# Structural pattern/tissue properties comparison between AD patients and control subjects

## Data

The analysis will be performed on volume maps extracted from multi-parameter (MPM) and T1-weighted MR data as well as on tissue property MPM data. We provide data of Alzheimer’s disease (AD) patients and healthy controls from the LREN-CLM database.

Besides demographic data (gender and age), we have also CSF biomarkers (amyloid beta, tau) and we have to calculate some global measures (individual total grey matter, white matter and CSF volumes),

## Goal

The project goal is to use the SPM framework for brain image analysis in order to achieve two main goals:

* To master technical skills required for anatomical MR data processing.
* To learn how to formulate scientific questions in the field of computational anatomy and address these with state-of-the-art statistical analysis.

### Technical goals

You will learn how to perform

* Structural MRI data pre-processing the framework of the most widely distributed software for brain image analysis - SPM. In particular, we will cover the topics of automated brain tissue classification, diffeomorphic spatial registration (DARTEL) and spatial smoothing.
* Group analysis statistic I: this step will cover the basic statistics steps (t-test) for group analysis.

### Scientific goals

Building on the acquired technical skills, you have to develop and formulate your own scientific question using the available data.We encourages you to investigate:

* Patterns of brain anatomy differences between patients and controls;
* Feature extraction from multi-contrast data - importance to discriminate between T1w and MPM data;
* Brain structure-CSF biomarkers relationships on the basis of the provided data

## Data pre-processing

Data can be found under directory: X:\LNDS\_ADexe\data\_ready

Data processing step:

### Unified segmentation:

1. Try alternative ways for tissue classification into grey, white matter, CSF and other tissue.
   1. With **spmTPM** 
      1. Click on Batch on the menu window
      2. within the Batch Editor window – SPM/Spatial/Segment
         1. Volumes: select image in data\_ready\whole\_brain\_single\spmTPM
            1. MT/PD
            2. T1w MPRAGE
         2. Tissue 1, 2 and 3:

Native Tissue - choose Native and Dartel imported

Warped Tissue – choose Modulated +

Unmodulated

* + - 1. Tissue 4, 5 and 6

Native Tissue - choose Native

* + - 1. Deformation fields – inverse and forward
    1. Save your batch as an .m file!
  1. With **eTPM** 
     1. Click on Batch on the menu window
     2. within the Batch Editor window – SPM/Spatial/Segment
        1. Volumes: select image in data\_ready\whole\_brain\_single\eTPM
           1. MT
           2. T1w MPRAGE
        2. Tissue 1, 2 and 3:

Tissue probability map – eTPM.nii.1-3

Native Tissue - choose Native and Dartel imported

Warped Tissue – choose Modulated +

Unmodulated

* + - 1. Tissue 4, 5 and 6

Tissue probability map – eTPM.nii.4-6

Native Tissue - choose Native

* + - 1. Deformation fields – inverse and forward
    1. Save your batch as an .m file!

Questions:  
How can you perform automatic tissue classification in the SPM framework? What are the differences between the two TPMs output? Why the difference btw MT and T1w segmentation?

1. Check the output with “Check Reg”
   1. What are c1, c2, c3, c4, c5 c6, wc1, mwc1 in segment?
   2. What are the output differences between Segment and OldSegment? (c1 vs. c1, wc1 vs. wc1, mwc1 vs. mwc1)
      1. Check Reg select c1 and c1 from both segmentation
         1. Right click on the first image: Blobs-> add colored image-> local and select the other c1
      2. To quantitatively explore the outputs differences create difference images with ImCalc within the Batch Editor window: SPM/Util/Image Calculator
         1. Input: 2 images: c1 in whole\_brain\_single\spmTPM and c1 in whole\_brain\_single\eTPM
         2. Output filename: diff\_c1.nii
         3. Output Directory: whole\_brain\_single\spmTPM
         4. Expression: **i1 – i2**
      3. Repeat the same with the MT and T1w segmentation output in the two TPM options
2. Check the 2 deformation fields with “check reg”
   1. Apply the forward deformation field to a c1 image using the Deformations tool within the Batch Editor window – SPM/Util/Deformations
      * 1. Composition: Deformations Field, select the iy image
        2. Output: Pushforward
        3. Apply to: c1 image
        4. Output destination: whole\_brain\_single\spmTPM\deformation
        5. Field of view: Image defined, select mwc1 image
        6. Preserve: Preserve Amount

Questions:  
What do “pushforward” and “pushback” mean? If you change the interpolation mode from the default mode to 4th order B-spline, what will happen and why?

### Dieffeomorphic registration – DARTEL

1. Run a “mock” 1 subject DARTEL with the existing imported rc1 and rc2 files.
   1. Choose Batch -> SPM -> Tools -> DARTEL Tools -> Run Dartel (create Template)
   2. Under Images – click 2x and enter rc1 under the 1st set of images and rc2 under the 2nd in your folder /single\_subject/segment
   3. Template basename: Template\_single\_subject
   4. Leave all other defaults and check reg the output – u\_rc1
2. In the folder: “whole\_brain\_single\spmTPM” we already created a Template with many subjects: Template\_0, 1, 2, 3, 4.nii
   1. Check the different Shoot output: Template \_0, 1, 2, 3, 4.nii,
   2. When the interactive window is asking you to select your images. type “1:2” under the Filt field followed by enter.
   3. 1 correspond to the grey matter template and 2 to the white matter
3. You can easily create a flow field (u\_) with the existing Template
   1. Click on Batch -> SPM -> Tools -> Shoot Tools -> Run Shoot (existing Template)
   2. Enter the different images (rc1, rc2) and the different templates from 1 to 4 one after the other
4. The output should begin with the prefix jd/v/y\_field (without the name of the template used)

Questions:

What are the differences between the Shoot Template\_0 and the Template\_4?

### Affine registration to MNI space

1. Click on Batch -> SPM -> Tools -> Shoot Tools -> Write normalised
   1. Shoot template: choose the 6th template
   2. Select according to few subjects(in the single subject case)
   3. Deformation field: select the y\_ image
   4. Images: be aware – choose the c1 and NOT the rc1 images for warping!!!
   5. Preserve: Preserve Amount
   6. Gaussian FWHM: let default
2. The output images are with the prefix smw\* (smooth, modulated, and warped). Check the images

Questions:  
What is the difference between preserve amount and preserve concentration? Try to perform the registration to the MNI space using different methods from DARTEL. How do you decide for the Gaussian kernel dimensions?

## Group analysis statistic I

In this workshop, you will use the pre-processed data, the meta-data information like gender, age, TIV, CSF biomarkers and the different morphometry output data to compare the GM estimation between AD and CN.

### Specification and estimation of the design matrix

Open the factorial design batch; insert the covariates used for the previous exercise. Find the design matrix that allows you to compare the GM maps of AD and insert the covariates.

### Specification and estimation of the design matrix

1. Open your Matlab program
2. Type” spm pet” in the MATLAB window
3. Create directory for your first stat analysis in the MATLAB command line
   1. mkdir(‘AD\_vs\_HC’)
4. Within the Batch Editor window – choose SPM/Stats/Factorial design specification (or click basic models on the menu)
   1. Select the right stats directory!!!
   2. Select the type of design you need (e.g. two-sample t-test)
   3. Enter the AD patients data (smwc1) in the AD folder, the controls data (smwc1)– in CTR folder
   4. Enter covariates: age, gender, TIV all are in the excel file: interactions with Factor 1
   5. level of absolute threshold: 0.2 (you can also choose an explicit mask instead)
5. Within the Batch Editor window – choose SPM/Stats/Model estimation (or estimate on the menu)
   1. Use the Dependency button to estimate in a “classic way” the design you specified beforehand
6. Save your batch as an .m file thru File/Save Batch
   1. Have a look at the saved batch in the Matlab Editor
7. Click on the green arrow when ready
8. Questions:
9. How could you restrict your statistical analysis to a well-defined region?
10. What are the outputs of this step? What does the SPM.mat file contains? What do the betas images represent?

### Results

1. Click on the Results button in the Menu panel
   1. Accordingly to your apriori hypothesis create differential contrasts showing differences between AD patients and controls:
      1. Volume differences
      2. Group by Age interaction
   2. Example1: Do AD have less volume than controls?
      1. Choose t-test
      2. Define new contrast
      3. Name: AD<HC
      4. Contrast: -1 1
      5. Submit
      6. Done
      7. Apply masking: None
      8. Title for comparison: enter
      9. p\_value: none
      10. Threshold: enter
      11. Extend threshold: enter
   3. Example2: is there an effect of diagnosis on volume?
      1. Choose F-test
      2. Enter 1 -1 and follow the previous steps
   4. Example 3: how can you look for the interaction of the diagnosis with age?
      1. Set your contrast to: 0 0 0 0 -1 1 and follow the previous steps
2. Explore the statistical parametric maps presented in the “glass brain”, have a look at the underlying table
   1. Click on whole brain
3. Overlay of stats results on an anatomical image
   1. Click on overlay -> section -> spm12 -> canonical -> single\_subject\_T1.nii
   2. Try to overlay your results after first calling an anatomical image thru the “check reg” function.
4. Further exploration of results
   1. Click on the most significant cluster. Right click on the result image (Upper part of the Graphics window) and click “go to global maxima”

Then click on plot -> Fitted response -> choose the current contrast -> click predicted -> scan