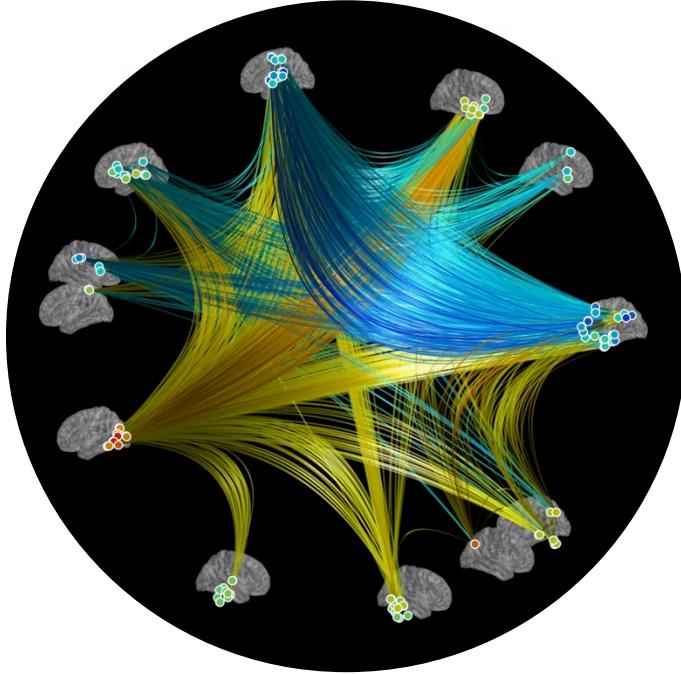


# CONN



Functional Connectivity  
SPM toolbox

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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, and voxel-to-voxel measures (intrinsic connectivity, local correlation maps, and others).

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, a*CompCor* strategy for control of physiological and movement confounds, first-level General Linear Model for correlation and regression 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*

*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:

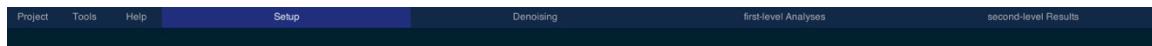
- a) **Functional data.** Either resting-state or task designs can be analyzed.
- b) **Structural data.** One anatomical volume for each subject (this is used for plotting purposes and to derive the gray/white/CSF masks used in the aCompCor confound removal method)
- c) **ROI definitions.** A series 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 is also provided with a series of pre-defined regions of interest that will be loaded automatically (these include a series of seed areas useful for investigating default network connectivity –FOX\_\*.img-, as well as a complete list of Brodmann areas obtained from the Talairach Daemon atlas –TD.img-; see the *utils/otherrois/* folder for additional roi files).

The toolbox operation is divided in four sequential steps:

1. **Setup:** Defines basic experiment information, data locations, regions of interest (seeds), temporal covariates, and second-level models.
2. **Denoising:** Define, explore, and remove possible confounds in the BOLD signal
3. **Analyses:** Perform first-level analyses. Define the seeds of interest and explore the functional connectivity of different sources separately for each subject
4. **Results:** Perform second-level analyses. Define and explore within- and between- subject contrasts of interest

Each of these steps can be defined interactively using the toolbox gui or by scripting using the *conn\_batch* functionality. The following sections describe the steps when using the gui in more detail (see the *conn batch manual* for additional information).

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



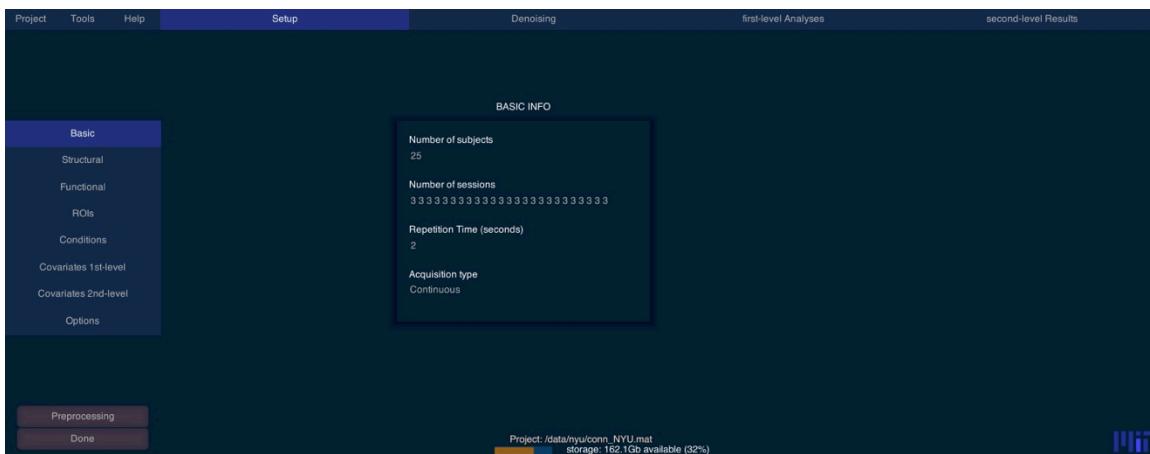
Click on the **SETUP** tab

Click on the **Project.New** button to start a new project. The toolbox will offer the option to spatially preprocess your data (segmentation, realignment, slice timing correction, coregistration, normalization, smoothing, and **outlier detection/scrubbing**) at this time using a wizard.

This wizard will also initialize some of the basic setup information (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). 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), or select ‘other’ to inspect or modify these pipelines (see “Notes on Structural, Functional, and ROI files coregistration” section below).

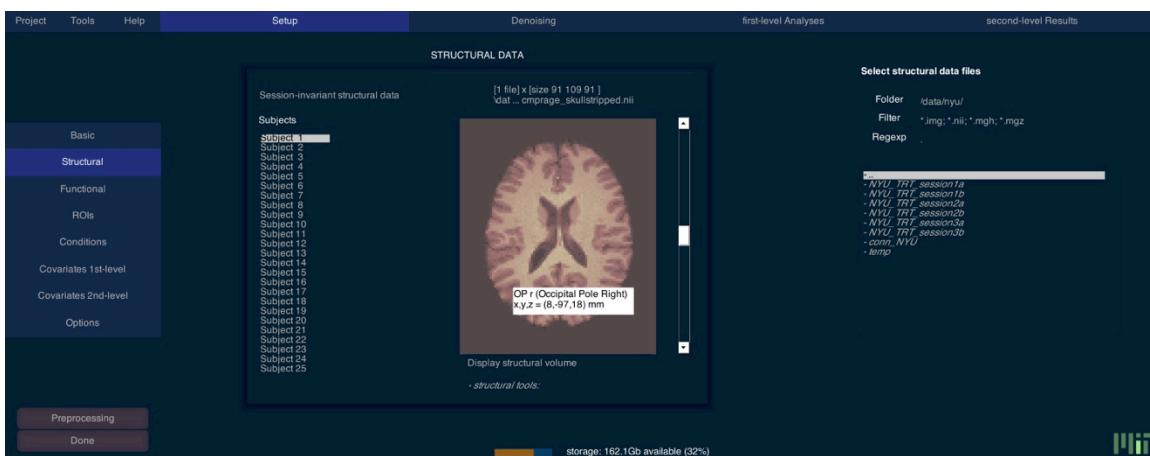
If your data is already spatially preprocessed, or if you prefer to run these or other spatial preprocessing steps at a later time (e.g. after defining the source of functional and structural files using the CONN gui), simply skip this step and continue with the steps below.

## 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:



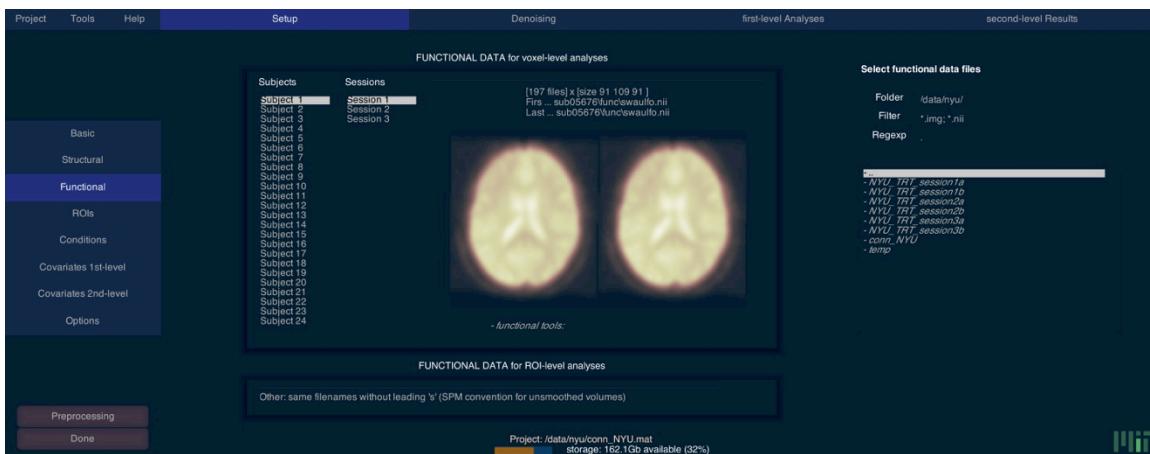
Click on the **Structural** button on the left side to load the structural images. Click sequentially on each subject and select the associated anatomical image (one anatomical volume per subject). If you have multiple anatomical volumes per subject (e.g. one per session/scan), select ‘Session-specific 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).

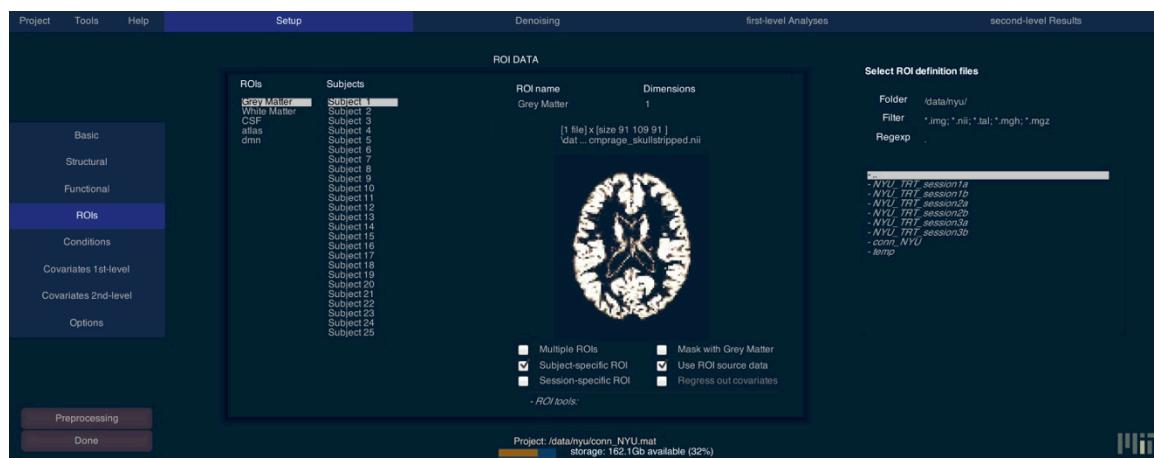
## Functional files Setup:



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. use unsmoothed 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 ‘*Functional data for ROI-level analyses*’ menu. By default the toolbox will use unsmoothed volumes for ROI-level extraction by using the SPM-convention for unsmoothed volumes (same files without the initial ‘s’ in the filename).

## 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). ROI files can be assigned separately to each subject (for subject-specific ROIs) or commonly across all subjects (normalized-space ROIs).

By default all files in the *rois* toolbox folder (*./conn/rois*) will be imported as initial regions of interest. This folder includes by default **four Fox ROIs** and all the cortical and subcortical structures from the **FSL Harvard-Oxford Atlas** as well as cerebellar areas from the **AAL atlas**. 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 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 here. 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 as 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**).

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).

## Notes on Structural, Functional, and ROI files coregistration:

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)<sup>1</sup>. 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 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).

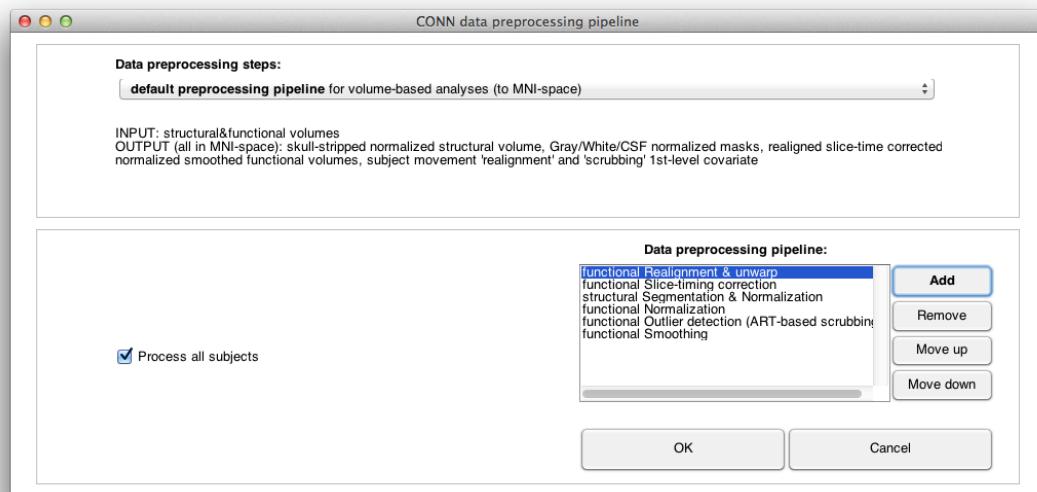
<i>Voxel-level analyses</i>	<i>Volume-based analyses on MNI-space</i>	<i>Surface-based analyses</i>
<i>Functional files</i>	Normalized and smoothed volumes (e.g. <i>swrFunct.nii</i> )	Subject-space non-smoothed volumes (e.g. <i>rFunct.nii</i> )
<i>Structural files</i>	Normalized structural (e.g. <i>wTl.nii</i> )	Subject-space structural (e.g. <i>mri/Tl.mgh</i> )

<i>ROI-level analyses</i>	<i>MNI-space ROIs</i>	<i>Subject-specific ROIs</i>
<i>Functional files (ROI extraction option)</i>	Normalized non-smoothed volumes (e.g. <i>wrFunct.nii</i> )	Subject-space non-smoothed volumes (e.g. <i>rFunct.nii</i> )
<i>ROI files</i>	Normalized-space (e.g. <i>wc1_Tl.img</i> )	Subject-space ROIs (e.g. <i>c1_Tl.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 intial 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*:

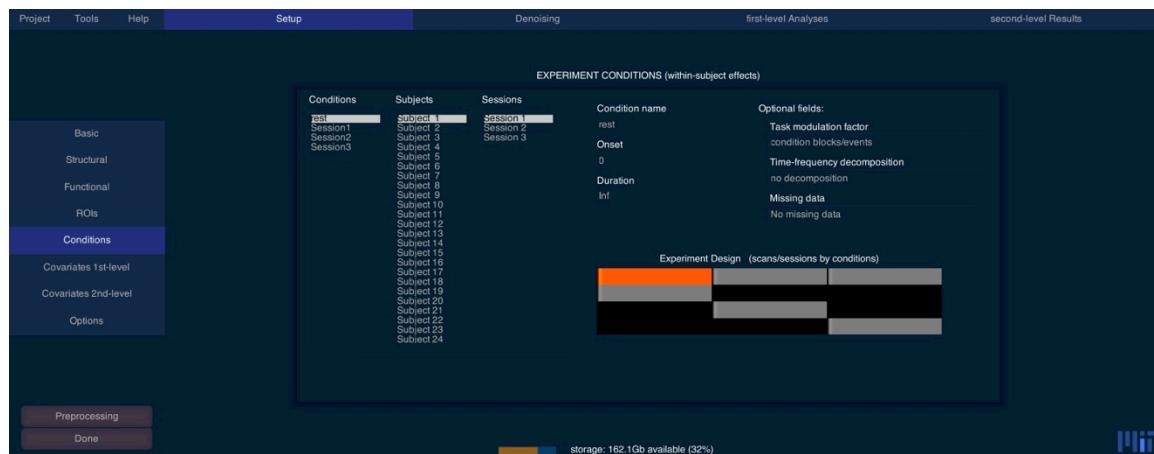
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<sup>1</sup> although of course not necessarily having the same dimensions or resolution (i.e. coregistered, but not necessarily resliced to the same reference space)



Using *Setup.Preprocessing* you may select individual spatial preprocessing steps or 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 run the entire sequence of spatial preprocessing steps on all of the defined subjects or any individual subject.

## Rest or task conditions Setup:



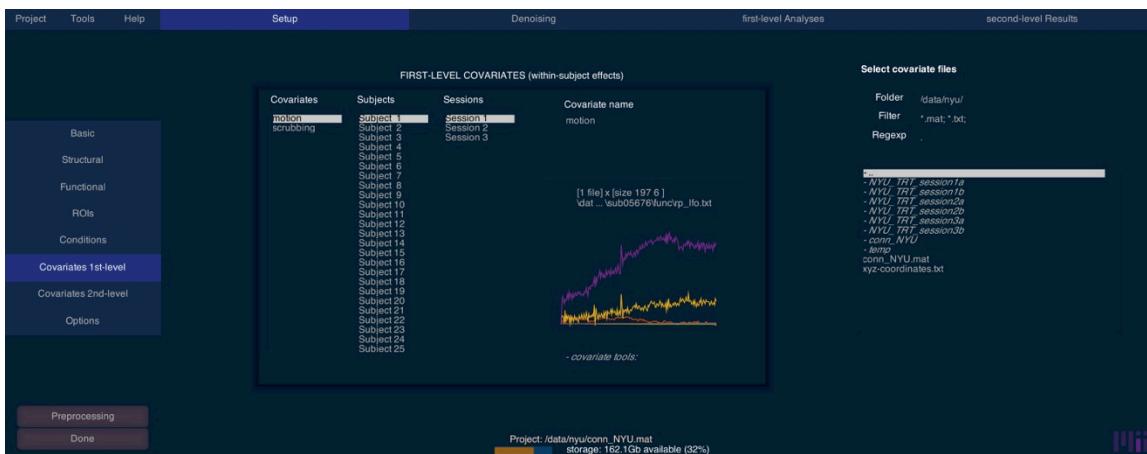
Click on the **Conditions** button on the left side to enter onsets and durations (in seconds) of each experimental condition (blocks/events). If resting state (no experimental conditions) enter a single condition with onset 0 seconds and duration the complete duration of each session (or *inf*). Right-click on the *conditions* list to remove some conditions, or to move a condition into the first-level covariates list instead (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 measures<sup>2</sup>.

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<sup>2</sup> 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 potential frequency-dependent differences in any of your fcMRI measures.

## First-level (within-subjects) covariates Setup:

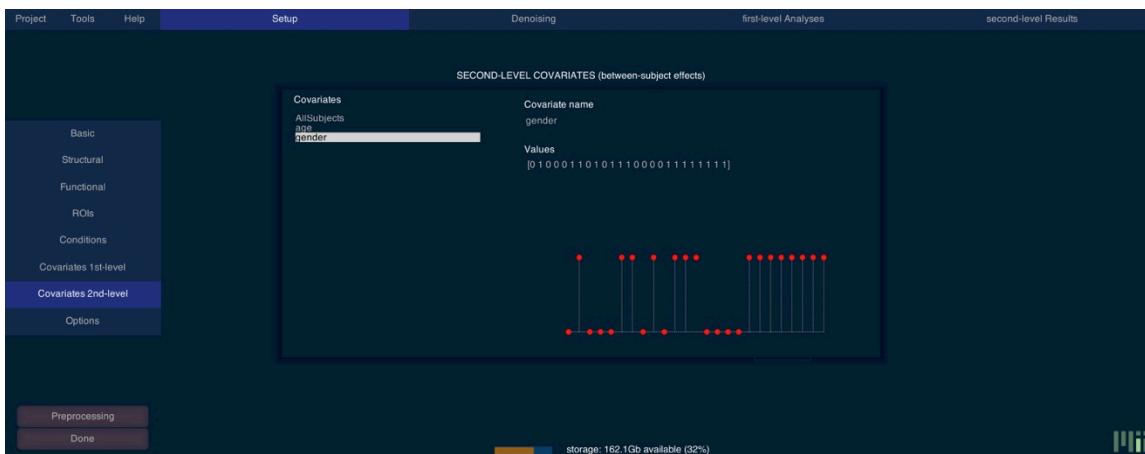


Click on the **Covariates:First-level** button to define first level covariates such as realignment parameters to be used in the first-level BOLD model. For each covariate select a .txt or .mat file (for each subject and session) containing the covariate time series (e.g. select the realignment parameters 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) you may do so by selecting each first-level measure in the *covariates* list and clicking on *covariate tools: subject-level aggregate*.

## Second-level (between-subjects) covariates Setup:



Click on the **Covariates:Second-level** button to define groups and subject-level regressors (e.g. behavioral measures). Use 1/0 to define subject groups, or continuous values to perform between-subject regression models). Click on the empty space below “All” to add a variable.

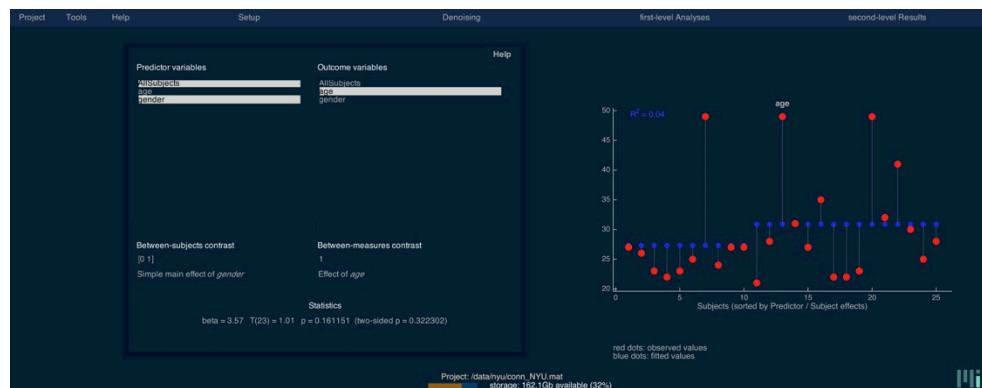
e.g.      AllSubjects: 1 1 1 1 1 1 1  
 Patients: 1 1 1 1 0 0 0  
 Controls: 0 0 0 0 1 1 1  
 Performance : 1 2 3 2 3 4 5 6

In addition to simple lists like the above, the *values* field will also accept any valid Matlab syntax (e.g. *[ones(1,5) zeros(1,5)]*). You may also refer to variables in Matlab workspace by name, or refer to other already-defined conditions by name (e.g. define in the example above the covariate *Controls* simply as *AllSubjects -Patients*).

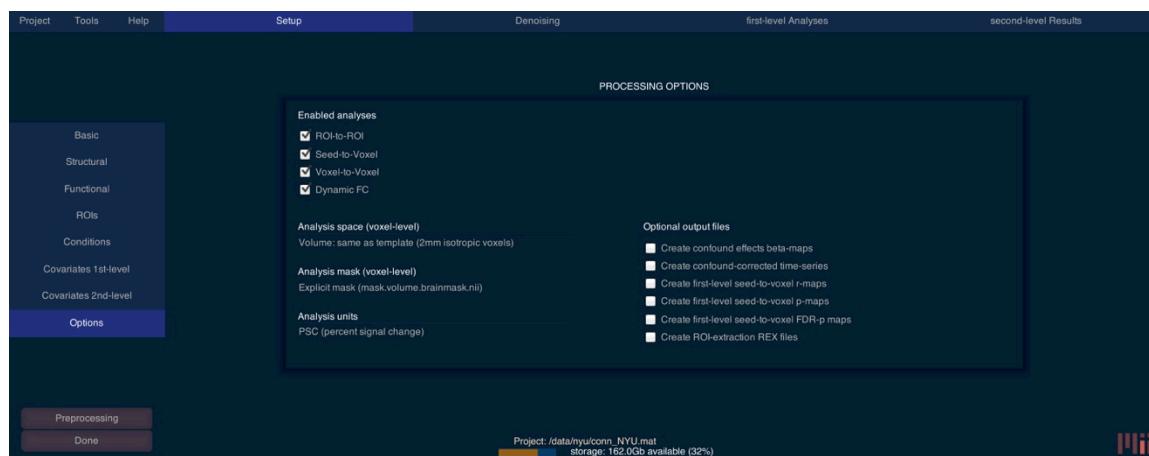
Note: Second-level covariates can be defined at any time in the analyses (changes to any of other “Setup” options requires rerunning all the analyses steps, while changes to the second-level covariates do not as they only affect the second-level analyses in the “Results” window)

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 2<sup>nd</sup>-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 2<sup>nd</sup>-level analyses in the calculator is the same as that of the main CONN gui 2<sup>nd</sup>-level results view (see Step 4 below)



## Options Setup:



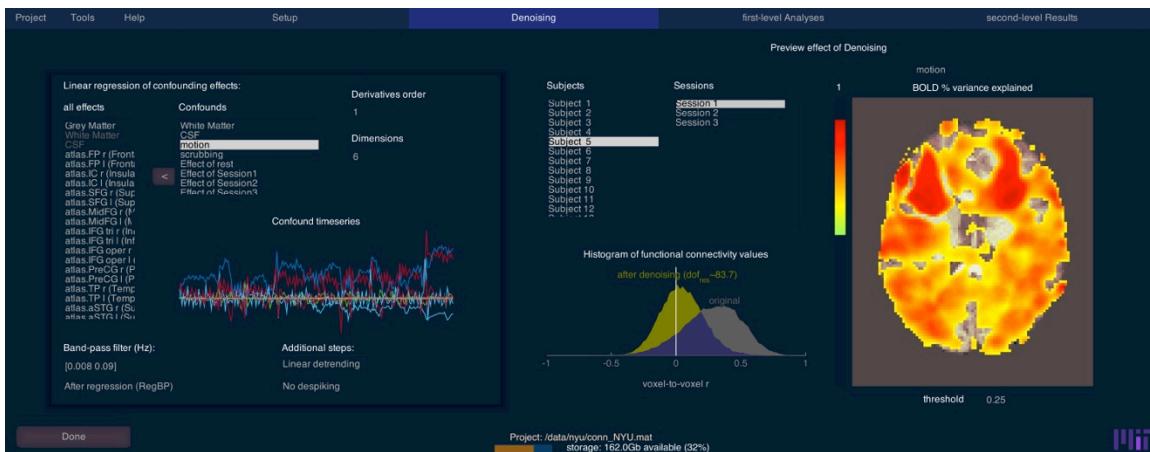
Click on the **Options** button for additional analysis options. Select the type of analyses that you wish to perform (ROI-to-ROI, seed-to-voxel, and voxel-to-voxel). For voxel-based analyses define the desired analysis space (by default volume-based analyses using isotropic 2mm voxels; you may indicate 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.

Note 1: If the data has initially been defined in SPM you can skip many of the above steps by clicking the **Import** button (right after entering the number of subjects in the **Basic** setup) 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.

## 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).

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 bottom 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. The dimensions of each confound can be changed up to the values entered in the “Setup” stage, to explore its effect on the total variance explained and on the histogram of voxel-to-voxel connectivity values. Images are displayed in neurological format. Click on the image to see the voxel locations and atlas areas<sup>3</sup>.

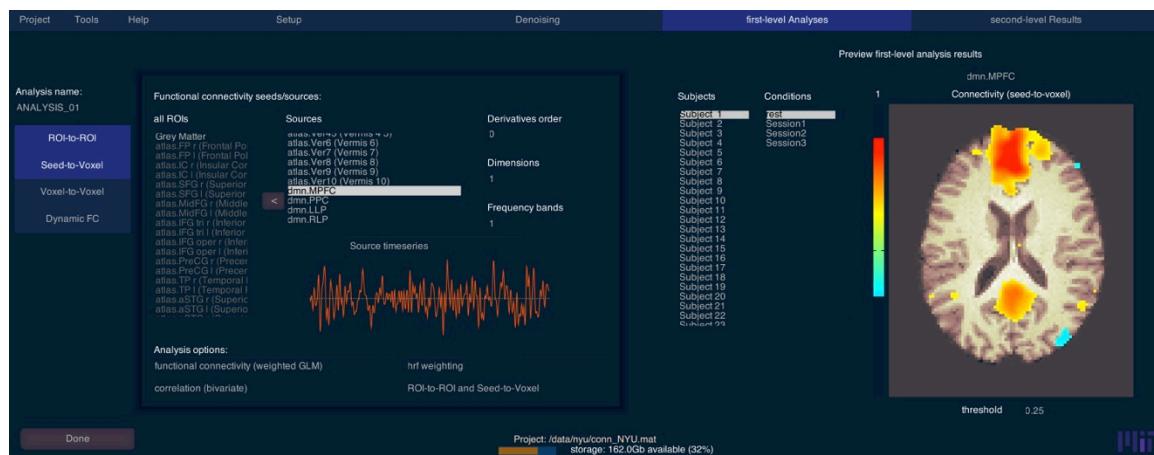
Enter the **band-pass filter** information in the bottom-left box (two numbers, in Hz, defining the band-pass frequency window of interest), as well as any additional desired denoising options (**detrending** removes linear/quadratic/cubic trends within each functional session, and **despiking** applies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

<sup>3</sup> 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 of different sources 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 PsychoPhyiological 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*. 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 you wish instead to use the same interaction factor for all of your conditions 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)<sup>4</sup>. Note that, although 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.

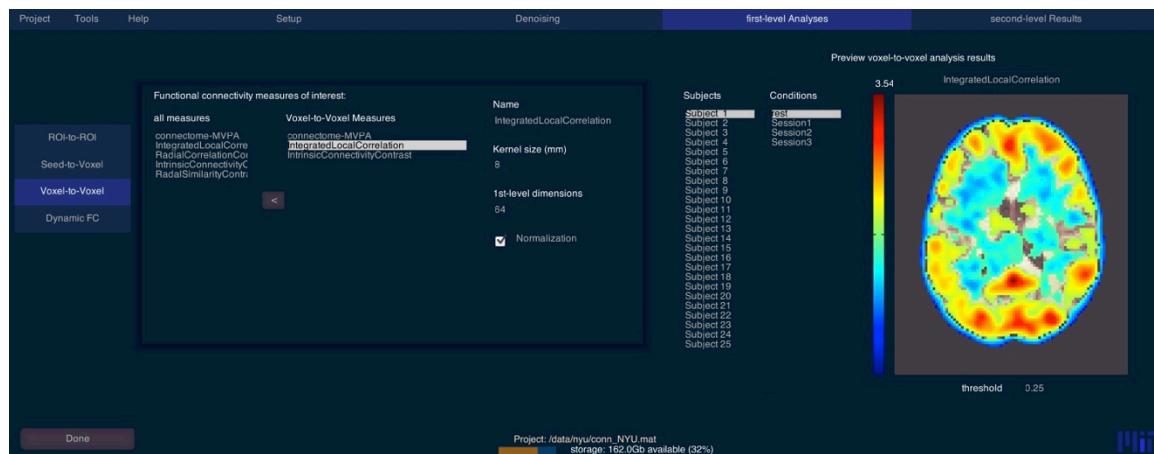
Last, click on **Analyses** to define the type of analyses to be performed (ROI-to-ROI, seed-to-voxel, or both). When finished defining/exploring the connectivity analyses press **Done**. This will perform the defined analyses for all subjects, constructing seed-to-voxel connectivity maps for each selected 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)

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<sup>4</sup> 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 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:

**Connectome-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 *2<sup>nd</sup>-level dimensions* field to modify the number of dimensions/components kept in this multivariate representation, and the *1<sup>st</sup>-level dimensionality* 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).

**Indexes:** These analyses create, separately for each voxel, specific indexes/measures each characterizing a different aspect of the connectivity pattern between this voxel and the rest of the brain. **Integrated Local Correlation** (ILC, Deshpande et al. 2007) characterizes the average local connectivity between each voxel and its neighbors (a single number for each voxel). You may define in *Kernel Size* the size of the local averaging (Gaussian FWHM in mm). **Radial Correlation Contrast** (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). **Intrinsic Connectivity Contrast** (ICC, Martuzzi et al. 2011) characterizes the strength of the global connectivity pattern between each voxel and the rest of the brain (a single number for each voxel). **Radial Similarity Contrast** 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.

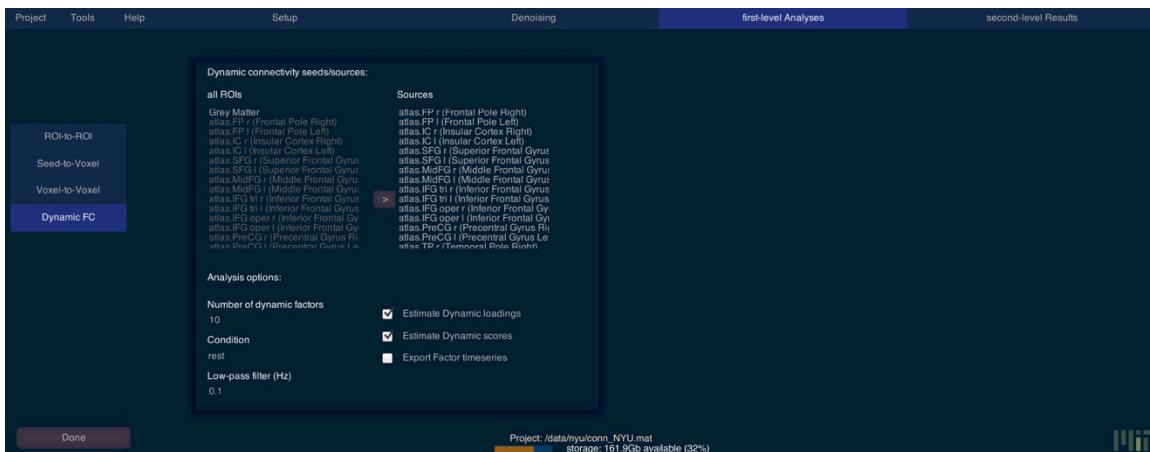
Select from the left-most list the **measures** of interest and click the right arrow to add these analyses/measures to the list of voxel-to-voxel analyses.

The right panel (“**Preview results**”) displays the resulting  $f(x)$  maps separately for each subject/session. Analyses here are performed in real-time (any changes in the “Define measure” definitions affect directly the results displayed in the “Preview” window, except for *connectome-MVAP* analyses for which the

preview display is not available). 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.

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 FC analyses:



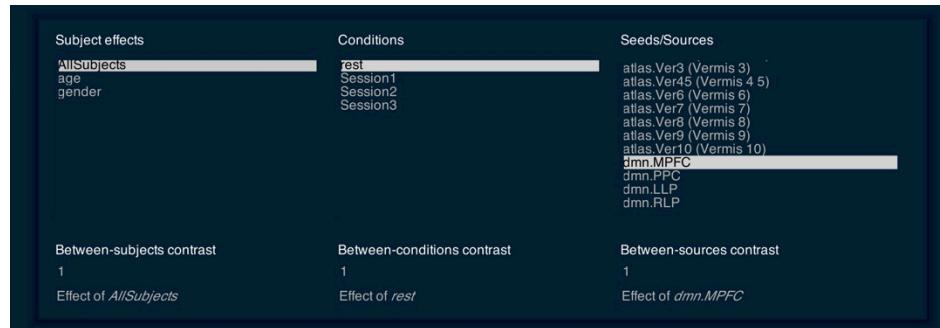
Select the ‘Dynamic FC’ tab if you want to define additional dynamic connectivity analyses.

Dynamic connectivity analyses explore dynamic properties (temporal modulation) of the ROI-to-ROI connectivity matrix. These analyses analyze the observed temporal modulation in ROI-to-ROI connectivity values and return a number of data-driven temporal factors characterizing the largest temporal modulation effects observed in this connectivity matrix 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 dynamic factors**. If there are multiple conditions in your design select in **Conditions** the resting condition (entire timeseries), and enter an optional low-pass filter threshold value (in Hz) in **Low-pass filter** in order to focus the estimated temporal modulation factors on the desired low-frequency range. Check **Estimate dynamic loadings** if you wish to obtain, for each estimated temporal modulation factor and for each subject, an associated ROI-to-ROI matrix characterizing the functional connectivity changes associated with this factor, check **Estimate dynamic scores** if you wish to obtain, for each estimated temporal modulation factor and for each subject, several summary measures characterizing this factor timeseries (average, variability, and rate of change). Check **Export factor timeseries** if you wish to export these timeseries as first-level covariates for additional analyses.

When finished defining the dynamic analyses press **Done**. This will estimate the data-driven temporal factors 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** window.

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).

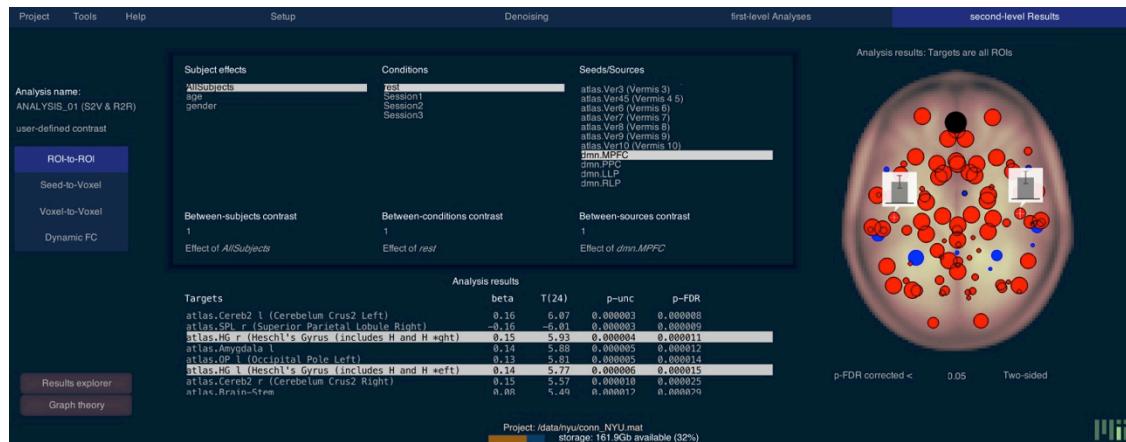
Multiple conditions can be selected simultaneously in order to analyze connectivity results across several conditions by specifying the contrast in “**Between conditions contrast**” (e.g. use 1 -1 to compare connectivity during rest vs. task). After selecting one or multiple effects of interest in the **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.

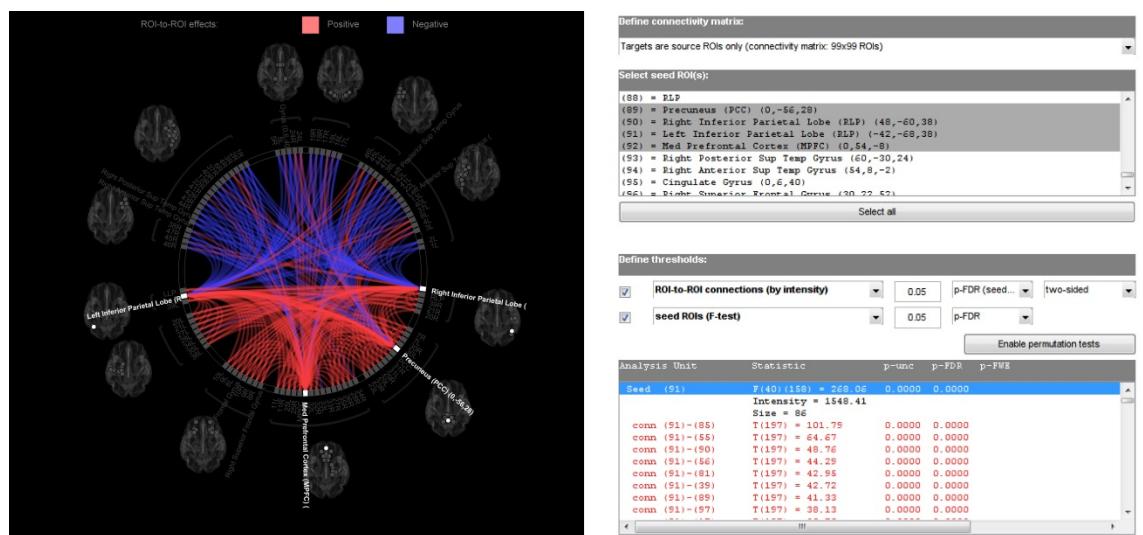
Each of these contrasts (between-subjects, between-conditions, and between-sources) can be defined as a single vector (e.g. [1,-1], for T-statistics) or as matrix (e.g. [1,0; 0,1], for F-statistics, where multiple contrasts are entered separated by ‘;’). In general, contrast matrices containing several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

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



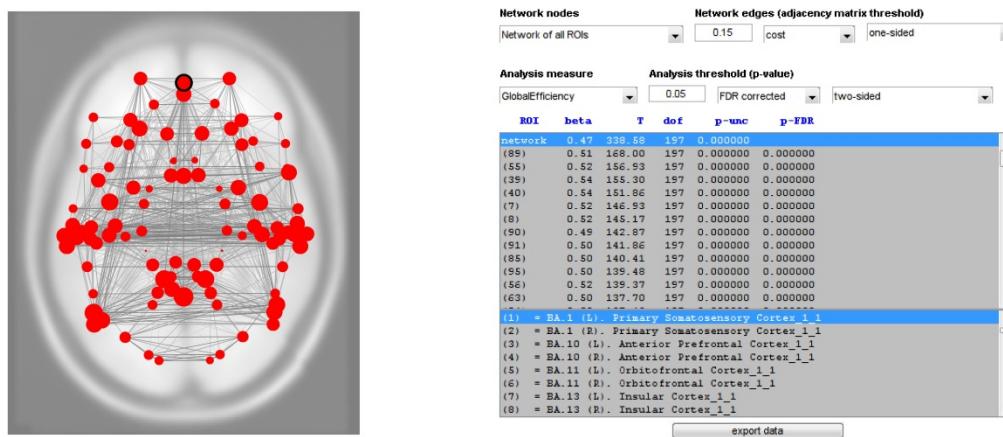
When selecting **ROI-to-ROI** analyses (from the leftmost tab in the figure), the brain display at the right shows an axial view of the roi-to-roi second-level analysis results estimated in real time for the selected second-level model and contrasts. These results can be thresholded at the desired **p-value** 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.



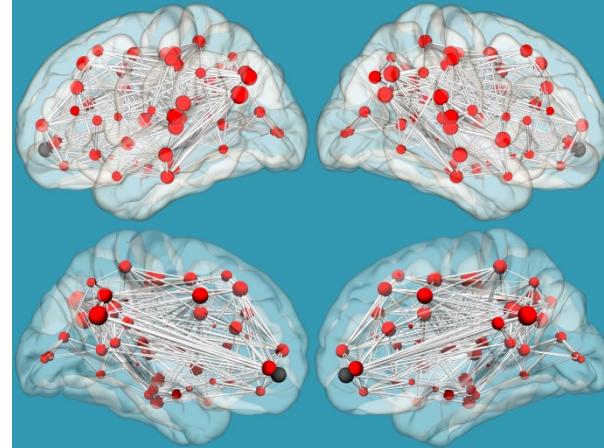
In addition by clicking **ROI-to-ROI results explorer** a graphical display of *all* of the ROI-to-ROI connectivity values (for the selected between-subjects and between-condition contrasts; note that the GUI between-sources contrast) is shown. The *define connectivity matrix* field allows you to define 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).

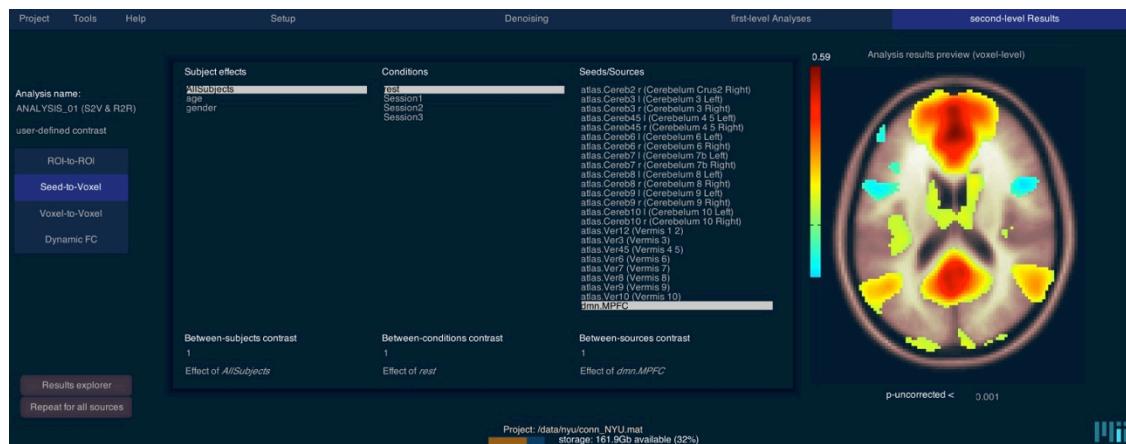
The top menu in this figure shows additional display options, including connectome, axial/coronal/sagittal, or 3d-rendered views of the results (note: the connectome view displays all of the ROIs on a circle, with individual suprathreshold connectivity lines between them; you may sort the ROIs on this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



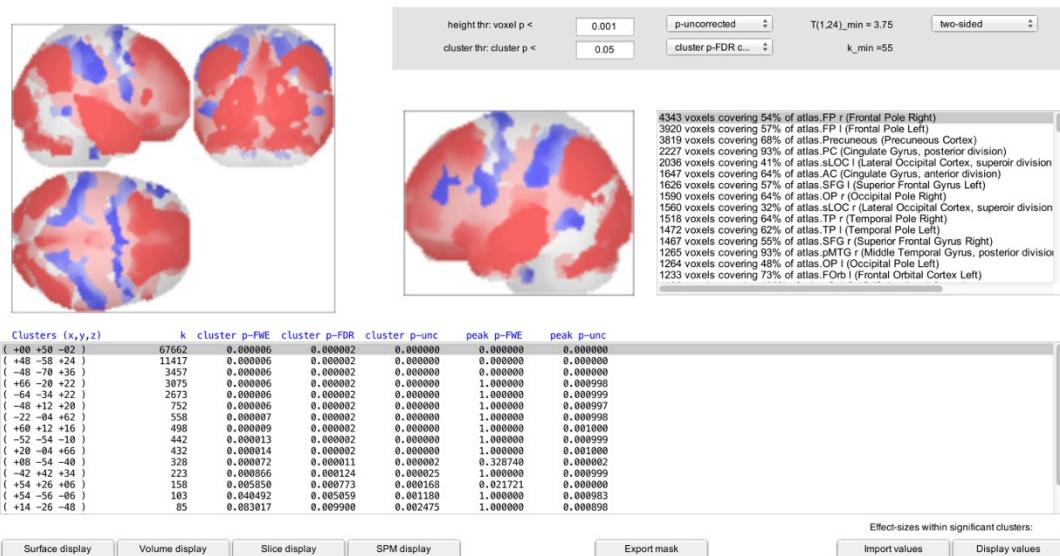
Last by clicking **graph-theory results explorer** a graphical display is shown allowing the users to test (for the selected between-subjects and between-condition contrasts) measures of efficiency, centrality, and cost/degree, associated with an ROI-to-ROI connectivity network. Click on 'Network nodes' 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.



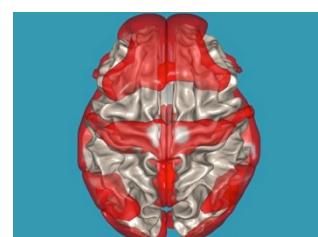
## Seed-to-Voxel analyses (functional connectivity maps):



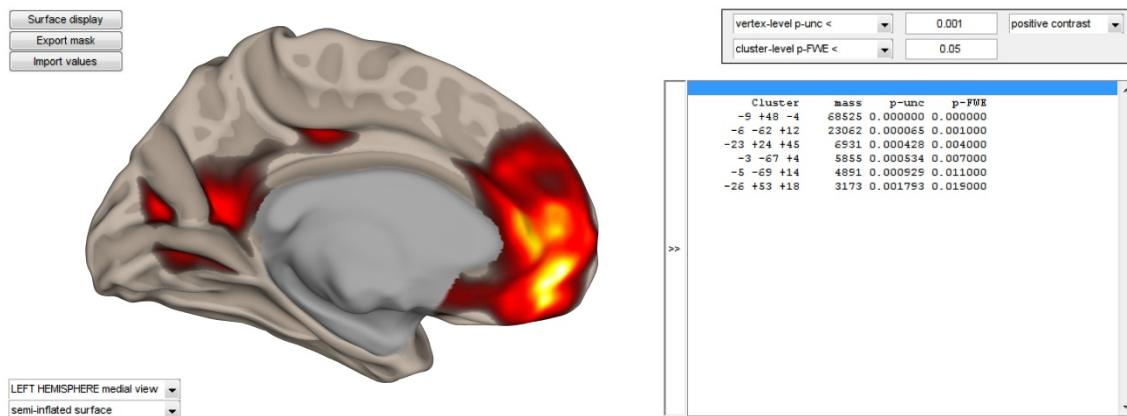
When selecting **Seed-to-Voxel** analyses (from the leftmost tab in the figure), the brain display at the right allows you to explore the second-level analysis results estimated in real time for the specified second-level model and contrasts. These results can be thresholded using a voxel-level uncorrected **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.



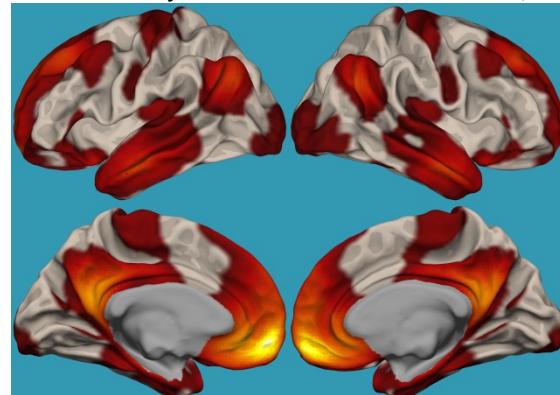
Clicking on **Seed-to-Voxel results explorer** button launches a graphical display showing the whole-brain results of these second-level analyses using a MIP view of supra-threshold voxels, and allowing you additional thresholding and analysis options. This display can be thresholded by a combination 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. From this graphical display you can also export the results as a text file containing the supra-threshold



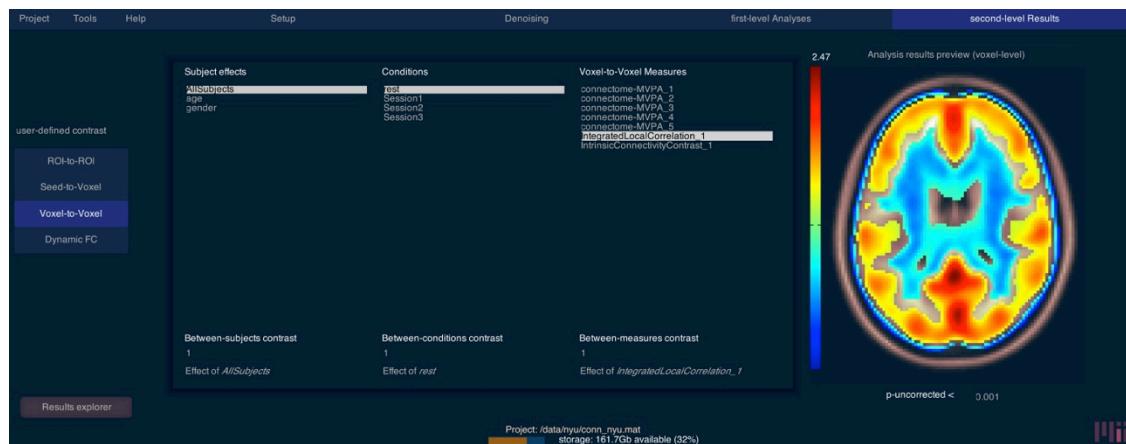
clusters and their statistics by right-clicking on the stats table, or as a .nii mask file defined by the results supra-threshold voxels (**export mask** button). These mask files can be re-entered directly in the toolbox as ROI files (e.g. for post hoc analyses using suprathreshold clusters as seeds/ROIs). 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**, and **volume 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.



When performing surface-based analyses, the **seed-to-voxel results explorer** button 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 permutation 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.

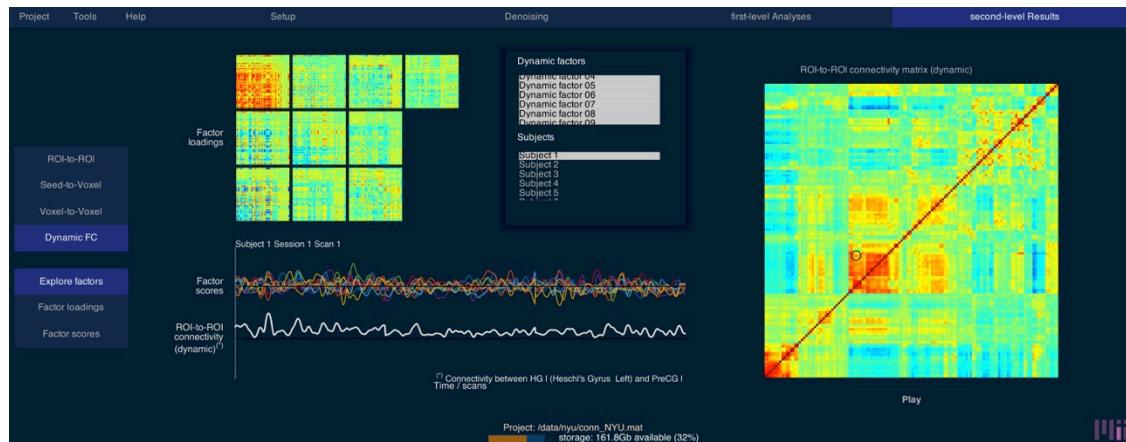


## Voxel-to-Voxel analyses (MVPA and other connectome-level measures):



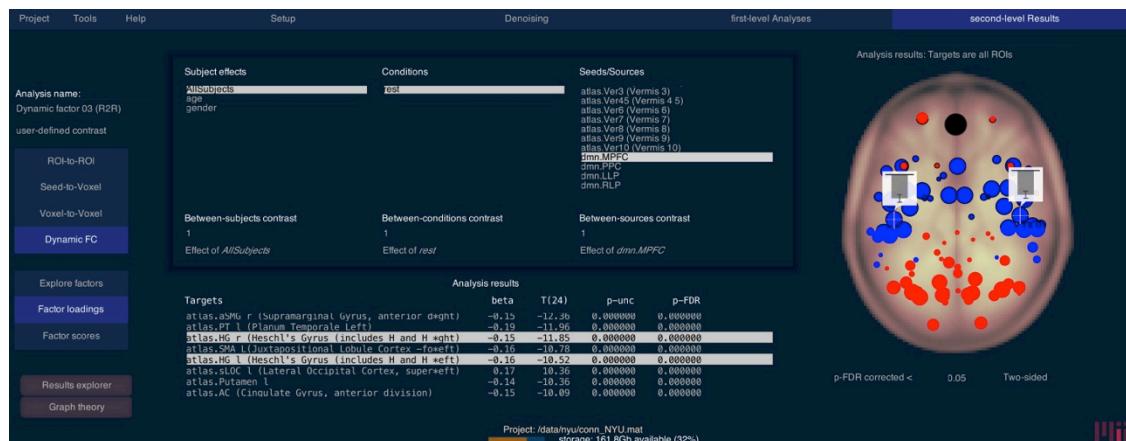
When selecting **Voxel-to-Voxel** analyses (from the leftmost tab in the main CONN gui), the gui options are the same as in the seed-to-voxel analyses above (but instead of multiple sources, users may now select across multiple measures; e.g. select all of the connectome-MVPA measures in the “**Measures**” 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 clicking the **voxel-to-voxel results explorer**. All the display and analysis options available for seed-to-voxel maps and described in the *seed-to-voxel analysis* section above are equally available for voxel-to-voxel measures, both for univariate measures such as Integrated Local Correlation and Intrinsic Connectivity, as well as for multivariate measures such as Multi-Voxel Pattern Analyses, Radial Correlation Contrast, and Radial Similarity Contrast.

## Dynamic FC analyses:

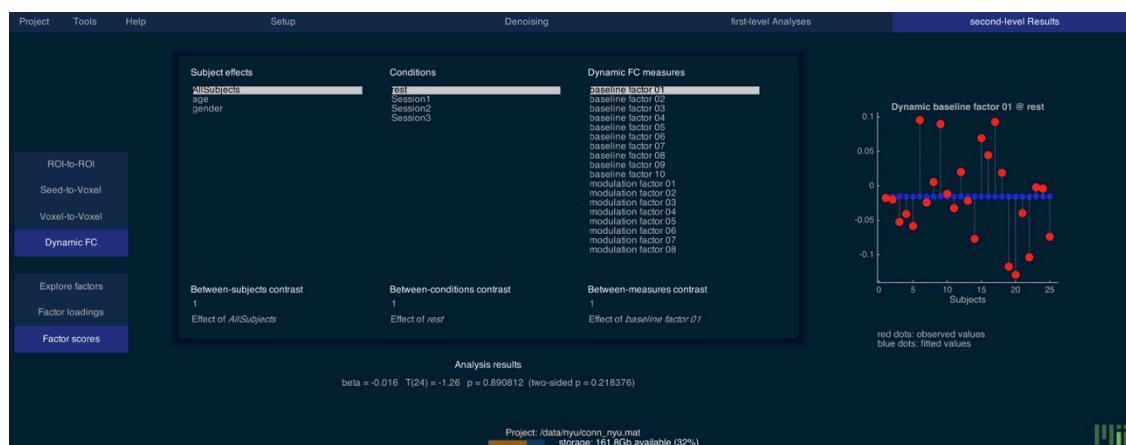


Select **Dynamic FC**. **Explore factors** to explore the estimated dynamic factors. The left-most displays show the estimated temporal factors (factor scores) and associated ROI-to-ROI connectivity modulation (factor loadings). The right display shows the estimated ROI-to-ROI connectivity matrix modulation over time (as approximated by the estimated factors), and the bottom plot shows the ROI-to-ROI connectivity values over time for any selected ROI pairs. Select in the ‘Subjects’ list the desired subject to display. Select in the ‘Dynamic factors’ list the desired factors to explore (when selecting individual factors, only the portion of the ROI-to-ROI connectivity matrix characterized by the selected factor is displayed in the ‘ROI-to-ROI connectivity’ display and the bottom ‘ROI-to-ROI connectivity’ plot). Click on ‘Play’ to

browse through the selected subject scans displaying the observed dynamic connectivity matrix at each timepoint.



Select **Dynamic FC. Factor loadings** for additional second-level analyses of the estimated factor loadings. Select the desired factor in the **Analysis name** top-left dropdown menu. Factor loadings are estimated for each subject using dynamic first-level ROI-to-ROI analyses where each factor timeseries is entered as the interaction factor in a standard PPI analysis. Second-level analyses work as standard ROI-to-ROI second-level analyses (see *ROI-to-ROI analysis* section above).



Select **Dynamic FC. Factor scores** for additional second-level analyses of the estimated factor scores summary measures. Select each measure in the 'Dynamic FC measures' list, and enter the desired between-subjects and between-conditions test in the 'Subject-effects' and 'Conditions' list and associated contrast fields. The bottom **Analysis results** display shows the selected test associated effect sizes, statistics, and p-values and the right display shows the observed and fitted values (right-click for additional display options).

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](http://www.nitrc.org/forum/forum.php?forum_id=1144)

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