https://docs.google.com/document/d/1srEpT1rqpJ3bX3Dkl1nZEPupeVf4T4_Z2rVUFEDS-3U/edit?usp=sharing

# FEAT 2 Practical

This is the version of the FSL practical 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.
- Remember that 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.

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This tutorial leads you through examples of higher-level group analysis in FEAT.

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## Paired t-test

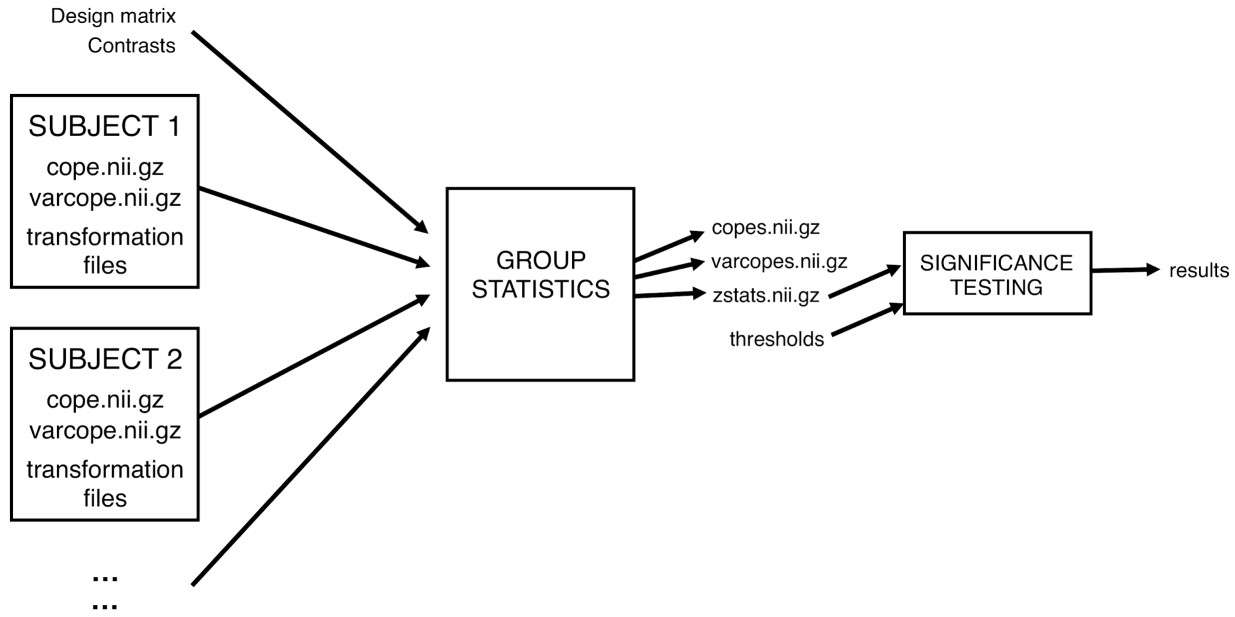
We have a group of six subjects, each scanned twice: once doing motor tasks with their left hand, and once with their right hand. This is a stroke study, and hence comparing left and right motor function is particularly interesting in this case. Within each run, subjects completed different blocks of index finger movement, sequential finger movement and random finger movement.

**Research question**: Is there a significant left vs right hand finger movement paired-difference, generalisable to the population from which the subjects are drawn?

To address this, we want the *left − right* paired mean difference within a mixed effects model, taking into account the within-subject fixed effects variances and the between-subject random effect variance. This is done as a two-level analysis with the following structure:

- **Level 1: Single-session analyses** There are 6 subjects × 2 sessions = 12 first-level FEAT analyses. These have already been done for you.
- **Level 2: Between-subject analysis** We do a separate second-level analysis for each of the first-level contrasts, and estimate the mean (paired) difference for each.

In FSL terminology, each contrast is represented by a COPE (contrast of parameter estimate), and it is these which we pass up to any higher-level analysis. Note that as well as the COPEs, FEAT passes the variance of these COPEs (VARCOPEs), and even the uncertainty in the variance of these COPEs (DOFs; degrees-of-freedom), between the different levels.




**QUESTION: What do the values in a COPE image obtained from the single-session analysis represent?**

**QUESTION: What do the values in a VARCOPE image obtained from the single-session analysis represent?**

## First-level analyses

Each first-level analysis contains 6 contrasts, each related to the different types of finger tapping performed in the scanner (e.g. mean response over the different conditions, index finger only, random finger tapping, sequential finger tapping). Thus there are 6 COPEs in the stats subdirectory of each first-level .feat directory.

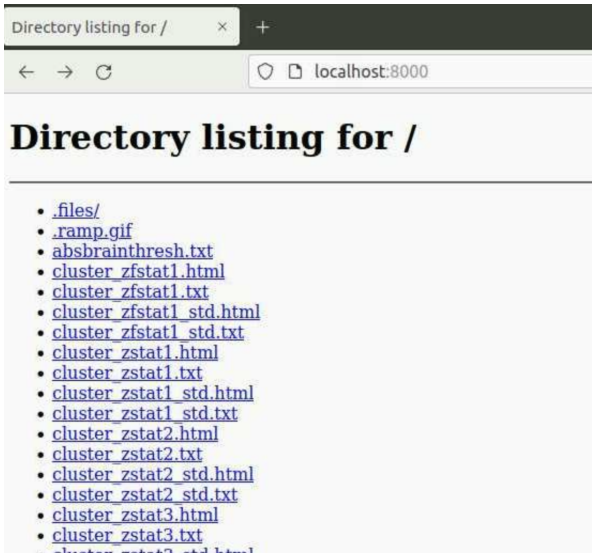
A higher-level FEAT analysis entails an independent analysis on each of these contrasts (i.e. a second-level analysis of all subjects' first-level *mean* contrasts, a separate second-level analysis of all first-level *index* contrasts, etc.). Each of these second-level analyses is performed simultaneously and will form a separate cope?.feat directory inside a newly-created .gfeat directory.

```
cd /storage/silver/pym0fm/<your DTS login>/fsl_course_data/fmri2/paired_ttest
```

The first-level analyses are held in 6 different directories within /storage/silver/pym0fm/<your DTS login>/fsl_course_data/fmri2/paired_ttest, one for each subject. The subject directories are ac at cm df dn eg. There are two first-level FEAT directories within each of these, and these have already been run for you.

Navigate to `ac/ac_left.feat` and type `ls` to see the outputs for the first level analysis. Among these is *report.html*, which summarizes the lower-level feat output. To take a look at this, type `python3 -m http.server &`.

Then open up firefox (make sure this is in your current VM, not the one 'above' it) and type in *localhost:8000* into the search bar and press enter. You will then be presented with the same directory listing you saw in the terminal.




Click on *report.html,* which will allow you to browse the lower level feat report. Navigate to the stats tab and take a look at the design matrix.

**QUESTION:** You will notice that the last few columns of this design matrix have the title 'conf' what could this mean?

**QUESTION:** Why do you think these 'conf' regressors are not included in any contrasts?

**QUESTION:** You can see that an F test has been specified. What hypothesis does this evaluate?

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

If you are waiting, please choose your preferred type of pizza here:
https://strawpoll.com/polls/GPgV35A9kZa

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## FEAT set-up

Now navigate back to the top level of the paired t-test directory.

```
cd /storage/silver/pym0fm/<your DTS login>/fsl_course_data/fmri2/paired_ttest
```

Or

```
cd ../..
```

Open FEAT (`Feat &`) and follow the instructions below to set up the higher-level analysis.

First, change **First-level analysis** to **Higher-level analysis** in the drop down box at the top.

**Data**

Here we are going to set up the input data for our higher level analysis. First, change the **Number of inputs** to 12 (i.e. 6 subjects × 2 sessions).

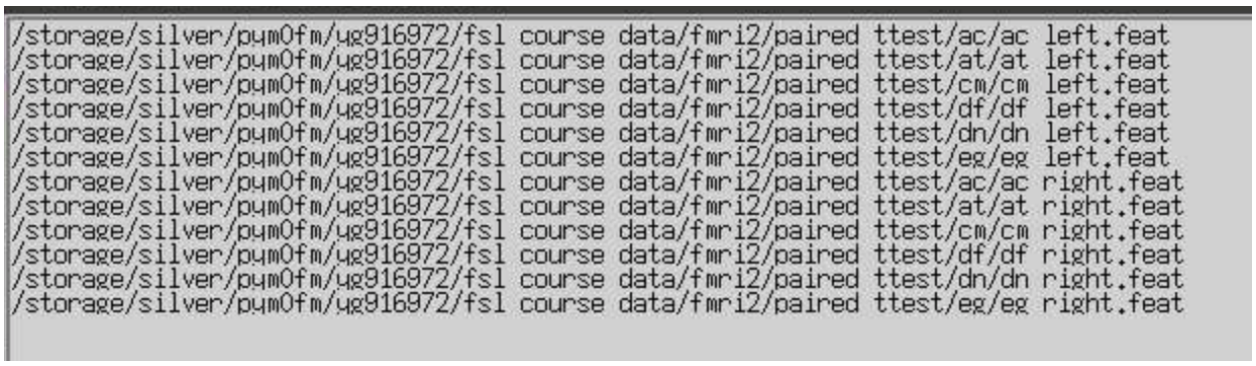
Press **Select FEAT directories**. At this stage, you need to decide on a *sensible* order for the first-level analyses. You could choose to group the analyses by subject (i.e. ac/ac_left.feat, ac/ac_right.feat, at/at_left.feat, etc.), or you could group by condition (i.e. ac/ac_left.feat, at/at_left.feat, …, ac/ac_right.feat, etc.). We recommend the latter option, because this matches the example paired t-test in the FEAT manual, and also matches the way the paired t-test is set up for you if you use the **Model setup wizard** (explained below).

You can often avoid having to tediously hand-select each of these first-level FEAT directories separately, using the **Paste** button. If you press this, a new free-text window comes up, within which you can paste text (in this case the list of first-level FEAT directories) which you can copy, e.g. from a list in a terminal. Press **Clear** to clear the text window. Then in your terminal, making sure you are inside the paired_ttest directory, type:

```
(ls -d "$PWD"/??/??_left.feat ;  ls -d "$PWD"/??/??_right.feat) | cat
```

This should give you a complete listing of the full pathnames of the FEAT directories in the right order. You can now highlight this list with the mouse copy it, and paste it into the FEAT paste window by clicking in the paste window and pressing control-y.

If you have done this correctly, it should look like this:




Press **OK**

To save time, we will only pass the *mean* contrast up to the top level. Make sure that **ONLY** contrast 1 is selected in the **Use lower-level copes** boxes.

When this is done, set the **Output directory** to *paired_ttest_ols* (the full path will end up as /home/$HOME/fsl_course_data/fmri2/paired_ttest/paired_ttest_ols.gfeat).

**Stats**

Select the **Mixed Effects: Simple OLS** option from the top drop down box. Also, make sure that the **Use automatic outlier de-weighting** button is **NOT** turned on. It is important that these two settings are chosen, otherwise the analysis will not be quick enough to be of use to you in the time that we have available for the practical. Normally, we recommend that the more accurate "Mixed Effects: FLAME 1" option. However, in the interest of speed, in this practical we choose the faster OLS option without outlier de-weighting.

With this design you can use the **Model setup wizard**, which provides an easy way of setting up a few simple designs. Select **two groups, paired** and press **Process**. You will now see the design matrix that has been created for you.

To understand how this is controlled in detail, click on **Full model setup**.

- The inputs (*Input 1* to *Input 12*) correspond to the order you entered the first-level FEAT directories—it is *essential* that your design matches the order you entered the lower level directories under the **Data** tab! Note also that the first column, labelled *Group*, corresponds to groupings of inputs that will share the same random effects (RE)

variance in this level of the model. Here, we let all subjects have the same RE variance (i.e. the *Group* column should be left as all 1s).
- There are 7 EVs: EV 1 models the *left − right* paired difference, and EVs 2-7 are 'confounds' which model out each subject's mean (this is what makes the design a paired t-test). By doing this, we 'model out' variation due to between subject differences, such that the remaining variation reflects between condition differences.
- Click on the **Contrasts & F-tests** tab. There are two contrasts set up for you by the wizard. EVs 2-7 are confounds of no interest and so do not appear in the contrasts. Hence, the contrasts only involve EV1. Change the **Titles** boxes to read *left* > *right* and *right* > *left*.
- Press **Done**.

**Post-stats**

Because we only use a small number of subjects in order to make it possible to run the analysis in the practical session, we will reduce the cluster threshold slightly. This will allow us to see some more results, but is **NOT** recommended for your own analyses. In the **Thresholding** box change the **Z threshold** to 2.3.

**Go!**

Press **Go**! The web browser that appears monitors the overall progress. This second-level analysis should take about 5 minutes. While you're waiting, either make a cup of tea (but do ***NOT*** add milk while the bag is still in the water) or familiarise yourself with the introduction to the next major section of the practical on group analyses with multiple sessions per subject.

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Let's discuss this next section.

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**Results**

Higher-level FEAT runs produce .gfeat directories. Once the analysis has finished, explore the web report (if this fails to load spontaneously, you can view it with the http.server method

described above). This top-level report provides links to the previous level reports, a registration summary page and links to the separate higher-level reports.

***LOOK AT YOUR DATA***! In particular it is always important to check the registration summary report page very carefully, to ensure that all lower-level registrations succeeded. If any of the lower-level FEATs look like the registration has failed badly, you need to fix this before re-running the higher-level FEAT analysis.

In the results page you get a link to the group results from running the group-level analysis on each first-level contrast. Within each contrast you get a group-level results page showing the standard post-stats output. However, note that the *time course* outputs in these higher-level results no longer refer to *time* (despite the heading). They refer to subject (or session) number. In this case that is the 12 sessions (6 subjects × 2 conditions) in the study, and it is effect size shown on the vertical axis, rather than normalised MRI signal.

As mentioned on Monday, this is because the Y of our second level GLM equation is composed of the parameter estimates from the first level.

Have a look at this and the other parts of the results webpage and make sure you understand what is being shown.

**Pre-baked analyses**

We have run a full analysis for you on this data (i.e. on all the contrasts, using FLAME for statistics, and with the recommended Z-thresholds). Take a quick look at this report as well.

```
firefox examples/flame.gfeat/report.html &
```

**QUESTION:** Looking at the results tab, can you spot any major differences between the two analyses?

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Lets discuss this next section.

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# Group analysis with multiple sessions for each subject

It is common to split a task up into multiple short scans instead of having one long scan. This can often help to reduce subject movement in the scans, and also to keep the attention of your participant. As a result, we need to combine data across multiple scanning sessions using a three-level FEAT analysis.

The data consists of a set of subjects, each scanned twice several months apart. For simplicity's sake, we will look for a simple mean effect across subjects and sessions. Hopefully this will help you understand how this analysis can be extended to more complex questions.

We want the mean group effect, within a mixed effects model, taking into account the within-subject fixed effects variances and the between-subject random effect variance. This is done in THREE levels:

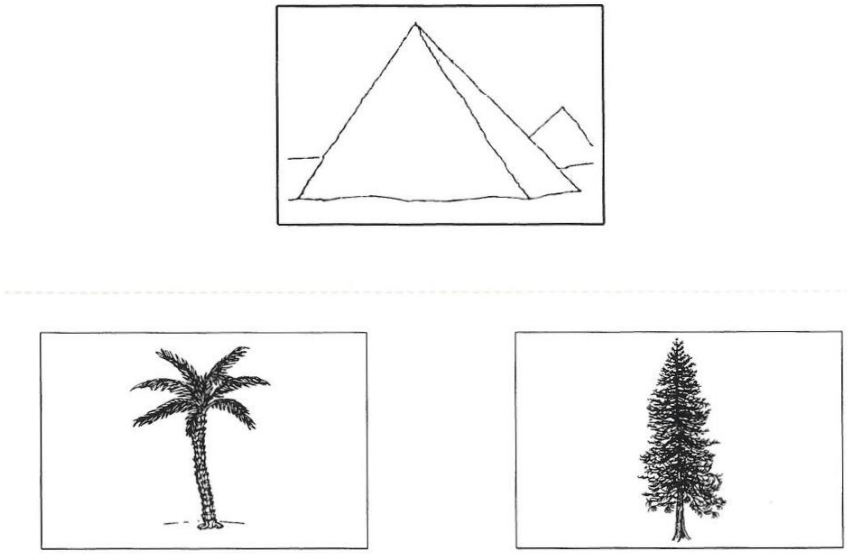
- **Level 1: Within-session analysis**. There are 5 subjects × 2 sessions = 10 of these first level FEAT analyses, which have already been done for you.
- **Level 2: Between-session analysis**. Here, we input the data from Level 1, and estimate each subject's mean response.
- **Level 3: Between-subject analysis**. We input the data corresponding to the subject means from Level 2, model the between-subject variances and estimate the group mean response.

Because each subject will typically only have a handful of sessions, we do *not* run a *mixed effects* second-level analysis to get an estimation of each subject's mean response. The reason for this is that we would not be able to get a good estimation of the within-subject session-to-session variance with a limited number of sessions. Hence we choose to ignore the session-to-session variance by using a *fixed effects* analysis at this second level.

In addition to this, the analysis cannot be combined into a single second-level analysis. This is tempting as a design matrix can easily be formed containing each subject's mean (across sessions) as a separate EV, and then contrasts can be formed to test the mean across all subjects. The problem with this model is that there are two separate sources of variability (session-to-session and subject-to-subject) but, within FSL, a single level cannot model more than one separate sources of variance.

**First-level analyses**

In both sessions, subjects performed the "Pyramids & Palm Trees" task (PPTT). Participants are presented with a target image, and are asked to select the image they most associate with the target from a pair of additional images. The canonical example is below:



This is meant to be a test of semantic memory, as the task requires reasoning about the links between objects. There is also a control condition, where participants have to match abstract line drawings. We are primarily interested in the *semantic* > *lines* responses. To begin with, familiarise yourself with the first-level design and typical responses in one of the session-specific FEAT analyses we have run for you:

```
cd /storage/silver/pym0fm/<your DTS login>/fsl_course_data/fmri2/3_levels
```

Take a quick look at one of the web reports within the run directories of the level_1/ directory.

**Second-level analysis**

We will now set up the second-level (i.e. within-subject) analysis. Open FEAT (`Feat &`) and follow the instructions below:

- Change **First-level analysis** to **Higher-level analysis**.
- Change the **Number of inputs** to 10 (5 subjects × 2 sessions).
- Press **Select FEAT directories**. Again, you need to specify the first-level FEAT directories in a sensible order: subject 1, sessions 1, 2; then subject 2, sessions 1, 2; etc. There are lots of ways we can enter these into the GUI: we can enter them individually into the GUI by hand, but this can be laborious for large studies; we can type out the names in a file and use the **Paste** window; or we could do some simple ls commands and then reorder the outputs as necesary in a text editor. Finally, if we have chosen a sensible naming convention we may be able to script the whole process.
  To save time, we will use a command to generate the names we need.
  To generate the list, use the command given here (if this is tricky to type out, you could

even enter this URL into firefox on the VM and copy-paste).
Select the text and right-click copy the generated list of names. Paste this text (control-y) in the **Paste** window as before.

- To save time, we will only pass the *semantic* > *lines* and *semantic* < *lines* contrasts up to the higher levels. Make sure that **ONLY** contrasts 1 & 2 are selected in the **Use lower-level copes** boxes.
- Set the **Output directory** to level_2
- Go to the **Stats** tab and select the **Fixed-effects** option.

**QUESTION:** Where will the fixed effects model take the variance estimate from?

- Press **Full model setup**. Remember that the *Inputs* (1-10) correspond to the order you entered the first-level FEAT directories. As this is a fixed effects analysis the *Group* column is ignored so leave all these entries as 1 (*if* we had lots of sessions and did a mixed effects analysis instead then we would use a unique number in this column for each subject (i.e. within each subject we would estimate a separate variance).
- We need 5 EVs: one for each subject mean. Change the 0s to 1s appropriately, in such a way that each EV models a different subject mean. We then need to pass the 5 parameter estimates (PEs) corresponding to the 5 subject means through to the third level as COPEs. To enable this, we need to have a contrast for each subject mean that just selects that parameter. Set the contrasts appropriately. Your design matrix should now match this design matrix
- Press **Done**. The default **Post-stats** are fine (in fact, the post-stats don't affect what gets passed up to third-level). You are now ready to run the second-level analysis so press **Go**!

If something has gone wrong with your analysis, or it is taking too long to run, there is a pre-baked version available: examples/level_2.gfeat. You can use this as the input to the third level analysis (the next section) if necessary.

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Let's discuss this next section.

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The analysis should only take a couple of minutes to run. Wait for the result web pages and then view them *carefully*. Check that the registrations are accurate, and then take a look at the results.

**Third-level analysis**

We are now ready to set up the third-level (i.e. between-subject) analysis. This will be valid for one of the contrasts we passed up to the second level (but it easy to repeat the analysis for the others). We will use the *semantic* > *lines* results, which corresponds to contrast 1. Reopen FEAT (Feat &) and follow the instructions below:

- Change **First-level analysis** to **Higher-level analysis**.
- Change **Inputs are lower-level FEAT directories** to **Inputs are 3D cope images from FEAT directories**. The inputs will be the 5 COPE images, one for each subject mean, from the second-level analysis.
- Change the **Number of inputs** to 5 (each corresponding to a subject mean).

Press **Select cope images** and enter the COPEs from the second level. These will be inside the cope1.feat/stats directory which is inside the second-level level_2.gfeat directory that you just created. The relevant command for pasting is:

```
ls -d "$PWD"/level_2.gfeat/cope1.feat/stats/cope?.nii.gz
```

- Set the output directory to *level_3*
- Go to the **Stats** tab and change to **Fixed effects**. Note that this is **NOT** recommended for group-level analyses, but we use it here to save time and because we are only analysing five subjects. Normally, mixed effects would be used.
- Use the **Model setup wizard** to generate a *single group average* design.

**QUESTION:** How do you interpret the design matrix? What will the only column model?

- Press **Go**!

Again, if something goes wrong, you can look at examples/level_3.gfeat if necessary with a pre-cooked example.

Again, this analysis should only take a couple of minutes to run. Wait for the result web pages and look at the results.

Again, if the browser window does not load spontaneously, you can load this by either starting the http server, as before, or typing

```
firefox level_3.gfeat/report.html
```

Do they look plausible?

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The end.