Imaging for Neuroscience Homework 3:
DMRI/fMRI Integration

The goal of this homework is to investigate the relationship between resting state functional MRI and diffusion MRI data. The following scans, collected in Verona, were acquired with a 3T MR scanner on a healthy subject.

The provided data are organized as follows in the **Data** folder.

- Subject's **Atlas** folder:
  - Hammers_2_T1_2mm_int.nii.gz = anatomical atlas, already resampled in the T1w space image.
  - Hammers_labels.pdf = file containing the labels of atlas anatomical regions. For example, regions 1 and 2 represent the right and left hippocampus respectively.

- Subject's **DMRI** folder:
  - diffusion_volumes.nii = the diffusion weighted volumes of the diffusion acquisition, resampled in the T1w space image.
  - diffusion_brain_mask.nii = the binary mask delineating the volume occpied by the brain in the diffusion scans.
  - bvals = txt file containing the bvalue with which each diffusion volume was acquired.
  - bvecs = txt file containing the gradient direction with which each diffusion volume was acquired.

- Subject's **FMRI** folder:
  - FMRI_2_T1_2mm.nii.gz = 4D resting state functional data (TR=2.6sec). These data have been already processed with the following procedures: slice timing, distortion correction and motion correction (using a rigid spatial transformation) and resampling in the T1w space image.
  - MOCOparams.mat = estimated motion parameters (matrix 225x6).
  - FDparams.mat = framewise displacement parameters (matrix 225x2).

- Subject's **Structural** MRI folder:
  - T1_2mm.nii = T1w structural image.

The **Matlab_tools** folder contains useful tools:

- Nifti_tools
- betweenness_wei.m
- fdr_bh.m
- save_3D_nii.m

AIMs of the study:

1. To perform some pre-processing steps on functional data.
2. To compute the subject's functional connectivity (FC) matrix and to derive graph measures.
3. To fit the diffusion tensor and to derive its most important metrics
4. To investigate the impact of various strategies of signal normalization on the computation of the DTI metrics (FA and MD)
5. To investigate at the ROI level whether there is a relationship between the DTI parameters and the computed connectivity measures.

Resting state fMRI analysis:

1. Data Preprocessing

a. Use SPM software (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) to segment the T1w structural image into GM, WM and CSF tissues, obtaining the tissues probability maps. (This step may take a few minutes)
b. Threshold the three tissues probability maps and create for each tissue a binary mask. Erode the masks (*imerode* matlab function) and compute the mean fMRI signal of WM and CSF.
   Provide a justification of the employed thresholds and of the erosion parameters.
c. Create the sumEPI image by summing the EPI volumes in the 4th dimension. Create a binary mask for the sumEPI image and provide a justification of the employed threshold.
d. Mask the Hammers atlas with two masks: the GM binary mask and the sumEPI mask.
e. ROI time activity curve extraction: for each masked ROI of the atlas, extract the mean fMRI signal. Using the *Hammers_labels.pdf* file, discard for the following analyses the masked ROIs with less then 10 voxels and those belonging from: amygdala, cerebellum, brainstem, corpus callosum, substantia nigra, ventricles.

2. Data Denoising

a. Noise regression:
   For each ROI, remove the non-neural undesidered fluctuations from its temporal dynamic using a linear regression approach. Perform the regression using eight regressors: the six parameters estimated during the motion correction step (provided in the *MOCOparams.mat* file) and the mean signals of white matter and cerebrospinal fluid. Before to be used, each regressor must be converted to Z scores. (Matlab functions: *zscore* and *lscov*)
   Visualize the regression matrix.
b. Temporal filtering:
   To take the slow components out, filter the signals obtained at point 2.a. with a high pass filter choosing reasonable cut-off frequencies. (Matlab functions: *buttord*, *butter* and *filtfilt*).
   Provide a justification of the selected filtering frequency cut-off.

3. Volume censoring

Discard the volumes that are affected from motion artefacts. These volumes have a framewise displacement greater than 3.5mm (the FD values in mm are reported in the first column of the Fdparams.mat matrix). Since motion artefacts could be present also in the neighboring volumes, discard also one volume before and two after the affected volume.

4. Check of preprocessing step

Plot the original time-course of the right hippocampus region and what obtained after each denoising step. Do you see a drift in the original signal? If so, is the denoising able to remove it?

5. **Static FC Matrix Computation**

   Compute the pairwise Pearson's correlation (and the relative *p*-value) between the time-series of the ROIs. Visualize the FC matrix after applying the Fisher's *z*-transform to the coefficients (*atanh* matlab function).

6. **Multiple Comparison Correction**

   Perform a multiple comparison correction with Bonferroni or False Discovery Rate approach and a significance level alpha=0.05. If you decide to use the FDR method, use the provided *fdr_bh.m* function. Provide a justification of the chosen correction method.

7. **Graph Measures**

   To summarize the functional connectivity in terms of node centrality, for each ROI compute the node degree, the node strength and the normalized betweenness centrality (with *betweenness_wei.m* provided function). In the metrics computation, consider only the statistically significative functional connections obtained after the multiple comparison correction at point 6. Plot the node degree, the strength and the normalized betweenness centrality of the ROIs using the *stem* matlab function. Which are for each metric the 10 ROIs with the higher metrics values? Provide the indices of these regions.

Diffusion MRI analysis:

1. **Diffusion signal visualization&understanding**

   a. Load the diffusion volumes, the bvals file and the bvecs file.
      How many different DWIs have been acquired?
      Excluding b=0, how many diffusion shells does this acquisition feature? (consider a small tolerance $\alpha=\pm 20$ s/mm^2 in the shell definition).
   b. Plot the diffusion signal of a voxel populated principally with cerebrospinal fluid (hint: you can skip steps 1a-1b and get back to them when you have computed the DTI metrics, which can aid you in the selection); is the diffusion signal ordered by its b-value? If not, sort it so that the signal points corresponding to the same shell are shown consequently (and shells are ordered in an ascending fashion).
      Provide both the plot of the unsorted signal and the sorted one.
   c. By visually inspecting the sorted signal, provide a brief comment both on the inter b-value and on the intra b-value variabilities. Why do these signal variations occur?

2. **Diffusion tensor computation**

   a. From the entirety of the diffusion volumes data, create a new 4D matrix containing only the volumes corresponding to b=0 s/mm^2 and to the shell closest to b=1000 s/mm^2 identified at the point 1a.
   b. Fit the voxel-wise diffusion tensor (using the linear least square approach seen in class) on the the whole brain diffusion data created at point 2a. When performing the log(S/S0) transformation of the signal, use as S0 the voxel-wise value of the first b=0 volume of the available dataset. Use the eigenvalue/eigenvector decomposition to recover the FA / MD indices.
   c. Fit the voxel-wise diffusion tensor again. This time, when performing the log(S/S0) transformation of the signal, use as S0 the voxel-wise mean value of all b=0 volumes of the available dataset.
      As in the previous points, recover the FA / MD indices.

d. Once you have computed the DTI indices in both cases (points 2.b and 2.c), compute the voxel-wise coefficients of variation (CVs) of both indices (FA example: 100*(FA_all-FA_one)/FA_all).
   Choose a single slice (report it) and provide visualization for both the CVs image, complete with the colorbar.
   Which metric is more affected by the different normalization choice?
e. Out of the two different diffusion tensors you fitted, choose the one you believe is the optimal one (provide justification); provide the visualization of the FA and MD maps for the same slice as the one you decided in point 2d. Additionally, save the two FA and MD volumes with the given *save_3D_nii.m* function.
f. Mask the FA and MD maps (as done for the Hammers atlas in fMRI: point 1.d), extract their mean values in each ROI (fMRI: point 1.e.).

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1. Visual inspection

Visualize the scatterplot of these variables:

- ROIs node degree versus ROIs FA
- ROIs node strength versus ROIs FA
- ROIs node normalized betweenness centrality versus ROIs FA
- ROIs node degree versus ROIs MD
- ROIs node strength versus ROIs MD
- ROIs node normalized betweenness centrality versus ROIs MD

2. Quantitative results

Compute and provide the Pearson's correlation between the six pairs of variables of point 1. Discuss the results: is there a statistically significant relationship between any pair of variables?

Submit you homework in the elearning page of the course in the Homeworks delivery section. The submission MUST be completed by 19/01/2020. You are required to do the homework in english (report, codes etc.).

In a .zip folder (named Hwnumber_Name_Surname) you are required to turn in a copy of your **own** commented code(s), as well as an electronic version of the complete homework solution which must include:

1. A brief and complete **report** of performed analysis (methods, problems and issues, results, discussions, …). Mandatory maximum 7 pages (including tables and figures).

2. The following **files**:
   a) *GM.nii :* the grey matter tissue probability map (FMRI: point 1.a.)
   b) *WM.nii :* the white matter tissue probability map (FMRI: point 1.a.)
   c) *CSF.nii :* the cerebrospinal fluid tissue probability map (FMRI: point 1.a.)
   d) *MD.nii* : the MD map (DMRI: point 2.e.)
   e) *FA.nii* : the FA map (DMRI: point 2.e.)

3. A **.mat file** with the following variables:

   a) *GC :* the regression matrix (fMRI: point 2.a.)
   b) a matrix with the ROIs fMRI signal after the denoising step (noise regression + temporal filtering) (fMRI: point 2.b.)
   c) *zFC* : the z-Fisher transformed functional connectivity matrix (fMRI: point 5.)
   d) *zFC_corr* : the z-Fisher transformed functional connectivity matrix after multiple comparison correction (fMRI: point 6.)
   e) *DEG* : a vector containing the ROIs node degree (fMRI: point 7.)
   f) *STR* : a vector containing the ROIs node strength (fMRI: point 7.)
   g) *BTW_NORM* : a vector containing the ROIs normalized betweenness centrality (fMRI: point 7.)
   h) *cv_MD* : the MD coefficients of variation (DMRI: point 2.d.)
   i) *cv_FA* : the FA coefficients of variation (DMRI: point 2.d.)