FMRI single subject analysis lab

Goals of this lab

After this first part of the lab you should:

1. Be able to examine data using SPM's single- and multi-volume display facilities.

2. Be able to characterize the susceptibility artifacts and signal voids in functional data, as compared to similar structural data.

3. Be able to evaluate the quality of functional image motion correction (realignment).

4. Be able to perform coregistration between the low-resolution and high-resolution structural images. You will understand the implications for an image to be the "Source" vs the "Reference" in terms of the "world space" of each image.

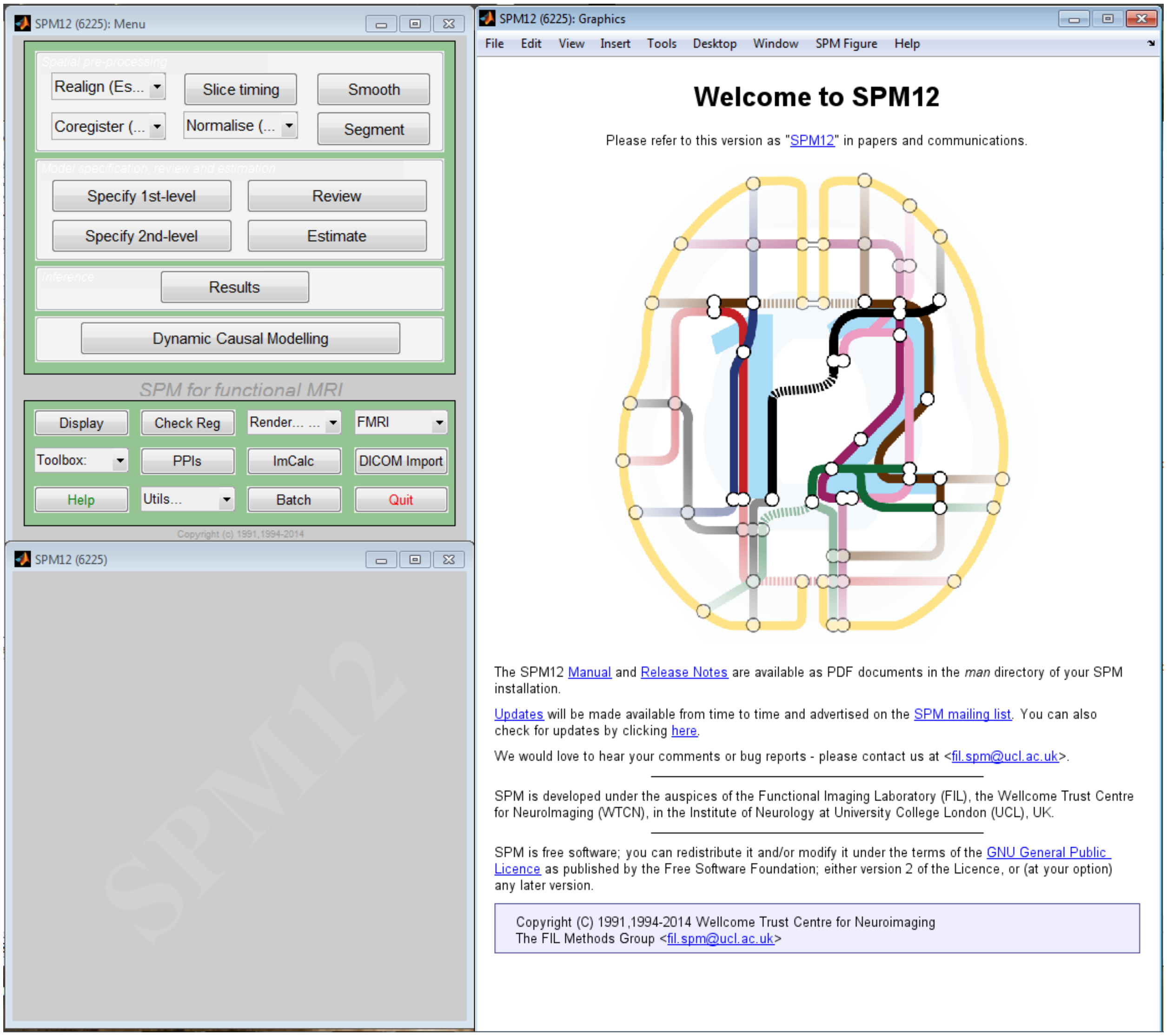
5. Be able to perform spatial normalization, check its success, and apply the transformation.

INTRODUCTION

1. Open **Matlab** software

2. Change the « **Current Folder** » and select the one with the data : **EF_mot**

3. In the command window, type ***spm fmri*** and press **Enter.** The following interface appears:



Welcome to SPM12

Please refer to this version as "SPM12" in papers and communications.

The SPM12 Manual and Release Notes are available as PDF documents in the *man* directory of your SPM installation.

Updates will be made available from time to time and advertised on the SPM mailing list. You can also check for updates by clicking here.

We would love to hear your comments or bug reports - please contact us at <fil.spm@ucl.ac.uk>.

SPM is developed under the auspices of the Functional Imaging Laboratory (FIL), the Wellcome Trust Centre for NeuroImaging (WTCN), in the Institute of Neurology at University College London (UCL), UK.

SPM is free software; you can redistribute it and/or modify it under the terms of the GNU General Public Licence as published by the Free Software Foundation; either version 2 of the Licence, or (at your option) any later version.

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The FIL Methods Group <fil.spm@ucl.ac.uk>

4. Take few minutes to discover the main interface (Menu). The top raws include the step needed for preprocessing the data, the central part is about statistical modelling, and the bottom part includes a certain number of useful tools.

Display and Check reg

5. Use the button "Display" to visualize one functional image first

Try to answer the following questions:

- What is the voxel size?
- What are the image dimensions?
- What are typical gray matter intensity values?
- On the lower right side of the display dialog you may see the kind of interpolation being used in visualizing the images. Trilinear interpolation is default. First select Nearest Neighbor interpolation and explore the image. Next select Sinc interpolation and again explore the image.

  a. Which interpolation do you prefer?
  b. Why? Can you see the differences?

6. Repeat the same steps and answer the questions after displaying one high resolution T1 image (MPRAGE acquisition)

7. Use the button "Check Reg" (Check registration) to visualize one functional and one high resolution anatomical image (you select two images in the same dialog box), are they superimposed? What about if you choose one functional and one T1 in the "Annex" folder?

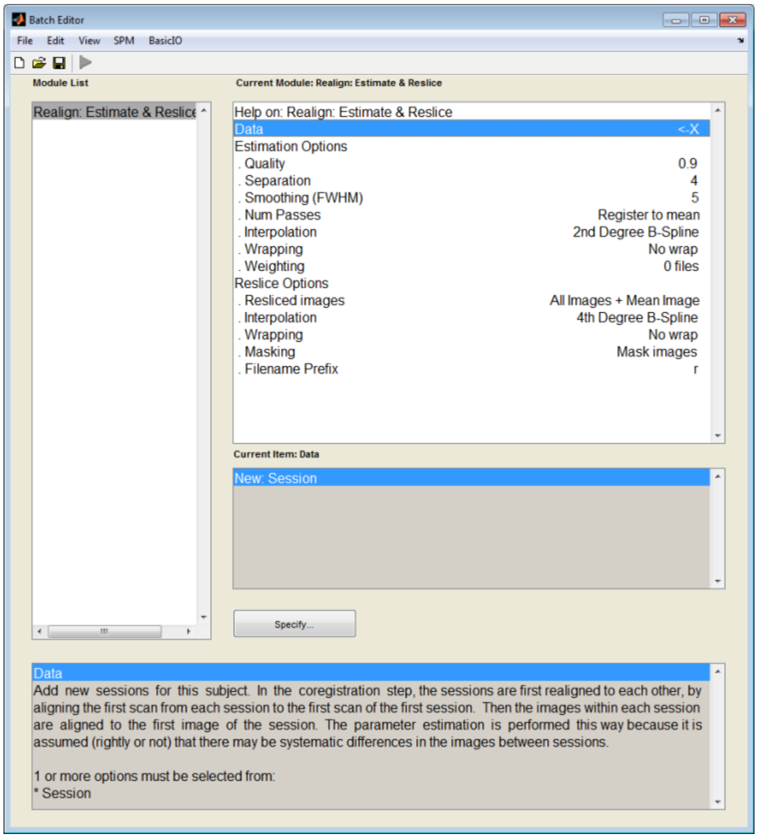
   Note that now you see both the anatomical and functional images in the same window. If you click around one, note that the crosshair in the other will also move. This is how one checks whether two images are correctly coregistered. Specifically, there are certain landmarks in the brain, which are used to ensure that all parts of the brain are correctly coregistered.
   Explore the pair of images, making sure to compare the following anatomical regions:
   i. Frontal pole
   ii. Occipital (posterior) pole
   iii. Left & Right sides (e.g. superior temporal gyrus)
   iv. Corpus callosum:
   a. Most anterior
   b. Most superior
   c. Most posterior extent

   Do the functional and structural line up well? If not, how so?

PREPROCESSING

REALIGMENT

To proceed with preprocessing, we start with the realignment in order to correct for subject's motion during the time of acquisition. Pressing the button "realign" and selecting "Estimate and reslice", the following interface appears:



Current Module: Realign: Estimate & Reslice

Help on: Realign: Estimate & Reslice	
Data	<-X
Estimation Options	
. Quality	0.9
. Separation	4
. Smoothing (FWHM)	5
. Num Passes	Register to mean
. Interpolation	2nd Degree B-Spline
. Wrapping	No wrap
. Weighting	0 files
Reslice Options	
. Resliced images	All Images + Mean Image
. Interpolation	4th Degree B-Spline
. Wrapping	No wrap
. Masking	Mask images
. Filename Prefix	r

Current Item: Data

New: Session

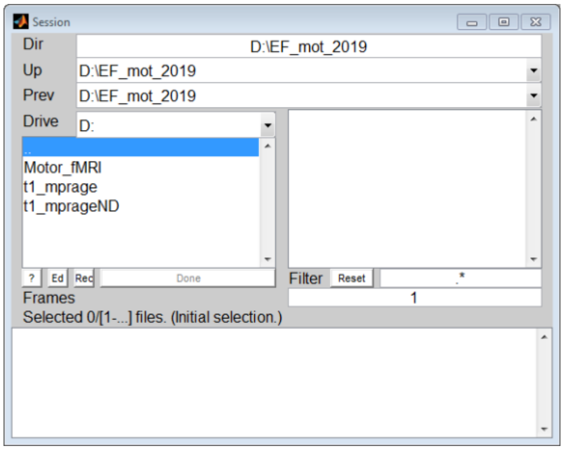
Data

Add new sessions for this subject. In the coregistration step, the sessions are first realigned to each other, by aligning the first scan from each session to the first scan of the first session. Then the images within each session are aligned to the first image of the session. The parameter estimation is performed this way because it is assumed (rightly or not) that there may be systematic differences in the images between sessions.

1 or more options must be selected from:
* Session

N.B: In all the interfaces, the parts where you have to interact and choose parameters before running, are marked with a <-X

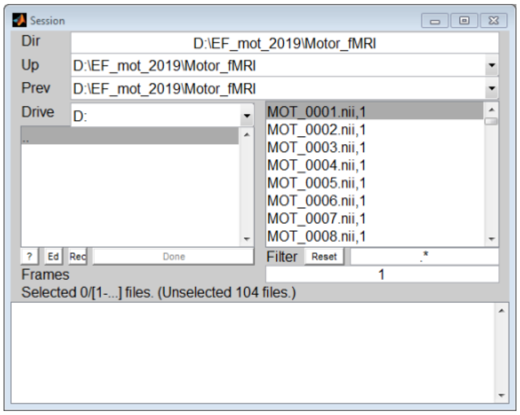
You must complete the selection of data by including all the functional images in the same order as they have been acquired (be careful files are 105 and should be selected in the right order)



Dir D:\EF_mot_2019
Up D:\EF_mot_2019
Prev D:\EF_mot_2019
Drive D:

..
Motor_fMRI
t1_mprage
t1_mprageND

Filter .*
Frames 1
Selected 0/[1-...] files. (Initial selection.)

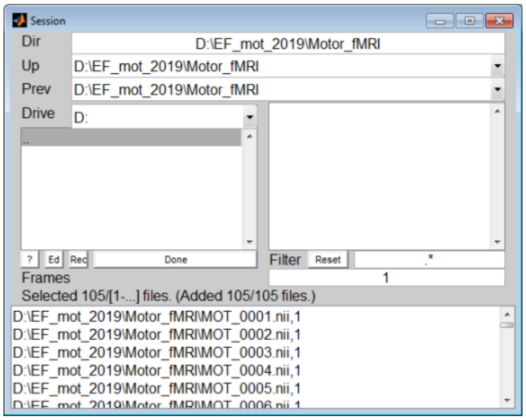


Dir D:\EF_mot_2019\Motor_fMRI
Up D:\EF_mot_2019\Motor_fMRI
Prev D:\EF_mot_2019\Motor_fMRI
Drive D:

..

MOT_0001.nii,1
MOT_0002.nii,1
MOT_0003.nii,1
MOT_0004.nii,1
MOT_0005.nii,1
MOT_0006.nii,1
MOT_0007.nii,1
MOT_0008.nii,1

Filter .*
Frames 1
Selected 0/[1-...] files. (Unselected 104 files.)



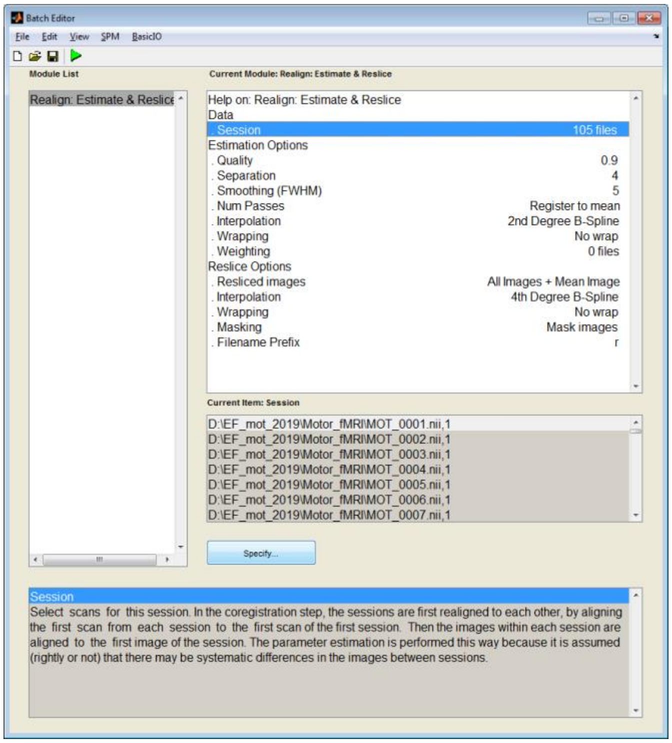
Dir D:\EF_mot_2019\Motor_fMRI
Up D:\EF_mot_2019\Motor_fMRI
Prev D:\EF_mot_2019\Motor_fMRI
Drive D:

..

Filter .*
Frames 1
Selected 105/[1-...] files. (Added 105/105 files.)

D:\EF_mot_2019\Motor_fMRI\MOT_0001.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0002.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0003.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0004.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0005.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0006.nii,1

Once that the data are selected, you can click on **Done** and you can run the “batch program” with the green arrow on the top raw of the batch window.



```
Help on: Realign: Estimate & Reslice
Data
. Session                                   105 files
Estimation Options
. Quality                                         0.9
. Separation                                        4
. Smoothing (FWHM)                                  5
. Num Passes                         Register to mean
. Interpolation                   2nd Degree B-Spline
. Wrapping                                    No wrap
. Weighting                                   0 files
Reslice Options
. Resliced images             All Images + Mean Image
. Interpolation                   4th Degree B-Spline
. Wrapping                                    No wrap
. Masking                                 Mask images
. Filename Prefix                                   r
```

```
D:\EF_mot_2019\Motor_fMRI\MOT_0001.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0002.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0003.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0004.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0005.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0006.nii,1
D:\EF_mot_2019\Motor_fMRI\MOT_0007.nii,1
```

Session

Select scans for this session. In the coregistration step, the sessions are first realigned to each other, by aligning the first scan from each session to the first scan of the first session. Then the images within each session are aligned to the first image of the session. The parameter estimation is performed this way because it is assumed (rightly or not) that there may be systematic differences in the images between sessions.

Once the realignement done, the movement parameters estimated are shown in the “Graphic window” and a new set of images with a “r” before the original filename are created in the folder where the input data are stored.

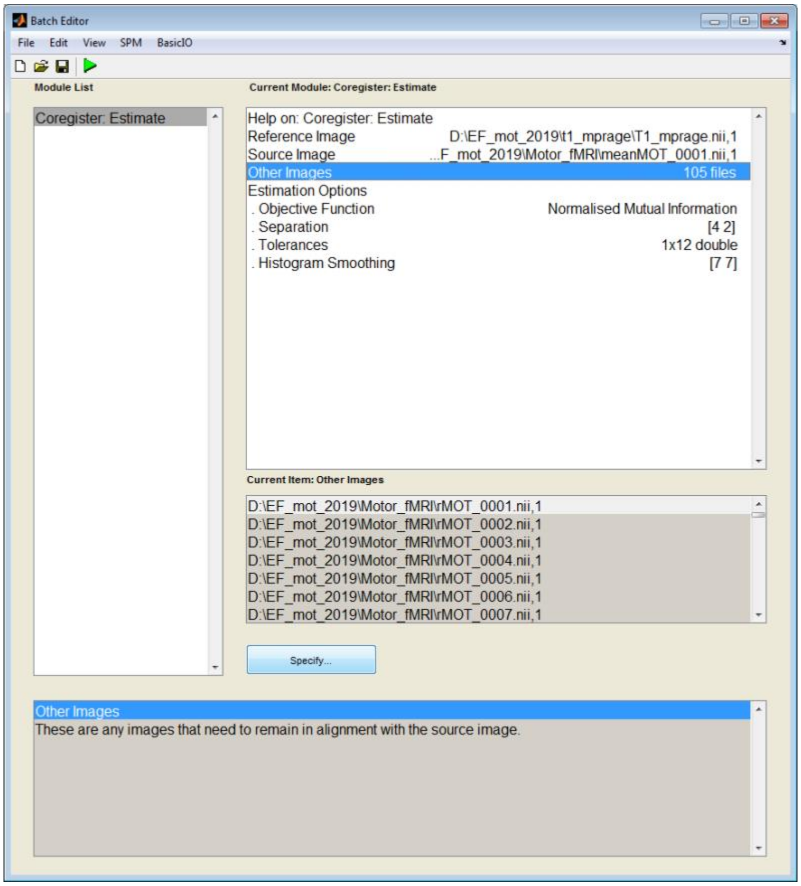
What is the maximum movement the subject has done? Do you think it is a lot?

COREGISTRATION

Press on the button “Coregister” and choose the option “Estimate”.

In this step you want to “move” the functional images exactly in the same space than the anatomical ones. To this end, you chose as a reference image the high-resolution anatomical image and as a source image the “mean functional image” created during the previous step.

You also select all the other “realigned images” (be careful, you should select the 105 images with the “r” before the original name).



Current Module: Coregister: Estimate

Help on: Coregister: Estimate	
Reference Image	D:\EF_mot_2019\t1_mprage\T1_mprage.nii,1
Source Image	...F_mot_2019\Motor_fMRI\meanMOT_0001.nii,1
Other Images	105 files
Estimation Options	
. Objective Function	Normalised Mutual Information
. Separation	[4 2]
. Tolerances	1x12 double
. Histogram Smoothing	[7 7]

Current Item: Other Images

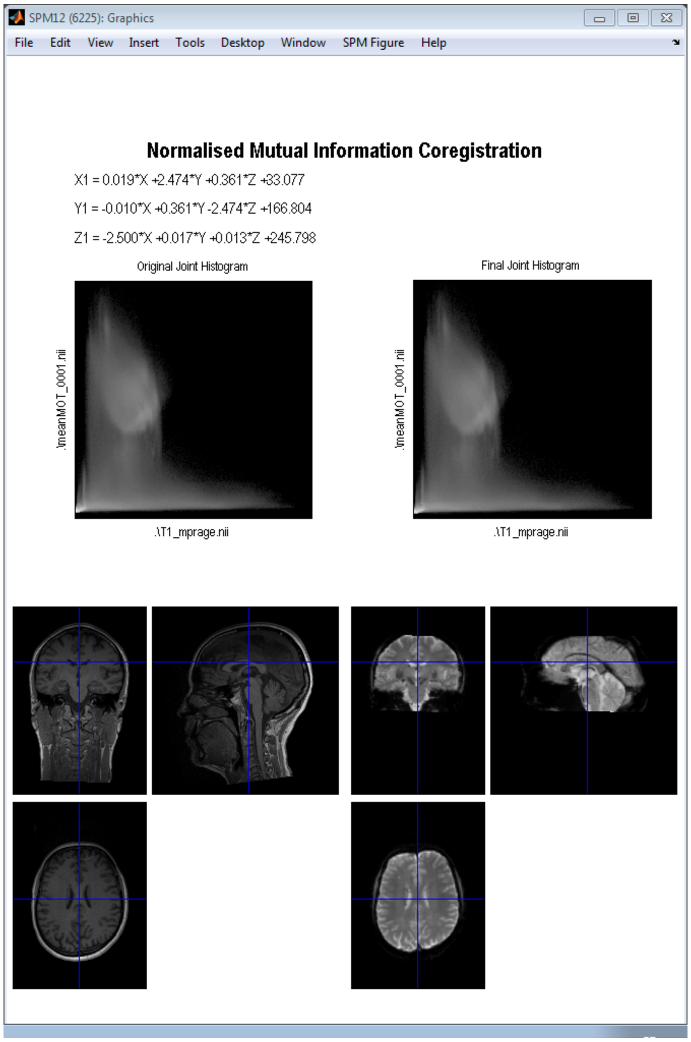
D:\EF_mot_2019\Motor_fMRI\rMOT_0001.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0002.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0003.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0004.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0005.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0006.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0007.nii,1

Other Images
These are any images that need to remain in alignment with the source image.

Then, you can run the script with the green arrow.

At the end of the script, you should see the result in the Graphical window. Navigate through the images to verify the goodness of the procedure.

With the “check reg” button, verify also that, for example, the first and the last functional images are well coregistered with the anatomical.



Normalised Mutual Information Coregistration

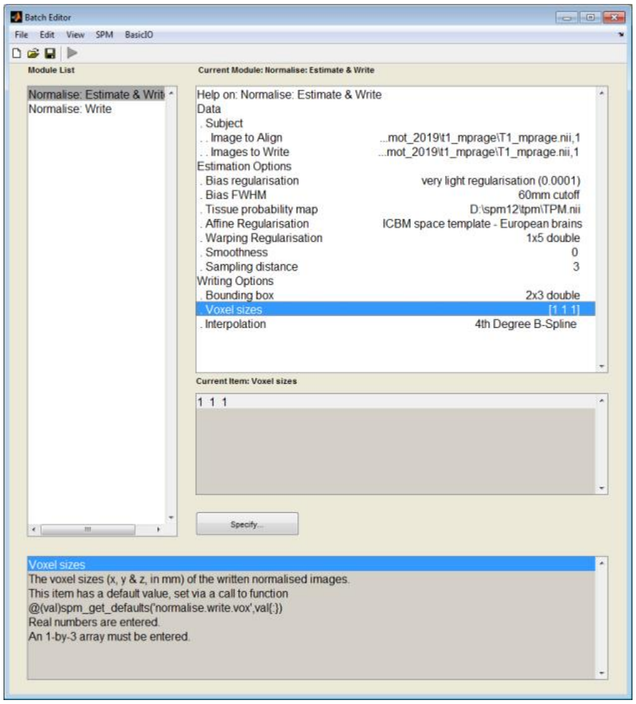
```
X1 = 0.019*X +2.474*Y +0.361*Z +33.077
Y1 = -0.010*X +0.361*Y -2.474*Z +166.804
Z1 = -2.500*X +0.017*Y +0.013*Z +245.798
```

NORMALIZATION

This part should be done in two steps.

You first estimate the parameters of normalization from the high resolution T1 anatomical image. Then you apply the same parameters to the functional ones.

An example of the script is the following.



Current Module: Normalise: Estimate & Write

```
Help on: Normalise: Estimate & Write
Data
. Subject
. . Image to Align                 ...mot_2019\t1_mprage\T1_mprage.nii,1
. . Images to Write                ...mot_2019\t1_mprage\T1_mprage.nii,1
Estimation Options
. Bias regularisation                very light regularisation (0.0001)
. Bias FWHM                                                 60mm cutoff
. Tissue probability map                            D:\spm12\tpm\TPM.nii
. Affine Regularisation         ICBM space template - European brains
. Warping Regularisation                                    1x5 double
. Smoothness                                                         0
. Sampling distance                                                  3
Writing Options
. Bounding box                                              2x3 double
. Voxel sizes                                                  [1 1 1]
. Interpolation                                   4th Degree B-Spline
```

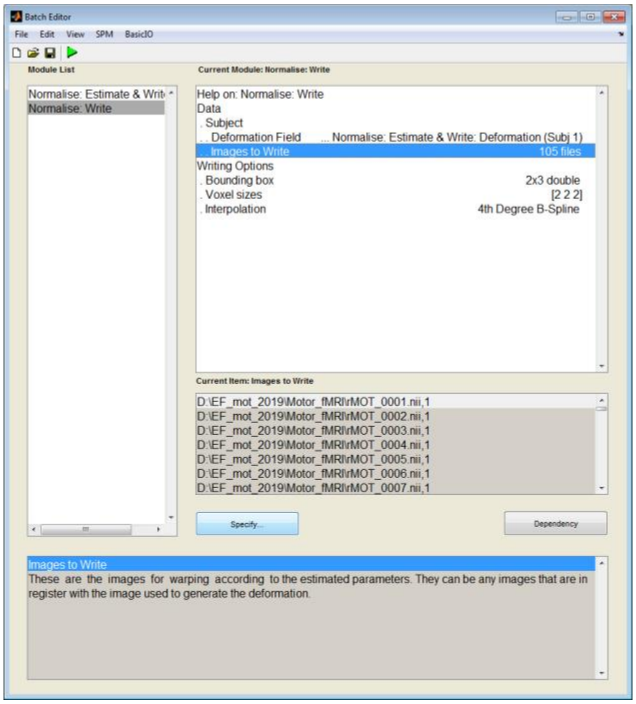
Current Item: Voxel sizes

```
1 1 1
```

Voxel sizes

The voxel sizes (x, y & z, in mm) of the written normalised images.
This item has a default value, set via a call to function
@(val)spm_get_defaults('normalise.write.vox',val{:})
Real numbers are entered.
An 1-by-3 array must be entered.

In the “write” part, the parameters estimated in the previous step are used to normalize functional data too. Since the file of parameters is still not available, you can “select it” by defining a “dependency” to the previous step.



Current Module: Normalise: Write

```
Help on: Normalise: Write
Data
. Subject
. . Deformation Field      ... Normalise: Estimate & Write: Deformation (Subj 1)
. . Images to Write                                                   105 files
Writing Options
. Bounding box                                                       2x3 double
. Voxel sizes                                                           [2 2 2]
. Interpolation                                            4th Degree B-Spline
```

Current Item: Images to Write

```
D:\EF_mot_2019\Motor_fMRI\rMOT_0001.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0002.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0003.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0004.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0005.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0006.nii,1
D:\EF_mot_2019\Motor_fMRI\rMOT_0007.nii,1
```

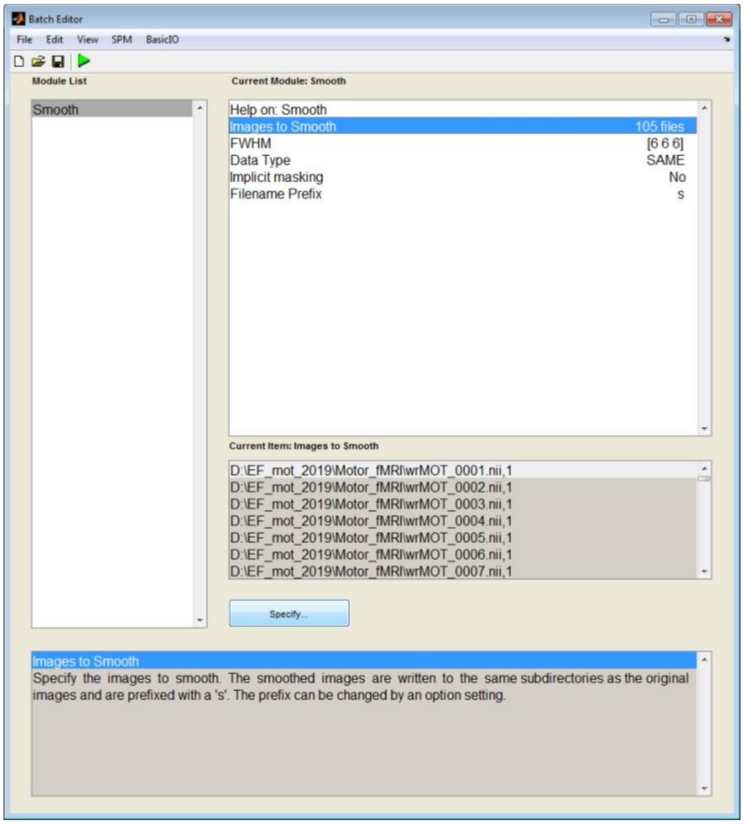
Images to Write

These are the images for warping according to the estimated parameters. They can be any images that are in register with the image used to generate the deformation.

Once the normalization completed, a new set of normalized images with the prefix "w" is available. Use the "check reg" button to verify that: a template (that can be found in -/SPM12/tmp folder), the normalized T1 image, and one normalized fMRI image are in the same space and are aligned.

SMOOTHING

In the "smoothing" batch interface you need just to select the normalized functional images that underwent all the previous steps (105 files with the prefix "wr") and change the FWHM to [6 6 6] before running the batch. A new set of images with an additional prefix "s" will be written in the same folder.



Help on: Smooth	
Images to Smooth	105 files
FWHM	[6 6 6]
Data Type	SAME
Implicit masking	No
Filename Prefix	s

Current Item: Images to Smooth

```
D:\EF_mot_2019\Motor_fMRI\wrMOT_0001.nii,1
D:\EF_mot_2019\Motor_fMRI\wrMOT_0002.nii,1
D:\EF_mot_2019\Motor_fMRI\wrMOT_0003.nii,1
D:\EF_mot_2019\Motor_fMRI\wrMOT_0004.nii,1
D:\EF_mot_2019\Motor_fMRI\wrMOT_0005.nii,1
D:\EF_mot_2019\Motor_fMRI\wrMOT_0006.nii,1
D:\EF_mot_2019\Motor_fMRI\wrMOT_0007.nii,1
```

Images to Smooth

Specify the images to smooth. The smoothed images are written to the same subdirectories as the original images and are prefixed with a 's'. The prefix can be changed by an option setting.

What do you think would be the impact of increasing a lot the FWHM or decreasing it to [2 2 2], for example?

You can also try to run the batch by changing the parameter and see what happens by visualizing the output images

Single Subject STATISTICS

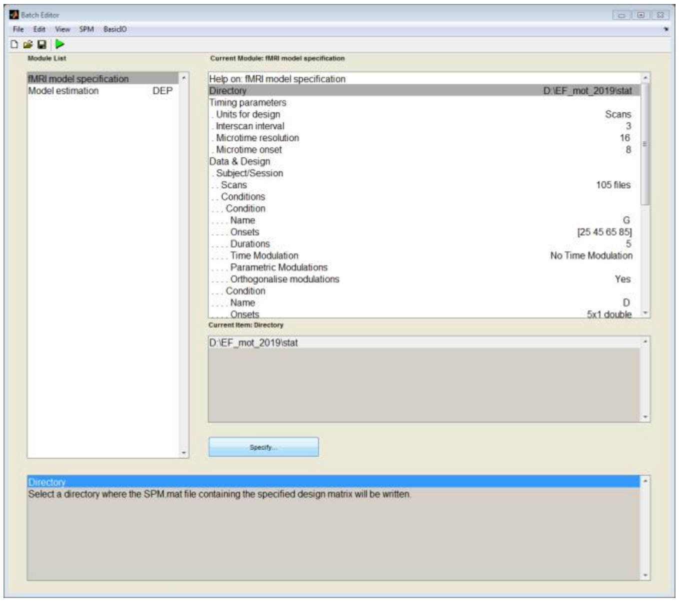
Now we have data ready for statistics. And we need information about the stimulation protocol the subject underwent.

INFO: The subject started at rest; then, after 15 seconds, he was asked to move the Left hand for 15 seconds, then his Right hand for 15 seconds, then rest again for 30 seconds and start again with the Left hand for 15 sec, Right Hand for 15 sec, and rest for 30 sec…and so on until the acquisition was finished.

The acquisition included 105 volumes and volumes were acquired one each 3 seconds.

1) Create the “onset vector” of the “left hand movement” – this means the list of the timepoints (in scans or seconds) when the movement begins.
2) Create the “onset vector” of the “right hand movement”
3) Define the “duration” of each condition.
4) The “rest” condition should not be explicitly defined: if a scan does not belong to one or the other condition, it will be associated with “rest”.

   Press on the “specify first Level” button to see the interface for setting up your model of analysis.
   Try to fill all the information needed in the batch; you can find some help in the following screenshots. However, be careful, there is a mistake.



fMRI model specification	
Model estimation	DEP

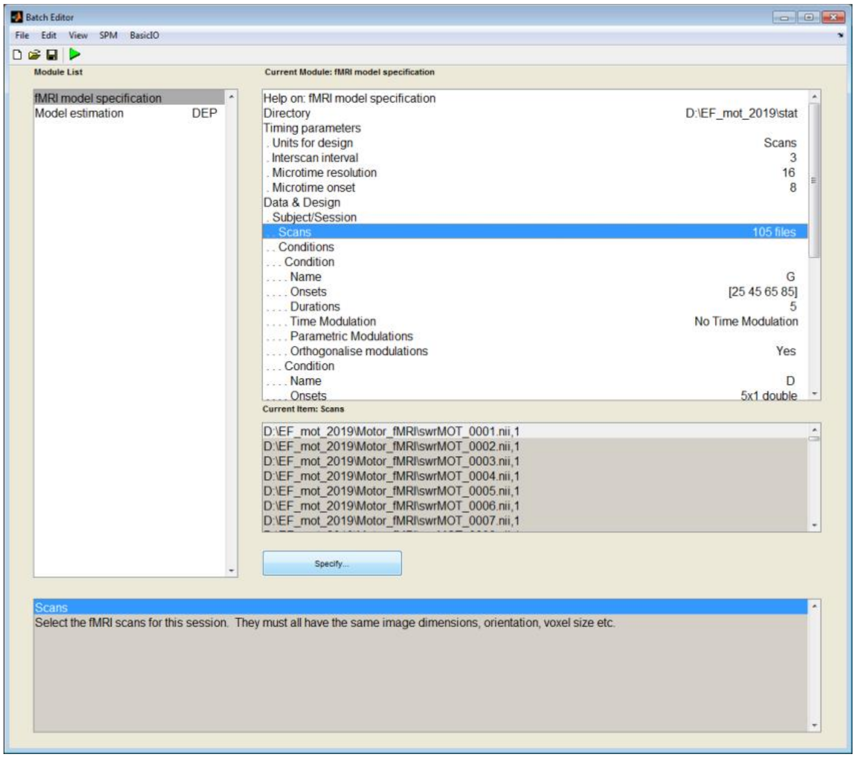
Help on: fMRI model specification	
Directory	D:\EF_mot_2019\stat
Timing parameters	
. Units for design	Scans
. Interscan interval	3
. Microtime resolution	16
. Microtime onset	8
Data & Design	
. Subject/Session	
. . Scans	105 files
. . Conditions	
. . . Condition	
. . . . Name	G
. . . . Onsets	[25 45 65 85]
. . . . Durations	5
. . . . Time Modulation	No Time Modulation
. . . . Parametric Modulations	
. . . . Orthogonalise modulations	Yes
. . . Condition	
. . . . Name	D
. . . . Onsets	5x1 double

Current Item: Directory

D:\EF_mot_2019\stat

Directory

Select a directory where the SPM.mat file containing the specified design matrix will be written.



fMRI model specification	
Model estimation	DEP

Help on: fMRI model specification	
Directory	D:\EF_mot_2019\stat
Timing parameters	
. Units for design	Scans
. Interscan interval	3
. Microtime resolution	16
. Microtime onset	8
Data & Design	
. Subject/Session	
. . Scans	105 files
. . Conditions	
. . . Condition	
. . . . Name	G
. . . . Onsets	[25 45 65 85]
. . . . Durations	5
. . . . Time Modulation	No Time Modulation
. . . . Parametric Modulations	
. . . . Orthogonalise modulations	Yes
. . . Condition	
. . . . Name	D
. . . . Onsets	5x1 double

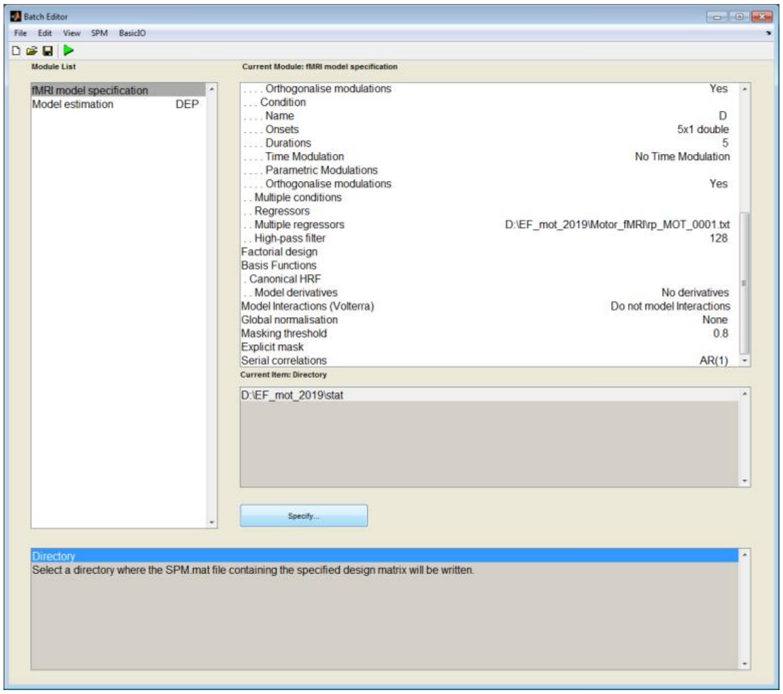
Current Item: Scans

D:\EF_mot_2019\Motor_fMRI\swrMOT_0001.nii,1
D:\EF_mot_2019\Motor_fMRI\swrMOT_0002.nii,1
D:\EF_mot_2019\Motor_fMRI\swrMOT_0003.nii,1
D:\EF_mot_2019\Motor_fMRI\swrMOT_0004.nii,1
D:\EF_mot_2019\Motor_fMRI\swrMOT_0005.nii,1
D:\EF_mot_2019\Motor_fMRI\swrMOT_0006.nii,1
D:\EF_mot_2019\Motor_fMRI\swrMOT_0007.nii,1

Scans

Select the fMRI scans for this session. They must all have the same image dimensions, orientation, voxel size etc.

As regressors (of no interest…but there is no distinction in setting up the model), you should include the movement parameters.



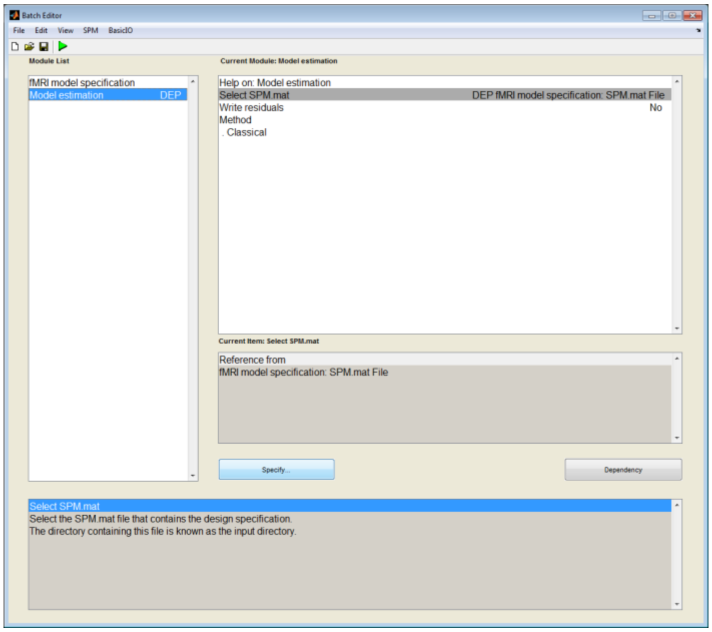
```
fMRI model specification
Model estimation                DEP
```

```
. . . . Orthogonalise modulations                                   Yes
. . . Condition
. . . . Name                                                          D
. . . . Onsets                                               5x1 double
. . . . Durations                                                     5
. . . . Time Modulation                                No Time Modulation
. . . . Parametric Modulations
. . . . Orthogonalise modulations                                   Yes
. . Multiple conditions
. . Regressors
. . Multiple regressors       D:\EF_mot_2019\Motor_fMRI\rp_MOT_0001.txt
. . High-pass filter                                                128
Factorial design
Basis Functions
. Canonical HRF
. . Model derivatives                                    No derivatives
Model Interactions (Volterra)                 Do not model Interactions
Global normalisation                                               None
Masking threshold                                                   0.8
Explicit mask
Serial correlations                                               AR(1)
```

```
D:\EF_mot_2019\stat
```

```
Directory
Select a directory where the SPM.mat file containing the specified design matrix will be written.
```

The last step requires the estimation of your model. Again, since the model is still not done, you must select it via a dependency.



```
fMRI model specification
Model estimation                DEP
```

```
Help on: Model estimation
Select SPM.mat                DEP fMRI model specification: SPM.mat File
Write residuals                                                       No
Method
. Classical
```

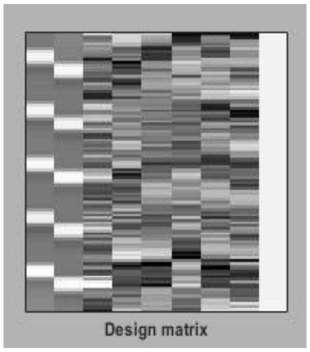
```
Reference from
fMRI model specification: SPM.mat File
```

```
Select SPM.mat
Select the SPM.mat file that contains the design specification.
The directory containing this file is known as the input directory.
```

After running this batch, the data are totally analysed, you need just to see the **"results".**

But…what about the contrasts? In what are you interested in? Which is your question? How can you answer to it?

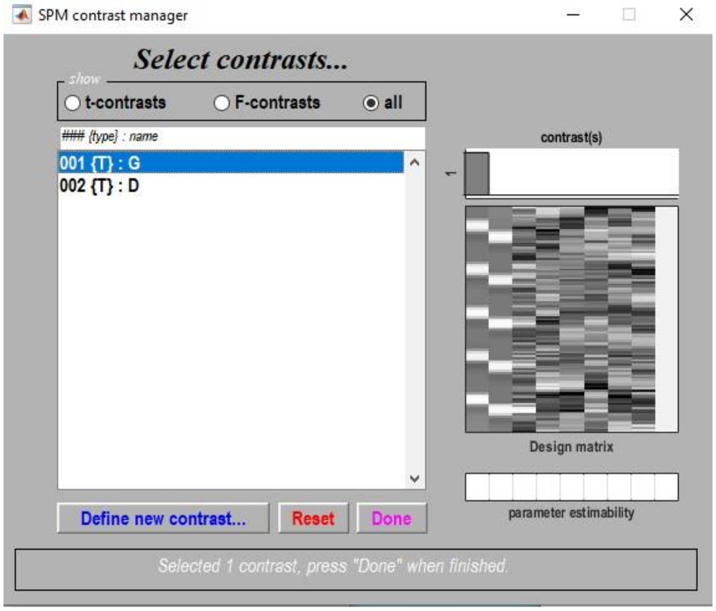
The first column of the design matrix, if you followed the hints given for the statistical model, should look like the following:



Where the first column represents the movement of the left hand.

If the question is: *which brain regions are more activated during the left hand movement then during rest*, the equivalent test will be:

- T test (because we say "more", so we define a "direction" of the difference we are interested in)
- The contrast will target the first column.



001 {T} : G
002 {T} : D

Selected 1 contrast, press "Done" when finished.

Some regions look like they activate while moving both hands; *which are the regions that are more activated during the left hand movement than during the right hand one*?

Which contrast answer to the question?