analyses. Define group analyses and perform population-level inferences from the resulting connectivity measures of each first-level analysis.

Each of these steps can be defined interactively using the toolbox GUI or programmatically using scripts and `conn_batch` functionality. In addition, and both when using the GUI or batch processes, all of the analyses can be performed locally on a single computer, or they can be distributed among multiple computers in a cluster environment. Currently CONN supports Sun Grid Engine (SGE), Open Grid Scheduler (OGS/GE) and other Grid Engine implementations, PBS/Torque, LSF, and Slurm batch queuing systems. The following sections describe the experiment definition and analysis steps when using the GUI on a local computer in more detail. See *Help.Documentation.Info:batch processing* for additional information on batch scripts, or see *Help.Documentation.Info:grid computing* as well as *Tools.Grid settings* for additional information on cluster environments.

General help resources

In the CONN GUI select *Help.Search* to easily search through a database of user questions/answers, select *Help.Updates* to check and update CONN to the latest available release, select *Help.Documentation* for general documentation, and *Help.Web* for additional resources, including access to the CONN forum.

Step one: Setup (Defines experiment information, file sources for functional data, structural data, regions of interest, and other covariates)



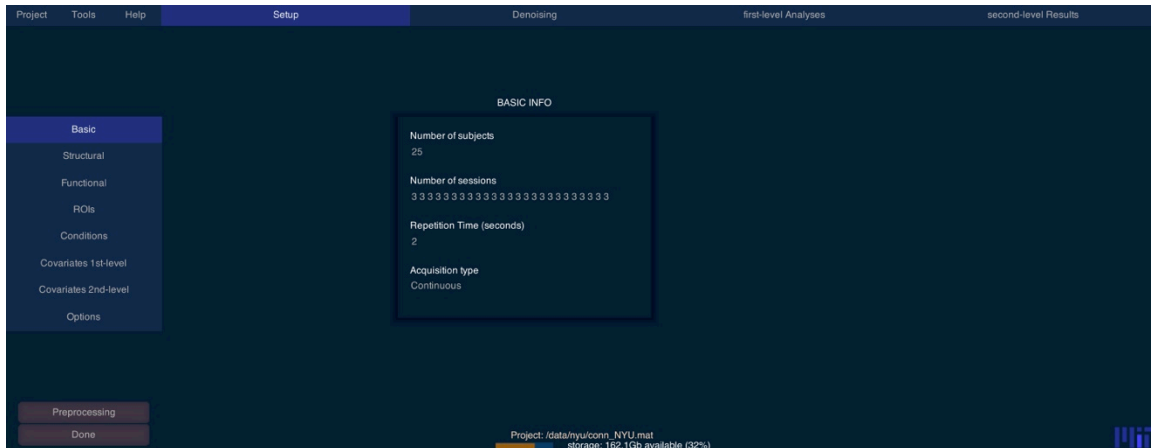
Click on the **SETUP** tab

Click on the **Project.New (blank)** button to start a new project.

Note 1: Alternatively, click on **Project.New (wizard)** to follow an initial simple step-by-step wizard that will allow you to import your functional, anatomical data, and optionally spatially preprocess your data using standard settings (segmentation, realignment, slice timing correction, coregistration, normalization, smoothing, and outlier detection/scrubbing; the wizard will offer a choice between two preprocessing pipelines: *defaultMNI* for analyses in MNI-space, and *defaultSS* for analyses in subject-space or surface-based analyses; see “Notes on Structural, Functional, and ROI files coregistration” section below). If using the wizard, this will also initialize some of the basic setup information that is described in the following sections (e.g. you can skip the **Basic**, **Functional** and **Structural** steps below, as well as some of the **Conditions**, and **First-level covariates** since those will be defined automatically by the spatial preprocessing steps).

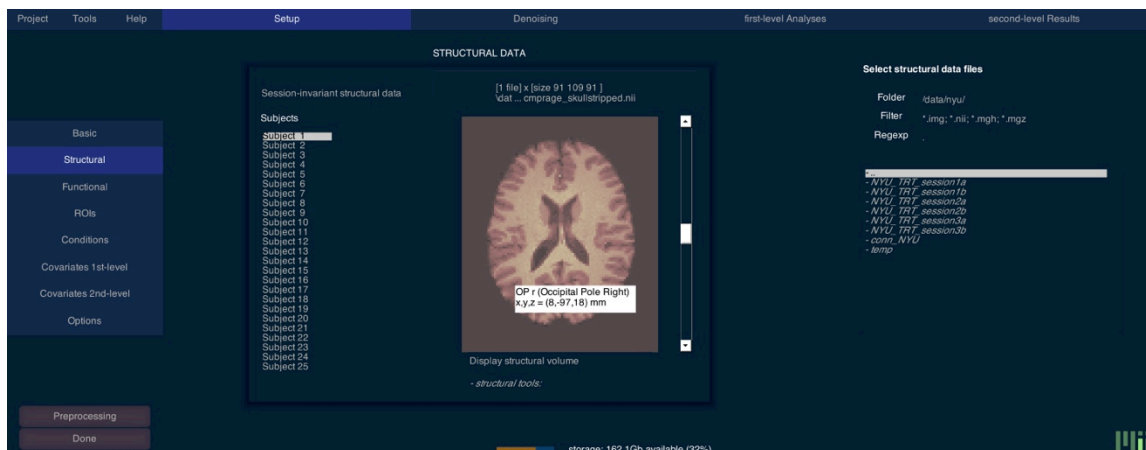
Note 2: If the data has initially been defined/analyzed in SPM you can similarly skip several of the steps below by using instead the **Import** functionality. Right after entering the number of subjects in the **Basic** setup, select **Project.Import** and specify one SPM.mat file for each subject. The program will extract the location of the functional data, the number of conditions per subject, the onset/length of the conditions of interest, and any specified first-level covariates from these SPM.mat files.

Basic experimental info Setup:



Click on the **Basic** button on the left side, enter experiment information (Number of subjects, TR, number of sessions per subject, and acquisition type). If the same number of sessions was acquired for every subject enter a single number in *Number of sessions*, otherwise enter the subject-specific values (one value per subject). If your data was acquired continuously select '*continuous*' in *Acquisition type*, and if you used sparse sampling select '*sparse*' (this will skip hrf-convolution when computing task effects)

Structural files Setup:



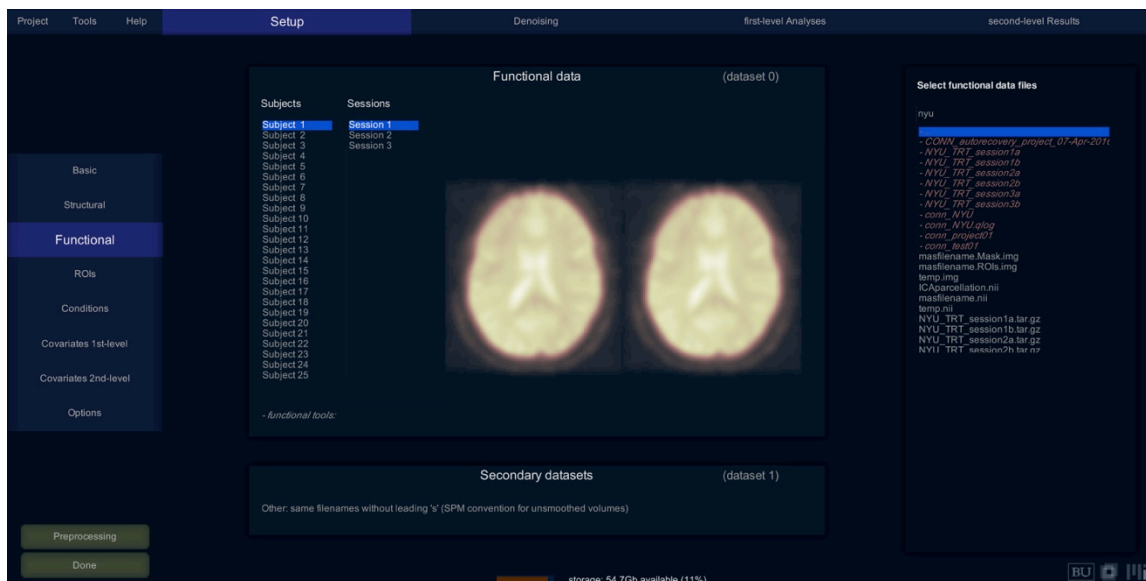
Click on the **Structural** button on the left side to load the structural images. Click sequentially on each subject and select the associated anatomical image (one anatomical volume per subject). If you have multiple anatomical volumes per subject (e.g. one per session/scan), select ‘*Session-specific structural data*’ and then enter the corresponding session-specific anatomical volumes. Anatomical volumes should typically be coregistered to the functional and ROI volumes for each subject (e.g. if using MNI-space normalized functional volumes you should enter here the normalized anatomical volume). Alternatively you may enter the raw (subject-space) anatomical volumes and transform them to your desired target space (e.g. MNI) using ‘*structural tools: individual preprocessing step*’ or ‘*Preprocessing*’ (see “Notes on Structural, Functional, and ROI files coregistration” section below).

GUI tip 1: The “Find” in the “Select functional data files” window can be used to search for all files within the target folder recursively. Change the “Filter” window to narrow the search.

GUI tip 2: If multiple subjects are selected in the “Subjects” list, and the number of files selected in the “Select functional data files” window matches the number of subjects, each subject is assigned one single file from the list

If your structural volumes have been preprocessed using Freesurfer (<https://surfer.nmr.mgh.harvard.edu/>), you may enter here the T1 or brainmask volumes in the subject-specific *mri* folder generated by Freesurfer. The toolbox will identify the associated *surf* folder containing the estimated cortical surfaces and it will display at this point a 3d-rendering of the pial surface (and this will allow you to obtain connectivity measures on the cortical surface; see *Setup.Options* below).

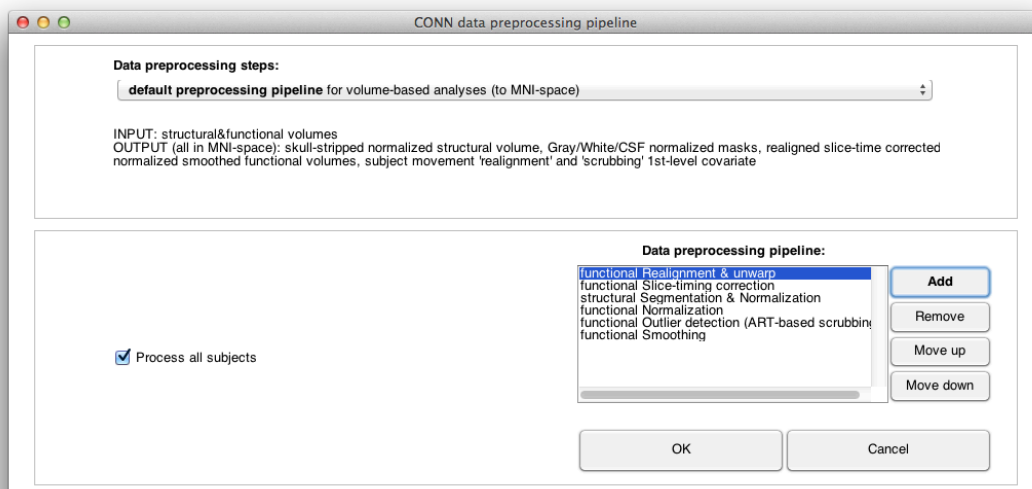
Functional files Setup:



Click on **Functional** button on the left side, from the right side panel, select the functional images (*img, or *nii, or 4d nii). This will take a second to load, check the middle panel (Functional data setup) to make sure the correct volumes are loaded. The brain display in the “Functional data setup” window shows the first (left) and last (right) scan for the selected subject/session (as in the figure above). The functional images are expected to be already pre-processed (realigned and smoothed), as well as coregistered with the structural and ROI volumes. If they are not, you may select ‘functional tools: individual preprocessing step’ or ‘Preprocessing’ to perform the appropriate preprocessing steps (see “Notes on Structural, Functional, and ROI files coregistration” section below). Clicking on ‘functional tools: check registration’ displays the coregistration between the functional and structural volumes for the selected subjects/sessions.

If you wish to use an alternative set of functional volumes specifically when extracting ROI-level BOLD signal estimates (e.g. extract ROI BOLD signals from unsmoothed functional volumes, in order to avoid “spillage” from nearby regions; or use subject-space volumes, in order to use subject-specific ROIs) you may indicate this in the ‘Secondary datasets’ menu. By default the toolbox will define an alternative ‘dataset 1’ pointing to the unsmoothed volumes (using the SPM-convention for unsmoothed volumes, same files without the initial ‘s’ in the filename) and this dataset will be the one used unless specified otherwise when extracting ROI-level BOLD signal timeseries (see *Setup.ROIs* section below).

Preprocessing functional & structural data:



After defining your raw Functional and Structural data, using *Setup.Preprocessing* you may run any combination of preprocessing steps (e.g. realignment, normalization, segmentation, outlier detection, etc.). Select individual spatial preprocessing steps or select one of the provided default pipelines (defaultMNI for analyses in MNI-space, and defaultSS for analyses in subject-space or surface-based analyses), add, remove, or resort individual steps if you wish to better tailor it to your data or planned analyses, and then click 'Ok' to run the entire sequence of spatial preprocessing steps on all of the defined subjects or on a subset of subjects. For reference, CONN's *defaultMNI* pipeline consists of the following steps: functional realignment and unwarping, slice-timing correction, structural segmentation&normalization, functional normalization, outlier detection, and smoothing. If your functional and structural data has been already preprocessed using SPM or other software you may simply skip all or any unnecessary preprocessing steps here.

Notes:

Typically, after selecting all structural, functional, and ROI files in the Setup step, and further applying any additional spatial preprocessing if necessary, all of the structural, functional, and ROI files should be coregistered to each other (in the same space; e.g. MNI-space)¹. This will happen, for example, when using CONN's initial spatial preprocessing pipeline (*defaultMNI*), which will result in both functional and structural files being in MNI-space (note that all of the ROIs provided in the toolbox `conn/rois` folder are also defined in MNI-space).

In some cases you might want to relax this condition and use structural/functional/ROI files that might not be all in the same space (e.g. using subject-specific ROI files, surface-based analyses, etc.). The toolbox uses separate ROI-level and voxel-level pipelines, and the tables below show typical configurations of Structural/Functional/ROI files that one would select depending on the desired analysis option within each

¹ although not necessarily having the same dimensions or resolution (i.e. coregistered, but not necessarily resliced to the same reference space)

² If you want to explore frequency-dependent variations in fMRI measures, but do not wish to specify a series of frequency filters manually, you may select *filter-bank* in the *Temporal filter* field, and enter there the desired number of frequency filters. This will partition the frequency band defined in the *Denoising* step into *n* equally-sized frequency regions, and it will automatically create one condition associated with each of these frequency bands. This allows you to use between-condition effects/contrasts as a way to analyzing potential frequency-dependent differences in any of your fMRI measures.

³ You may click on *Tools->Gui settings* to change the default atlas used when displaying labels associated

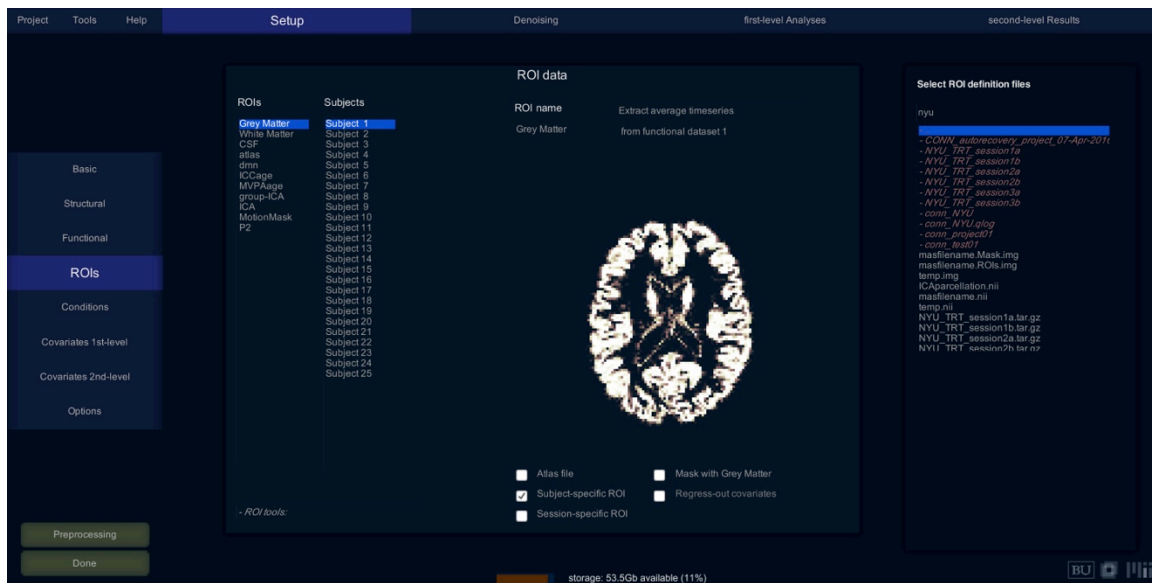
of these pipelines. For example, if you want to perform volume-based analyses on MNI-space for voxel-level analyses, but use subject-specific ROIs for ROI-level analyses, you will only need to have the functional files coregistered to the structural files (both normalized in MNI-space), and separately the functional files (ROI extraction option) coregistered to the ROI files (typically in subject-space).

<i>Voxel-level analyses</i>	<i>Volume-based analyses in MNI-space</i>	<i>Surface-based analyses</i>
<i>Functional files (dataset 0)</i>	Normalized and smoothed volumes (e.g. <i>swauFunct.nii</i>)	Subject-space non-smoothed volumes (e.g. <i>auFunct.nii</i>)
<i>Structural files</i>	Normalized structural (e.g. <i>wT1.nii</i>)	Subject-space structural (e.g. <i>mri/T1.mgh</i>)

<i>ROI-level analyses</i>	<i>MNI-space ROIs</i>	<i>Subject-specific ROIs</i>
<i>Functional files (dataset 1)</i>	Normalized non-smoothed volumes (e.g. <i>wauFunct.nii</i>)	Subject-space non-smoothed volumes (e.g. <i>auFunct.nii</i>)
<i>ROI files</i>	Normalized-space (e.g. <i>wc1_T1.img</i>)	Subject-space ROIs (e.g. <i>c1_T1.img</i>)

If your structural or functional files are not in the desired space or they have not been yet spatially preprocessed, you may (during the Setup step) apply any combination of structural segmentation, structural segmentation&normalization, functional removal of initial scans, functional realignment, functional realignment&unwarp, functional realignment&unwarp&B0correction, functional slice timing correction, functional coregistration to structural volumes, functional normalization, functional outlier detection (ART-based scrubbing), and functional smoothing steps to your structural/functional files using the *tools: individual preprocessing step* menu on each of the *Structural* and *Functional* tabs, or from *Setup.Preprocessing*:

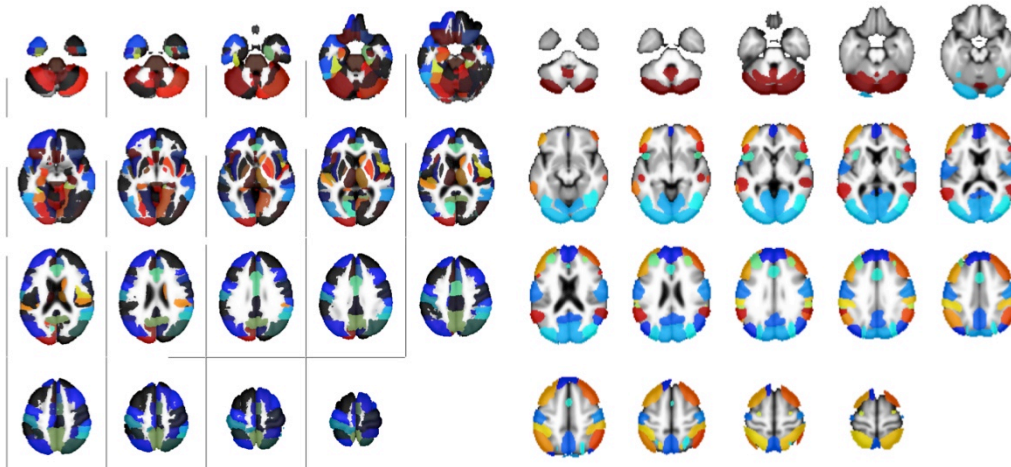
ROI files Setup:



Click on the **ROIs** button on the left side to load ROI mask files (.img or .nii volumes), MNI coordinate files (.tal text files), or atlas files (.nii files with multiple labels, or 4d files with multiple masks). ROI files can be assigned separately to each subject (for subject-specific ROIs) or commonly across all subjects (normalized-space ROIs). From each ROI, CONN may extract an average BOLD signal timeseries within the ROI voxels (*extract average timeseries* option), multiple timeseries from a Principal Component decomposition of the BOLD signal within ROI voxels (*extract PCA decomposition* option), or a weighted sum of the BOLD signal within ROI voxels (weighting each voxel timeseries by a different factor; *extract weighted sum timeseries* option). Also you may indicate which dataset, among potentially multiple alternative functional datasets, each ROI BOLD signal is to be computed from ('*from functional dataset #*' field).

Running the structural or functional segmentation *Preprocessing* step will automatically fill-in the first three ROIs in this tab (Gray/White/CSF) with the corresponding subject-specific masks. By default all new projects in CONN are set up to extract a Principal Component decomposition from both the White and CSF regions which can later be used during the *Denoising* step to implement aCompCor (removal of White/CSF noise components).

When starting a new project all files in the *rois* toolbox folder (./conn/rois) will be imported as initial regions of interest. This folder includes: 1) **atlas.nii/.txt/.info**, an atlas of cortical and subcortical areas from the FSL Harvard-Oxford Atlas, as well as cerebellar areas from the AAL atlas; and 2) **networks.nii/.txt/.info**, an atlas of a few commonly used networks and areas (e.g. Default Mode MPFC/PCC/RLP/LLP areas)



Default ROIs. atlas.nii (left, 132 ROIs) and networks.nii (right, 31 ROIs across 8 networks).

To import new ROIs, click below the last ROI listed and enter the appropriate information. To remove an existing ROI from this list right-click on an ROI and select “*remove*”. When importing subject-specific ROIs click sequentially on each subject and select the corresponding ROI file. When importing subject-independent ROI files, select all subjects simultaneously and then select the corresponding ROI file or simply uncheck the *Subject-specific ROI* option before selecting a single ROI file.

Select “*Subject-specific ROI*” and/or “*Session-specific ROI*” to specify whether you would like to enter different ROI files for each subject and/or each session.

Load the grey matter, white matter and CSF mask for each subject if they already exist (if left blank, the toolbox will generate these masks by performing segmentation on the structural image for each subject)

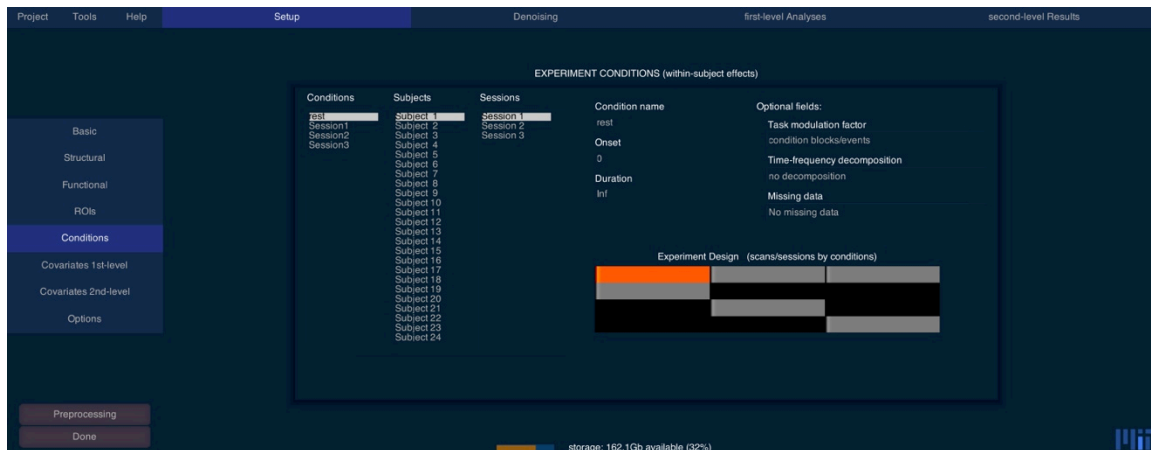
ROI files should be coregistered to the provided structural and functional volumes of this subject (they could be defined in normalized space, or they could be defined in subject-space). Click on *ROI tools: check registration* to display the coregistration between the selected ROI(s) and the structural or functional volumes (see “Notes on Structural, Functional, and ROI files coregistration” section below for additional options).

The default dimensions (number of PCA components to be extracted) for each ROI can be changed when selecting the *extract PCA decomposition* option. In the following steps (denoising, analyses), you can later select the number of components among the extracted ones you wish to use in the connectivity analyses. If one dimension is chosen for a ROI the time-series of interest is defined by the average BOLD activation within the ROI voxels. If more than one dimension is chosen for a ROI the time-series of interest are defined as the principal eigenvariates of the time-series within the ROI voxels (note: PCA decomposition is performed after removal of task-effects and first-level covariates when selecting the *regress-out covariates* option).

Select “*mask with grey matter*” on selected ROIs to further restrict these ROIs’ voxels to those voxels within the estimated grey matter mask for each subject.

Select “*multiple ROIs*” if the ROI file contains multiple labels (e.g. atlas file TD.img provided in *roi* folder), or multiple disconnected sets (e.g. functionally-defined ROI composed of multiple clusters, and you wish to use each cluster as a separate seed).

Rest or task conditions Setup:



Click on the **Conditions** button on the left side to enter onsets and durations (in seconds) of each experimental condition (blocks/events). Enter *0* and *inf* in the onset and duration fields respectively to indicate that the selected condition(s) encompass the entire scan time during the selected session(s). Leave empty (or enter [] –empty brackets-) in the onset and duration fields to indicate that the selected condition(s) was not present at all during the selected session(s). See the table below for a few examples of condition onset/duration specification:

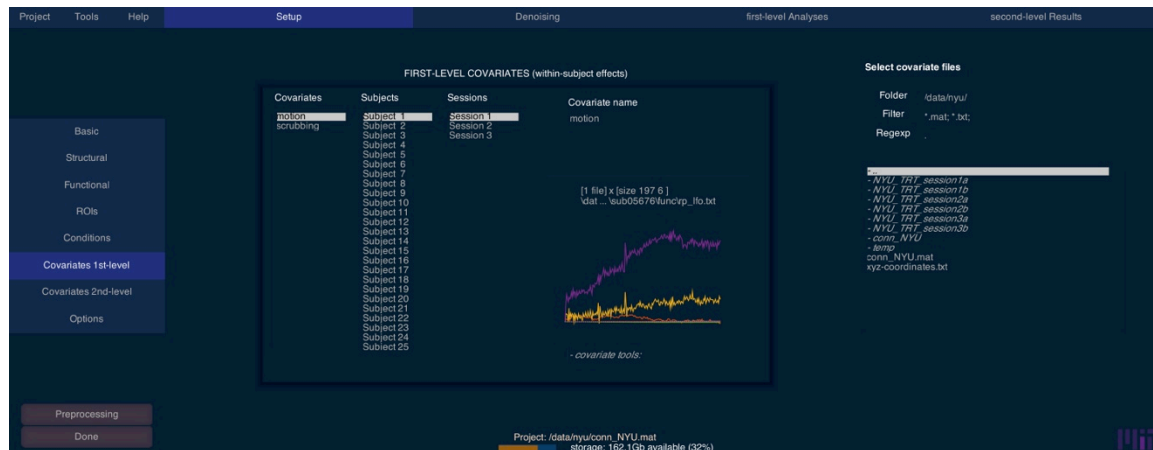
	Condition name	Session 1		Session 2	
		Onset	Duration	Onset	Duration
<i>Resting state, single condition</i>	rest	0	inf	0	inf
<i>Resting state, pre-post- design</i>	pre	0	inf	[]	[]
	post	[]	[]	0	inf
<i>Block design (30s blocks, 10s silence)</i>	taskA	0 80	30	40 120	30
	taskB	40 120	30	0 80	30
	rest	30 70 110 150	10	30 70 110 150	10

Condition design examples

Additional options: Right-click on the *conditions* list to delete or replicate a condition; select *condition tools* to enter all condition information simultaneously from a text file, or to copy or move a condition to the first-level covariates list (e.g. when you are not interested in obtaining task-specific measures of functional connectivity, but want instead to use Fair et al. method for estimating resting-state connectivity from block- or event-related designs). Optionally, you may also define a temporal modulation factor (in *condition-specific temporal modulation*; this defaults to a timeseries defined by hrf-convolution of the condition blocks/events) in order to perform gPPI analyses for task-related designs, or analyze potential temporal-modulation in fcMRI measures. You may also enter for each condition a condition-specific band-pass filter (in *condition-specific filter*) as a way to explore potential frequency- modulation of fcMRI measures².

² If you want to explore frequency-dependent variations in fcMRI measures, but do not wish to specify a series of frequency filters manually, you may select *filter-bank* in the *Temporal filter* field, and enter there the desired number of frequency filters. This will partition the frequency band defined in the *Denoising* step into n equally-sized frequency regions, and it will automatically create one condition associated with each of these frequency bands. This allows you to use between-condition effects/contrasts as a way to analyzing

First-level (within-subjects) covariates Setup:



Click on the **Covariates:First-level** button to define first level covariates such as realignment parameters to be used in the first-level BOLD model. For each covariate select a .txt or .mat file (for each subject and session) containing the covariate time series (e.g. select the realignment parameters file `rp_*.txt` to include the movement parameters as covariates).

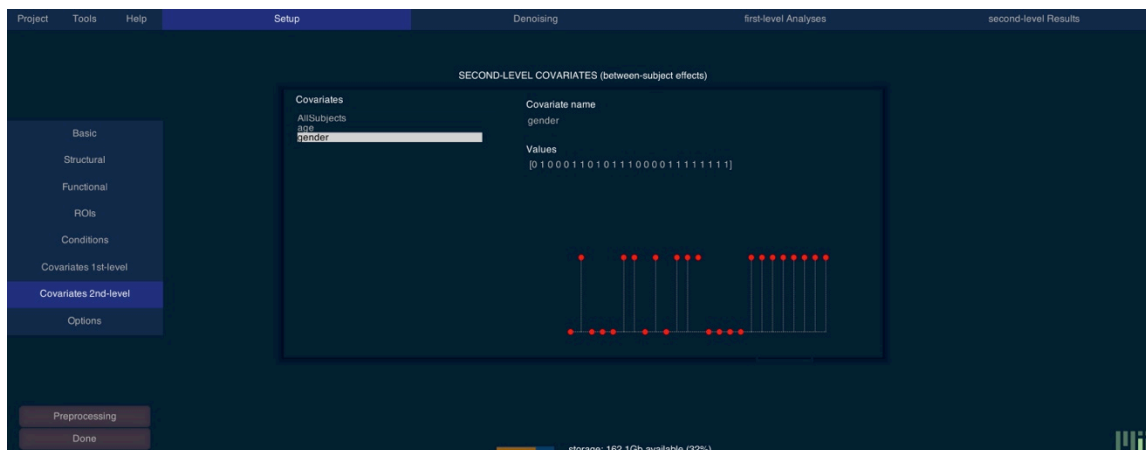
When importing *.txt* files they should contain as many rows of number as scans for any given subject/session, and an arbitrary number of columns (columns are separated by spaces). When importing *.mat* files they should contain a single variable (arbitrarily named) consisting of a matrix with as many rows as scans for any given subject/session and arbitrary number of columns.

If you want to obtain aggregated subject-level measures of some of your first-level covariates (e.g. to compute the maximum amount of movement for each subject, or the number of outlier scans for each subject) you may do so by selecting each first-level measure in the *covariates* list and clicking on *covariate tools: compute summary measures*.

Running some of the functional *Preprocessing* steps will automatically create or fill-in several first-level covariates in this tab. In particular, running the outlier scan identification *ART-based scrubbing* step will automatically create a first-level covariate named '**scrubbing**' containing the offending scans for each subject/session (this variable can later be used during the *Denoising* step to perform scrubbing), and running any of the *functional realignment* steps will automatically create a first-level covariate named '**realignment**' containing the 6 rigid-body parameters characterizing the estimated subject motion for each subject/session (this variable can also be later used during the *Denoising* step to perform regression of residual movement-related effects).

potential frequency-dependent differences in any of your fMRI measures.

Second-level (between-subjects) covariates Setup:



Click on the **Covariates:Second-level** button to define groups and subject-level regressors (e.g. behavioral measures). Click on the empty space below the last variable in the *Covariates* list to add a new variable. For each variable enter the corresponding subject values in the *values* field (one number per subject). Use 1/0 to define subject groups, or continuous values for between-subject regression models. Missing values may be indicated using the special symbol *NaN* (second-level analyses that contain a variable with missing values will automatically disregard the corresponding subjects' data). Some examples of second-level covariates definitions are below:

Name	Values	comment
<i>AllSubjects</i>	1 1 1 1 1 1 1 1 1 1	Used to identify the entire group of subjects
<i>Patients</i>	1 1 1 1 1 0 0 0 0 0	Used to identify a subset of subjects
<i>Controls</i>	0 0 0 0 0 1 1 1 1 1	
<i>Age</i>	23 13 19 25 90 66 12 83 74 72	Used for regression analyses or to control for covariates of no interest
<i>AgePatients</i>	23 13 19 25 90 0 0 0 0 0	Used for regression analyses on subset of subjects (or covariate by group interactions)
<i>Severity</i>	3 5 4 3 1 3 nan 2 1 nan	Nan's are used to indicate missing-values

Second-level covariates example

Tip: In addition to simple lists of numbers like the examples above, the *values* field will also accept any valid Matlab syntax (e.g. `[ones(1,5) zeros(1,5)]` or `(1:10)<=5` would produce the same values as the *Patients* example above). You may also refer to variables in Matlab workspace by name, or refer to other already-defined second-level covariates by name (e.g. in the example above the covariate *Controls* could also be defined simply entering in the values field *AllSubjects -Patients*).

By default the variable '*AllSubjects*' is always created when starting a new project. In addition, running some of the *Preprocessing* or *Denoising* steps will automatically create or fill-in several second-level covariates in this tab. In particular, running the outlier scan identification *ART-based scrubbing* step will automatically create three second-level covariates named '*QA_MaxMotion*', '*QA_ValidScans*', and '*QA_InvalidScans*' containing the maximum inter-scan movement (framewise displacement), number of valid scans, and number of invalid scans, respectively, observed for each subject/session, and running the *Denoising* step will automatically create a second-level covariate named '*QA_GCOR*' containing the

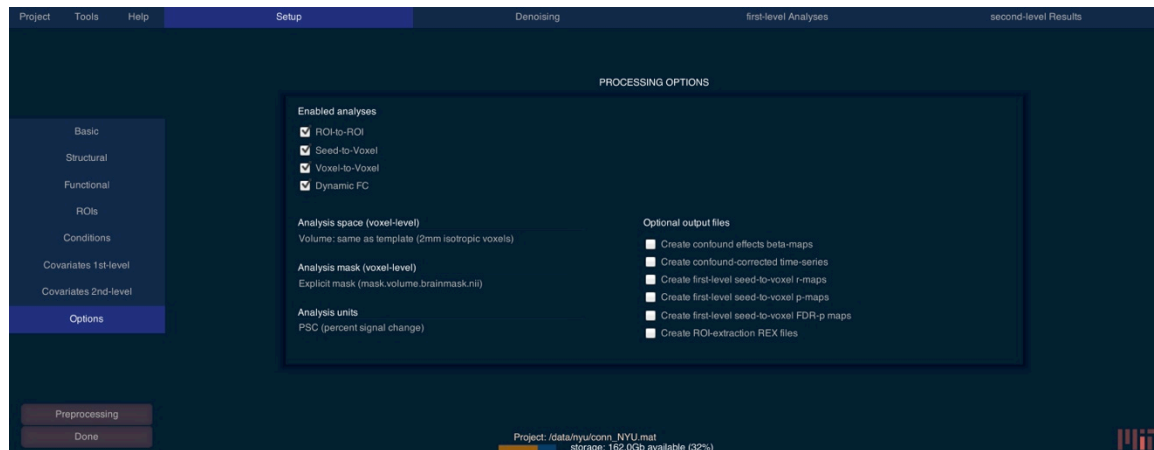
global correlation index (average correlation coefficient between every pair of voxels across the entire brain) for each subject/condition. Any of these variables can later be used during the *Second-level analyses* step for additional control over potential between-subject differences in motion/outliers, or to help identify and remove potential outlier subjects.

Second-level covariates can be defined at any time in the analyses (changes to any of other “Setup” options requires rerunning all the analyses steps, while changes to the second-level covariates do not). Right-click on the *covariates* list to delete a covariate, or select *orthogonalize covariate* to orthogonalize it against one or several of your other covariates (e.g. you may ‘center’ a between-subjects variable by orthogonalizing it against the constant –all subjects- term)

GUI tip: Use Tools->Calculator on the main CONN gui to visualize and analyze your current 2nd-level covariates (e.g. look at potential between-group differences in subject movement, levels of association between your different subject-level measures, etc.). The way to define 2nd-level analyses in the calculator is the same as that of the main CONN gui 2nd-level results view (see Step 4 below)



Options Setup:



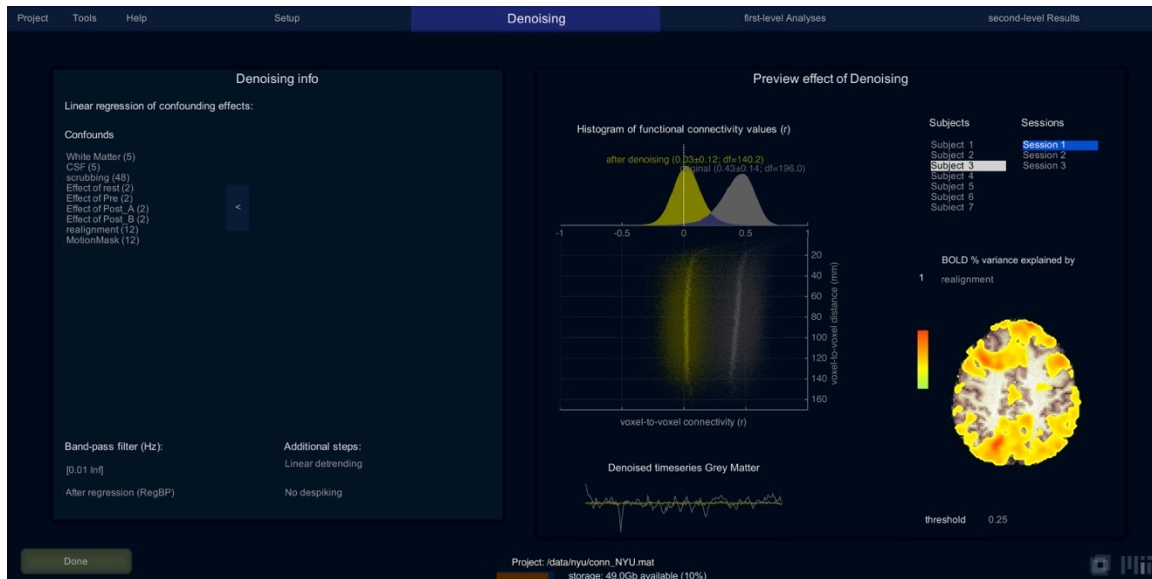
Click on the **Options** button for additional analysis options. Select the type of analyses that you wish to perform (ROI-to-ROI, seed-to-voxel, and voxel-to-voxel). For voxel-based analyses define the desired analysis space (by default volume-based analyses using isotropic 2mm voxels; you may indicate surface-based analyses only if you have selected Freesurfer generated structural volumes for all of your subjects) as well as the type of analysis mask to be used (by default a gray matter template mask).

Last select if you wish optional/additional output files to be created by the toolbox during the Setup and Denoising steps (these are files that are not necessary for any of the CONN-based analyses, but could be useful for example if you plan to export some of the toolbox intermediate results to other packages; e.g. confound-corrected BOLD timeseries, first-level seed-to-voxel r- or p-maps, etc.).

When finished defining the experiment data press **Done**. This will import the functional data for each session. If the gray/white/CSF ROIs have not been defined before, this step will perform segmentation of the structural data in order to define these masks (in this case, after this process is finished come back to Setup to inspect the resulting ROIs for possible inconsistencies). Last it will extract the voxel-level and ROI-level time-series for each session (performing PCA on the within-ROI activations when appropriate). A .mat file and a folder of the same name will be created for the project.

The **Save / Save as** button will save the setup configurations in a .mat file, which can be loaded later (**Load** button). Note that in addition to your manually saving your project, this .mat file will also be automatically saved each time the “*Done*” button is pressed on any of the Setup, Denoising, First-level analyses, or Second-level results tabs.

Step two: Denoising (Define, explore, and remove possible confounds)



Click on the **DENOISING** tab. This step applies linear regression and band-pass filtering in order to remove unwanted motion, physiological, and other artifactual effects from the BOLD signal before computing connectivity measures. By default the system will start with three different sources of possible confounders: 1) BOLD signal from the white matter and CSF masks (5 dimensions each); 2) any previously-defined within-subject covariate (realignment and scrubbing parameters); and 3) the *main* condition effects (condition blocks convolved with *hrf*). For each of the selected possible confounds you may change the number of dimensions (specifying how many temporal components are being used), and the derivatives order (specifying how many successive orders of temporal derivatives are included in the model). For example, the *realignment* confound (derived from the estimated subject motion parameters) is defined by default by 6 dimensions. You can change the *derivative order* to 1 indicating that in addition the first-order temporal derivative of the motion parameters should also be used as covariates. Similarly, the *White Matter* confound is defined by default by 5 dimensions and 0 derivative order (indicating that 5 PCA temporal components are being used, with not additional temporal derivative terms).

By default and unless specified otherwise all new projects that use the default CONN preprocessing steps will be automatically set up to use a combination of aCompCor (White and CSF ROIs, 5 components each), scrubbing (as many regressors as identified invalid scans), motion regression (12 regressors: 6 motion parameters + 6 first-order temporal derivatives), and filtering in the Denoising step. In addition for task designs the main effect of task (direct BOLD signal changes associated with the presence/absence of a task) are also regressed out during this step unless specified otherwise. Additional/alternative denoising steps can be specified by adding/substituting effects listed in the ‘confounds’ list. For example GSR is performed when introducing the GrayMatter ROI as an additional regressor; ICA denoising is performed when entering in the confounds list the ROIs associated with all noise components identified during ICA (see ICA analyses below, method (3) of ROI definition).

The “**Preview results**” window in the right panel shows the total variance explained (r-square) by each of the possible confounding sources (for the selected subject/session data). The histogram plot at the center displays the voxel-to-voxel connectivity (r values) before, and after confound removal. Typically confounds introduce a positive bias in connectivity measures so the histogram of original connectivity values can appear “shifted” to the right. After confound removal the distribution of connectivity values appears approximately centered. Clicking on the “**Show all**” button will compute and display these histograms for every subject for quality control and to easily identify potential outlier subjects. All of the settings in the *Denoising* tab can be modified and the associated changes in the observed histograms and spatial maps are estimated and displayed in real time. Images are displayed in neurological format,

hovering over the image will display voxel locations and reference areas³.

Enter the ***band-pass filter*** information in the bottom-left box (two numbers, in Hz, defining the band-pass frequency window of interest), as well as any additional desired denoising options (***detrending*** removes linear/quadratic/cubic trends within each functional session, and ***despiking*** applies a squashing function to reduce the influence of potential outlier scans).

When finished, press the **Done** button. This will filter the functional data and remove the effect of the defined confounds on all brain voxels and regions of interest, and create condition-specific timeseries (voxel- and ROI- level) by concatenating the samples/scans for each condition across all sessions.

³ You may click on *Tools->Gui settings* to change the default atlas used when displaying labels associated with each spatial location (*background reference atlas*), as well as the default background image used in these displays (*background anatomical image*)

Step three: first-level Analyses (Define and explore functional connectivity measures for each subject)

Seed-to-voxel and ROI-to-ROI analyses:



Click on the ‘**ROI-to-ROI**’ or ‘**Seed-to-voxel**’ tab to define source of interest (seed ROIs, for seed-to-voxel as well as ROI-to-ROI analyses).

Select from the left-most list the **sources** (seeds) of interest and click the right arrow to perform connectivity analyses using the selected sources as seeds.

For each seed/source you may optionally also indicate the number of *dimensions* to be analyzed (e.g. when extracting multiple components from a ROI), whether you want to include *derivative* terms, and the number of *frequency bands* if you wish to obtain for this particular ROI/seed a simple decomposition of the connectivity estimates power spectrum.

The right panel (“**Preview results**”) displays the connectivity measures for each subject/condition/source. Analyses here are performed in real-time (any changes in the “Define sources” definitions affect directly the results displayed in the “Preview” window). The measures displayed in the “Preview results” brain image correspond to the *connectivity measure* selected (*r* if correlation is selected, *beta* if regression is selected). The threshold value is also defined in the same units (in the figure above, voxels with correlation coefficients above 0.25 are shown/colored). For volume-based analyses images are displayed in neurological format (click on the image to see the voxel locations and Brodmann areas). For surface-based analyses images are displayed on the fsaverage brain.

In **Analysis type** you may select the type of first-level model to be used. The default behavior for functional connectivity analyses is to use a weighted General Linear Model for weighted regression/correlation measures of the condition-specific association between the seed/source BOLD timeseries and each voxel or target ROI BOLD timeseries. This model is appropriate both for resting state as well as task-related designs. When using weighted GLM click on **Weights** to change the relative weighting of the scans within each condition (by default condition-specific weights are defined from the hrf-convolved blocks/events for each condition). In addition to the standard bivariate correlation measure for functional connectivity analyses, you may also select regression measures, as well as define whether you want to compute bivariate measures –analyzing individual seed/sources separately-, or semipartial/multivariate –where all of the sources/ROIs are entered jointly into the general linear model to estimate their unique contributions-.

For task-related designs (or for temporal-modulation analyses) you may also select in **Analysis type** the

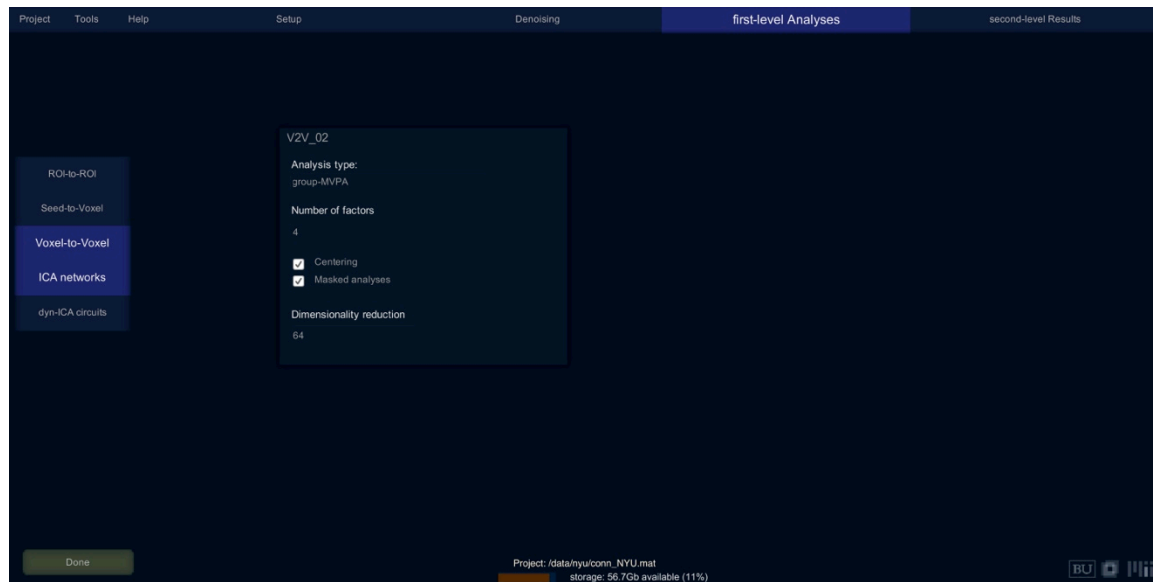
option to perform gPPI analyses (generalized PsychoPhysiological Interaction). PPI analyses compute the interaction between the seed/source BOLD timeseries and a chosen condition-specific interaction factor when predicting each voxel or target ROI BOLD timeseries. In addition, generalized PPI will include the interaction factors from all conditions simultaneously in the estimation model in order to better account for between-condition overlaps. When choosing *task-modulation effects (gPPI)* the interaction factor is defined (individually for each condition) in *Setup.Conditions.TaskModulationFactor* (this factor defaults to the standard hrf-convolved task effect). You will be prompted to select which conditions to be included simultaneously in the gPPI model. You may choose all **task** conditions, for example, to perform standard gPPI analyses, or you may select no conditions (or click Cancel) to perform standard PPI (single-condition) analyses. If instead you wish to use the same interaction factor for all of your conditions (e.g. PhysioPhysiological interaction model) you may choose instead *other temporal-modulation effects* and select when prompted the desired factor (e.g. select an ROI to perform standard physiophysiological interaction analyses)⁴. Note that, while the toolbox will allow you to use any of the correlation or regression measures in the context of gPPI analyses as well, we recommended to use gPPI in combination of regression measures only (either univariate or multivariate) in line with the most common implementation of this type of analyses.

Last, click on *Analyses* to define the type of analyses to be performed (ROI-to-ROI, seed-to-voxel, or both). When finished defining/exploring the connectivity analyses press **Done**. This will perform the defined analyses for all subjects, constructing seed-to-voxel connectivity maps for each selected source (as well as complete ROI-to-ROI connectivity matrices for these sources) for each subject and for each condition. First-level results (beta maps and correlation maps when appropriate) are also exported as nifti volumes (one per Subject/Condition/Source combination) in the *results/firstlevel* folder.

If you wish to perform a new set of first-level analyses without overwriting the existing one click on the *ANALYSIS_01* dropdown list and select *new* to define a new first-level analysis (or an existing one to edit/modify those first-level analyses)

⁴ Note that the PPI/gPPI models used by CONN follow the implementation in FSL, where the interaction factor is convolved with the hemodynamic response function and the linear interactions are modeled on the resulting BOLD-level signal; c.f. SPM-style implementation which deconvolves the BOLD timeseries instead and the interactions are modeled on the resulting "neural"-level signal. Also note that PPI effects (interaction terms in PPI model) are always relative to the baseline state (the baseline state is defined by the zero values of the interaction term), so they provide a *relative* measure of connectivity characterizing differential task-specific effects, rather than an absolute measure of connectivity such as that estimated using standard functional connectivity analyses (e.g. weighted correlation measures).

Voxel-to-voxel & ICA analyses:



Select the ‘**voxel-to-voxel**’ tab if you want to define additional voxel-to-voxel analyses

Voxel-to-voxel analyses are analyses that take into account the entire matrix of voxel-to-voxel connectivity values. These are useful when you do not want to restrict your analyses to one or several a priori seeds/ROIs and rather want to investigate possible connectivity differences across the entire brain.

The implemented analyses/measures are:

group-ICA (Independent Component Analyses): These analyses identify a number of networks of highly functionally-connected areas. CONN’s implementation uses Calhoun’s group-level ICA approach (Calhoun et al. 2001), with variance normalization pre-conditioning, optional subject-level dimensionality reduction, subject/condition concatenation of BOLD signal data along temporal dimension, group-level dimensionality reduction (to the target number of dimensions/components), fastICA for estimation of independent spatial components, and GICA1 backprojection for individual subject-level spatial map estimation.

Edit the *number of factors* field to modify the number of independent components estimated. Optionally edit the 1st-level *dimensionality reduction* field to specify the degree of subject-level dimensionality reduction desired (number of subject-specific SVD components retained when characterizing the voxel-to-voxel connectivity matrix for each subject/condition; set to *inf* for no dimensionality reduction). Optionally specify a mask file for masked-ICA analyses (in masked ICA the data is masked to include only within-mask BOLD signal timeseries in all analyses up to fastICA and the associated group-level spatial component estimation; the final backprojection step is still performed using the general/unmasked data in order to also be able to characterize the associations between the resulting networks/areas and the rest of the brain)

group-PCA (Principal Component Analyses): These analyses are identical to group-ICA above, but skipping the fastICA reorientation of group-level components and using instead the maximal-variance spatial components determined by the group-level dimensionality reduction step. All other steps, including backprojection, are performed identically to group-ICA.

group-MPVA (Multi-Voxel Pattern Analyses): These analyses create, separately for each voxel, a low-dimensional multivariate representation characterizing the connectivity pattern between this voxel and the rest of the brain (this representation is defined by performing separately for each voxel a Principal Component Analysis of the variability in connectivity patterns between this voxel and the rest of the brain across all subjects and conditions). The resulting representation optimally characterizes the observed

variability in connectivity patterns across subjects/conditions, and it allows you to investigate connectivity differences across subjects directly using second-level multivariate analyses.

Edit the *number of factors* field to modify the number of dimensions/components kept in this multivariate representation. Optionally edit the 1st-level *dimensionality reduction* field to modify the degree of dimensionality reduction used to characterize the voxel-to-voxel connectivity matrix for each subject (number of subject-specific SVD components retained when characterizing this matrix; set to *inf* for no dimensionality reduction). Optionally specify whether to use *centering* (by default average effects are removed before SVD step so that the dimensionality reduction step focus on the between-subjects/conditions covariance).

Intrinsic Connectivity (Intrinsic Connectivity Contrast, ICC, Martuzzi et al. 2011) is a measure of network centrality at each voxel. It characterizes the strength of the connectivity pattern between each voxel and the rest of the brain (root mean square of the correlation coefficient values).

Global Correlation is another measure of network centrality at each voxel. It characterizes the strength and sign of the connectivity pattern between each voxel and the rest of the brain (average of the correlation coefficient values).

Local Correlation (Integrated Local Correlation, ILC, Deshpande et al. 2007) is a measure of local coherence at each voxel. It characterizes the average correlation between each voxel and its neighbors. Neighbourhood is defined as a probabilistic region (isotropic Gaussian with user-defined width). Enter in the *Kernel Size* field the size of the local averaging window (Gaussian FWHM in mm).

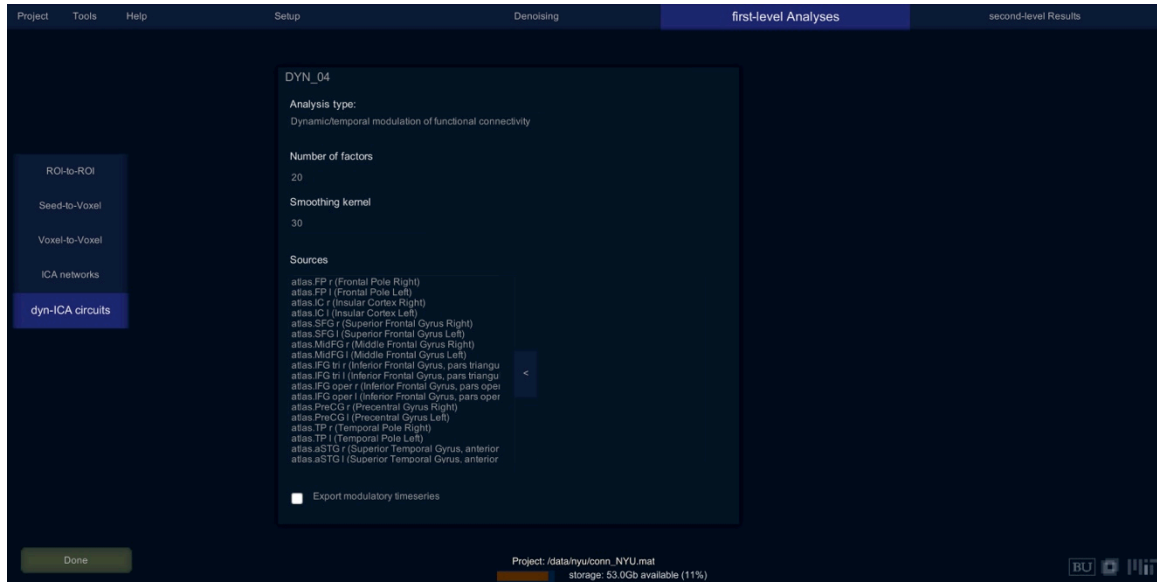
Radial Correlation (RCC, Goelman, 2004) characterizes the spatial asymmetry of the local connectivity pattern between each voxel and its neighbors (a 3d vector for each voxel). You may define in Kernel Size the size of the local asymmetry computation (Gaussian FWHM in mm).

Radial Similarity characterizes the global similarity (Kim et al. 2010) between the connectivity patterns of neighboring voxels (a 3d vector for each voxel).

For all of these measures, checking the *Normalization* checkbox will normalize the computed measures across all voxels to a $N(0,1)$ Gaussian distribution separately for each subject and condition. This is useful, for example, when computing centrality measures and wishing to control for potential network-cost differences between subjects or conditions.

When finished defining/exploring the connectivity analyses press **Done**. This will perform the defined analyses for all subjects, estimating the resulting maps from the voxel-to-voxel connectivity matrix for each subject and for each condition. First-level results (beta maps) are also exported as nifti volumes (one per Subject/Condition/Measure combination) in the *results/firstlevel* folder.

Dynamic ICA analyses:



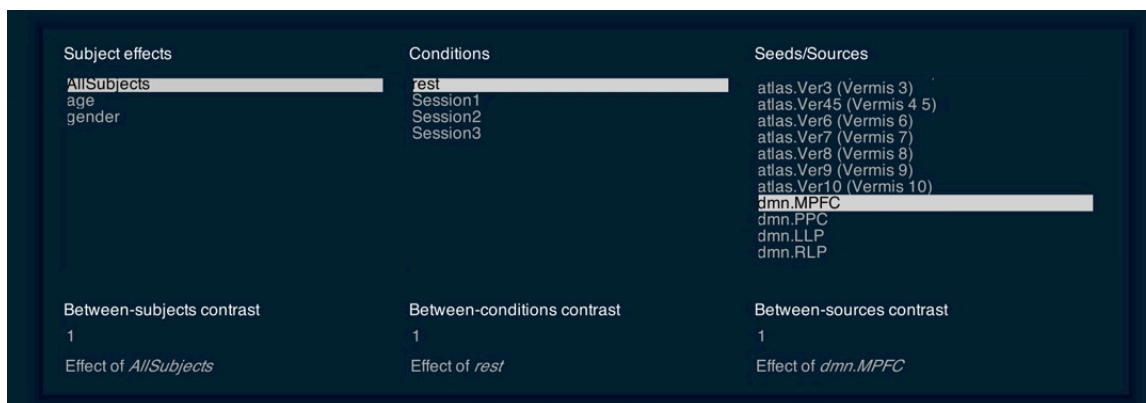
Select the **'Dynamic ICA'** tab if you want to define additional dynamic connectivity analyses.

Dynamic connectivity analyses (Nieto-Castanon 2016) explore dynamic properties (temporal modulation) of the ROI-to-ROI connectivity matrix identifying a number of circuits of similarly-modulated connections. Dyn-ICA performs an Independent Component Analysis of the connectivity timeseries (strength of connectivity between each pair of ROIs at any given timepoint), returning a number of independent components/circuits best characterizing the observed functional connectivity modulation across time.

Select the ROIs to be included in this analyses in the allROIs list, and click on '<' to enter these into the **Sources** list. Define the number of desired factors in *Number of factors*. Enter an optional low-pass filter threshold value (in seconds) in *Low-pass filter* in order to focus the estimated temporal modulation factors on the desired low-frequency range (similar to window-length in sliding-window approaches; set to 0s for no filtering). Optionally check *Export modulatory timeseries* if you wish to export the estimated connectivity modulation timeseries of each circuit as first-level covariates for additional analyses.

When finished defining the dynamic analyses press **Done**. This will estimate the dynamic-ICA components and create the selected measures for additional second-level analyses.

Step four: second-level Results (Define and explore contrasts of interest and second-level results)



At this point, second level analysis can be defined in the **RESULTS** tab.

A second-level model is defined by selecting one or multiple elements in the “**Subject effects**” list and specifying the desired “**between-subjects contrast**”. For example, if we have two groups defined (in the “covariates:second level” setup step) : patients, and controls, simply select both of them in the “Subject effects” list, and enter 1 -1 in the “Between-subject Contrast” window to compare the connectivity between the two groups (or enter 1 0 or 0 1 in the “Between-subject Contrast” window to look at the simple main effects showing the connectivity within each of the groups separately). After selecting one or multiple effects of interest in the **Subject effects** list right-clicking on the **Between-subjects contrast** field will show several default contrast vector/matrices (e.g. *patients > controls* in the example above).

Multiple conditions can be selected simultaneously in order to analyze connectivity results across several conditions by specifying the contrast in “**Between conditions contrast**” (e.g. use 1 -1 to compare connectivity during rest vs. task). After selecting one or multiple effects of interest in the **Conditions** list right-clicking on the **Between-conditions contrast** field will show several default contrast vector/matrices.

Similarly multiple ROIs/sources can be selected simultaneously in order to analyze connectivity results across several ROIs by specifying the contrast in “**Between source contrast**” (e.g. select both “PCC” and “Angular Gyrus” sources and enter a [1 -1] contrast to compare the connectivity between these two seeds, or enter [1,0; 0,1] contrast to investigate regions functionally connected to any of these two seeds). Again after selecting one or multiple effects of interest in the **Sources** list right-clicking on the **Between-sources contrast** field will show several default contrast vector/matrices.

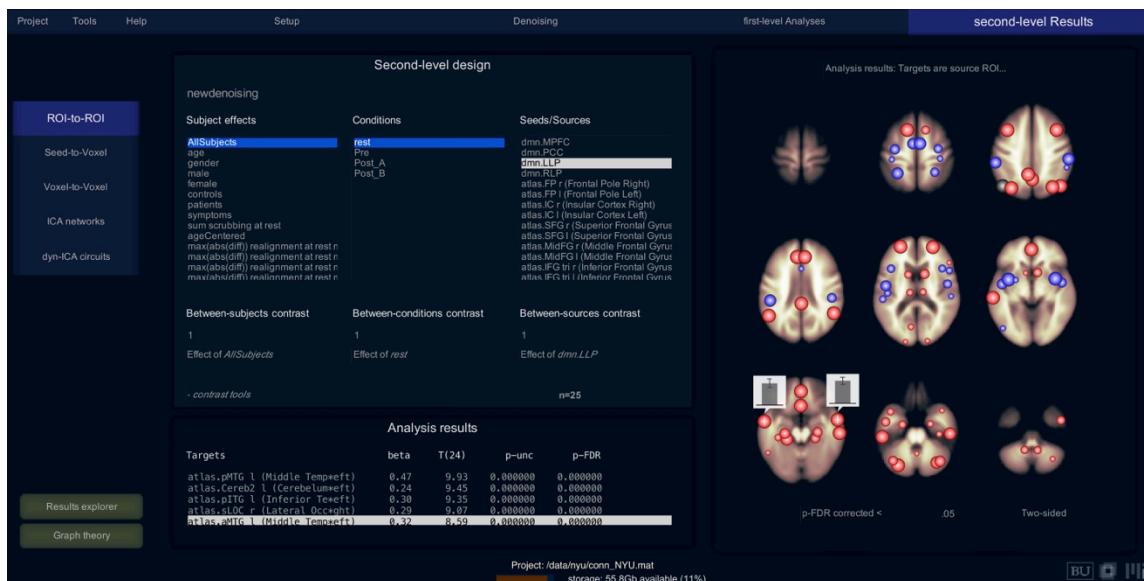
Each of these contrasts (between-subjects, between-conditions, and between-sources) can be defined as a single vector (e.g. [1,-1], for T-statistics) or as matrix (e.g. [1,0; 0,1], for F-statistics, where multiple contrasts are entered separated by ‘;’). In general, contrast matrices containing several rows are equivalent to an OR conjunction test on any of the individual row contrasts.

Both between-condition and between-source contrasts represent within-subject effects and the second-level analyses will correspond to multivariate/repeated-measures analyses of the selected effects. Specifically the second-level model will be a general linear model that includes as regressors the selected terms in the “**Subject effects**” list. The outcome variable will be the within-subjects linear combination(s) of effects specified by the “*between-conditions*” and “*between-sources*” contrasts, applied to the first-level connectivity-measure volumes (for the seed-to-voxel analyses) or to the first-level connectivity-measure matrix (for the ROI-to-ROI analyses). For F-contrasts, voxel-level analyses are implemented as repeated-measures analyses using ReML estimation of covariance components and evaluated through F-statistical parameter maps, and ROI-level analyses are implemented as multivariate analyses and evaluated through F- or Wilks lambda statistics depending on the dimensionality of the within- and between- subjects contrasts. The following table illustrates a few common second-level design examples:

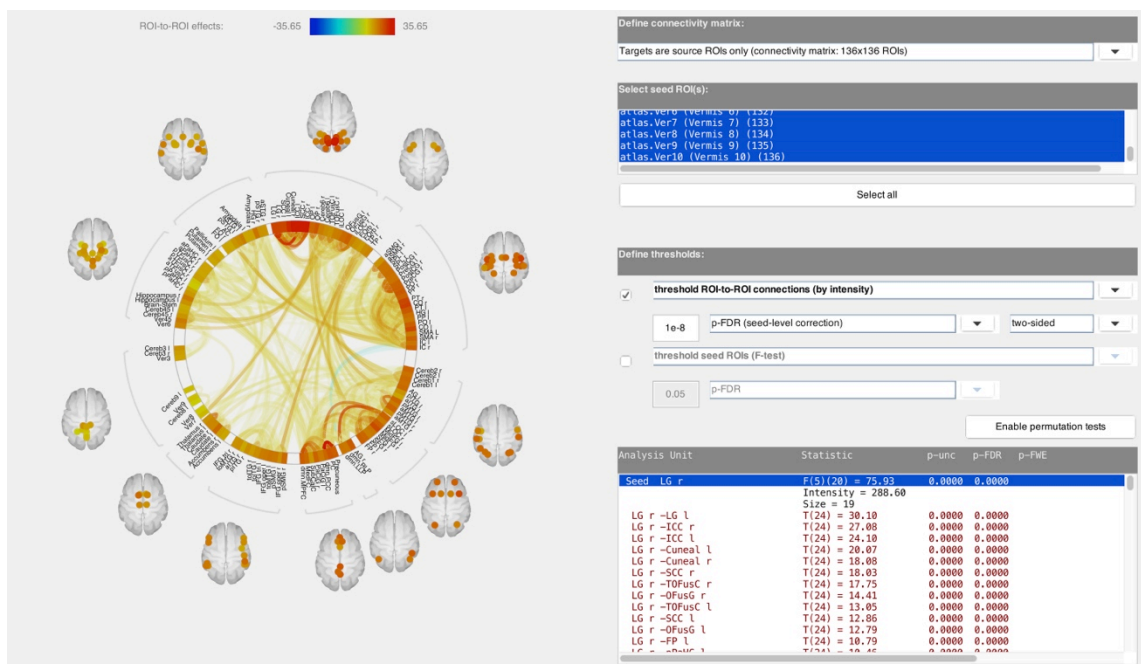
Analysis type	Subject-effects [Between-subjects contrast]	Conditions [Between-conditions contrast]
<i>one-sample t-test</i>	AllSubjects [1]	Rest [1]
<i>two-sample t-test</i>	Patients, Controls [1 -1]	Rest [1]
<i>paired t-test</i>	AllSubjects [1]	Pre, Post [-1 1]
<i>regression</i>	AllSubjects, behavioral [0 1]	Rest [1]
<i>multiple regression (individual/unique effect)</i>	AllSubjects, behav, age [0 1 0]	Rest [1]
<i>multiple regression (joint effect)</i>	AllSubjects, behav1, behav2 [0 1 0; 0 0 1]	Rest [1]
<i>2x2 between-subjects ANOVA interaction</i>	PatientsTreat, PatientsSham, ControlTreat, ControlSham [1 -1 -1 1]	Rest [1]
<i>2x2 within-subjects / repeated measures ANOVA interaction</i>	AllSubjects [1]	PreTask, PreRest, PostTask, PostRest [-1 1 1 -1]
<i>2x2 mixed ANOVA interaction</i>	Patients, Controls [1 -1]	Pre, Post [-1 1]
<i>3x2 mixed ANOVA interaction</i>	PatientsA, PatientsB, PatientsC [1 -1 0; 0 1 -1]	Pre, Post [-1 1]
<i>2x2x2 mixed ANOVA interaction</i>	PatientsTreat, PatientsSham, ControlTreat, ControlSham [1 -1 -1 1]	Pre, Post [-1 1]
<i>one-way ANCOVA covariate control</i>	Patients, Controls, age [1 -1 0]	Rest [1]
<i>one-way ANCOVA covariate interaction (comparing regression between groups)</i>	Patients, Controls, behavPatients, behavControls [0 0 1 -1]	Rest [1]
<i>General Linear Model data fit: $Y = X*B$ hyp test: $C*B*M' = 0$</i>	Columns of X (effects) [C]	Columns of Y (conditions) [M]

Second-level design examples

ROI-to-ROI analyses (ROI-to-ROI functional connectivity matrices):



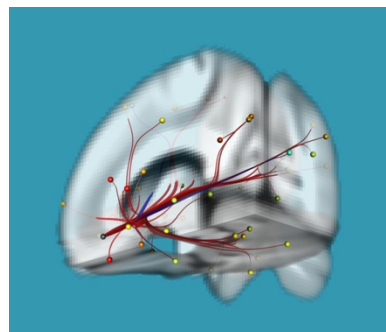
When selecting **ROI-to-ROI** analyses (from the leftmost tab in the figure), the brain display at the right shows an axial view of the roi-to-roi second-level analysis results estimated in real time for the selected second-level model and contrasts. These results can be thresholded at the desired **p-value** threshold, using uncorrected p-values or FDR-corrected p-values, and for one- or two-sided inferences. The dropdown menu above this figure allows you to modify the list of target ROIs being displayed (this choice affects the FDR-corrected p-values of the results; FDR correction is applied over the set of target ROIs chosen). Images are displayed in neurological format. Select **Display 3d** to view a 3d-rendered display of the supra-threshold ROI-level results. For each target ROI the list at the bottom of the figure displays the connectivity contrast effect sizes (between the selected source –or linear combination of sources- and each target), as well as T/F/X- values, uncorrected p-values, and FDR-corrected p- values for the specified second-level analysis. Right-clicking on this table allows you to export this table to a .txt, .csv, or .mat file. Select **Display Values** or **Import Values** to display/import the estimated ROI-to-ROI connectivity values for each subject and for each selected condition between the selected seed and target ROIs. Importing these values will create new second-level covariate containing these connectivity values for each subject for further analyses.



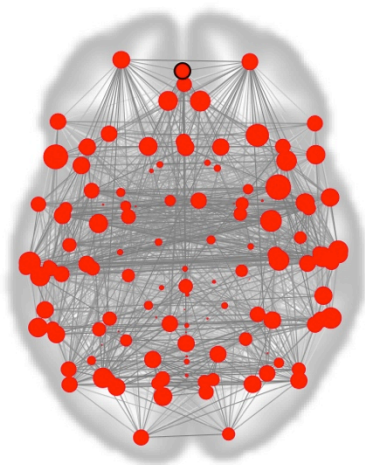
ROI-to-ROI results explorer window

In addition by clicking **results explorer** a graphical display of *all* of the ROI-to-ROI connectivity values (for the selected between-subjects and between-condition contrasts; note that the GUI between-sources contrast) is shown. The *define connectivity matrix* field allows you to define the specific ROIs to be included in these ROI-to-ROI connectivity matrix analyses. You can then select one or multiple seed ROIs in the *select seed ROIs* field, and the bottom-right table will display the ROI-to-ROI connectivity values between the selected source(s) and all of the other ROIs. Right-clicking on this table allows you to export the table or sort it by different criteria. The statistical results can be thresholded using a combination of a connection-level threshold (e.g. uncorrected or FDR-corrected p- values on the individual ROI-to-ROI connections) and a seed- or network- level thresholds (e.g. F-test on the multivariate connectivity strength for each seed ROI; or network-based statistics (NBS) on the number or strength of individual connections in each connected subnetwork of ROIs). In order to use any of the network-based statistic measures you need to first *enable permutation tests* (NBS measures are obtained using permutation tests which may take a few minutes to run, depending on the complexity of your between-subjects and between-conditions contrasts).

The top menu in this figure shows additional display options, including connectome, axial/coronal/sagittal, or 3d-rendered views of the results (note: the connectome view displays all of the ROIs on a circle, with individual suprathreshold connectivity lines between them; you may sort the ROIs on this ring using several criteria by selecting one of the *ROI display order* options)



ROI-to-ROI 3d-view figure



Network nodes: Network of all ROIs

Network edges (adjacency matrix threshold): 0.15 cost one-sided (po...)

Analysis measure: GlobalEfficiency

Analysis threshold (p-value): 0.05 FDR correc... two-sided

ROI	beta	T	dof	p-unc	p-FDR
network	0.47	73.84	24	0.000000	
IC r	0.56	90.10	24	0.000000	0.000000
IFG oper l	0.51	88.46	24	0.000000	0.000000
FOrb r	0.54	81.51	24	0.000000	0.000000
pMTG l	0.56	78.86	24	0.000000	0.000000
PreCG r	0.58	77.09	24	0.000000	0.000000
toMTG r	0.55	76.12	24	0.000000	0.000000
PostCG r	0.55	73.39	24	0.000000	0.000000
PaCiG r	0.54	73.17	24	0.000000	0.000000
TP r	0.62	73.15	24	0.000000	0.000000
PT r	0.55	71.91	24	0.000000	0.000000
sLOC l	0.57	71.90	24	0.000000	0.000000
pMTG r	0.57	70.89	24	0.000000	0.000000
aSMG l	0.48	70.52	24	0.000000	0.000000
PaCiG l	0.54	69.81	24	0.000000	0.000000
toMTG l	0.54	68.78	24	0.000000	0.000000
IFG oper r	0.50	68.49	24	0.000000	0.000000
PreCG l	0.50	67.48	24	0.000000	0.000000
PP r	0.56	66.93	24	0.000000	0.000000
Cuneal l	0.49	66.58	24	0.000000	0.000000

dmn.MPFC x,y,z = (-8,48,-4) mm

dmn.PCC x,y,z = (-6,-52,40) mm

dmn.LLP x,y,z = (-46,-70,36) mm

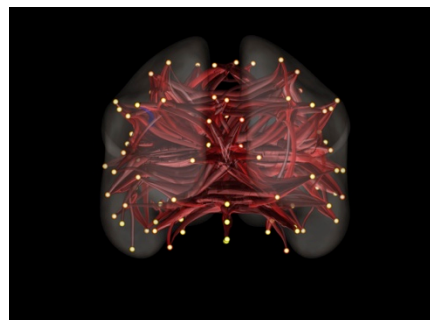
dmn.RLP x,y,z = (46,-70,36) mm

atlas.FP r (Frontal Pole Right) x,y,z = (26,52,8) mm

Export Adjacency matrices Export data

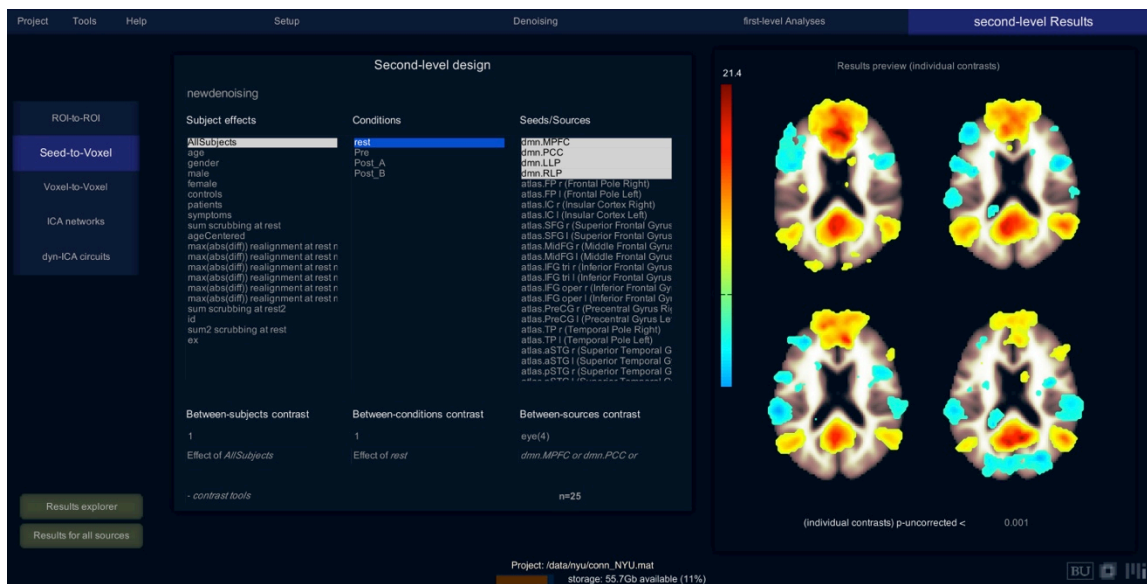
graph theory results explorer window

Last by clicking **graph-theory** a graphical display is shown allowing the users to test (for the selected between-subjects and between-condition contrasts) measures of efficiency, centrality, and cost/degree, associated with an ROI-to-ROI connectivity network. Click on 'Network nodes' to limit the ROI-to-ROI network analyzed to that defined by a subset of ROIs. The 'Network edges' option allows the definition of the connectivity threshold above which two ROIs are considered connected, and it can be defined based on correlation scores, z-scores, or cost values. For each ROI the list at the bottom of the figure displays the corresponding measure effect size (global efficiency, local efficiency, or cost), as well as T-values, uncorrected p-values, and FDR-corrected p-values for the specified second-level analysis. The graphical display and second-level results can be thresholded using either uncorrected or FDR-corrected p-values, and it can be set to display one-sided or two-sided results. Right-clicking on the brain display shows additional display options, including 3d-rendered views of the analyzed network of connectivity.

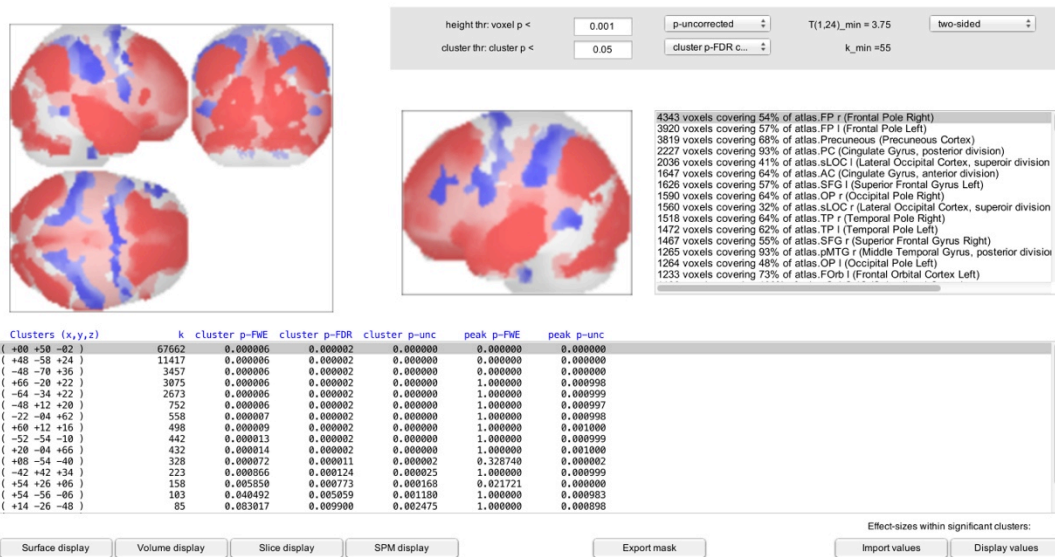


graph theory 3d-view figure

Seed-to-Voxel analyses (functional connectivity maps):



When selecting **Seed-to-Voxel** analyses (from the leftmost tab in the figure), the brain display at the right allows you to explore the second-level analysis results estimated in real time for the specified second-level model and contrasts. These results can be thresholded using a voxel-level uncorrected **p-value** threshold (e.g. $p < .001$ uncorrected in the gui image above; see below for additional thresholding options). For volume-based analyses images are displayed in neurological format, while for surface-based analyses images are displayed on the fsaverage surface.

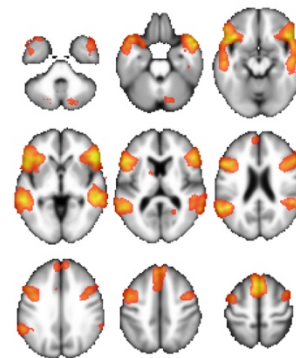


seed-to-voxel results explorer window

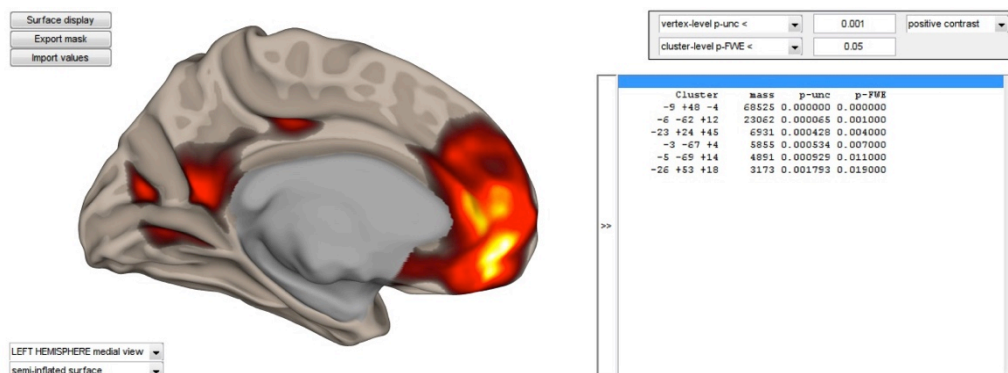
Clicking on **results explorer** launches a graphical display showing the whole-brain results of these second-level analyses using a MIP view of supra-threshold voxels, and allowing you additional thresholding and analysis options. This display can be thresholded by a combination of height (voxel-level) and extent (cluster-level) thresholds. Clusters are listed below together with their peak-voxel location (in mm), number of voxels (k), uncorrected, FWE-corrected (Guassian fields theory, Friston et al. 94), and FDR-corrected cluster-level p-values, and uncorrected and FDR-corrected peak-level p-values (topological FDR,

Chumbley et al., peak voxel within each cluster). Statistics are one-sided, select **negative contrast** in the dropdown menu at the top right to view the results for the reverse directionality of the contrast, or **two-sided** to perform two-sided analyses. Select **non-parametric** if you wish to perform these same analyses using instead non-parametric permutation/randomization statistics: **parametric statistics** uses parametric distributions (Random Field Theory) for cluster-level statistics, assumes similar between-conditions or between-sources covariance across voxels (equal up to scaling factor; estimated using SPM's ReML) and homogeneous spatial covariance across voxels (equal up to scaling factor; estimated using SPM's residual smoothness estimation); **non-parametric statistics** uses non-parametric analyses (residual permutation/randomization tests) for cluster level statistics, as well as multivariate between-conditions or between-sources covariance estimation for voxel-level statistics (multivariate analyses separately for each voxel).

From this graphical display you can also export the results as a text file containing the supra-threshold clusters and their statistics by right-clicking on the stats table, or as a .nii mask file defined by the results supra-threshold voxels (**export mask** button). These mask files can be re-entered directly in the toolbox as ROI files (e.g. for post hoc analyses using suprathreshold clusters as seeds/ROIs). In addition you may click on **explore clusters** to look at the effect sizes within each cluster, or **import values** to directly import the average connectivity values for each subject within each supra-threshold cluster as a new second-level covariate into the CONN toolbox. Last, the **surface display**, **volume display**, and **slice display** options allow you to access different 3d-rendered views of the results, and the **SPM display** option allows you to load these results in the standard SPM results viewer.

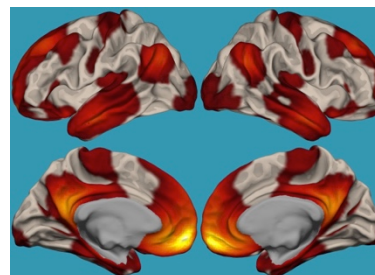


results explorer slice-display figure



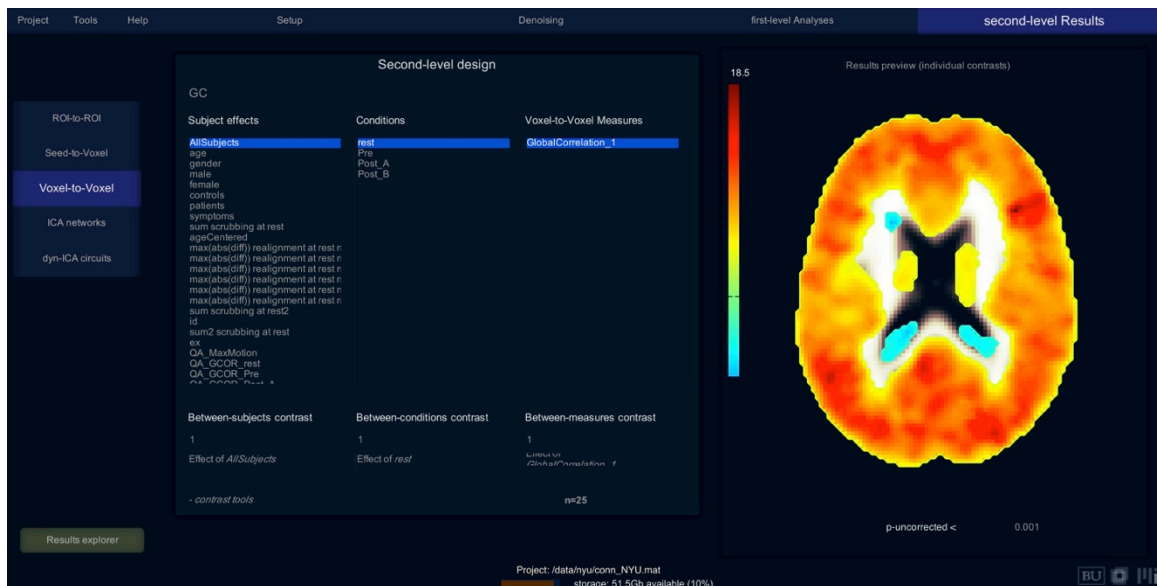
seed-to-voxel results explorer window (for surface-based analyses)

When performing surface-based analyses, **results explorer** will launch a similar graphical display showing the results of these second-level analyses on the fsaverage brain. The gui allows you to threshold the results using a combination of a vertex-level “height” threshold (e.g. uncorrected p-values) and cluster-level “extent” threshold (e.g. FWE-corrected cluster extent). Cluster-level statistics for surface-based analyses are obtained using non-parametric statistics (permutation/randomization tests) which may take from a few minutes to run, for simple T-contrast, to a few hours, for more complex F- or Wilks’ lambda statistics. If permutation tests have not been run for the chosen vertex-level height threshold, the button **compute cluster-level statistics** will allow you to run them now and obtain cluster-level p-uncorrected and p-FWE measures. The **Surface display** button allows you to access the default 3d-rendering display and options for these results. Use **export mask** to export the resulting supra-threshold mask to a .nii file, or **import values** to import the average connectivity values for each subject within each of the supra-threshold clusters as new second-level covariate into the CONN toolbox.

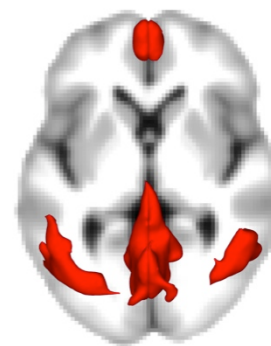


results explorer surface-display figure

Voxel-to-Voxel analyses (MVPA and other connectome-level measures):



When selecting **Voxel-to-Voxel** analyses (from the leftmost tab in the main CONN gui), the gui options are the same as in the seed-to-voxel analyses above (but instead of multiple sources, users may now select across perhaps multiple measures; e.g. select all of the connectome-MVPA measures in the “Measures” list to perform a second-level multivariate analysis of these measures). As before, results can be thresholded using a voxel-level uncorrected **p-value** threshold, and further explored selecting **results explorer**. All the display and analysis options available for seed-to-voxel maps and described in the *seed-to-voxel analysis* section above, such as using a combination of height- and extent- thresholds, non-parametric analyses, etc. are equally available for voxel-to-voxel measures, both for univariate measures such as Local Correlation and Intrinsic Connectivity, as well as for multivariate measures such as MVPA, RCC, etc.

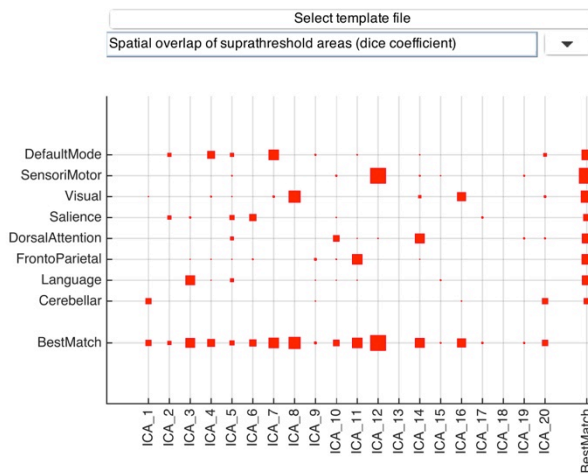


results explorer 3d-view figure

ICA analyses:



Select **ICA.Summary** to explore the estimated ICA networks/components. The top-left table lists several properties of each network (spatial variability/kurtosis/skewness, and temporal variability/frequency). The top-right display shows the group-level maps (*ICA spatial components*) for the selected networks/components. These maps may be thresholded based on a z-score threshold for display purposes (factor loadings are centered and normalized across all voxels to compute z-scores). The bottom display shows the BOLD signal timeseries associated with each network (*ICA temporal components*) for the selected subject/condition. By default ICA components are sorted by their relative overlap with gray matter areas (last networks/components may be loading more heavily on white or CSF areas, which are often identified as noise components)



networks match-to-template summary display

In addition selecting **ICA tools. Spatial match to template** computes the correlation between each group-level spatial map and a CONN's default networks file or a user-defined mask file, which can be used to identify networks of interest. In addition, hovering over the top-right plot displays the factor loadings at each voxel, as well as the networks/components with the highest loadings at each voxel, which is another

common way to identify networks of interest. The top-right display can also be selected to show the associated *ICA parcellation* maps. Parcellations are computed by finding, among the selected networks/components, the one that shows the highest factor loading at each voxel, and thresholding the resulting maps using the chosen z-score threshold. Parcellations may be computed across all networks/components or only across a subset of the estimated networks simply by selecting the desired subset of networks in the top-left table. The resulting parcellations can also be exported to a file (e.g. to be used as ROIs) by selecting the ***ICA tools. Create ICA parcellation ROI file*** option.

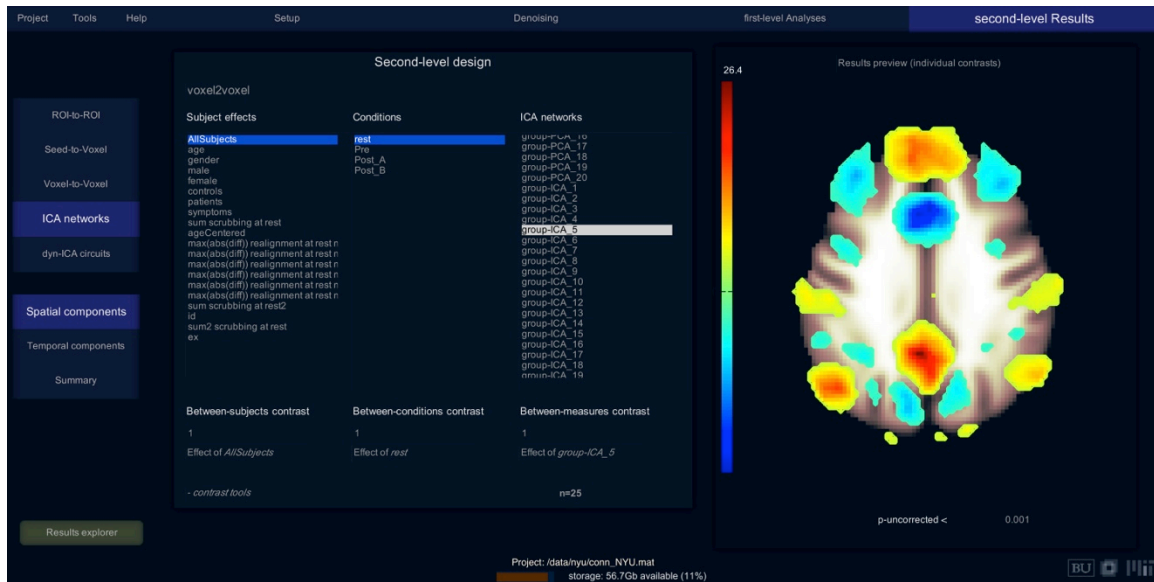
Tip: some times it is desirable to define ROIs from some or all of the ICA networks for additional analyses (e.g. within- and between- network connectivity analyses, ICA denoising, etc.) There are three ways in CONN to define meaningful ROIs from ICA results (and all of these methods are commonly used in the literature):

1) thresholded second-level analyses: from *ICA.SpatialComponent* analyses (see section below), in the *results explorer* window define the desired thresholds (e.g. in terms of T- statistics from a one-sample t-test of the individual subject-level maps) and use '*export mask*' to create a mask of suprathreshold areas, then in *Setup.ROIs* import the resulting mask.ROI.nii file as a new ROI atlas file

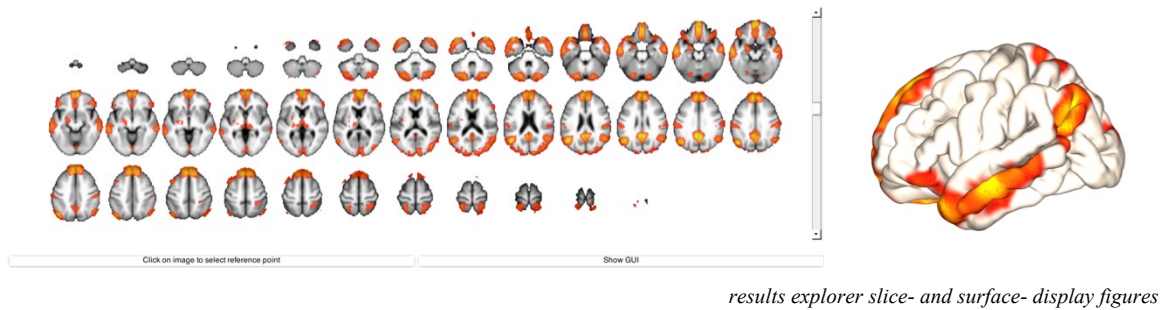
2) thresholded group-level maps: from the *ICA.Summary* tab, select one or several networks, define the desired z-score threshold, and use '*ICA tools. Create ICA parcellation file*' to create a new ROI file, then again import the resulting file in *Setup.ROIs* as a new ROI atlas file

and 3) weighted/unthresholded group-level maps: in *Setup.ROIs* select '*ROI tools. Add ICA-network ROIs*' to define a new set of ROIs directly from the group-level spatial masks, using these maps as weighted/probabilistic-ROIs (one ROI per network)

Generally the advantage of method (3) is that the resulting ROI timeseries and connectivity maps more closely represent the properties of the networks identified in the ICA procedure (when using this method, the extracted ROI timeseries for each network will be exactly the same as the ICA temporal component timeseries, and if using these ROIs as seeds in multivariate analyses the resulting seed-to-voxel maps will be exactly the same as the individual subject-level spatial maps estimated in the ICA procedure for each network), while the advantage of methods (1) and (2) is that they allow breaking individual networks into potentially multiple separate clusters/areas (e.g. for additional within-network or between-network connectivity analyses).

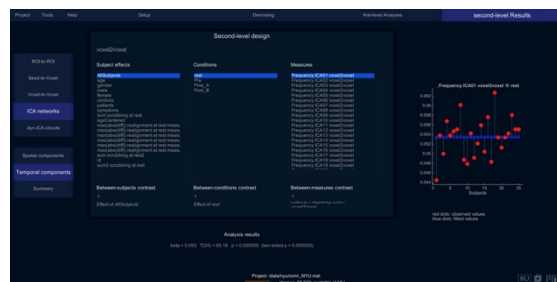


Select **ICA.SpatialComponents** to analyze the backprojected subject-level maps for each network/component. Second-level analyses can be defined in the same way as for any other voxel-level measure (e.g. seed-to-voxel connectivity maps). These analyses enter the estimated subject-level spatial maps for each subject/condition/network into standard second-level analyses in order, for example, to display the average map across all subjects for a given network, or compare these networks across subjects and/or conditions. Select **Results Explorer** to perform the desired second-level analyses, threshold the resulting statistical maps using a combination of height- and extent- thresholds, perform non-parametric analyses, or additional display and analysis options.



results explorer slice- and surface- display figures

Select **ICA.TemporalComponents** to analyze properties of the BOLD signal timeseries associated with each network/component. For each network/component, the temporal variability as well as the frequency (center of mass in spectral power) of these BOLD signal timeseries are computed for each subject and for each condition. Second-level analyses can be defined in the same way as for any other individual measure to, for example, compute the average of these measures across all subjects or compare these measures across subjects or conditions.



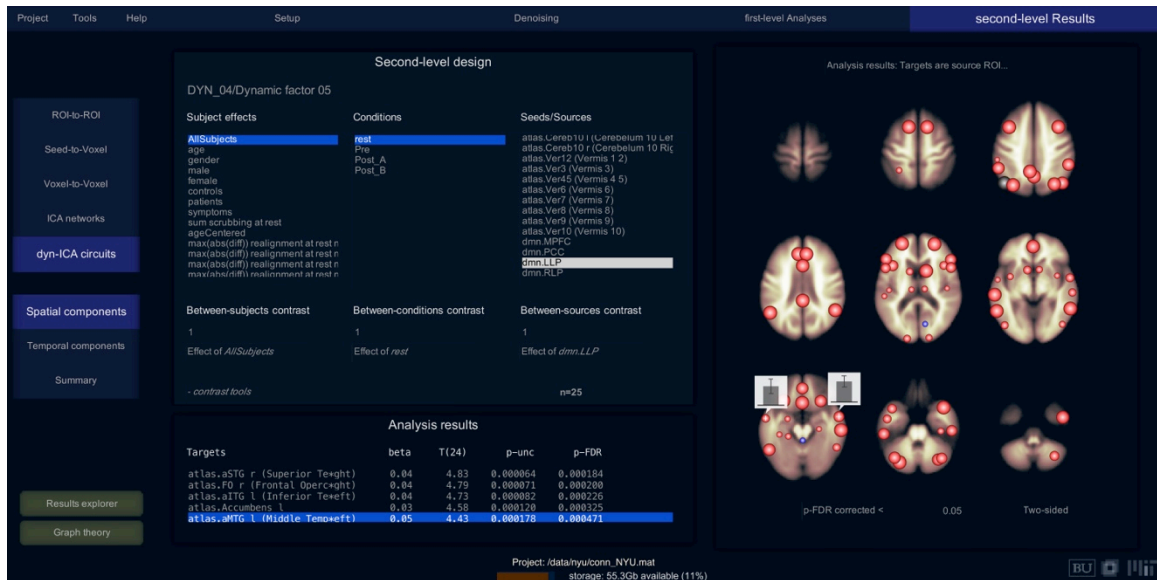
Dynamic ICA analyses:



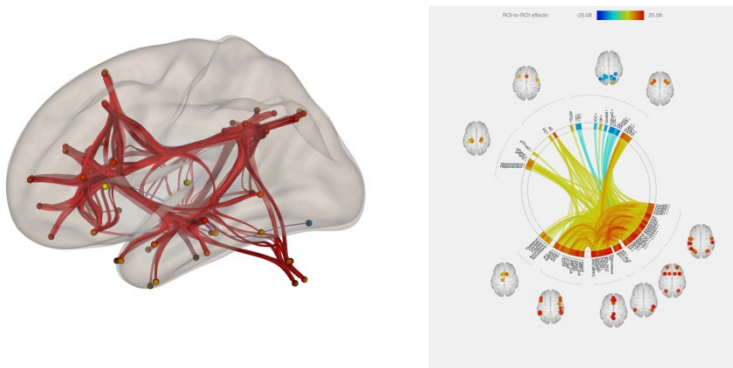
Select **dyn-ICA.Summary** to explore the estimated dynamic circuits/components. The top-left table lists several properties of each circuit (spatial variability/kurtosis/skewness, and temporal variability/frequency). The top-right display shows the group-level maps (*ICA spatial components*) for the selected circuits/components. These maps represent an ROI-to-ROI connectivity matrix with the factor loadings associated with each individual connection for each circuit color coded. These maps may be thresholded based on a z-score threshold for display purposes (factor loadings are centered and normalized across all connections to compute z-scores). The bottom display shows the connectivity modulation timeseries associated with each circuit (*ICA temporal components*) for the selected subject/condition.

Hovering over the top-right plot displays the factor loadings at each connection, as well as the circuits/components with the highest loadings for each connection, which can be used to identify circuits of interest. The top-right display can also be selected to show the associated *estimated dynamic connectivity*. From the estimated ICA decomposition the connectivity timeseries can be reconstructed showing the ROI-to-ROI connectivity matrix at each timepoint during each subject scan. The top-right display shows in this case the estimated ROI-to-ROI connectivity matrix modulation over time, and the bottom plot shows the ROI-to-ROI connectivity values over time for any selected connection (ROI pair). Select in the 'Subjects' list the desired subject to display. You may switch between displaying the full connectivity at each timepoint or only the contribution from one or a subset of circuits/components (by selecting the '*display the selected circuit(s) contribution only*' option). Click on 'Play' to browse through the selected subject scans displaying the observed dynamic connectivity matrix at each timepoint.



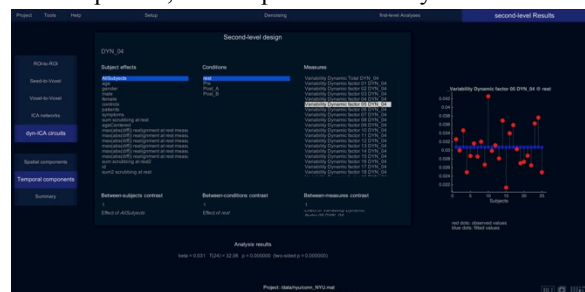


Select **dyn-ICA.SpatialComponents** to analyze the backprojected subject-level maps for each circuit/component. Second-level analyses can be defined in the same way as for any other ROI-to-ROI measure. These analyses enter the estimated subject-level spatial maps (ROI-to-ROI connectivity modulation matrices) for each subject/condition/circuit into standard second-level analyses in order, for example, to display the average map across all subjects for a given circuit, or compare these circuits across subjects and/or conditions. Select **Results Explorer** to perform the desired second-level analyses across all ROIs, threshold the resulting statistical maps using a combination of connection- and seed- or network-level thresholds, perform non-parametric analyses, or additional display and analysis options. You may also select **Graph Theory** to perform additional analyses of network-theoretical measures of the resulting circuits across subjects and/or conditions.



ROI-to-ROI results explorer 3d-view figure and connectome display

Select **dyn-ICA.TemporalComponents** to analyze properties of the connectivity-modulation timeseries associated with each circuit/component. For each circuit/component, the temporal variability as well as the frequency (center of mass in spectral power) of these connectivity timeseries are computed for each subject and for each condition. Second-level analyses can be defined in the same way as for any other individual measure to, for example, compute the average of these measures across all subjects or compare these measures across subjects or conditions.



For more information about the CONN toolbox visit:

NITRC conn site: <http://www.nitrc.org/projects/conn>

Help forum: http://www.nitrc.org/forum/forum.php?forum_id=1144

FAQ: <http://www.alfnie.com/software/conn>