Multimodal Imaging Brain Connectivity Analysis (MIBCA)

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Abstract

In recent years, connectivity studies using neuroimaging data have increased the understanding of the organization of large-scale structural and functional brain networks. However, data analysis is time consuming as rigorous procedures must be assured, from structuring data and pre-processing to modality specific data procedures. Until now, no single toolbox was able to perform such investigations on truly multimodal image data from beginning to end, including the combination of different connectivity analyses. Thus, we have developed the Multimodal Imaging Brain Connectivity Analysis (MIBCA) toolbox with the goal of diminishing time waste in data processing and to allow an innovative and comprehensive approach to brain connectivity. The MIBCA toolbox is a fully automated all-in-one connectivity toolbox that offers pre-processing, connectivity and graph theoretical analyses of multimodal image data such as diffusion-weighted imaging (DWI), functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). It was developed in MATLAB environment and pipelines well-known neuroimaging softwares such as Freesurfer, SPM, FSL, and Diffusion Toolkit. It further implements routines for the construction of structural, functional and effective or combined connectivity matrices, as well as, routines for the extraction and calculation of imaging and graph-theory metrics, the latter using also functions from the the Brain Connectivity Toolbox. Finally, the toolbox performs group statistical analysis and enables data visualization in the form of matrices, 3D brain graphs and connectograms. In this manual the MIBCA toolbox is presented by illustrating its capabilities using a multimodal image sample with volumetric T1-weighted, diffusion tensor imaging, and resting state fMRI data, and 10 subjects with 18F-Altanserin PET data also.

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Part I

Setting-up MIBCA

Chapter 1

Installation & Main Interface

Contents

1.1 Download & Installation

The Multimodal Imaging Brain Connectivity Analysis (MIBCA) toolbox was developed in MATLAB and pipelines well-known neuroimaging softwares such as SPM, Freesurfer, FSL, and Diffusion Toolkit. Currently, all the above software are required to properly run MIBCA. A list of the software required for each instance of MIBCA is provided in Table 1.1.

Although MIBCA does not require any installation procedure, the required third-party software do. The specifications of where to obtain and how to install them, can be seen here:

- **MIBCA**: http://www.mibca.com/downloads/
- SPM: http://www.fil.ion.ucl.ac.uk/spm/software/
- Freesurfer: http://freesurfer.net/fswiki/DownloadAndInstall
- FSL: http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/
- Diffusion Toolkit: http://trackvis.org/download/
- BCT toolbox: https://sites.google.com/site/bctnet/

Table 1.1: Software required for each step of the MIBCA toolbox.

Processes	MATLAB	SPM	Freesurfer	FSL	D. Toolkit	BCT
1. Pre-processing	X	X	X	X	X	X
1.1. aMRI	X	-	X	-	-	X
1.1.1 Image Conversion	X	-	-	-	-	-
1.1.2 Registration	X	-	X	-	-	-
1.1.3 Segmentation	X	-	X	-	-	-
1.1.4 Atlas mapping	X	-	X	-	-	-
1.1.5 Connectivity matrices	X	-	-	-	-	X
1.2. dMRI	X	X	X	X	X	X
1.2.1 Image Conversion	X	-	-	-	-	-
1.2.2 Diff. Tensor Estimation	X	-	-	X	X	-
1.2.3 Registration to aMRI	X	X	-	-	-	-
1.2.4 Fibre Tracking	X	-	-	-	X	-
1.2.5 Connectivity matrices	X	-	-	-	-	X
1.3. fMRI	X	X	-	X	-	X
1.3.1 Image Conversion	X	-	-	-	-	-
1.3.2 Filtering	X	-	-	X	-	-
1.3.3 Registration to aMRI	X	X	-	-	-	-
1.3.4 Connectivity matrices	X	-	-	-	-	X
1.4. PET	X	X	X	X	-	X
1.4.1 Image Conversion	X	-	-	-	-	-
1.4.2 Spatial Filtering	X	X	-	X	-	-
1.4.3 rSUV Estimation	X	-	-	X	-	-
1.4.4 Registration to aMRI	X	X	-	-	-	-
1.4.5 Connectivity matrices	X	-	-	-	-	X
2. Visualization	X	-	-	-	-	X
2.1. Matrices	X	-	-	-	-	-
2.2. Connectogram	X	-	-	-	-	X
2.3. 3D-Graph	X	-	-	-	-	X

1.2 Main Interface

After the download and installation of the required software, MIBCA is ready to be used.

To start using the MIBCA toolbox, first open an instance of MATLAB, and run the function ***mibca.m***. The toolbox is not required to be manually added to the searched path of MATLAB. The main interface (Figure 8.6) should now be visible. This interface presents only three buttons: ***General Paths***, ***Processing***, and ***Visualization***. To run either the ***Processing*** or ***Visualization***, the ***General paths*** must first be run.

After clicking on the ***General Paths*** button, the following window is presented to the user (Figure 1.2).

To reduce the complexity of setting-up the different paths of the third-party software used by MIBCA, an automatic procedure was developed, where the specific software location are searched in the local computer. This step is only required the first time MIBCA is initialized, or if the location of these software change after setting-up MIBCA. This step can take up to 5 minutes and **may fail** to find the necessary paths. If the paths are known they can be overwritten by the user. Therefore, the first time this step is run the first 7 paths present the message "waiting...", and change after the respective paths are found.

The remain fields need to be completed by the user and refer to the study to be analysed.

- ***Data location***: Location of the raw data. All the data to be analysed need to be on this folder.
- ***Subject folder identifier***: To allow the user to have other data on the same folder (yet not to be analysed), an identifier of the folders to be analysed must be given. This can be anything that distinguish these folders from the remaining.

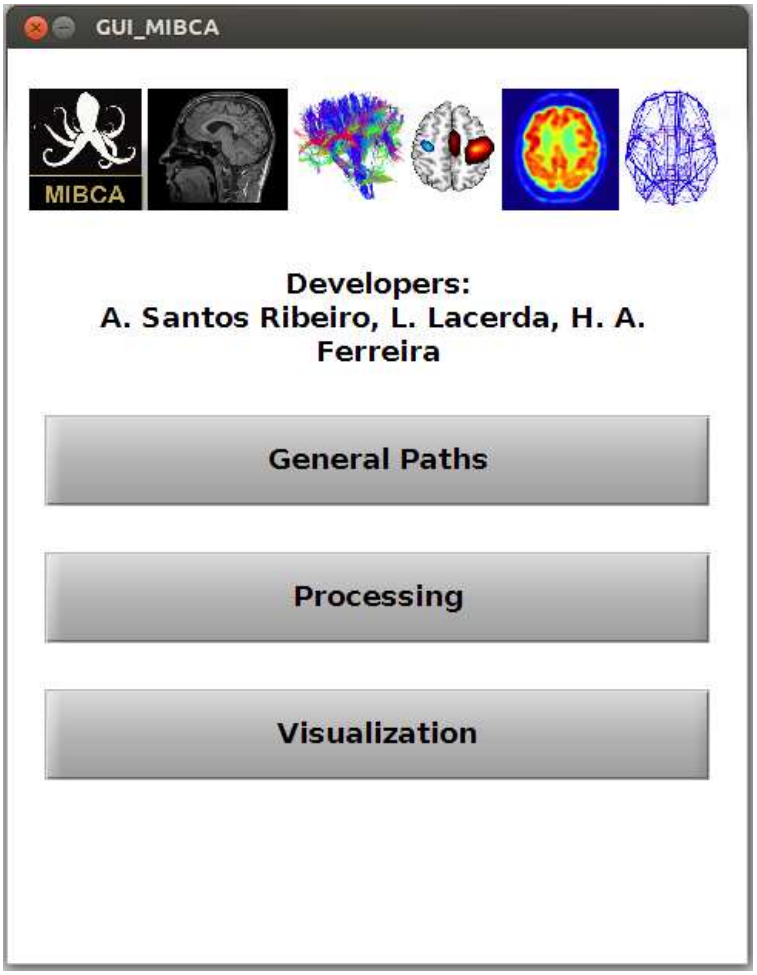

Figure 1.1: Main interface of the MIBCA toolbox.

- ***Subfolder sMRI identifier***: Most of the times the identification of the different modalities can be really complex. This is specially due to the amount of different techniques and image modalities. For example, only taking into account anatomical MRI, the technician may have acquired different Fields-Of-View (FOV), different contrasts, or repetitive measures. This makes a global automation truly complex. As such, the user needs to provide a identifier to the specific anatomical image to be used (ideally a high resolution T1-weighted image should be provided).
- ***Subfolder fMRI identifier***: If existent the user needs to provide a identifier for the fMRI data.
- ***Subfolder DTI identifier***: If existent the user needs to provide a identifier for the DTI data.
- ***Subfolder PET identifier***: If existent the user needs to provide a identifier for the PET.

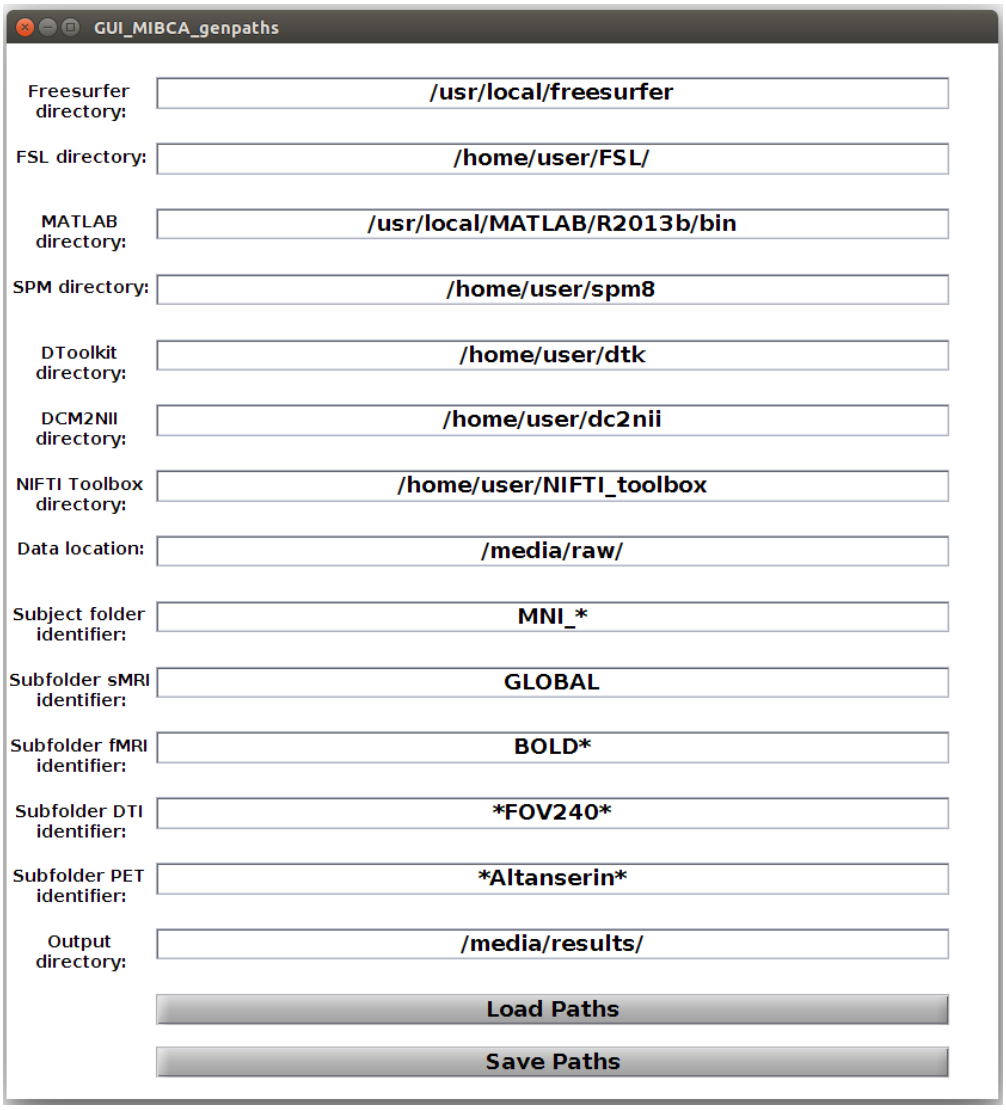

Figure 1.2: General Paths interface of the MIBCA toolbox.

Part II

Pre-Processing

Chapter 2

Processing interface

The pre-processing of all modalities by the toolbox is performed by selecting the processing button on the main interface. This leads the user to the main processing interface.

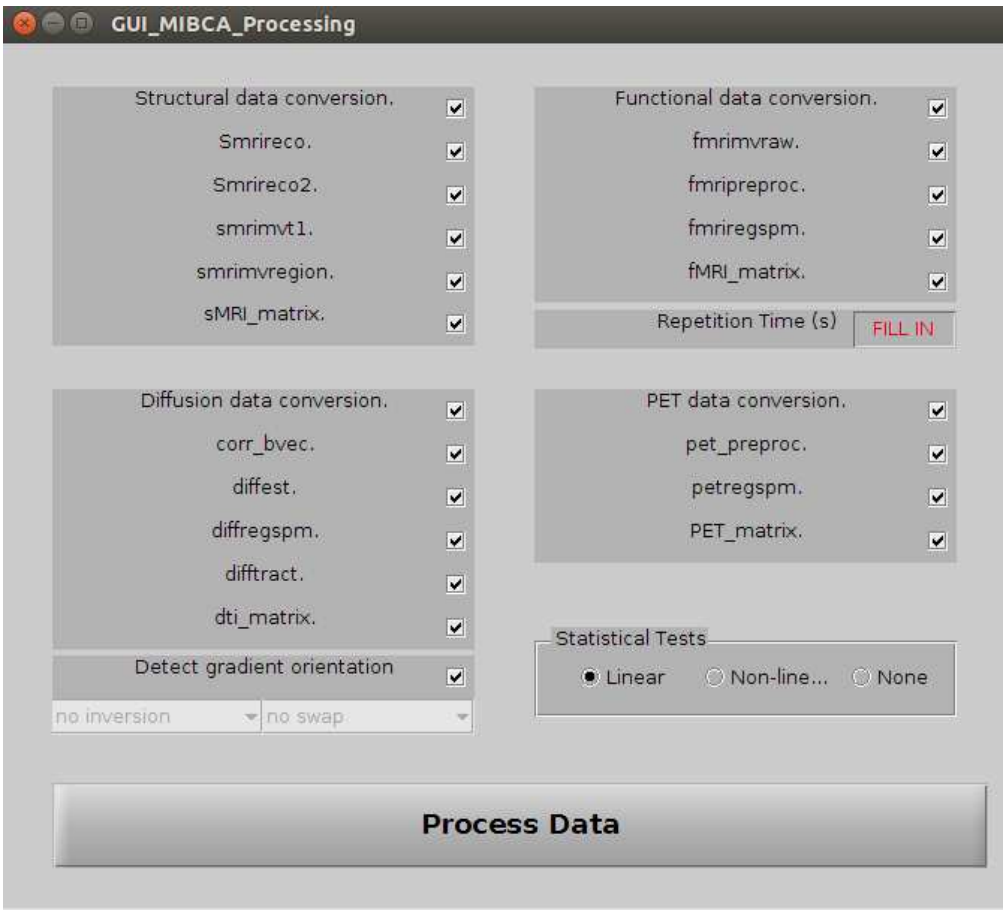

Figure 2.1: Processing interface of the MIBCA toolbox.

In this window the user must select which modalities to analyse, which pre-processing should be run for each, and which type of second analyses should be performed (group-level analysis).

Five main blocks are presented: Structural MRI, Diffusion MRI, functional MRI, and PET data analysis, and Statistical tests.

Chapter 3

Anatomical Magnetic Resonance Imaging

Contents

3.1 Image type Conversion

Converts different types of medical images to Nifti format. This is an essential step of the toolbox and must be performed prior to any pre-processing of the data.

To perform this step select the first checkbox of the processing window and leave the remain unchecked. An example data is provided in example_data and can be used to test this step. After selecting the appropriate box click on the process data button. The anatomical image should have been converted from the DICOM format in the folder ***raw*** to the Nifti format in the folder ***results***.

Table 3.1: Files before (Top) and after (Bottom) image conversion.

./raw/MNI_0590/GLOBAL/2006-09-28_13_48_11.0/S60766/
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125850304_1_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125850554_2_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125850695_3_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125850804_4_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125850945_5_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125851086_6_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125851554_7_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125851664_8_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125851804_9_S60766_I130046.dcm
ICBM_MNI_0590_MR_GLOBAL_br_raw_20081210125851898_10_S60766_I130046.dcm
./results/MNI_0590/sMRI/s002a001/
s002a001.nii.gz

3.2 Affine Registration, Segmentation, Intensity normalization

This step first registers the anatomical image to the Tailarach space, performs brain extraction, corrects for intensity inhomogeneities, segments into GM, WM and CSF and normalizes WM intensity to 150. This step takes approximately 20-30 minutes.

Images to be analysed must have already been converted to Nifti format and must be located in the target folder. Once images are in place select the second checkbox of the processing window (and the first one if not done already) and leave the remain unchecked. After running the user should see a brain.nii.gz file in the subject folder. Check the resulting file using any medical imaging visualization tool. The image produced should be a skull stripped, intensity normalized version of the original image, Figure, 3.1.

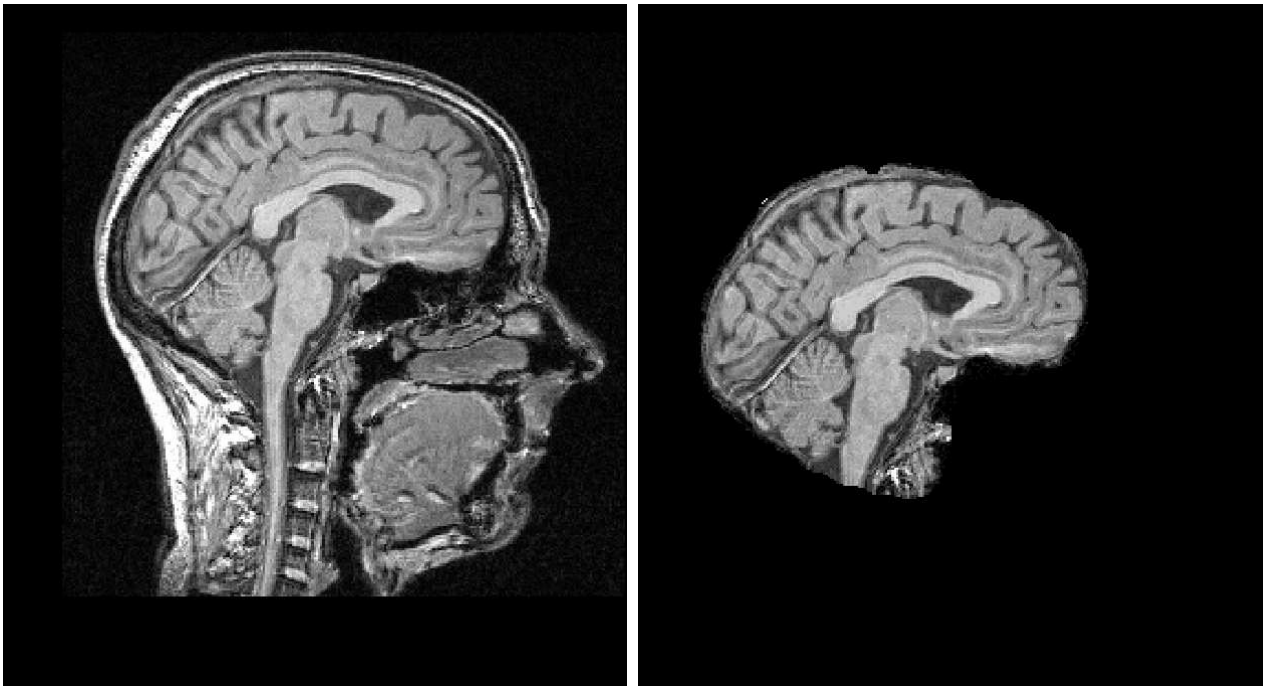

Figure 3.1: Left - Image after conversion to Nifti. Right - Image after Intensity normalization and skull stripping.

3.3 Non-linear Registration, Atlas mapping

This step performs non-linear volume and surface registration of the different subjects to the Freesurfer template, and uses this information for mapping a manual segmented atlas to each individual. This is an important step for every group-level analysis as it maps the different subjects to the same space. This is the most time consuming step of the toolbox and takes approximately 12-24 hours per subject.

As before, to run this step the previous processing lines must have been performed already. After running this step an $aparc+aseg.nii.gz$ file should have been created in the subjects folder. This image corresponds to the mapped brain regions into each subject's anatomy.

Due to the time consumption of this step, this image (along with the remain processed data) is provided on the example_dataprocessed folder.

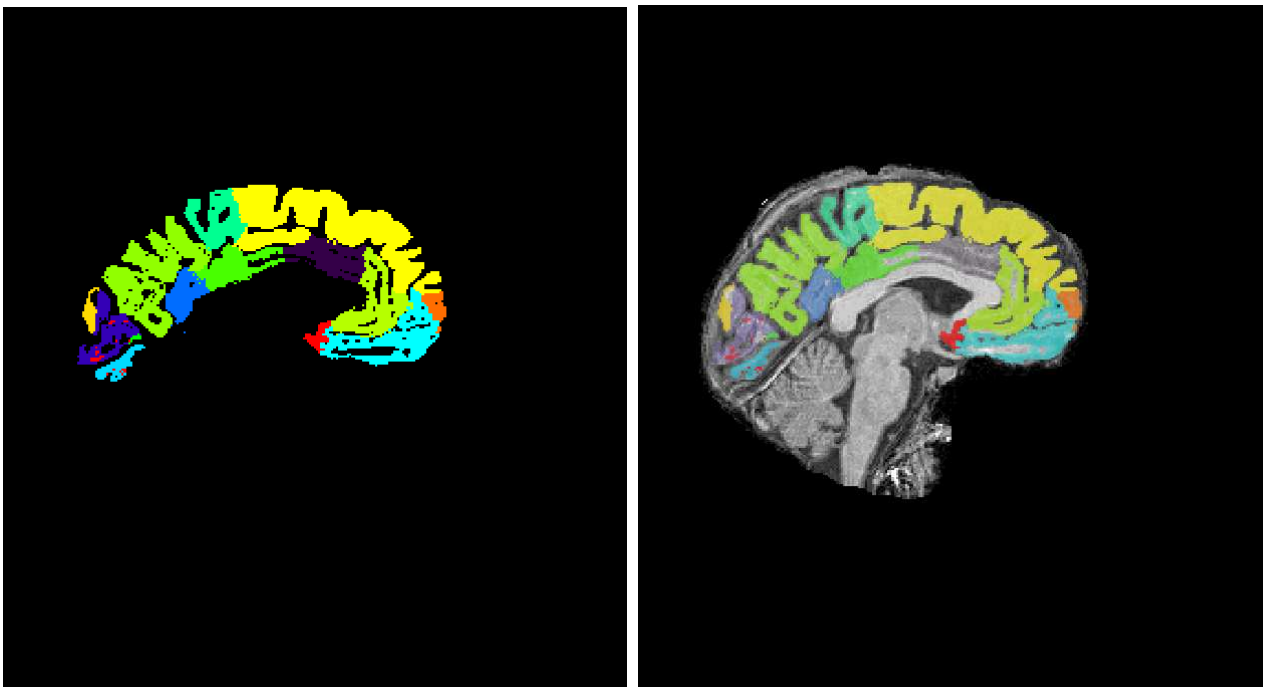

Figure 3.2: Atlas registered to the original space of the Anatomical T1w image.

3.4 Create Anatomical connectivity matrices

Along with the mapping of the atlas, the thickness, volume and area of the cortical regions, and the volume of the sub-cortical regions are calculated for each individual. This data is here used to produce anatomical connectivity matrices.

As before, to run this step the previous processing lines must have been performed already.

Chapter 4

Diffusion Weighted Magnetic Resonance Imaging

Contents

4.1 Image type Conversion

This step is similar to the Anatomical Image type conversion block (see above). The major difference is that DWI must further present a bval and a bvec files for the posterior analysis of the data. This is obtained directly from the DICOM files and must be given for all other image types with the following names X.bval, X.bvec.

Run this step with the example data to learn the structure of these files. **In the dataset provided the function used to convert dicom to nifti format *dcm2nii* is unable to identify the first 5 b0 in the data. In this case manually replace the first 5 values of the bval file to 0.**

4.2 Diffusion Tensor Estimation

MIBCA performs motion and eddy current correction prior to diffusion tensor estimation, through the FSL command *eddy_correct*. Diffusion tensor estimation is then performed with diffusion toolkit's *dti_recon* yielding apparent diffusion coefficient (ADC), Mean Diffusivity (MD), Fractional Anisotropy (FA), main eigenvector maps. Additionally an image with no diffusion weighting (B0) is also saved.

Table 4.1: Files before (Top) and after (Bottom) image conversion.

./raw/MNI_0590/ep2d_diff_susumu_FOV240/2006-09-28_15_07_47.0/S58093
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102354646_1_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102354755_2_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102354880_3_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102355021_4_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102355146_5_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102355286_6_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102355427_7_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102356239_8_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102356380_9_S58093_I124595.dcm
ICBM_MNI_0590_MR_ep2d_diff_susumu_FOV240__br_raw_20081030102356536_10_S58093_I124595.dcm
./results/MNI_0590/DTI/s016a001
s016a001.bval
s016a001.bvec
s016a001.nii.gz

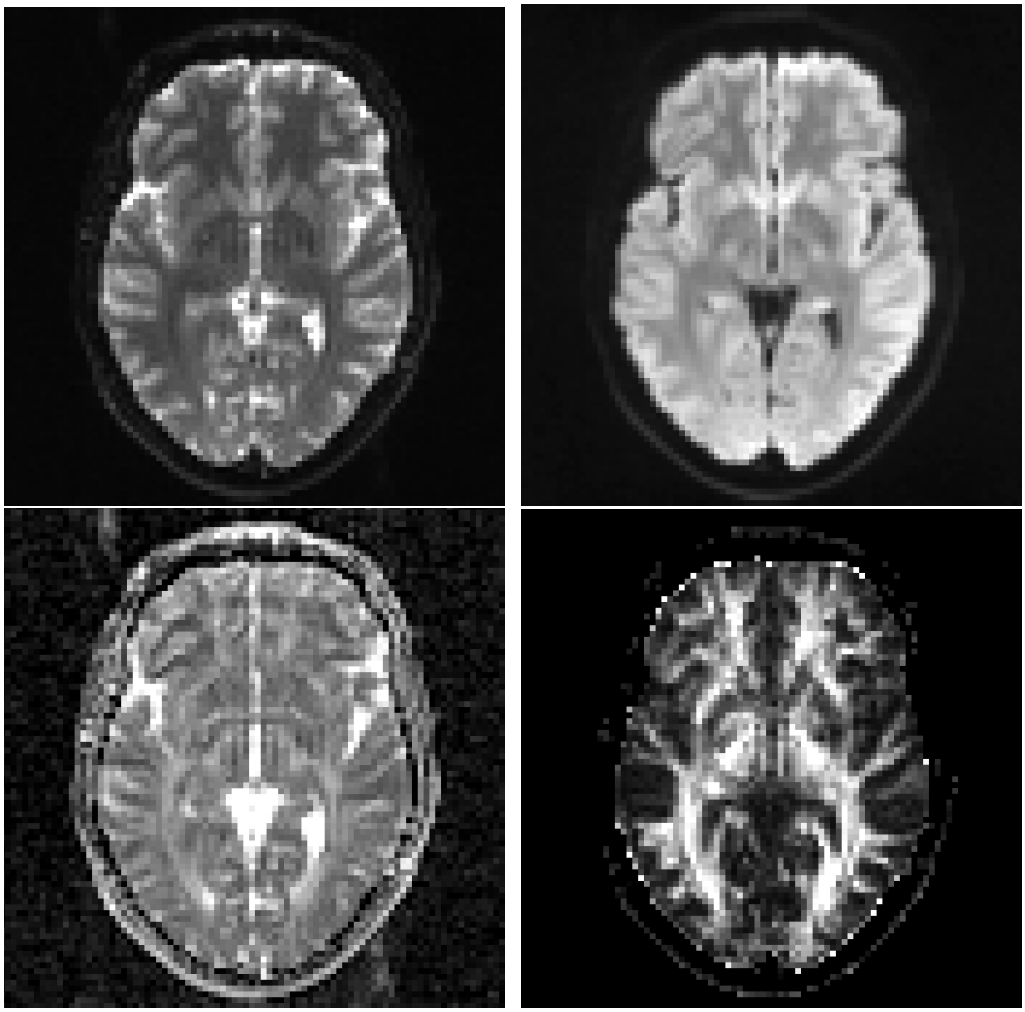

Figure 4.1: Some of the images generated by the Diffusion Tensor Estimation block. Top Left - B0; Top Right - DWI; Bottom Left - 1^{st} Eigenvector; Bottom Right - FA.

4.3 Registration to Anatomical MRI

The analyses performed by the toolbox are in the native space of each subject and modality. As such, the anatomical MRI and corresponding atlas are affine registered to the first B0 of the DWI data. This provides the atlas in the DWI space, therefore allowing further analysis for each ROI. This step is performed before the Fibre Tracking as a brain mask is created using the registered atlas, and provided to the Fibre Tracking algorithm.

After running this step the file $aparc + aseg2DTI.nii.gz$ should be present in the subjects folder.

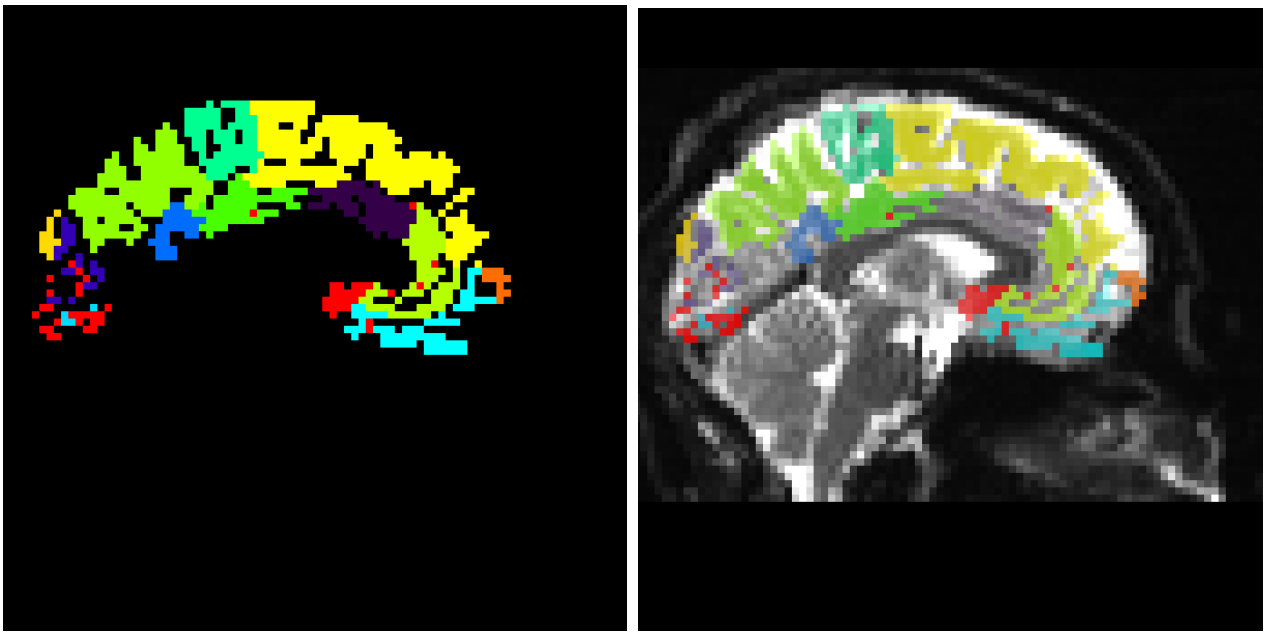

Figure 4.2: Structural atlas registered to the B0 image.

4.4 Fibre Tracking

Once again using diffusion toolkit, fibre tracking is achieved by applying the function *dti_tracking*. An interpolated streamline method of fixed step-length and a deterministic tractography algorithm were chosen for this particular analysis. To account for inversion or swapping of the gradient table used for tensor estimation and consequent tractography reconstruction, this information can be either provided by the user, or estimated by an in-house algorithm. The generated track file is then smoothed with the *spline_filter* (Diffusion Toolkit) function and loaded into MATLAB.

4.5 Create Diffusion connectivity matrices

The loaded track file is used to calculate the number of fibers, mean fiber length and mean fiber orientation between pairs of ROIs, thus providing 3 different matrices. The matrix of the number of fibers is defined as the structural connectivity matrix (DTI, in this occasion). From the obtained connectivity matrices, graph theory metrics can be extracted: node degree, cluster coefficient, and small-worldness.

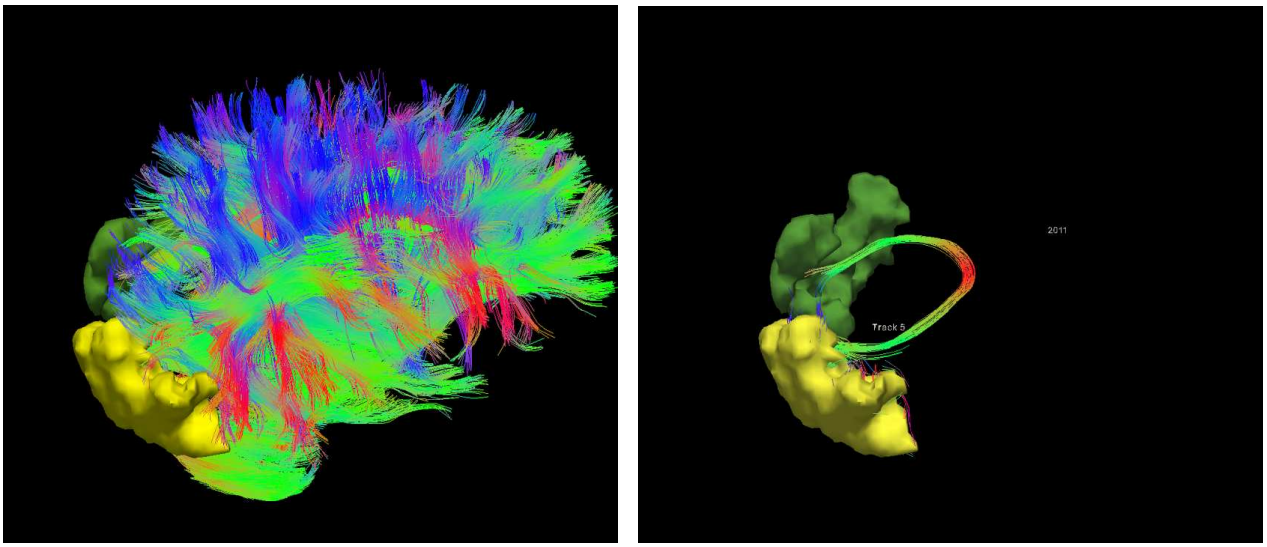

Figure 4.3: Fibre tracking derived from the processed data. These images were created by the TrackVis visualization tool, using the track information and the registered atlas generated by MIBCA.

Chapter 5

Functional Magnetic Resonance Imaging

Contents

5.1 Image type Conversion

This step is similar to the Anatomical Image type conversion block (see above). The major difference is that for fMRI (as well as DWI) a 4D Image is generated where the 4th component corresponds to the number of images of the acquired timeseries (number of TR's).

Run this step with the example data to learn the structure of these files.

Table 5.1: Files before (Top) and after (Bottom) image conversion.

./raw/MNI_0590/BOLD_MOSAIC_64_resting_st./2006-09-28_13_48_11.0/S59595
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854142_1_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854282_2_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854376_3_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854516_4_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854609_5_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854718_6_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854812_7_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153854921_8_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153855030_9_S59595_I127412.dcm
ICBM_MNI_0590_MR_BOLD_MOSAIC_64_resting_st._br_raw_20081118153855138_10_S59595_I127412.dcm
./results/MNI_0590/fMRI/s013a001
s013a001.nii.gz

5.2 Spatial Smoothing & Temporal Filtering

MIBCA first performs spatial smoothing of the fMRI data with an 8mm Gaussian kernel. This step is important to increase the signal-to-noise ratio, as well as, to improve the posterior rigid registration to the structural image. Temporal filtering is further applied to the data to remove non-brain signal, such as cardiac and respiratory signals.

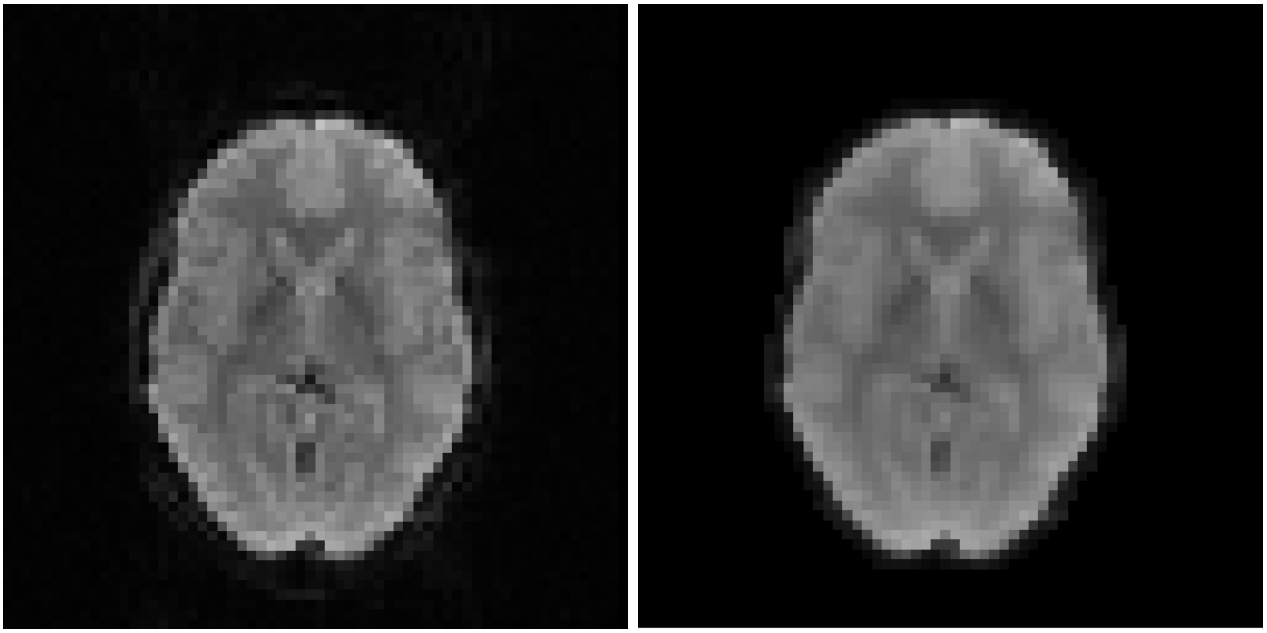

Figure 5.1: Left - fMRI before spatial and temporal filtering; Right - After filtering.

5.3 Registration to Anatomical MRI

As well as in the DWI analysis, the fMRI analysis is performed in the original space of the acquired fMRI. As such, the anatomical MRI and corresponding atlas are affine registered to the time average BOLD signal. This provides the atlas in the fMRI space, therefore allowing further analysis for each ROI.

After running this step the file $aparc + aseg2fMRI.nii.gz$ should be present in the subjects folder.

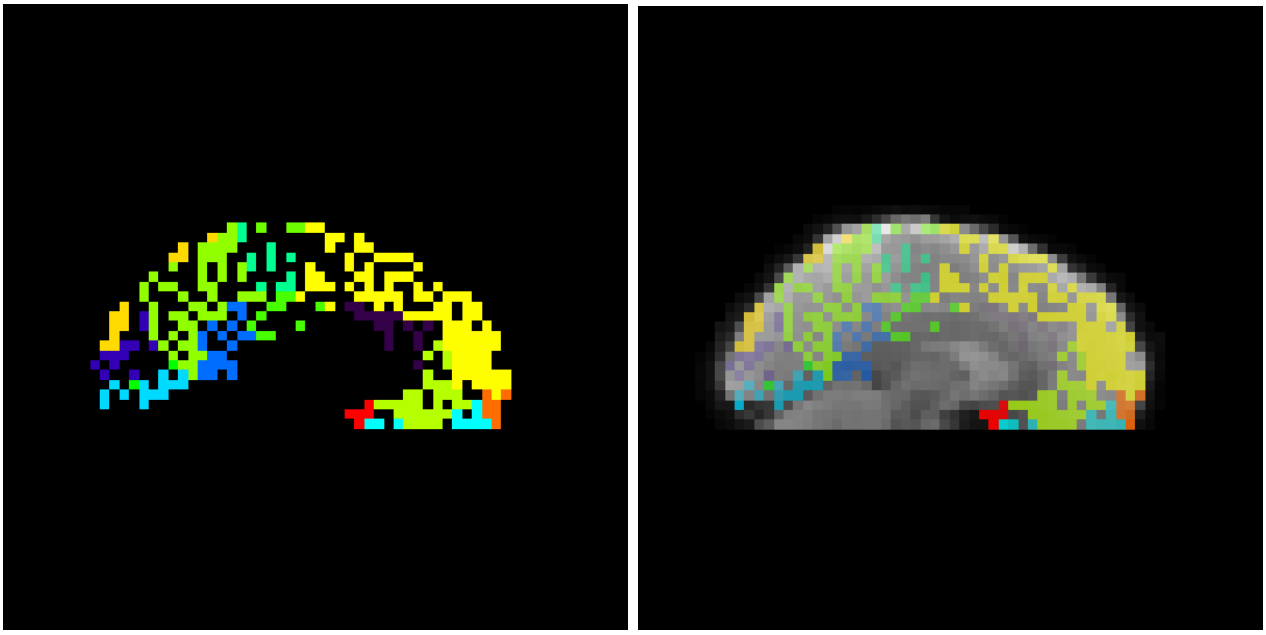

Figure 5.2: Structural atlas registered to the fMRI space.

5.4 Create fMRI connectivity matrices

The $filtered\_fmri.nii.gz$ and the $aparc + aseg2fMRI.nii.gz$ are used to calculate the mean activity for each ROI, pairwise ROI correlation (functional connectivity), and pairwise ROI Granger causality (effective connectivity).

From the functional connectivity matrices, the graph theory metrics: node degree, cluster coefficient, and small-worldness are further calculated.

Chapter 6

Positron Emission Tomography

Contents

6.1 Image type Conversion

This step is similar to the fMRI Image type conversion block (see above). The major differences is that instead of the TR's the 4D component refers to the number of reconstructed temporal volumes.

Run this step with the example data to learn the structure of these files.

6.2 Spatial Smoothing

MIBCA performs spatial smoothing of the PET data with an 8mm Gaussian kernel. This step is important to increase the signal-to-noise ratio, as well as, to improve the posterior rigid registration to the structural image.

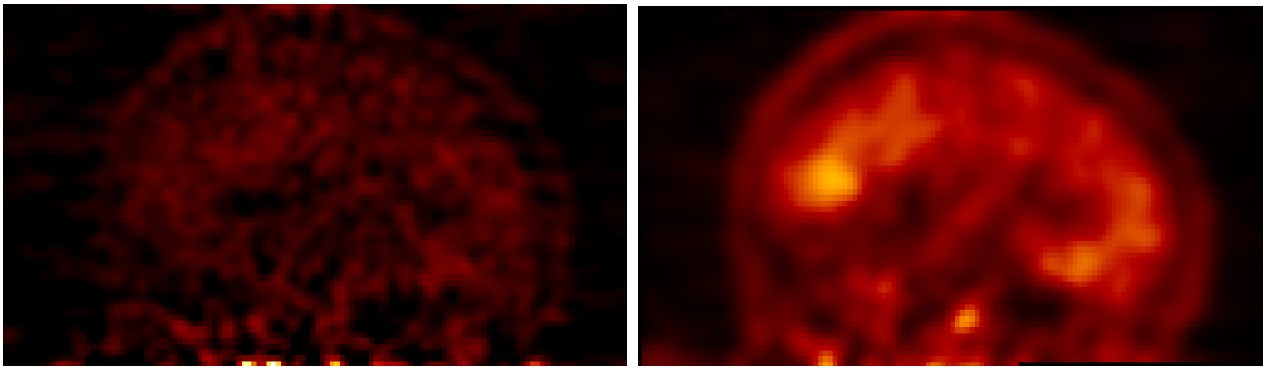

Figure 6.1: Left - PET before smoothing and motion correction; Right - After smoothing and motion correction.

6.3 rSUV Estimation

MIBCA first calculates the summed temporal PET image to obtain the total activity. Next, the relative Standard Uptake Values (rSUV), are calculated based on a predefined reference region. By default the cerebellum is used as the reference region.

6.4 Registration to Anatomical MRI

As well as in the previous analysis, the PET analysis is performed in the original space of the acquired PET image. As such, the anatomical MRI and corresponding atlas are affine registered to the summed PET image. This provides the atlas in the PET space, therefore allowing further analysis for each ROI.

After running this step the file $aparc + aseg2PET.nii.gz$ should be present in the subjects folder.

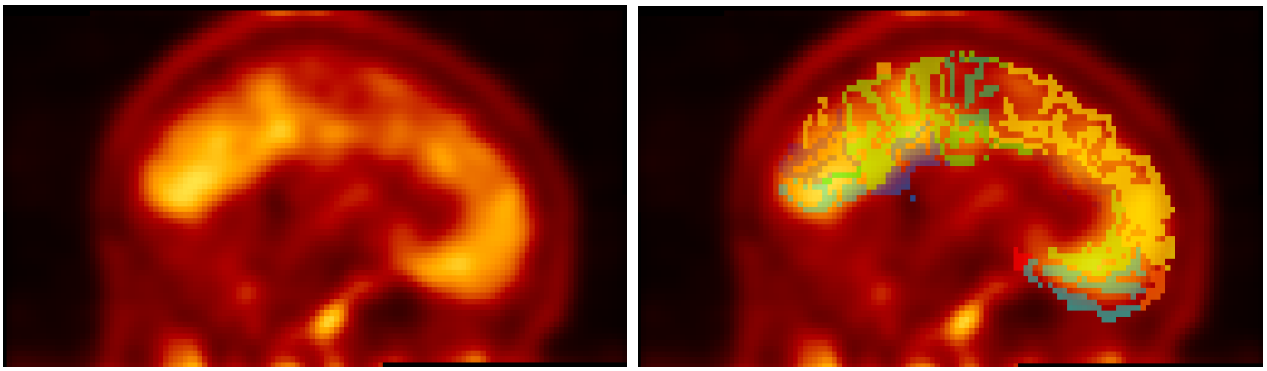

Figure 6.2: Left - Summed PET image; Right - Atlas registered to summed PET.

6.5 Create PET connectivity matrices

The $summed\_PET.nii.gz$ and the $aparc + aseg2PET.nii.gz$ are used to calculate the mean activity, and rSUV for each ROI.

From the functional PET connectivity matrices, graph theory metrics can be obtained but are not given by default.

Part III

Visualization

Chapter 7

General visualization interface

Contents

Figure 7.1: Main GUI of the visualization tool.

7.1 Loading data

The data produced by the processing interface can further be analysed and visualized by the MIBCA toolbox. The data is located at the main directory of the *output directory* defined in the *General Paths*. To load the data, one first needs to select the processed ROIs and then the data itself. To select the labels, click on the *load labels* field in the *File* menu and choose the file *labels_MIBCA.mat* in the main directory of the MIBCA toolbox. Afterwards, select the desired data that you wish to visualize, through *load data* field in the *File* menu.

In the example data the file *all_group_data.mat* corresponds to the individuals connectivity analysis, the *mean_groups_data2all.mat* to the average group connectivity analysis, and the *ttest_groups_data2Old − Youth.mat* to the statistical analysis performed. In the example provided, we will analyse the average group

connectivity of the following metrics: number of fibers (DTI), DTI node degree, fMRI functional connectivity, fMRI node degree, cortical thickness, and cortical and subcortical volume.

First select the average group connectivity file *mean_groups_data2all.mat*. Then click:

- DTI → fibre (2D matrix window)
- DTI → fibre threshold(2D matrix window)
- DTI → node degree (1D matrix window)
- fMRI → functional connectivity (2D matrix window)
- fMRI → functional connectivity threshold(2D matrix window)
- fMRI → node degree (1D matrix window)
- aMRI → cortical thickness (1D matrix window)
- aMRI → all volume (1D matrix window)
- Finalize the loading phase by clicking on the *Done* button.

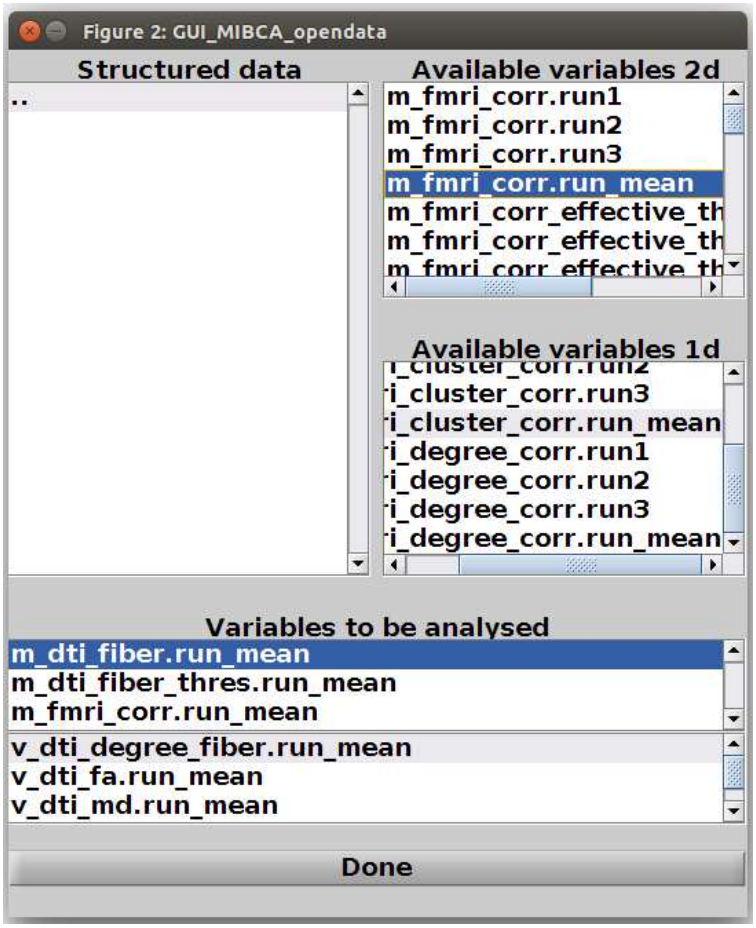

Figure 7.2: Loading data interface.

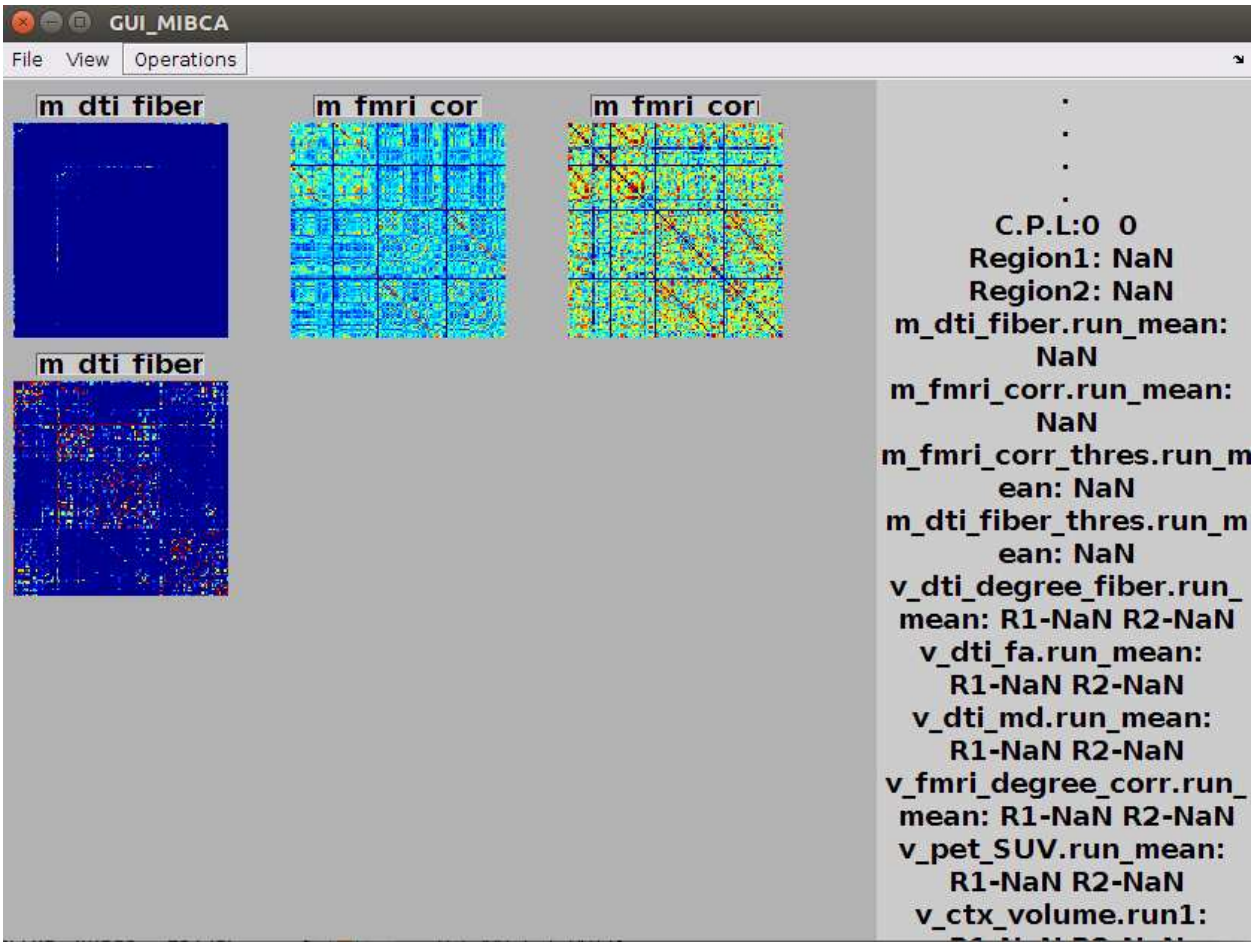

Figure 7.3: Default loaded data from the provided example dataset.

7.2 ROI selection

MIBCA loads by default all the ROIs generated by freesurfer. Most of the times we are only interested in the cortical ROIs, or even a specific subset of these. To confine the analysis to the desired ROIs, the user can remove the undesired ROIs through the ROI selection in the tool menu.

In the example data, after loading the matrices, go to the *Operations* menu and select the *Regions* subfield. Now on the right window select the following ROIs to exclude: To make selection easier the user can further use the CTRL and ALT shortkeys.

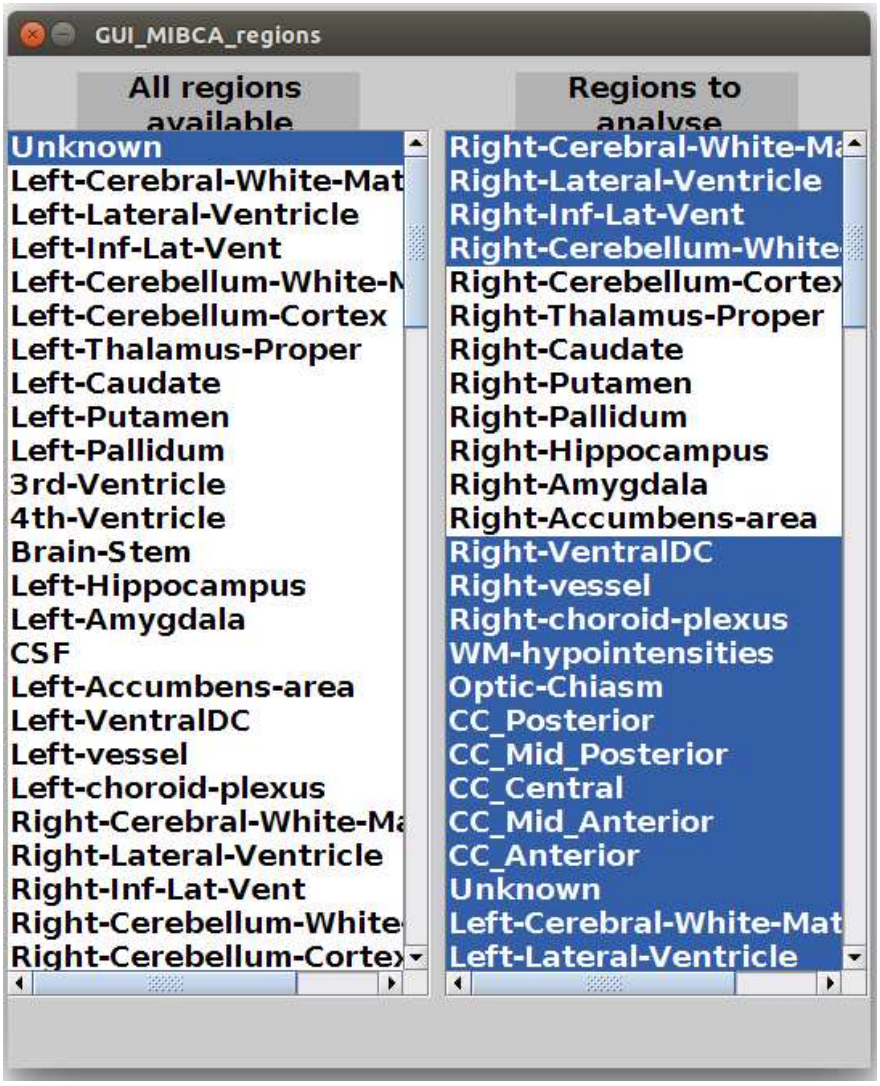

Figure 7.4: ROI selection interface.

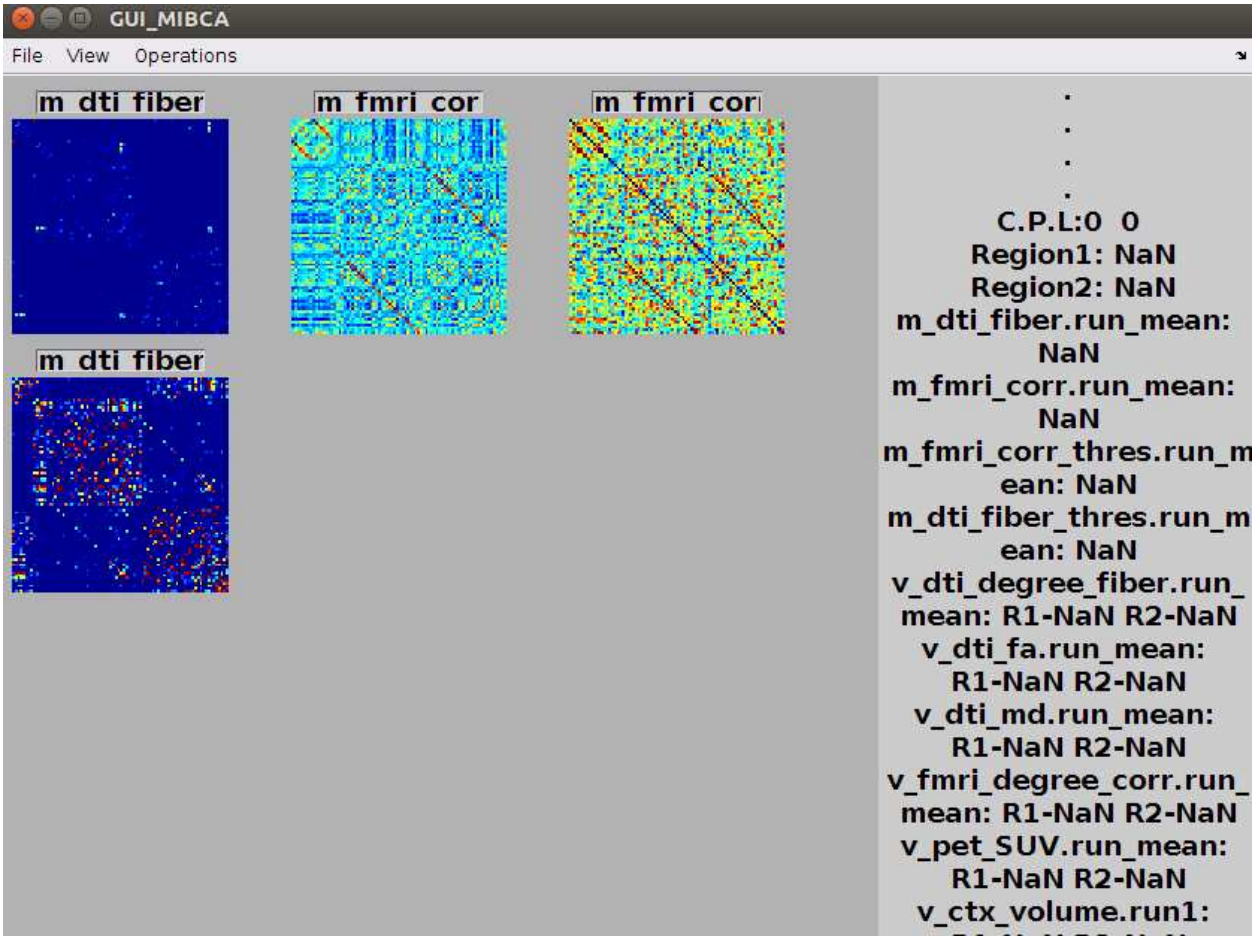

Figure 7.5: Data after removing undesired ROIs.

7.3 Renaming matrices

MIBCA automatically assigns the field names when loading the different matrices to the respective matrix. This can be confusing and therefore these matrices can be renamed after loading. To accomplish this click on the name of the matrix to be renamed and write a new one.

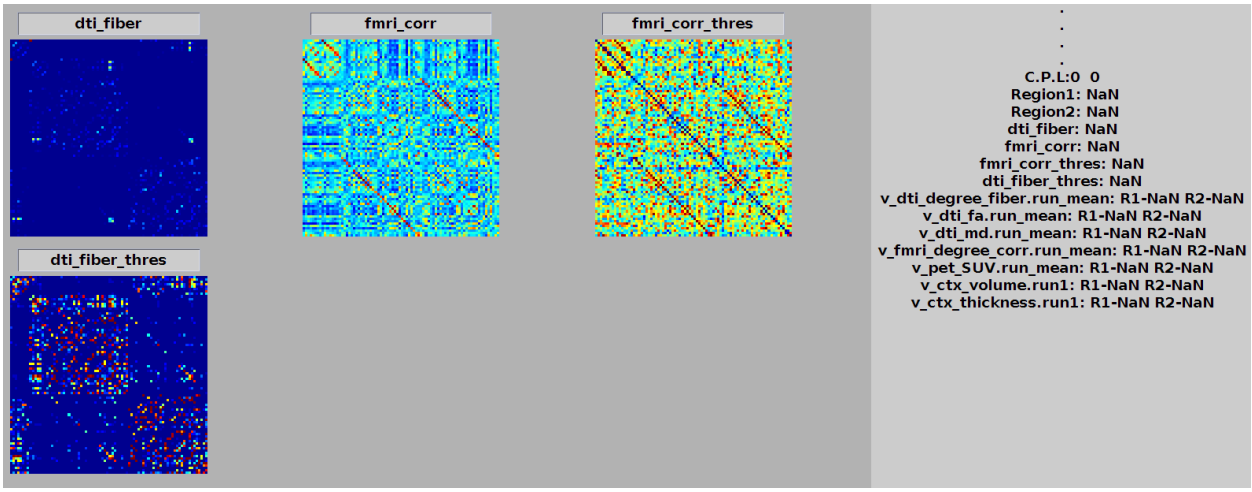

Figure 7.6: Renamed matrices.

7.4 Matrices operations

The visualization tool of MIBCA allows further combinations of the original matrices obtained through the processing interface. For example the user may create an anatomical-functional matrix by combining both the anatomical matrix derived by DTI and the functional matrix derived by fMRI, or binarize a certain matrix. To accomplish this, in the *Operations* menu a *Operations* sub-field is provided.

In this example we will binarize the *DTI mean matrix* and the *fMRI mean matrix* such as structural connection and functional connection, respectively, is present in at least 80% of the subjects between each two ROIs. Further, these two matrices will be combined to study the fMRI connectivity that can be explained through direct or mediated connections.

1. Select the sub-field *Operations* from the *Operations* menu.
2. On the bottom text-field insert the following instructions followed by the Enter key:
   (a) $dti\_fiber\_thres > 0.8$
   (b) $fmri\_corr\_thres > 0.8$
3. Rename these two matrices to $dti\_fiber\_0.8$ and $fmri\_corr\_0.8$, respectively.
4. Select the sub-field *Operations* from the *Operations* menu again.
5. On the bottom text-field insert the following instructions followed by the Enter key:
   (a) $(dti\_fiber\_0.8).*(fmri\_corr\_0.8)$
   (b) $(\ dti\_fiber\_0.8).*(fmri\_corr\_0.8)$
6. Rename these two matrices to $fmri\_direct$ and $fmri\_mediated$, respectively.

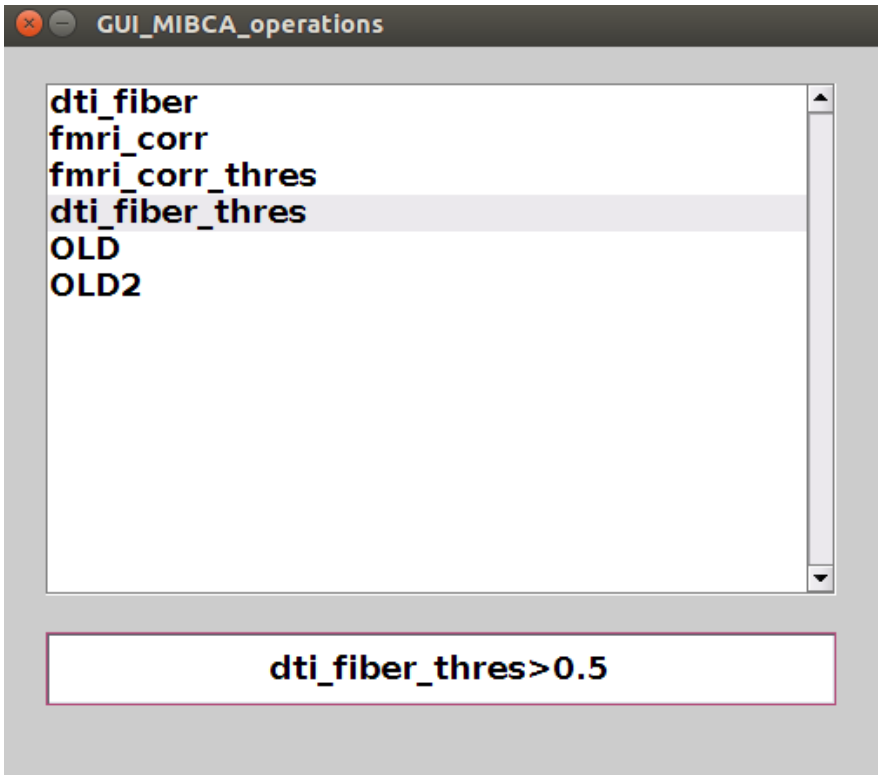

Figure 7.7: Operations interface.

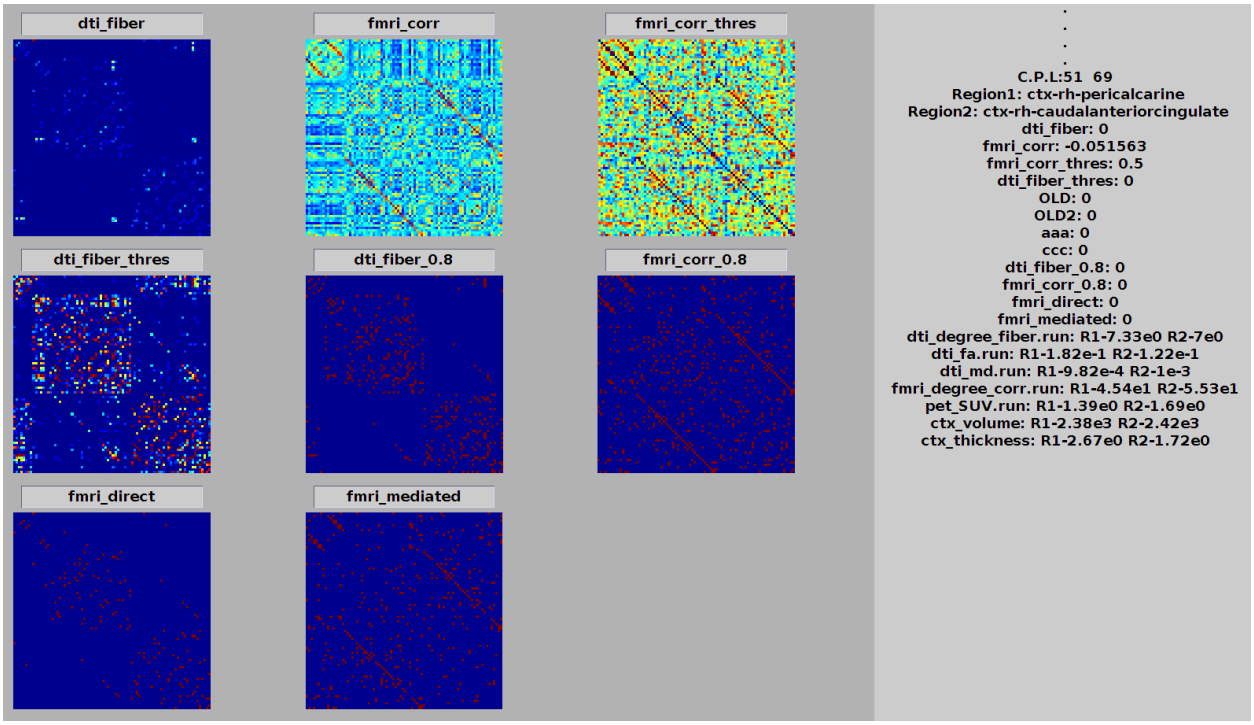

Figure 7.8: Matrices generated through the operations interface.

7.5 Matrices visualization

Matrices can be visualized through the matrix interface, connectogram or 3D-graph (see below). The matrix visualization interface is the most simple of the three visualizations and is intended to provide a quick visualization of the general shape of the connectivity matrices. By moving the mouse over a desired matrix position, the corresponding connectivity metrics for all the generated metrics are presented at the right.

To hide/show matrices from view, the user can check/uncheck the respective matrix in the sub-field *2D data* under the *View* menu. A jet color scheme is presented for all matrices, where cooler colours refer to low values, while warmer colours refer to high values. Different colormaps can further be selected in the *View* menu, Figure 7.9.

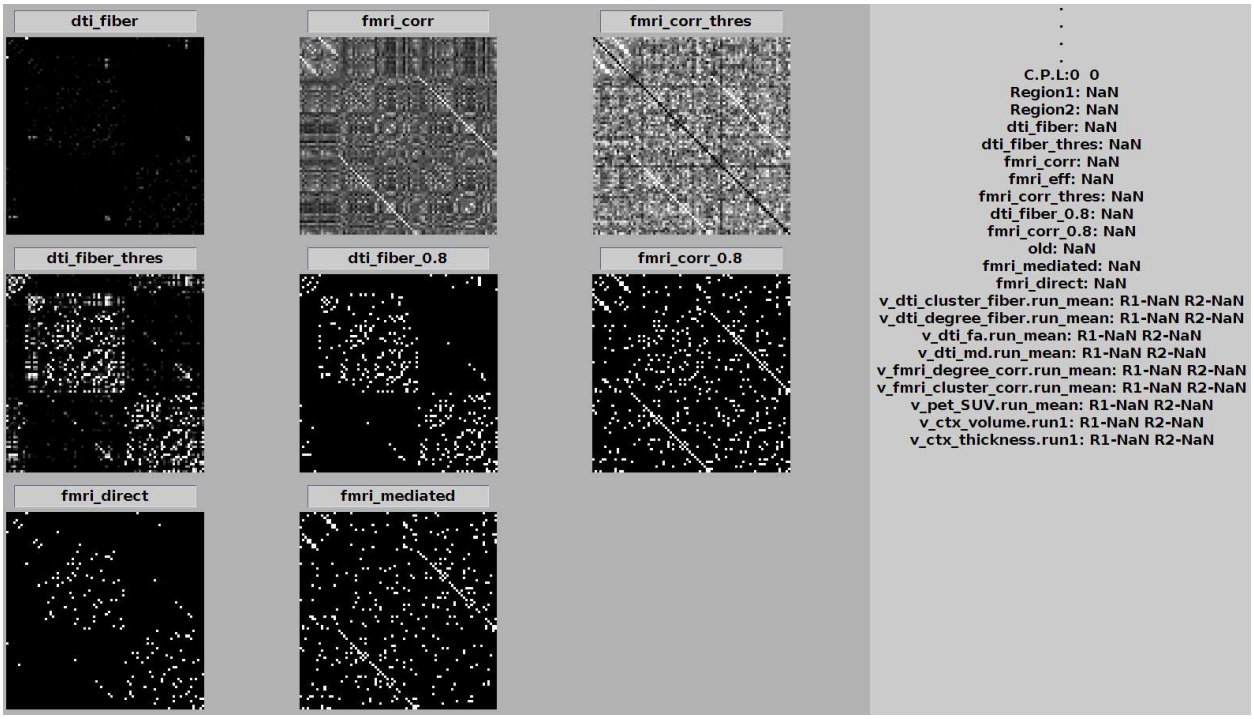

Figure 7.9: Matrix visualization with a gray colormap. Other colormaps are also available: Jet, Hsv, Hot, Cool, Spring, Summer, Autumn, Winter, Gray, Bone, Copper, Pink, Lines. See matlab colormap function for further description of each.

Chapter 8

Connectogram visualization

Contents

The connectogram visualization allows the user to transform and combine in the same interface, both the 2D metrics (connectivity matrices) and the 1D metrics. This interface allows an easier and specific visualization of connections between different ROIs. The default connectogram is organized as follows: the lower left ring presents the subcortical regions, the lower right ring the left cortical regions, and the top ring the right cortical regions.

In here we will analyse the previous generated matrices, namely, $dti\_fiber\_0.8$, $fmri\_corr\_0.8$, $fmri\_direct\_0.8$ and $fmri\_mediated\_0.8$, and the connectivity metrics $ctx\_thickness$, ctx_volume, PET_SUV, dti_md, dti_fa, dti_degree.

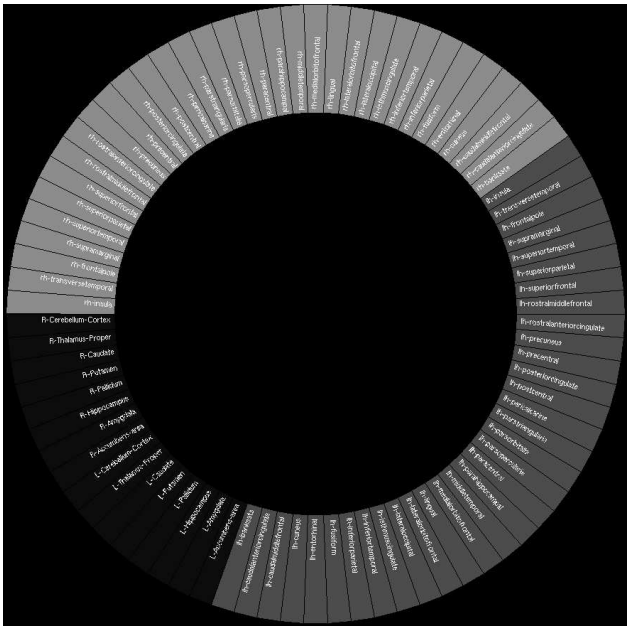

Figure 8.1: Connectogram view.

8.1 Modality-specific MRI connectivity data

In this example we will analyse the DTI and fMRI connectivity matrices independently.

1. Second click on the mouse and in functional data select $dti\_fiber\_0.8$
2. Second click on the mouse and in *add circle* select in the following order: $ctx\_thickness$, ctx_volume, pet_SUV, dti_md, dti_fa, dti_degree.

A full connectogram containing three rings and lines connecting the different ROIs should now be visible.

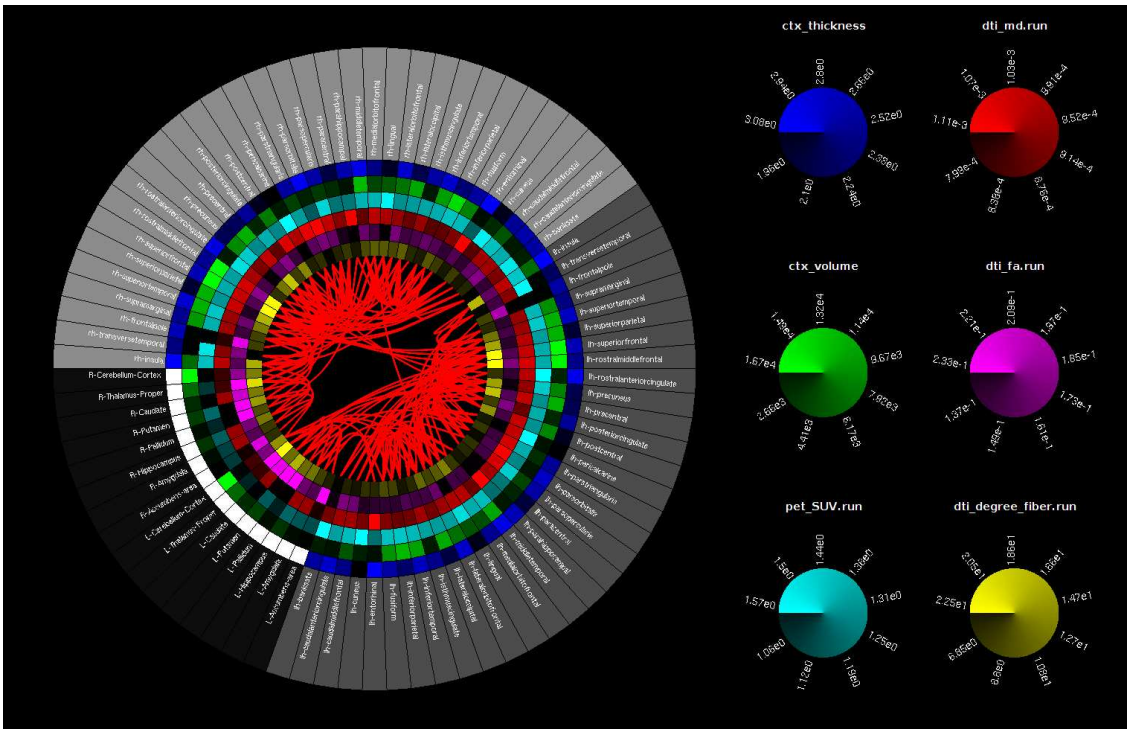

Figure 8.2: Structural connectogram with 1D rings: $ctx\_thickness$, ctx_volume, pet_SUV, dti_md, dti_fa, dti_degree, and 2D lines: $dti\_fiber\_0.8$.

At a first glance it can be seen that structural connectivity has an intra-hemispherical configuration (i.e. high number of intra-hemispherical connections with fewer inter-hemispherical connections). On a less extent, brain symmetry can be observed in the connectivity metrics (e.g. a high node degree is observed for both rostralmiddlefrontral and superiorfrontral bilaterally with lower values for the remain ROIs, or a low cortical thickness for both pericalcarine and postcentral bilaterally).

To further enhance the description of the connectivity matrix the user can observe the clusters (modules) within the matrix, by second click on the mouse and in *Add Modularity* select $dti\_fiber\_0.8$. Here we can observe that 5 modules are present within the structural connectivity matrix (5 colours), with modules dark blue and purple mainly represented in the left hemisphere, modules green and light blue mainly representing the right hemisphere, and module red shared between the two hemispheres.

To further analyse which ROIs and modules are connecting both hemispheres, the user can hover the mouse over a particular ROI and observe the respective connections. In this example, the identified inter-hemispherical connections are: *superiorparietal*, *superiorfrontal*, *rostralmiddlefrontal*, *precuneus*, *posteriorcingulate*, *superiororbitrofrontal*, and the connecting modules are the blue, red and purple.

To analyse the functional data, second click on the mouse and in *Add Connection* select $fmri\_corr\_0.8$. To observe the modules select $fmri\_corr\_0.8$, in the *Add Modularity* submenu. Here we can see that both intra- and inter-hemisphericas ROIs are well represented. Further, modules seem to be inter-hemispherical, instead of intra-hemispherical as seen in the structural data.

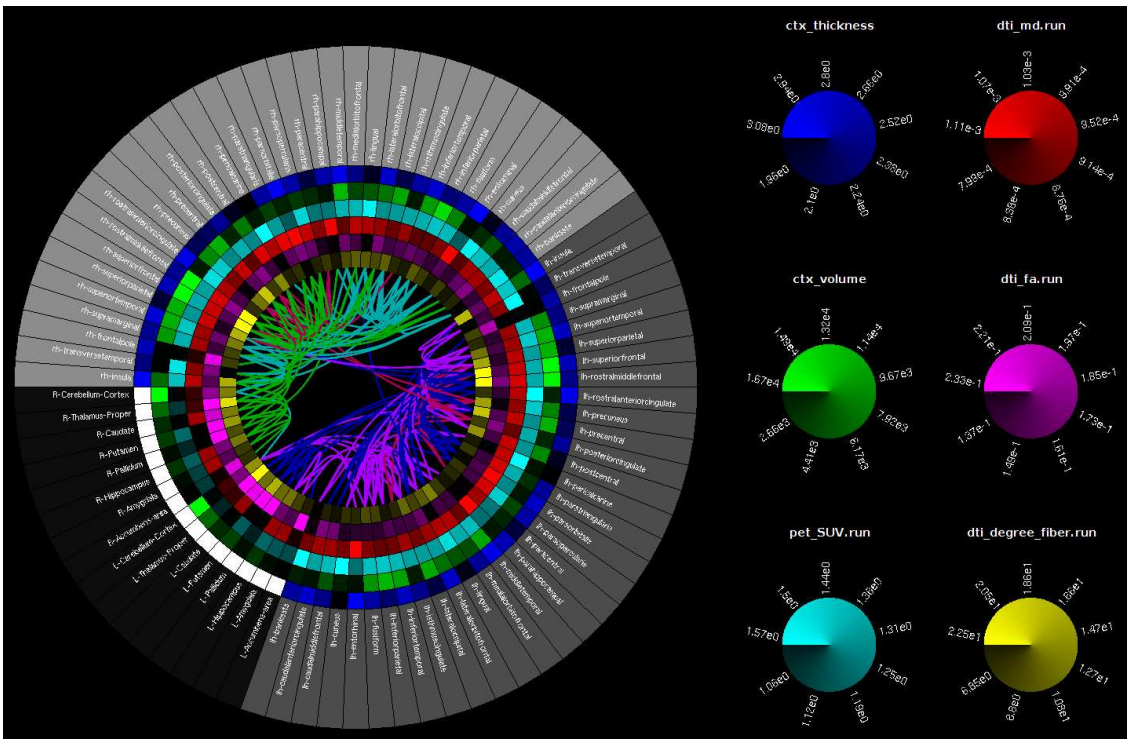

Figure 8.3: Modularity of the structural connectogram.

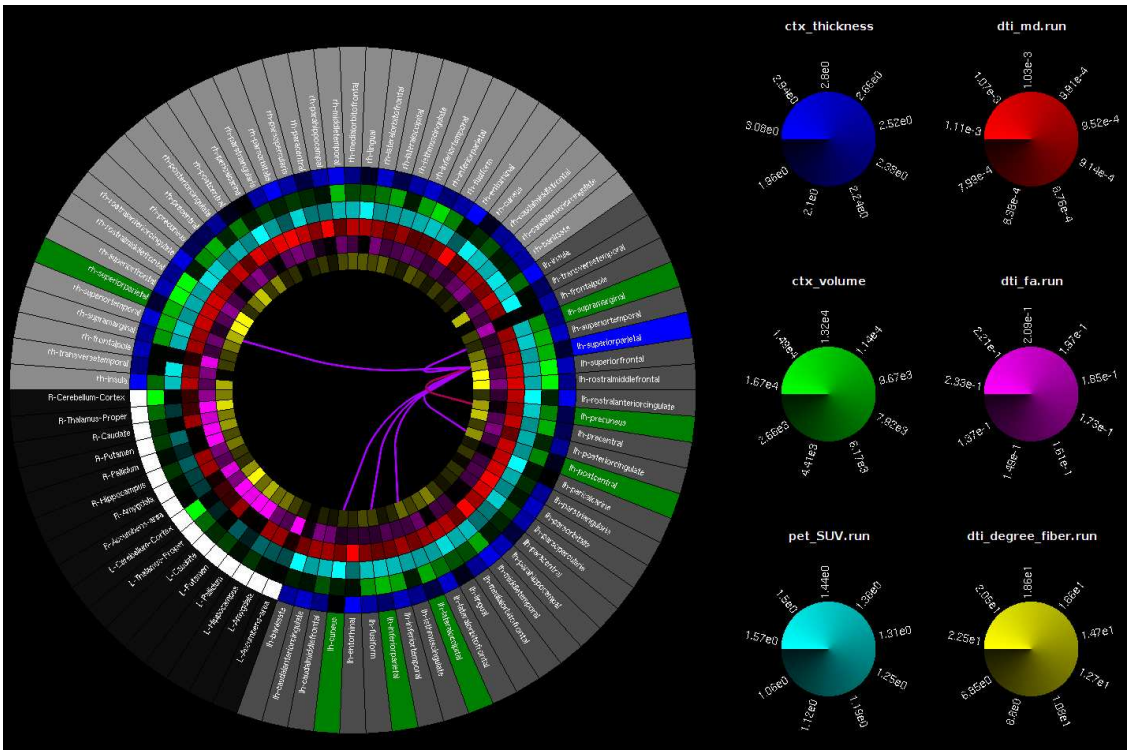

Figure 8.4: Structural connectogram with the mouse positioned over the superiorfrontal region (blue ROI). Green ROIs highlight regions that are structurally connected with the superiorfrontal region.

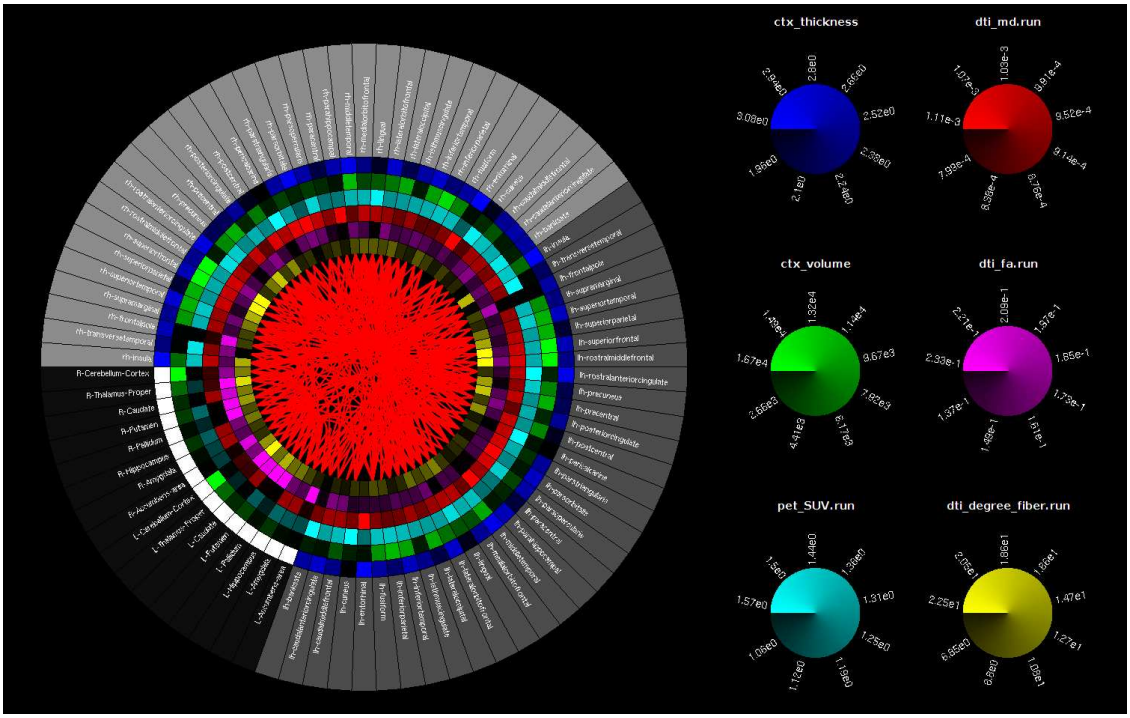

Figure 8.5: Functional connectogram.

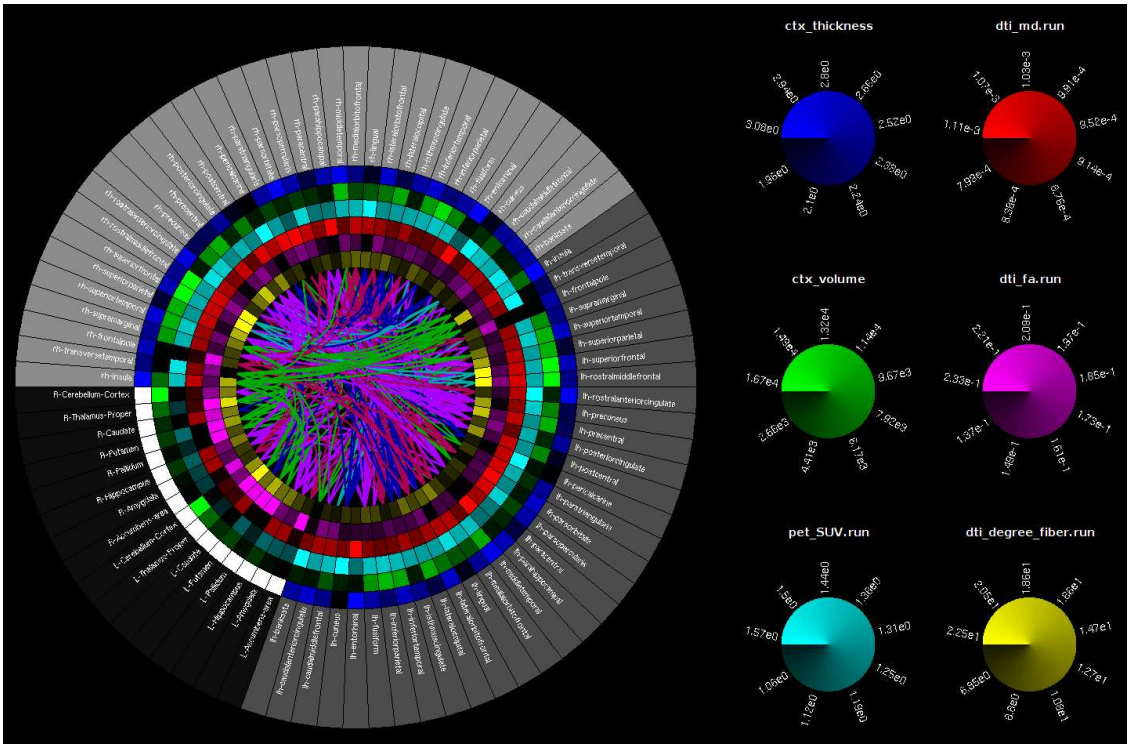

Figure 8.6: Modularity of the functional connectogram.

8.2 Multimodal MRI connectivity data

To decompose the functional connectivity matrix into ROIs directly connected through fibres, and mediated through different regions, in the previous chapter the matrices $fmri\_direct\_0.8$ and $fmri\_mediated\_0.8$ were generated. Here we analyse these matrices through the connectogram. As can be seen intra-hemispherical functionally connected regions tend to directly connected to each other, while inter-hemispherical functionally do not present this direct connection, suggesting that they may be mediated through other regions. This supports the idea of small-worldness of neural networks.

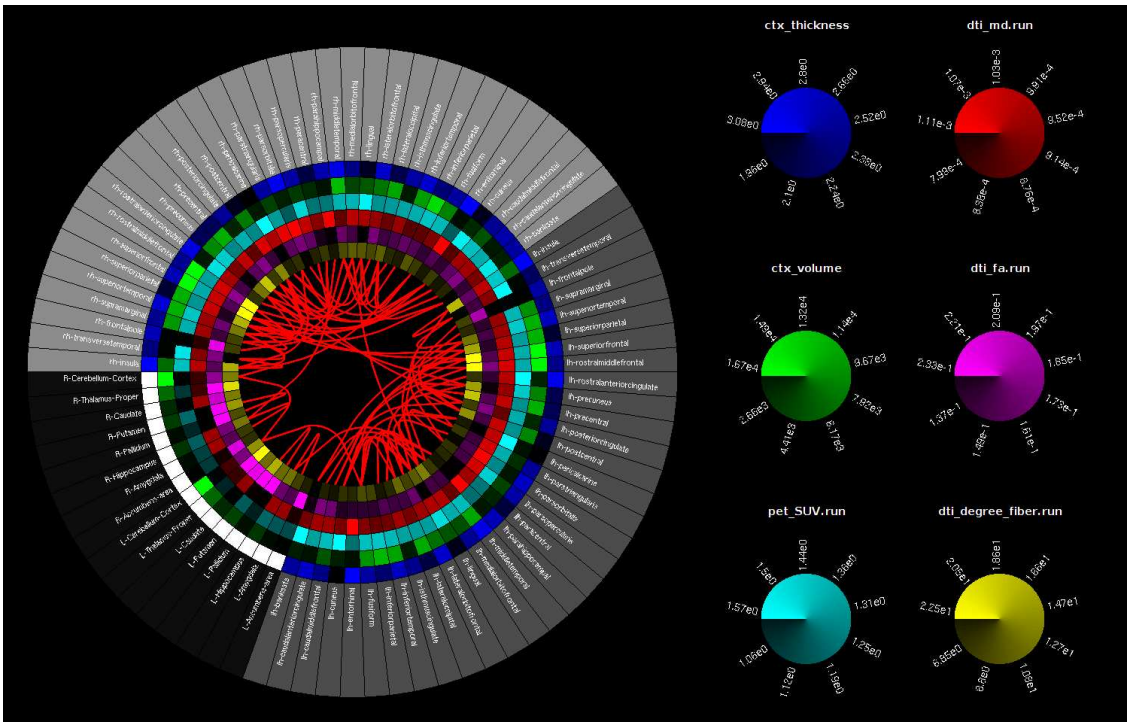

Figure 8.7: Functionally directed connectogram.

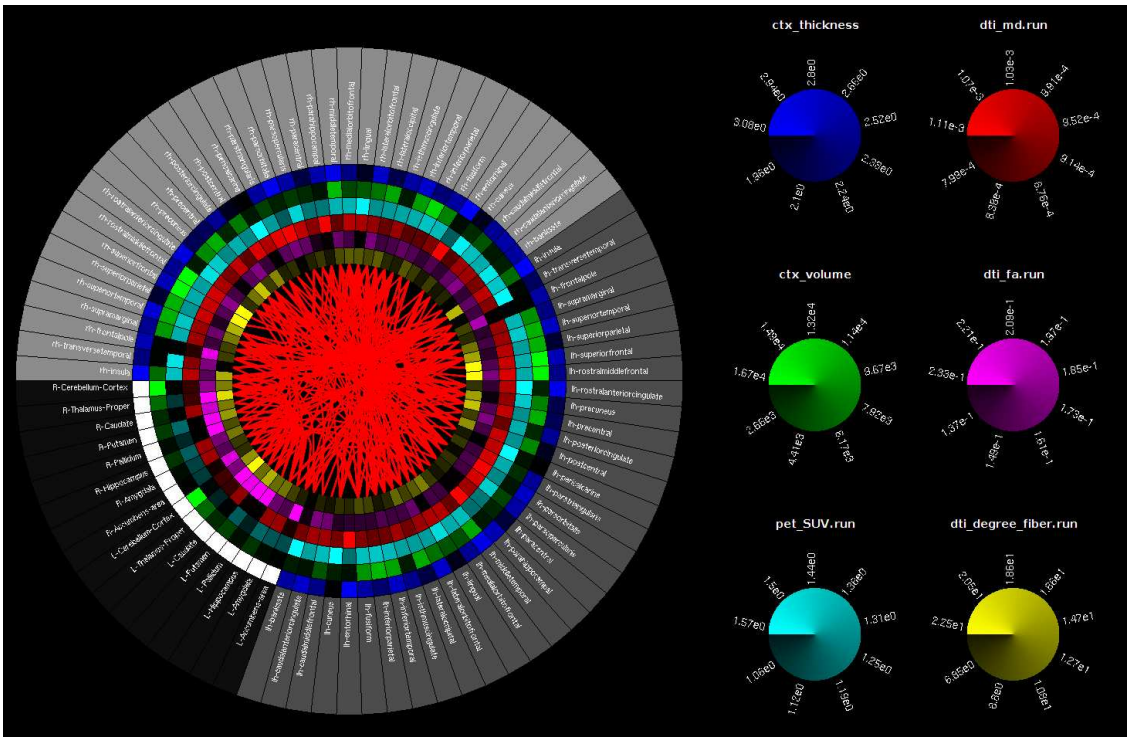

Figure 8.8: Functionally mediated connectogram.

Chapter 9

3D-Graph visualization

Contents

To further enhance visualization a 3D-Graph view can also be generated with the processed data. This visualization allows a better spatially visualization of the connectivity between different ROIs, comparatively with both the matrix and connectogram views.

9.1 Loading data

To use this interface the user first needs to select the anatomical reference to position the different ROIs. This can be any of the processed subjects. To select the reference volume click on *Load* and choose the appropriate volume (any of the aparc+aseg.nii.gz images).

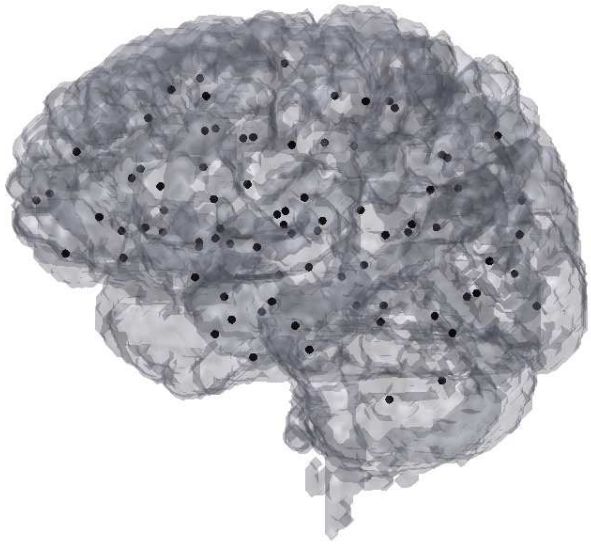

Figure 9.1: 3d Graph view interface.

9.2 Modality-specific MRI connectivity data

In this example we will extend the previous analysis with the 3D graph view. Here we will analyse the different generated metrics in a 3D anatomical map. To load the structural connectivity matrix $dti\_fiber\_0.8$, right click on the mouse and on *Add connections* select $dti\_fiber\_0.8$.

The previous loaded ROIs should now be connected through lines, generated through the $dti\_fiber\_0.8$. The nodes size is related with the node degree, while the lines thickness with the connection strength. To change the 3D view, select the *Rotate 3D* tool from the *image toolbox*

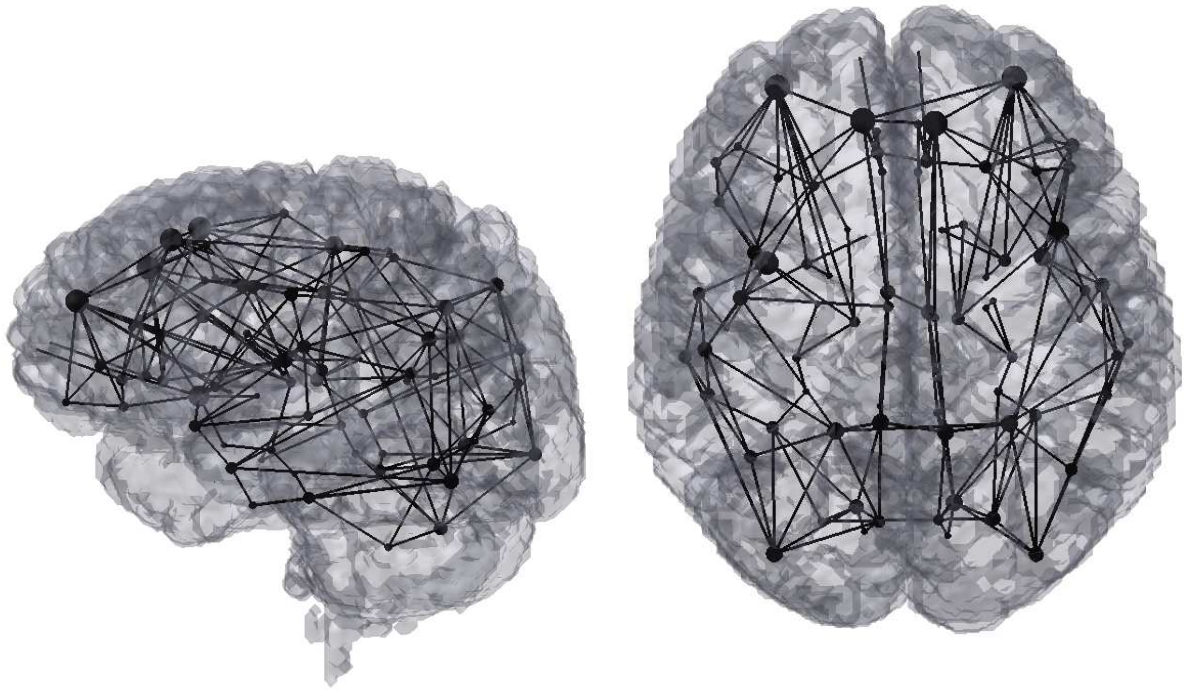

Figure 9.2: Structural 3d Graph. Left - Sagittal view. Right - Axial View.

In a first glance we can observe the inter-hemispherical symmetry of the structural connectivity in the brain. This observation is greatly increase when compared with both the matrix and the connectrogram views. We can further observe that the highest connected ROIs are located at the frontal and superior regions of the brain. To identify to which nodes they correspond, select the *Data cursor* tool from the *image toolbox* and choose one of the nodes. The node name as well as a full description of its the metrics should be presented in a new textbox. To select multiple nodes right click on the mouse, and click on *Create New Datatip*, and then select the new node to analyse. On this example the strongest ROIs are the rostralmiddlefrontal, and superiorfrontal bilaterally.

To analyse the functionally connected ROIs, first remove all the text boxes by right clicking on the mouse and select *Delete all datatips*. Then, deselect the *Data cursor* tool, right click on the mouse and on *Add connections* select the $fmri\_corr\_0.8$. At this point you should be able to see the difference between the structural connected ROIs and the functional connected ones. While the former presented an organized intra-hemispherical behaviour, the functionally connected ROIs present a higher inter-hemispherical behaviour.

To further analyse the local clusters (modules) within each matrix, right click on the mouse and on *Add modularity* select $dti\_fiber\_0.8$, and then make the same procedure and select $fmri\_corr\_0.8$. The user should now be able to reproduce the following images.

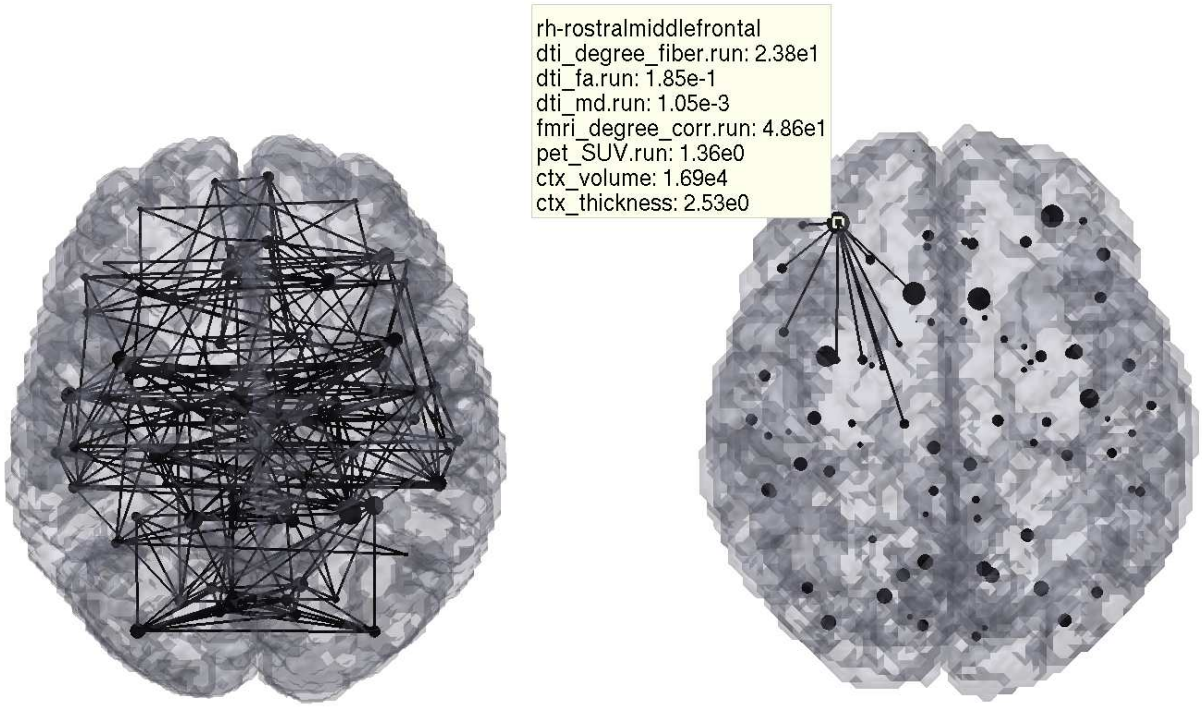

Figure 9.3: Functional 3d Graph. Left- Full graph. Right - Selected ROI.

9.3 Multimodal MRI connectivity data

To further analyse the direct and mediated functional connections, the previously generated matrices can be here analysed. Right click on the mouse and on *Add functionality* select $fmri\_combined\_0.8$. Here we can see in red the direct connections and in blue the mediated connections. We can observe that the direct connections are primarily intra-hemispherical and tend to be between close ROIs. On the other hand, mediated connections are both intra- and inter- hemispherical and present connections between more distant ROIs.

This leads to an interesting question: What is the shortest path that connects two functionally connected ROIs that are not directly connected? To achieve this the user can select two ROIs of interest and the tool calculates the shortest number of ROIs that connect those two ROIs together (if possible).

In this example we will analyse what is the shortest path that connects the functionally connected lh-caudalmiddlefrontal to the rh-caudalmiddlefrontal. To do this first right click on the mouse and on *Add connections* select $fmri\_direct$. This will load only the functional connections that are also directly connected (through fibres). Next, right click on the mouse and select *Find connection*. Now select the lh-caudalmiddlefrontal, and then the rh-caudalmiddlefrontal. The following images should be generated.

To further analyse if these connections are uni-directed or bi-directed an effective connectivity matrix can be used[1]. To accomplish this, right click on the mouse and on *Add directionality* select $fmri\_effective$. You should now be able to see the directionality of each connection (from blue to red for uni-directed connections, and black for bidirected connections).

[1]Take into attention that although Granger Causality was implemented here as default it may not be reliable in fMRI studies. Results from this analysis should be taken with care.

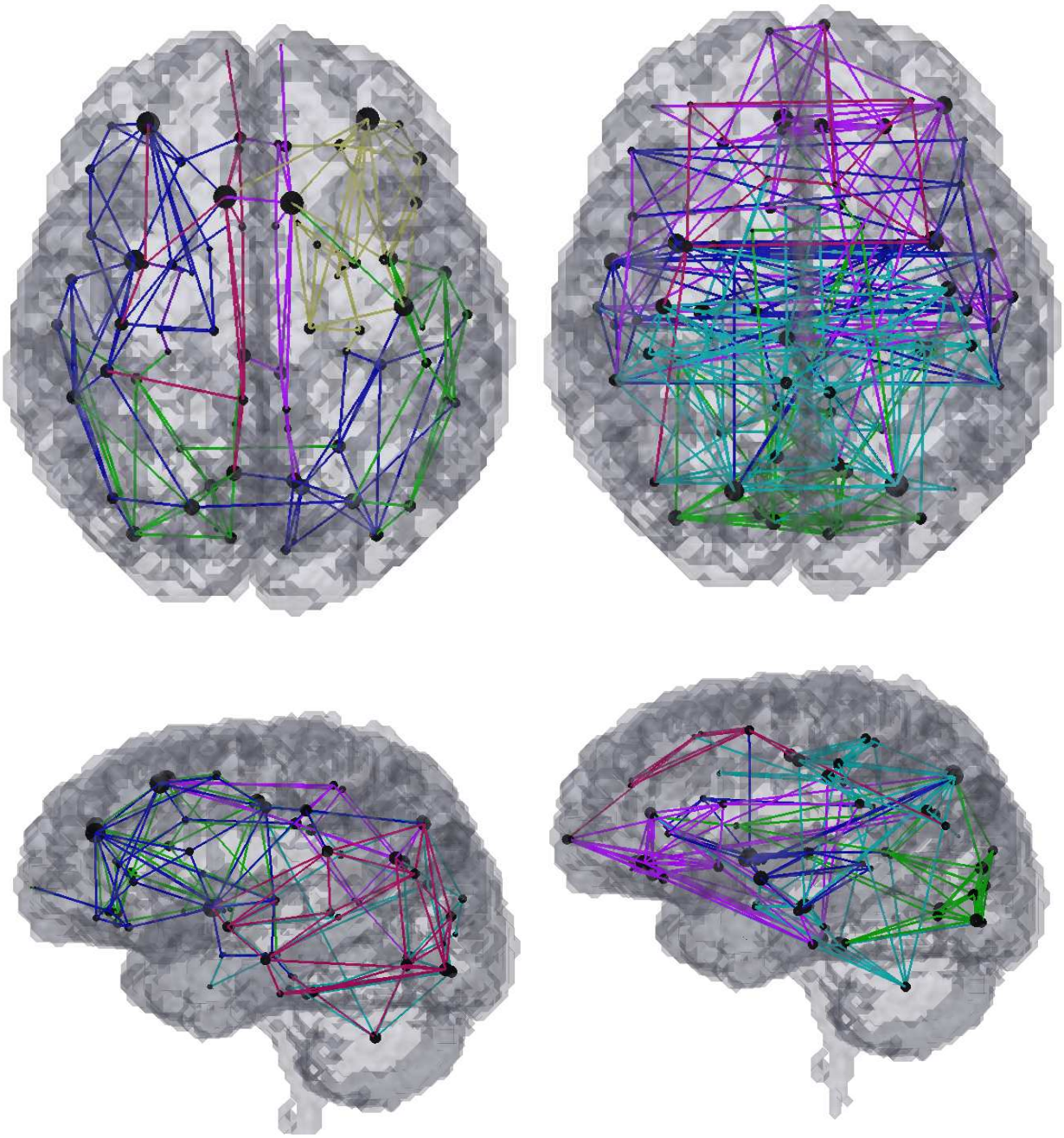

Figure 9.4: 3d Graph modularity. Left - Structural Graph. Right- Functional Graph.

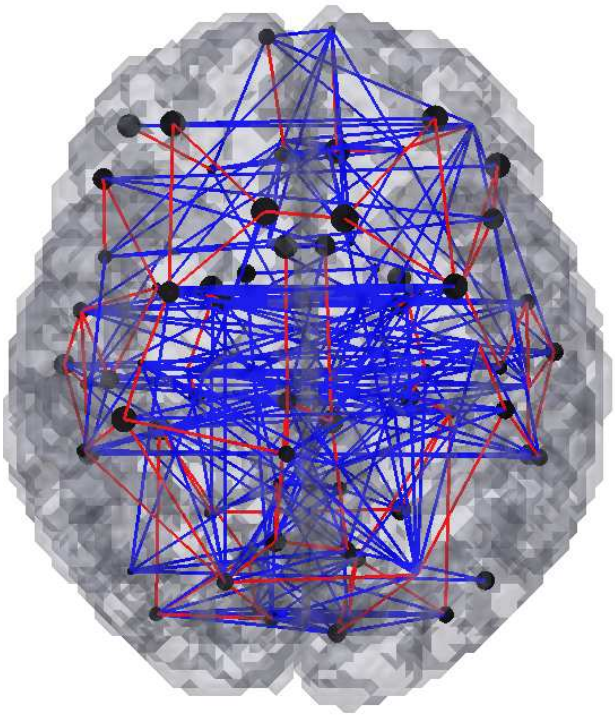

Figure 9.5: Hybrid functional 3dGraph. Red - Direct Connections. Blue - Mediated Connections.

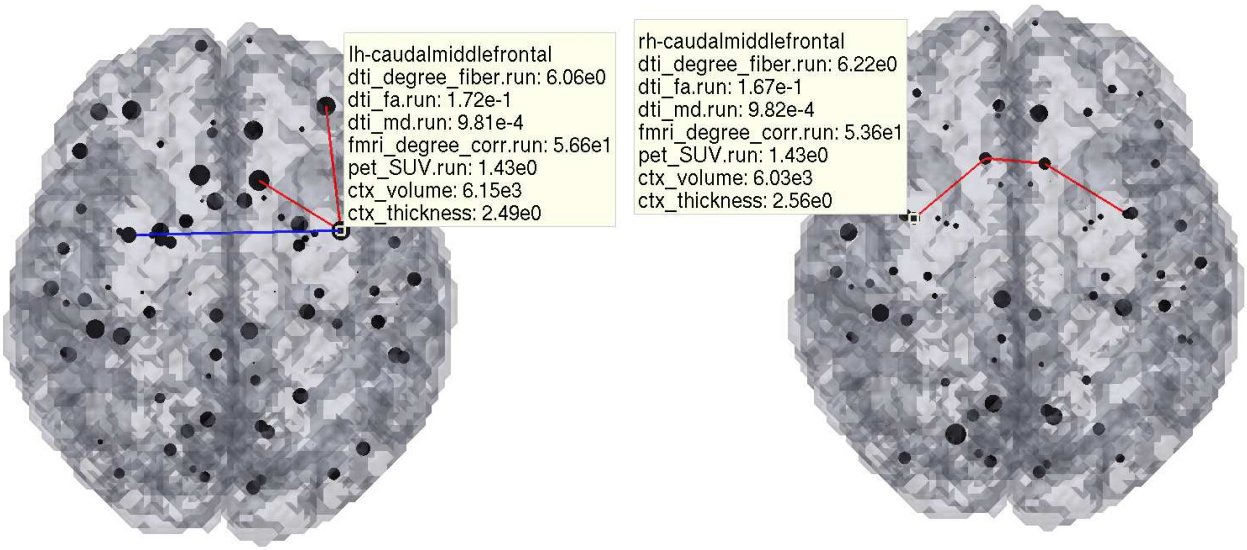

Figure 9.6: ROI selection of hybrid functional 3d Graph.

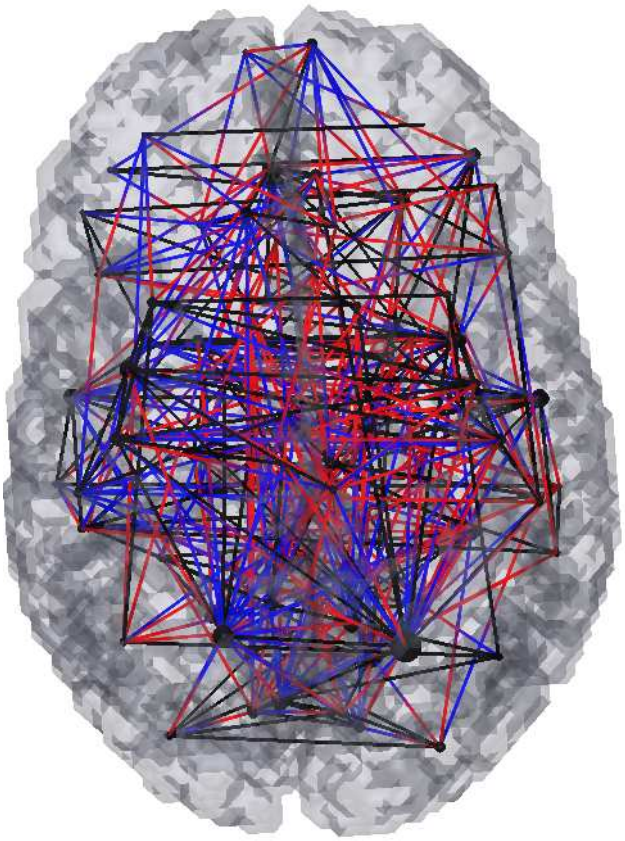

Figure 9.7: 3d Graph of effective connectivity calculated through Granger causality. Note that for fMRI Granger Causality may not provide reliable results without strong hypothesis.

Chapter 10

Multimodal Statistical tests

MIBCA is further able to process and visualize group differences and statistical tests. The latter, allows the user to compare different metrics in the same normalized scale. In this toolbox we use the *p values* as the normalized metric, yet other metrics such as the t score will be further available in a future release.

In this example we will analyse the statistical differences between young and eldery in the example data provided. Take into attention that this is a dataset composed of only 3 young and 3 eldery subjects and the results may not be reliable.

To do this first load the *ttest_groups_data2Old-Youth.mat* file in the results folder, as explained in Chapter 7. Then select the following metrics:

1. pval → DTI → dti_fibre
2. pval → DTI → md
3. pval → DTI → dti_degree
4. pval → fMRI → fmri_corr
5. pval → sMRI → ctx_volume
6. pval → sMRI → ctx_thickness

Rename the two loaded matrices to *dti_fibre* and *fmri_corr* and remove the list of ROIs as presented in section 7.2.

As the loaded pval matrices are not threshold (i.e. both significant and non-significant values are presented) we first need to define a cut-off value and then apply to these matrices. Further, these matrices are composed of both positive and negative statistical differences, where $pval > 0$ and $pval < 0$ encode each one of the legs of the statistical test.

To threshold these matrices use the operation toolbox as described in section 7.4 and perform the following operations:

1. $(abs(dti\_fibre) < 0.05). * sign(dti\_fibre)$
2. $(abs(fmri\_corr) < 0.05). * sign(fmri\_corr)$

Finally, rename the generated matrices to *dti_fibre_c* and *fmri_corr_c*

As explained in Chapters 8 and 9 create the connectograms and 3d graphs for visualization. The connectograms and 3d graphs should look similar to the ones presented in Figure 10.1. From the connectograms we can see that the cortical thickness, and the gray matter volume decrease between the young and eldery groups. The mean diffusivity on the other hand increases. The structural connections between the different ROIs seem to be increased, while the functional connections seem to both increase and decrease. These results are as explained before based on a very small dataset and serve only as example data to the toolbox.

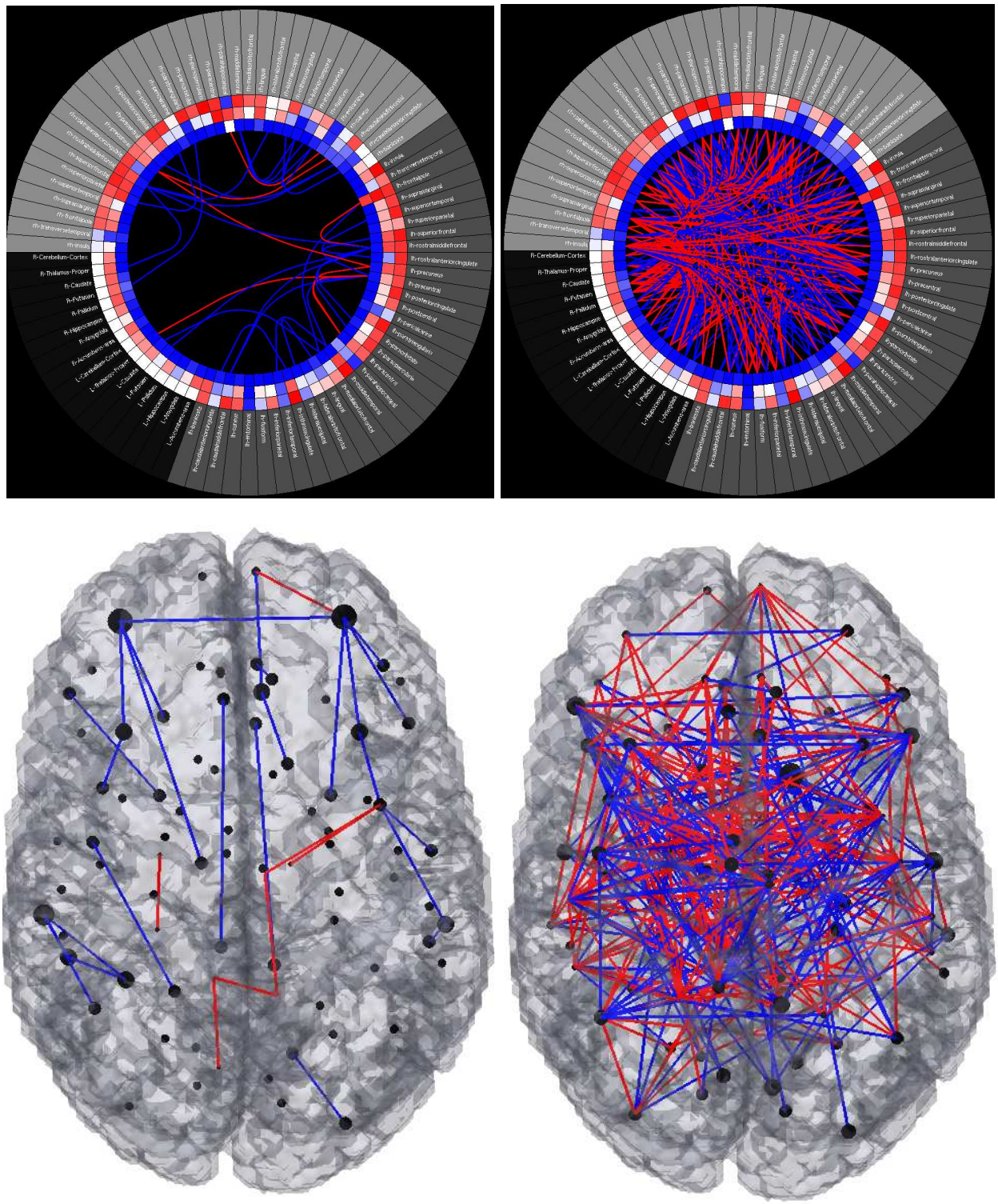

Figure 10.1: Statistical connectograms and 3d graphs. Left- Statistically different structural connectogram. Right - Statistically different functional connectogram. Red - Decreases; Blue - Increases.