

Analysis of fMRI data in FSL FEAT

This document provides a concise description of the steps for processing fMRI data using the FSL GUI. More verbose descriptions of each step are available through the FSL documentation, which should be used as supplement to this abridged protocol.

Required software.

1. FSL fsl.fmrib.ox.ac.uk/fsldownloads/fsldownloadmain
2. MRICron mccauslandcenter.sc.edu/mricro/mricron/install
3. Mango ric.uthscsa.edu/mango/download

Convert DICOM data to NIFTI: This stage will change file formats to NIFTI which is universal and easy to handle.

- Download mricron suite if not done already
- Open dcm2nii application within mricron suite
 - File-> Dicom to NIFTI (browse for folder containing DICOM files)
- Repeat for all anatomical and functional scans.
 - Anatomicals will produce three copies. Use the one prefixed “co”.

Startup: This stage starts fsl

- Arrange data in some sensible way.
- Make sure there is no white space in the filepath.
- Open Terminal and navigate to a convenient place in the file hierarchy

>cd Documents/MyProject/data

- run FSL

>fsl_gui

Note: Receive help by hovering mouse over any option.

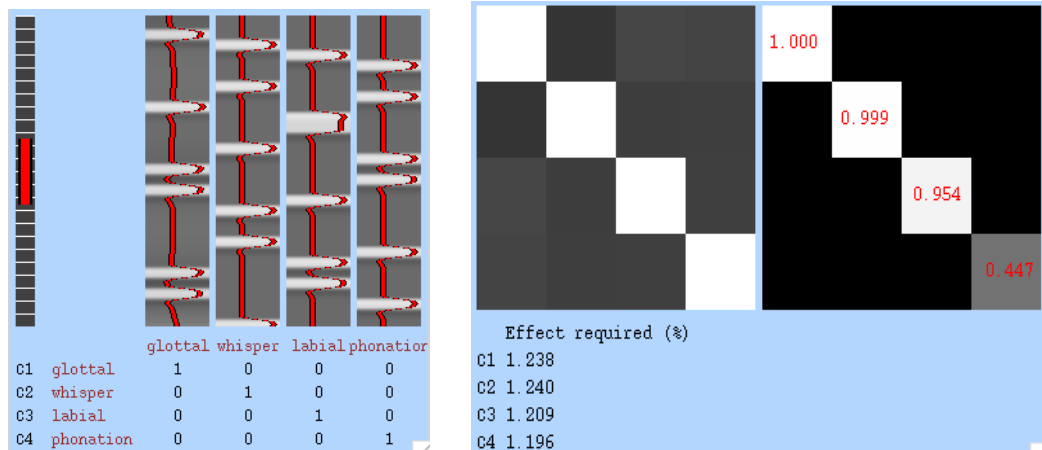
BET Brain extractions: This stage will process the anatomical scans and remove skull and dura

- Click BET brain extraction. This will open a new window.
- Input image = anat.nii
- Output image is automatically filled.
- Switch to robust brain center estimation in dropdown.
- Click '*Advanced options*'
 - Check output brain-extracted image
 - Check binary brain mask image
 - Fractional intensity threshold = 0.25 (varies by scanner)
 - Threshold gradient = 0.25 (varies by scanner)
- Check results. Modify either of the latter parameters and try again if result was not satisfactory.
- Repeat for all anatomical scans.
- Optional: use bet to make a mask of EPI data to apply to analyses.
- Close window

First level analysis with FEAT: This stage will perform first level fixed effects analyses on all subjects. Highly recommend using the batch function and checking individual results afterwards. NOTE: First level outputs are registered to native space only. Transformation matrices for registration to standard space are

computed at this stage, but not applied until second level mixed effects analysis. Running this step takes about 1-2 hours/scan.

- From dropdown menu select '*First-level analysis*' and '*Full analysis*'
- Data tab
 - Number of inputs = number of scans to batch process
 - Click select 4D data->in each line browse for raw functional data in .nii
 - Set TR, total volumes and volumes to delete. Ignore high pass filter for now.
- Pre-stats tab
 - Set desired preprocessing parameters.
 - Recommend BET, FWHM = 8mm. intensity normalization, Highpass
- Stats tab
 - Standard motion parameters in dropdown box
 - Click model setup wizard. This will open a new window.
 - **For simple block designs** that alternate between task and rest, click Model Setup Wizard, select an option & block duration.
 - **For complex designs** click Full Model Setup.
 - Set number of explanatory variables (EVs)
 - Name each EV (and note the names).
 - For each EV, make a .txt fill with a single vector giving the value of that EV at each TR.
 - EVs indicating the presence/absence of a task will be a string of 0s and 1s.
 - EVs for a continuous variable (i.e., measured in the scanner) are also allowed.
 - Do NOT specify an EV for rest.
 - Contrasts must be set in the Contrasts tab assigning 0s and 1s to build a design matrix
 - Click View Design for a plot of the design timecourse.
 - Both the red line and greyscale represent the predictor variable. Left red bar is related to the highpass filter.
 - Click Efficiency for correlation matrix of predictors.






- Apply temporal filtering if this was done is pre-stats
 - Why are temporal derivatives recommended?
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- Post-stats tab
 - Select a threshold. Voxel ~ FDR?. Cluster = Cluster based thresholding (less conservative).
 - Pre-threshold mask should be brain extracted mask of EPI produced by BET. Must be same spatial dimensions as EPI scans.
 - This decision will not affect the higher-level analysis
- Registration tab
 - Select main structural images->Browse for anat_brain.nii created by BET in earlier processing. anat.nii must also be in the same folder.
 - If batch processing select the appropriate anatomical to match each functional image to be processed. The anatomical scan list must have one to one correspondence with the functional scan list.
 - Leave Normal search and BBR.
 - Standard space->Browse for standard space template with brain removed. Check Non-linear and leave warp at default 10mm.
- Analysis outputs to folder labeled with extension .feat
 - Check reg/example_func2standard.png to assess coregistration.
 - Top row shows outline of standard on EPI
 - Bottom row shows outline of EPI on standard
 - Example_func2highres and highres2standard show other steps
 - If it's bad try again with 3DOF registration to highres and 12DOF registration to the template brain.
 - thresh_zstat.nii is the thresholded image.
 - rendered_zstat.png shows axial slices
 - **All first level image outputs are in native space.**
 - There are no tables here. They need to be calculated via script later.

Higher level analysis with FEAT: This level takes the output images from the previous step for random effects testing. You'll need to run this for each contrast (ie., each vs. rest and each high-level contrast). This step runs for about 10 minutes/contrast

- From dropdown menu select '*Higher-level analysis*' and '*Stats+Post-stats*'
- Data tab
 - Set number of inputs to the number of scans required
 - Select FEAT dorectproes->browse for output folders (.feat)
 - If .feat caontains several contrasts (cope1,cope2...) these can be selected or deselected with the numbered radioboxes.
 - Set output directory (.gfeat)
- Stats tab
 - Mixed effects: FLAME1
 - Click full model setup wizard (see FSL users guide for contrasts) <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT/UserGuide>
 - For a within subject design, leave the group column as '1'
 - Columns are conditions, rows are input images
 - Set one 'EV' per condition in the design.
 - For each EV set the relevant input images to 1, otherwise 0
 - In the contrast tab, set the number of contrasts (including vs. rest)
 - Set contrast vectors (e.g., [1 0] for EV1 > Rest or [1 -1] for EV1 > EV2)
 - Click 'View Design' to see a summary, then 'Done'
 - Note: With careful planning this can all be done in one step. Load all the .feat files as input, specify all the conditions and all the contrasts together. This would mean using the same threshold for each condition though.
- Post-Stats tab
 - Set thresholds as before
 - Use standard space template as background image for group data
- FSL will output 1 image for each contrast specified in the stats tab. These images are numbered in the order of the contrasts with no relevant names. Keep notes about which contrasts are which.

Make slice views

- Open Mango
- Open->Open Image...->Browse for Colin1.1.nii (available @brainmap.org)
-  |   (set to stereotaxic instead of native mango space)
- File->Add Overlay...->Browse for thresh_ztest.nii.gz of each contrast
- Edit-> Update to image range (sometimes this looks like it does nothing)
- To view multiple maps on a single brain simply repeat the process
- If some maps aren't visible try View->All Overlays
- Plugins->Generate Layouts (row=6, col=5, start=10, skip=3, slice labels=true)
 - For first level analyses, which are always in native space

- Plugins->Generate Layouts (row=6, col=5, start=4, skip=3, slice labels = true)
 - For second level analysis in a standard space
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- For single slices ->Viewers (row=1, col=1, start=0, skip=0)
- Click update, then full preview to see the image
- Save as... to screen capture

Psychophysiological Interactions (PPI)

- Define functional ROI
 - Terminal
 - `>FSLview_deprecated` or FSLeyes
 - File-> open->browse for epi (to provide anatomy at same resolution)
 - File-> add ->browse for thresh_zstate for contrast of interest
 - Bottom panel->click 'T' for info panel->choose a colour
 - Use min/max slider to adjust zthreshold as needed
 - Draw large ROI over target region
 - File->create mask
 - Top Panel->click pencil icon
 - Use pencil to draw over the cluster you want, save as *penmask*
 - Toggle pen value to find a contrasting colour
 - Toggle pen size to something convenient for drawing
 - Draw over the activation you want to capture
 - Get every voxel
 - Is ok to spill over as long as not into another blob
 - Terminal
 - Navigate to folder with image
 - `>cd Docs/wherever`
 - Make a mask from activations (Z is your Z threshold)
 - `>fslmaths thresh_zstat1.nii.gz -thr Z -bin thresh_zstat1_funcmasks.nii.gz`
 - Restrict functional map to the space you drew by multiplying
 - `> thresh_zstat1_funcmask.nii.gz -mul thresh_zstat1_penmask.nii.gz roi_name.nii`
- Get time course
 - Terminal
 - `Fslmeans -i filtered_func_data.nii.gz -o roiname_timecourse.txt -m roi_name.nii.gz`
- Run a GLM
 - EV1 = main task regressor
 - EV2 = my_timecourse.txt
 - EV3 = PPI
 - Set basic shape to interaction
 - Select EV1 and EV2
 - Zero Center for EV1

- Zero Mean for EV2
- Do not orthogonalise, temporal derivative, or temporal filter
- Also include all other EVs from full GLM model to improve fit.