https://docs.google.com/document/d/1Zg4TsdPD-wzLH0xjsj8myBlzCLpRq-4vnu9Yok53o_o/edit?usp=sharing

FEAT & GLM Practical

Last week, we conducted a single-subject GLM using python. This week, our job is to perform a similar task, instead using the functionality of FSL and FEAT.

This worksheet borrows material from the FSL course, adjusted for use in PYM0FM, to be used on the CINN Nutanix Platform. Consequently, there are a few things you need to keep in mind:

- The data is not stored in your home folder (~/fsl_course_data), and instead it is stored in the shared pym0fm drive, located at /storage/silver/pym0fm/<your DTS login>/fsl_course_data.
- We are using virtual machines that are preconfigured for neuroimaging work. Our researchers typically use both FSL verion 5 and 6, and to avoid conflict we are containing version of the software in modules. When you start a new terminal session, whether you start a new analysis, or you just closed one terminal, you will have to type module load fsl6.0, each time.

This tutorial leads you through a standard single-subject analysis with FEAT. There may be moments when you are waiting for programs to run; during those times take a look at the FEAT manual (in particular go to the **User Guide** and look at the *FEAT in Detail* section). We also suggest that you do read it carefully after the course, before using FEAT for analysing your own data.

Example real fmri-fluency dataset

```
cd /storage/silver/pym0fm/<your DTS login>/fsl_course_data/fmri1/fluency_task
```

The *dataset fmri.nii.gz* is from a language experiment. The TR is 4.2 seconds. The experiment is event-related and has three different types of events:

1. **Word-generation events (WG)**: Here the subject is presented with a noun, say for example "car" and his/her task is to come up with a pertinent verb (for example "drive") and then "think that word in his/her head". The subject was explicitly instructed never to say or even mouth a word to prevent movement artefacts.
2. **Word-shadowing events (WS)**: Here the subject is presented with a verb and is instructed to simply "think that word in his/her head".

3. **Null-events (N)**: These are events where nothing happens, i.e. the cross-hair remains on the screen and no word is presented. The purpose of these "events" is to supply a baseline against which the other two event types can be compared.

Note that there were no additional "instruction events" as part of the experiment. Each event was "its own instruction" in that the class of the word determines the task. This means that even the "shallow" word-shadowing events contain an element of grammatical decoding.

Within one session, the events were presented at a constant ISI (Inter Stimulus Interval) of 6 seconds. For example, the first 72 seconds (twelve events) in this session may have looked like:

N-WS-N-WS-N-WS-N-WG-N-WS-WG-N

The randomisation of event types was "restricted" in the sense that there was an equal number (24) of each event type. In other words, at any given ISI each type of event was equally likely.

The main question for this experiment was to see if the "deeper" language processing in the word-generation task would yield activations over and above that of the shallower processing in the word-shadowing task. But there are also other interesting questions you can ask of the data.

Now we have introduced the experiment, let's discuss everything so far.

FEAT

So, let us get started with the analysis.

`Feat &`

(Type `Feat_gui &` if you are on a Mac). The FEAT GUI will open.

Data

Feat starts by displaying the **Data** tab. Press **Select 4D data** and select **fmri.nii.gz** (don't just type "*fmri.nii.gz*" in the file select popup or you probably won't end up setting the full pathname; use the file-select icon on the right to select the input data).

FEAT now knows how many time points (volumes) you have (106 in this dataset). The GUI will set the TR (time between 3D volumes) using information in the NIFTI file, however this information is not always correct (depending on how the conversion to NIFTI was done). So you should always check that the value for TR is correct after the FMRI data is loaded. For these data it should be 4.2 seconds (you might get 4.199, and that is ok).

The **High pass filter cutoff** is preset to **100secs**. This is chosen to remove the worst of the low frequency trends, and is also long enough to avoid removing the signal of interest. In general you need to ensure that this is not set lower than your maximum stimulation period. For a random-order event-related design there is no clear "stimulation period" so in order to assess what the cutoff should be one needs to analyse the frequency-content of our expected activations (remember that the design matrix embodies what we expect to see in the brain). Leave it at the default for now and we'll come back to it when we have specified the design.

Pre-stats and Stats

Press the **Pre-stats** tab to look at the preprocessing steps. For this experiment we will change **Spatial smoothing FWHM (mm)** to 7mm, which is slightly more than we normally recommend. All the other default pre-processing steps are fine for this dataset.

QUESTION: What does FWHM refer to? How might we usually choose the FWHM? (Think back to the preprocessing lecture).

Setting up the design matrix

Select the **Stats** tab and press **Full model setup** to setup the GLM details.

Change the **Number of EVs** to **2** (we have two conditions to model separately - Word-generation and Word-shadowing).

Setup **EV1** (Word-generation): First chose a sensible name like for example **Gen** for it and change **Basic shape** to **Custom (3 column format)** and select the file *word_generation.txt*. Later (when you wait for FEAT to finish the analysis) we will return to this file and make sure you understand what is in it. Next set **Convolution** to *Double-Gamma HRF* which corresponds to the HRF you saw in the talks. Leave the setting for **Phase**, to the default but *unset* **Add temporal derivative**. We would normally recommend leaving it set (and we will come back to set it) but in order to obtain a very simple initial design we will unset it for now.

Setup **EV2** (Word-shadowing): Chose a name (for example **Shad** and change **Basic shape** to **Custom (3 column format)** and this time select the file *word_shadowing.txt*. Same as for **EV1** set **Convolution** to *Double-Gamma HRF*, unset **Add temporal derivative** and leave everything else as the defaults.

QUESTION: Here we are giving FSL the information it needs to construct our predictors. Thinking back to last week - what pieces of information do you anticipate might be contained in the .txt files?

Setting up contrasts

We introduced the concepts of **contrasts** last week. As you may recall, these are linear combinations of our estimated parameters **β** that we can use to evaluate a hypothesis. You may also recall that they are the numerator (the 'effect') of a t statistic, with the standard error (the 'uncertainty') being the denominator.

$$t_{\hat{\beta}} = \frac{\hat{\beta}}{\mathrm{SE}_{\hat{\beta}}}$$

To introduce a bit more terminology - FSL refers to the images produced by these contrasts as **COPES.** By contrast, the variance of the contrast is referred to as a **VARCOPE.**

Hence, from this, you may be able to reason that a t-statistic image produced by FSL is created as follows:

$$t = \frac{COPE}{\sqrt{VARCOPE}}$$

Now set up these Contrasts (click on the **Contrasts & F-tests** tab. Set the **Number of contrasts** to 5 and enter the following contrasts: (try to have a guess as to how to input these before looking at the answer).

1. Name the first contrast **Generation**. Set the contrast to be sensitive to the activation in word-generation above and beyond that in rest. show answer.
2. Name the second contrast **Shadowing**. Set the contrast to be sensitive to the activation in word-shadowing above and beyond that in rest. show answer
3. Name the third contrast **Mean**. Set the contrast to be sensitive to the mean activation in word-generation and word-shadowing being larger than in rest show answer.
4. Name the fourth contrast **Shad > Gen**. Set the contrast to be sensitive to the activation in word-shadowing above and beyond that in word-generation. show answer
5. Name the fifth contrast **Gen > Shad**. Set the contrast to be sensitive to the activation in word-generation above and beyond that in word-shadowing. show answer

Let’s talk about this next phase.

Setting up F tests.

F-tests enable you to investigate several contrasts at the same time, for example to see whether any of them (or any combination of them) is significantly non-zero. If you recall learning about an ANOVA from your stats classes, an F test is also known as an *omnibus* test, since it tests multiple parameters of a model simultaneously.

In this context, the F-test allows you to compare the contribution of each contrast to the model and decide on significant and non-significant ones. F-tests are non-directional (i.e. test for "positive" and "negative" activation).

Let's set up an **F-test**. Set the **Number of F-tests** to 1 and select the first two contrasts. This spans both conditions and will show you any areas where there is significant activation by Word-generation *AND/OR* Word-shadowing. Thus the output image produced by this F test will show where either generation *or* shadowing activation (or both) occurs; i.e. it will show both on a single image.

Press **View design**. Make sure you understand the resulting design matrix. Time goes down the page, with every 10 TRs ticked off on the left. The red bar shows the width of the highpass filter (any signal much longer than it will be filtered out). There are 2 columns in the design corresponding to our predictions about BOLD activity from Word-generation and Word-shadowing respectively. Hopefully this will all be familiar to you since we manually made something like this using python last week. The contrasts appear at the bottom of the image, with the F-test to the right of the contrasts. Note that you can make the design matrix display disappear just by clicking on it once. For now, leave the design matrix display up (Press **View design** again if necessary).

QUESTION: What part of the GLM equation is this design matrix?

Temporal derivatives

Now, return to the **EVs** tab and FOR BOTH EVs select **Add temporal derivative**.

Press **View design** again. You now see 4 columns with columns 1 and 3 being the same as before and column 2 and 4 being "new". These are the temporal derivatives that are used to correct for timing errors caused either by slight experimental errors in synchronising the times of the scanner with the stimulus presentation and/or inter-subject differences in the delay inherent in the HRF. Now press "Done" and dismiss the view of the design matrix.

Do you remember that we said we should return to the issue of **High pass filtering** once we knew the design (and with that the expected frequency content of the signal we expect/hope to see)? Now the time has come. Press the **Data**-tab to make sure that **High pass filter cutoff (s)** is set to 100. Next press the **Misc**-tab where there will be a button saying **Estimate High Pass Filter**. Press this button and then go back to the **Data**-tab to see what has happened. This should now have changed to 90 seconds.

QUESTION: How might FSL have estimated the best high-pass filter based on the information we gave it?

Look at the **Post-stats** section - the defaults are fine; cluster-based thresholding will be carried out. We will review the concept of cluster-based thresholding in a future session.

QUESTION: What does high pass filtering do to your data? Why do we want to do it?

Registration

Select the **Registration** tab. By default FEAT will register the middle-timepoint FMRI image (saved as example_func in the .feat output directory) to the standard space template. We recommend in general turning on the **Main structural image** option so that the lowres FMRI image is first registered to a brain-extracted highres structural image from the same subject; this highres image is then registered to the standard space template, and then the two registrations are combined to give an *example_func2standard.mat* transform which can be used later to resample the FMRI stats into standard space.

Set the **Main structural image** file to *structural_brain.nii.gz* with 7 DOF (note that we have already run BET on this, and in order to save time in this practical session we are not using the BBR method, but we *strongly* recommend that BBR is used generally). Leave **Standard space** turned on with *MNI152_T1_2mm_brain.nii.gz* selected and set the DOF to 12. Instead of the linear (12 DOF) registration we are using here, the more accurate nonlinear registration is normally recommended for registration to MNI space by selecting the "nonlinear" option, but we use linear here as it is faster for this practical.

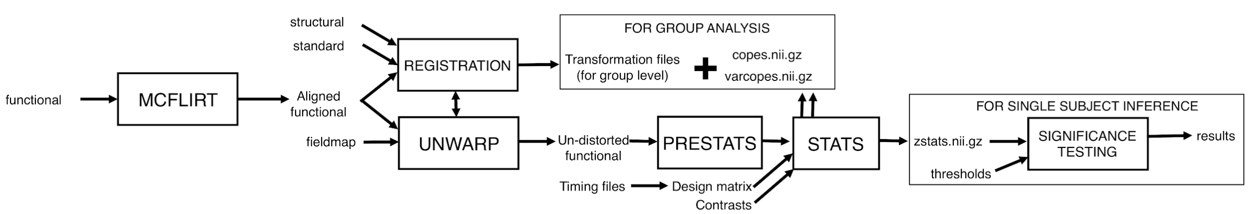
Go!

You are now ready to run FEAT. Press **Go**. A web browser should appear, and as FEAT completes the different stages of processing, you will see messages appear in the **Log** section. Whether the web browser (and indeed the FEAT GUI) is left displayed or is closed, FEAT will continue to run in the background. For now, leave the web browser open so that you can monitor FEAT's progress. FEAT will take 2-5 minutes to complete.

Ok, let's discuss this phase.

While you wait

Take a look at the flow diagram below which summarizes all of the steps that you just set up in Feat. Hopefully it will help you to see the big picture, and avoid getting bogged down in the details:



Whilst FEAT is running, run FSLeyes to have a quick look at the different images mentioned above: start by looking at *structural_brain.nii.gz*, and then view fmri.nii.gz. Note that when viewing the 4D image you can see the image time series as a movie by pressing on the movie icon (), and you can also see time series plots by pressing *View* > *Timeseries*.

Create a mask to probe statistics

Whilst FEAT is still running, we will now use FSLeyes to create a mask in standard space that will be used later to find out about activation statistics from within the mask.

1. Reopen FSLeyes and load the standard space template image $FSLDIR/data/standard/MNI152_T1_2mm ($FSLDIR is an environment variable indicating the directory in which FSL is installed, you can type `echo $FSLDIR` to see what this is set to). Inside FSLeyes you can use the *File -> Add standard* menu option to find these standard space images quickly.
2. Open the atlas panel via *Settings -> Ortho View 1 -> Atlas panel*, and enable the *Harvard-Oxford cortical*, *Harvard-Oxford subcortical* and *Juelich Histological* atlases. Move the cursor around a little in the standard brain and see how the labels and numbers in the atlas tool window changes. If you have a favourite part of the brain and you happen to know where it is you can move the cursor there and see if the atlas tool agrees with you.
3. Now select the *Atlas search* tab in the atlas panel, and choose the *Juelich Histological Atlas* from the list on the left. You will now see a list of all the structures in that atlas on the right.
4. Type ba4 in the text box above the structure list to filter the structures that are shown. Click the check boxes next to **GM Broca's area BA44 L** and **GM Broca's area BA45 L**.
5. You will notice that in the FSLeyes overlay list, two images have been added with name corresponding to the regions you just selected. If you select one of those and then move the cursor around you will notice its intensity values in changing between 0 and 100. These values reflect the probability that a given voxel (cursor position) is indeed part of that structure.
6. Press the save icon ( ) next to the image **juelich/prob/GM Broca's area BA44 L** in the overlay list and save the image to a file called *BA44*. Repeat this process for *BA45*. (**note:** make sure you save these to a location where you have write access (for example */storage/silver/pym0fm/<your DTS login>/fsl_course_data/fmri1*)
7. What we will do next is to create a mask which has the value 1 for each voxel that has a 50% or greater chance of belonging to BA44 and/or BA45. We do this by typing (in the terminal window):
   `fslmaths BA44 -add BA45 -thr 50 -bin Broca`
   The end result of this is a file named *Broca.nii.gz* that we will later use as a mask to plot time-series of our results. If you want to convince yourself that this file indeed contains what it should you can type
   `fsleyes -std Broca -cm red`
   and have a look.

Look at the EV specification

Lastly, whilst FEAT is running take a look at the files that we used to specify our design, *i.e. word_generation.txt* and *word_shadowing.txt*. We do this by typing (still in the terminal window):

`more word_generation.txt`

The more command will show you the contents of the file (type q to quit if the terminal doesn't give you your prompt back). Once you've finished looking at word_generation.txt, run `more word_shadowing.txt` to look at the timing information for the word shadowing task.

QUESTION: There are three columns in these files, what information does each column contain?

QUESTION: Why is the final column all ones in both files?

While FEAT is running it will display **STILL RUNNING** in the main FEAT report page, which is replaced by **Finished at ...** when it is done. Once FEAT has finished, look carefully at the various sections of the web page report, including motion correction plots in the Pre-stats section, the colour-rendered activation images and timeseries plots in the Post-stats section, and the Registration results. Note that if you click on the activation images you get a table of cluster co-ordinates.

Ok, let’s discuss this phase

Featquery

Featquery allows you to calculate certain data statistics, either at a voxel of interest, or averaged over a region of interest using a mask. We will use the standard-space mask which we created earlier. Start up Featquery from the terminal:

`Featquery &`

Select the *fmri.feat* directory created by your first analysis on the fmri-fluency dataset.

Featquery automatically reads the FEAT directory and gives you the appropriate options as to which statistics you can choose to investigate.

1. Select the following statistics for the contrast that looks at activation for word-generation (*i.e.* the **[1 0]** contrast), the contrast that looks at activation for word-shadowing (*i.e.* the **[0 1]** contrast) and the contrast that looks at activation for word-generation over and above word-shadowing (*i.e.* the **[1 -1]** contrast), *i.e.* contrasts number 1, 2 and 5:
    - **stats/cope** (unthresholded contrast of parameter estimate)
    - **stats/tstat** (unthresholded t statistics)
    - **stats/zstat** (unthresholded z statistics)
    - **thresh_zstat** (thresholded z statistics)
2. In the **Input ROI selection** panel, enter the mask that you created earlier (*i.e. Broca.nii.gz*) as the **Mask Image** (note - Featquery can take either a standard-space mask OR a lowres one in the original dataspace OR a mask in the space of the structural image)
3. In the **Output options** section, select the **Convert PE/COPE values to %** option
4. Select the **Do not binarise mask (allow weighting)** option
5. Select an atlas (inside the Output options section), for example the **Harvard-Oxford Cortical Structural Atlas**. The local maxima voxels reported by Featquery will be related to structures in the selected atlas.
6. Press **Go** and a web browser showing the estimated statistics should popup shortly (possibly after a minute or two).

The resulting web page will contain a table summarizing each of the statistics that you asked Featquery to report on in step 1. The first column gives the statistic name. The second column gives the number of non-zero voxels in the mask. The next group of columns gives a summary of the distribution of values within the mask. Finally, the last group of columns contains the position of the maximum in voxel space, in mm space, and in the atlas space selected in step 5. Plots of the timeseries at the maximum z-stats are available by clicking the link labeled "Masked time series plot" just below the image of the mask at the top of the page.

QUESTION: What does the second column ("# voxels") tell you for the copes and t-stats images?

Ok, now we have finished. Let's discuss all this.


STOP