

# SPM99 fMRI Data Analysis Workbook

This file is a description of the steps needed to use SPM99 analyze a fMRI data set from a single subject using a simple on/off activation paradigm. There are two parts to this analysis: (i) pre-processing, and (ii) analysis.

The original documents were obtained from the FIL's web-site with original authors Andrew Holmes & Geriant Rees (Thu May 13 12:15:10 BST 1999), and comments have been added by Terry Oakes relevant to the file locations at the LfAN, as well as to extend the analysis/display hints from the original authors.

The experiment referenced in this description was conducted by Geriant Rees under the direction of Karl Friston and the FIL methods group. The purpose was to explore new equipment and techniques. As such it has not been formally written up, and is freely available for personal education and evaluation purposes. Those wishing to use these data for other purposes, including published evaluation of methods, should contact the methods group at the Wellcome Department of Cognitive Neurology. <methods@fil.ion.ucl.ac.uk>

The original data, together with the results of performing each of the following steps, are stored in:

/d8b/oakes/spm\_data\_fmri/fM00223

/d8b/oakes/spm\_data\_fmri/sM00223

If you want to run the pre-processing yourself, make a new directory (e.g. on /scratch) and copy the original fMRI data into it. You need the fMRI files in /d8b/oakes/spm\_data\_fmri/fM00223/fM00223\_\*.img, .hdr}, and also the structural MRI image in

/d8b/oakes/spm\_data\_fmri/sM00223\_002.{img, hdr}. This is a large data set, so you should delete the files after you are done playing with them. Ignore all of the other files in these directories- these are the product of my earlier analysis, and are available if you want to skip the tedious number-crunching.

## Pre-Processing Overview

The following is a step by step recipe for a basic spatial preprocessing stream.

- (1) Realignment (motion correction)
- (2) Spatial normalisation
- (3) Spatial smoothing
- (\*) ...plus some additional spatial processing techniques on the structural image

These whole brain BOLD/EPI images were acquired on a modified 2T Siemens MAGNETOM Vision system. Each acquisition consisted of 64 contiguous slices (64x64x64 3mm x 3mm x 3mm voxels). Acquisition took 6.05s, with the scan to scan repeat time (TR) set arbitrarily to 7s.

96 acquisitions were made (RT=7s), in blocks of 6, giving 16 42s blocks. The condition for successive blocks alternated between rest and auditory stimulation, starting with rest. Auditory stimulation was bi-syllabic words presented binaurally at a rate of 60 per minute. The functional data starts at acquisition 4, image fM00223\_004. Due to T1 effects it is advisable to discard the first few scans (there were no "dummy" lead-in scans).

A structural image was also acquired: sM00223\_002

## Data Description:

fM00223\_[004-099].{img,hdr}

Analyze format functional images.

64x64x64 3mmx3mmx3mm voxels, TR = 7 seconds.

Epochs of rest alternating with auditory stimulation (60 words/min).

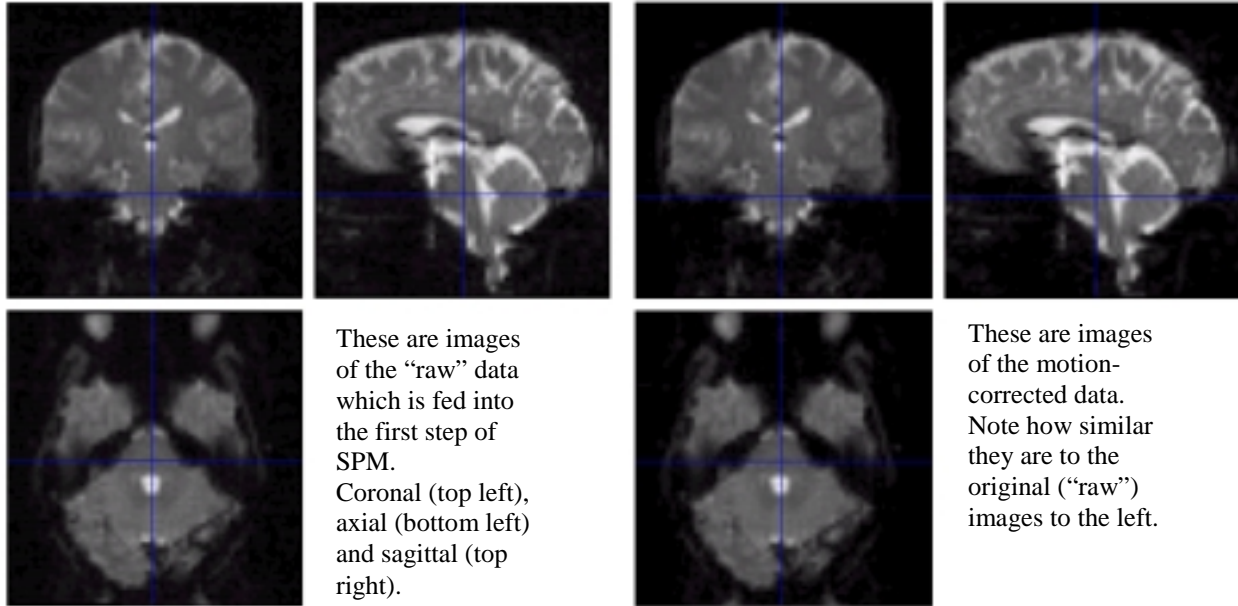
Epochs are 42 seconds long (6 TR), sequence begins with a rest epoch.

96 acquisitions were made (RT=7s), in blocks of 6, giving 16 42s blocks.

The functional data starts at acquisition 4, image fM00223\_004.

sM00223\_002.{img,hdr}

Analyze formatted MRI structural image (1x1x1 mm)



These are images of the “raw” data which is fed into the first step of SPM. Coronal (top left), axial (bottom left) and sagittal (top right).

These are images of the motion-corrected data. Note how similar they are to the original (“raw”) images to the left.

## **SPM'99 Spatial Pre-processing Steps:**

### **START SPM99:**

Log into a SUN or linux computer.  
`% cd /d8b/oakes/spm_data_fmri/fM00223/`  
 Make a new directory (e.g. `mkdir /scratch/mytest`) and copy files into it to actually run the analysis. `cd` to your new directory, or to “anal” if you just want to see how the menus work etc.  
`% matlab52`  
`>> spm99`  
 Select “fMRI time-series” from the small pop-up menu. The main SPM menus (3) should appear.

### **MOTION CORRECTION:**

To REALIGN with SPM99:

Press <REALIGN> in the upper left SPM window  
 'Number of subjects' - 1  
 'Num Sessions for subject 1' - 1  
 Select all fM00223\_004.{img} (96 images total) and press 'Done'  
 Pull down menu - select 'Sinc Interpolation'  
 'Adjust sampling errors' - no

This step took about 1 hour and 25 minutes on lan175.

Output: r\* files - Realigned functional images  
 - rfM00223\_004.{img,hdr} & meanfM00223\_004.{img,hdr}  
 - All 96 images were realigned to the first (rfM00223\_004)  
 fM00223\_[005-099].mat  
 - affine transformation matrices mapping for each image, mapping from voxel co-ordinates to the mm co-ordinates, such that in mm co-ordinates, each image is in alignment with the first. These are written alongside the input images.  
 spm99.ps - printout of realignment parameters  
 meanfM00223\_004.img - mean realigned image.  
 realignment\_params\_fM00223\_004.txt  
 - text-file containing 6-parameter or rigid body parameters for each input image.  
 - each row contains the x, y, z translations (mm) and the x, y, z rotations (degrees).

**SPATIAL NORMALIZATION to MNI (~Talairach) SPACE:**

To NORMALISE with SPM99:

Press <NORMALIZE>

Pull down menu

Number of subjects

'Image to determine parameters'

'Images to write Normalised'

'Template image'

Pull down menu

- 'Determine Parameters and Write Normalised'

- 1

- meanfM00223\_004.img

- meanfM00223\_004.img AND all rfM00223.{img}

- EPI.img

- 'Bilinear interpolation'

This step took approx. 1 hour on lan175.

Output: nr\* files - the spatially normalised realigned functional images.

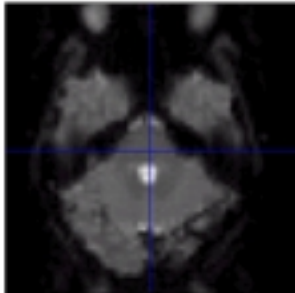
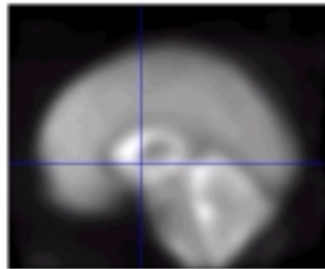
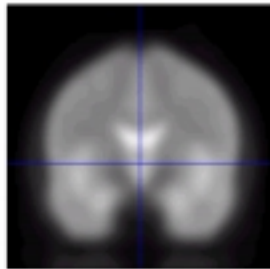
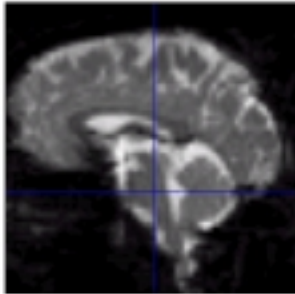
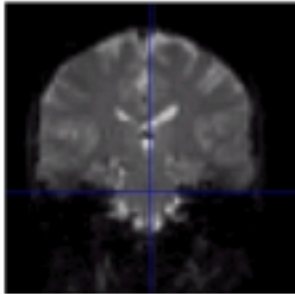
- nrfM00223\_004.{img,hdr} & meanfM00223\_004\_sn3d.mat

- Parameters generated from mean realigned functional image, normalised to EPI template.

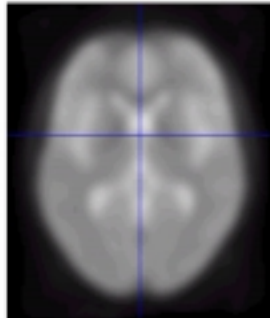
nmeanfM00223\_004.img - mean normalized image.

meanfM00223\_004\_sn3d.mat - the estimated parameters describing  
the spatial transformation

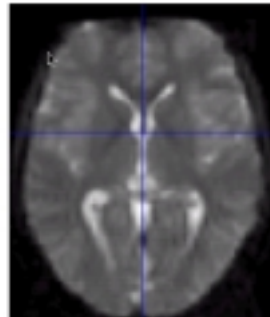
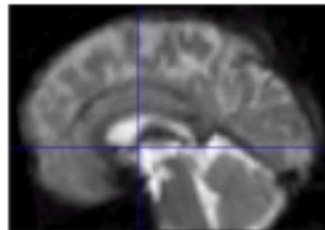
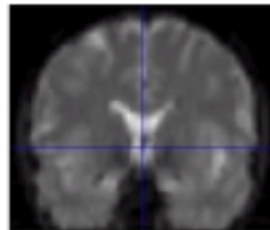
spm99.ps - printout of spatial normalisation parameters



Mean EPI images,  
the average of 96  
separate EPI image  
volumes.



EPI.img: template  
images supplied  
with SPM99. This  
is the "target" data  
set to which your  
data will be  
coregistered.



Normalized data:  
The mean EPI  
image volume after  
it has been  
coregistered into  
the coordinate  
system of the  
EPI.img template.

## SPATIAL SMOOTHING:

To SMOOTH with SPM99:

Press <Smooth>

'smoothing {FWHM in mm}': 6 or 6 6 6

'select scans'

- select all n\*.img scans: the nrf\*.img series, and also the nmean...img.

Approx 25 minutes on lan175.

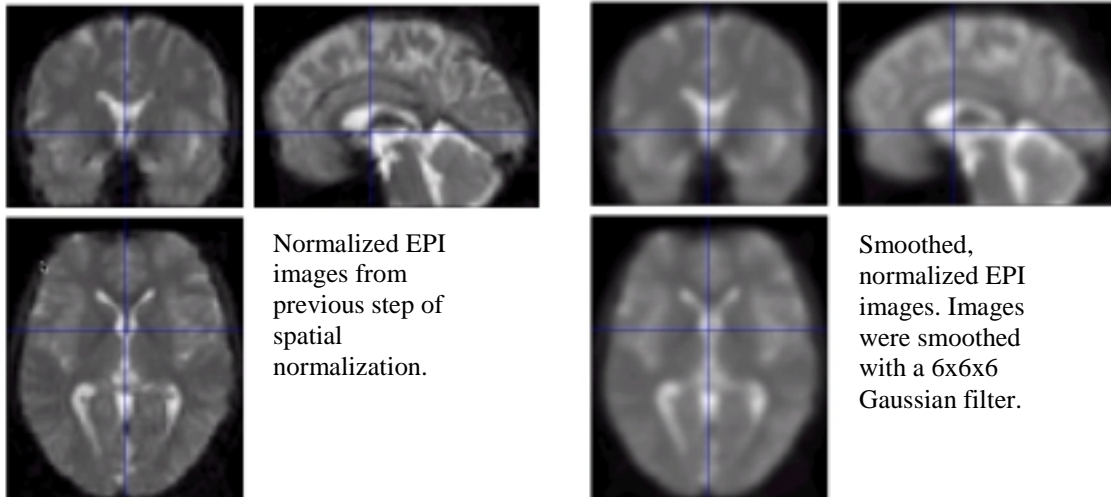
Output:

- snr\* files: Smoothed normalised realigned images

- snrfM00223\_004.{img,hdr}

- Smoothed with an isotropic Gaussian kernel with FWHM = 6mm

- These images are suitable for voxel-by-voxel statistical analysis



## SPATIALLY NORMALIZED ANATOMIC REFERENCE IMAGE:

Operations:

Press <DEFAULTS>

Pull down menu - 'Spatial Normalization'

Pull down menu - 'Defaults for Writing Normalized'

Pull down menu - '-78:78 -112:76 -50:85 (Default)'

Pull down menu - '1 1 1'

Press <NORMALIZE>

Pull down menu - 'Write Normalized Only'

'# Subjects' - '1'

'subj 1 - Normalisation parameter set'

- meanfM00223\_004\_sn3d.mat

'subj 1 - Images to write normalised'

- sM00223\_002.img

- Note that this image is in the structural directory!

Pull down menu - 'Bilinear Interpolation'

Press <DEFAULTS>

Pull down menu - 'Reset All'

Output:

nsM00223\_002.img - the normalized image

- Contains the normalized structural image normalized using meanfM00223\_004\_sn3d.mat with the default bounding box, 1mm isotropic resolution and trilinear interpolation.

- Results can be superimposed on this image via "Slices" and "Sections".

## **Analysis Overview**

This section explains in a step by step manner one way of statistically analysing the example auditory epoch fMRI activation data, once the data have been spatially pre-processed. The analysis procedure has three stages, all of which can be done separately.

- (1) Specify a design matrix (model)
- (2) Estimate the (previously specified) model
- (3) Inspect the results and specify t or F contrasts

To run the example analysis here you need the smoothed, normalised, realigned analyze images

```
snrfM00223_[004-099].{hdr,img}  
obtained from the previous section, or else look in  
/d8b/oakes/spm_data_fmri/fM00223  
/d8b/oakes/spm_data_fmri/sM00223
```

The analysis results can be found in  
/d8b/oakes/spm\_data\_fmri/fM00223/anal/

If you want to run the analysis yourself, make a new directory and cd into it before starting matlab, otherwise you may overwrite the existing example data set!

## **Data Description:**

- \* Analyze format functional images - ready for SPM
- \* 79x95x68 (2mm x 2mm x 2mm) voxel images
- \* RT (time between scans) = 7s
- \* Rest vs. Passive word listening @ 60wpm
- \* Epochs of 6 scans (42s/epoch => HPF cut of of  $2*2*42 = 168s$ )
- \* 8 cycles (=16 epochs), starting with rest
- \* 96 scans, image files 04-99

To avoid T1 effects in the initial scans of an fMRI time series acquisition with low RT, we recommend discarding the first few scans. To make this example simple, we'll discard the first complete cycle (12 scans, 04-15), leaving 84 scans, image files 16-99. The extra scans were put into a sub-directory to hide them:

```
/d8b/oakes/spm_data_fmri/fM00223/snrf_discards/
```

## **0) Preliminaries**

SPM organizes statistics by directory, so move to an empty directory for the analysis, using either MatLab's `cd` command, or the CD facility on the SPM MenuWil "Utils" PullDown menu. You will usually be alerted if you are about to overwrite stuff!

The SPM file selection GUI will lump large numbers of similarly named files together for easy selection. Thus, it's easiest to remove scans that won't be analysed from the data directory. For this example, consider moving scans 04-15 elsewhere, to leave the 84 scans (16-99) we will analyse. At the LfAN, these scans have already been moved to a subdirectory "snrf\_discards".

## **1) fMRI MODEL SETUP**

The first stage is to specify the design matrix for the analysis:

Press "fMRI models"	
Pull down menu: Would you like to...	- "specify a model"
Interscan interval	- 7 (seconds)
Scans per session	- 84
Number of conditions or trials	- 1
Condition or trial name	- 'active'
Stochastic design	- "no"
SOA (Stimulus Onset Asynchrony)	- "Fixed"
SOA (scans) for active	- 12 (=6+6, for 1 complete on/off cycle)

time to 1st trial (scans)	- 6
Parametric modulation	- "none"
Are these trials	- "epochs"
Select type of response... (PullDown)	- fixed response (Box-Car)
Convolve with HRF	- "yes"
add temporal derivatives	- "no"
epoch length {scans} for active	- 6
interactions among trials (Volterra)	- "no"
user specified regressors	- '0'
setup trial-specific F-contrast?	- "no"

At all stages the little green "?" in the bottom right of the SPM Interactive window will display the help of the appropriate function (spm\_fmri\_spm\_ui.m, spm\_fMRI\_design.m or spm\_get\_ons.m).

Model specification is now complete, and a small interface for exploring the design launches. (You can click & surf the design matrix to find out the values.) This design is saved in SPM\_fMRIDesMtx.mat in the current working directory, and can be reviewed again by pressing "Explore design". Note that we haven't as yet selected the scans – this design file can be re-used for another identical experiment.

## **2) MODEL ESTIMATION**

(You need to have completed step 1)

Press "fMRI models"	
Pull down menu: Would you like to...	- "estimate a specified model"
Select the fMRIDesMtx.mat from (1) and press "Done"	
Select the 84 snrfM00023_0??.{hdr,img} scans numbered 16-99	
Remove Global effects	- "scale"
High-pass filter?	- "specify"
Cutoff period	- '168' (= 2*84)
Low-pass filter?	- "hrf"
Model intrinsic correlations	- "none"

SPM will then compute the globals (short wait, with progress reported in the MatLab command window), and then display a summary of the design matrix and parameters. At this point, the SPM statistics configuration is saved in the SPMcfg.mat file.

Estimate model?	- "now"
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The Matlab window will then show the progress of the estimation procedure. (Estimation doesn't produce any graphics.) If any errors occur then they will appear in this window and you will need to diagnose the problem, fix it, reset SPM (by pressing the clear button on the Graphics window) and restart the analysis from the SPMcfg.mat file using the "Estimate" button on the MenuWindow (or the "Estimate" option on the design menu created by the "Explore design" button..

This took ~10 minutes on lan175.

### 3) RESULTS

When 'Use the Results Section for assessment' appears in the Matlab window, estimation procedure is finished and you can inspect the results. Unlike SPM96-97, no spm.ps results file is automatically produced. Rather, specification of contrasts is done interactively once the parameters have been estimated for each voxel. This means you can return to the same analysis and dynamically explore further contrasts:

#### Defining a contrast:

Press "Results" in the SPM MenuWindow.

Select the SPM.mat that was produced in (2) and press 'Done'.

The contrast manager will appear.

In the right hand panel is the design matrix (epochs+globals).

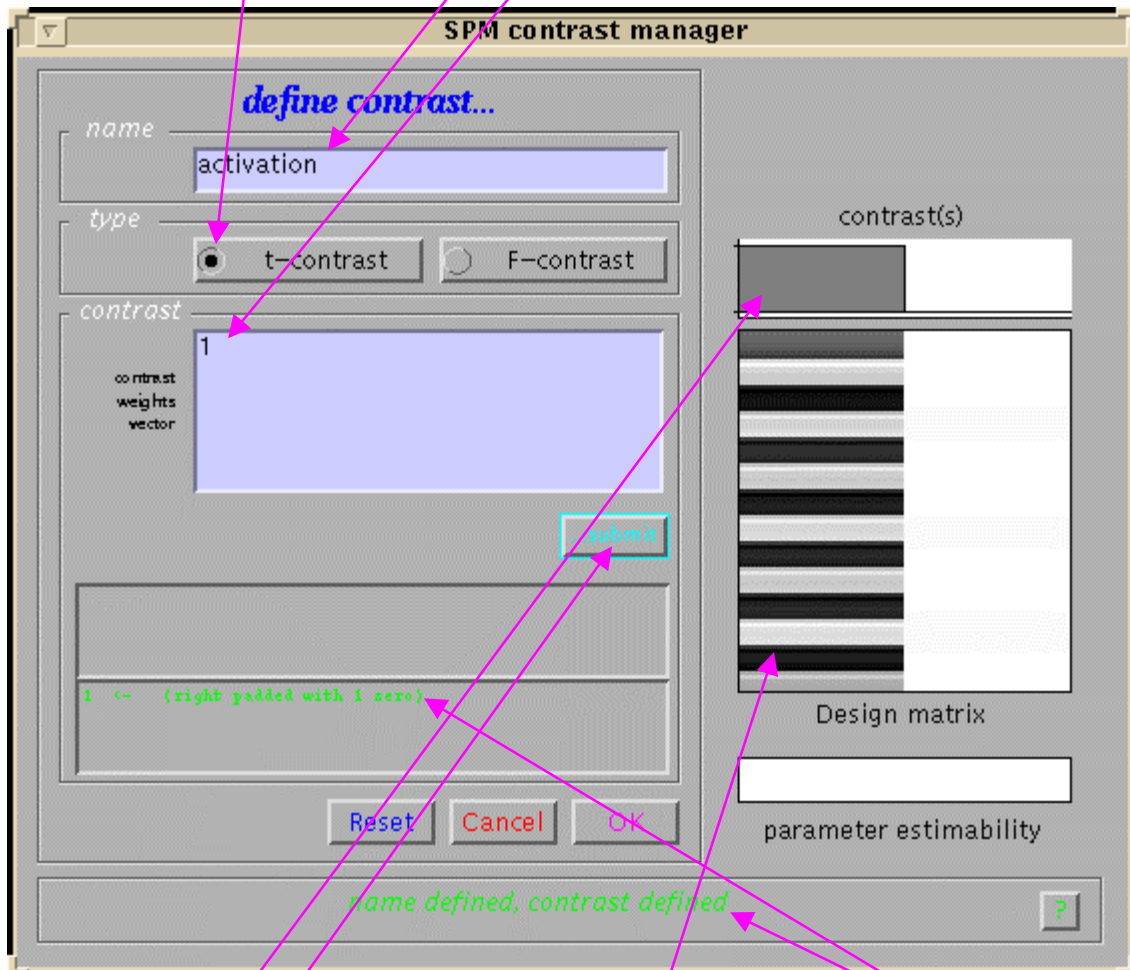
One contrast has been predefined in this example dataset, an F-contrast testing for "effects of interest", in this case any activation or deactivation. (Select "F-contrasts" to see it.)

To specify more, select 'define new contrast':

Type 'activation' in the name area (at the top)

Select "t-contrast"

Type a '1' in the contrast area (in the middle). You could also type '1 0', but in this simple case a 0 is assumed by SPM and automatically put into the design matrix if not specified.



The contrast should be depicted above the design matrix.

Press "submit". Make sure that SPM accepted the contrast by checking the message windows.

Press "OK" to leave the contrast definition menu.

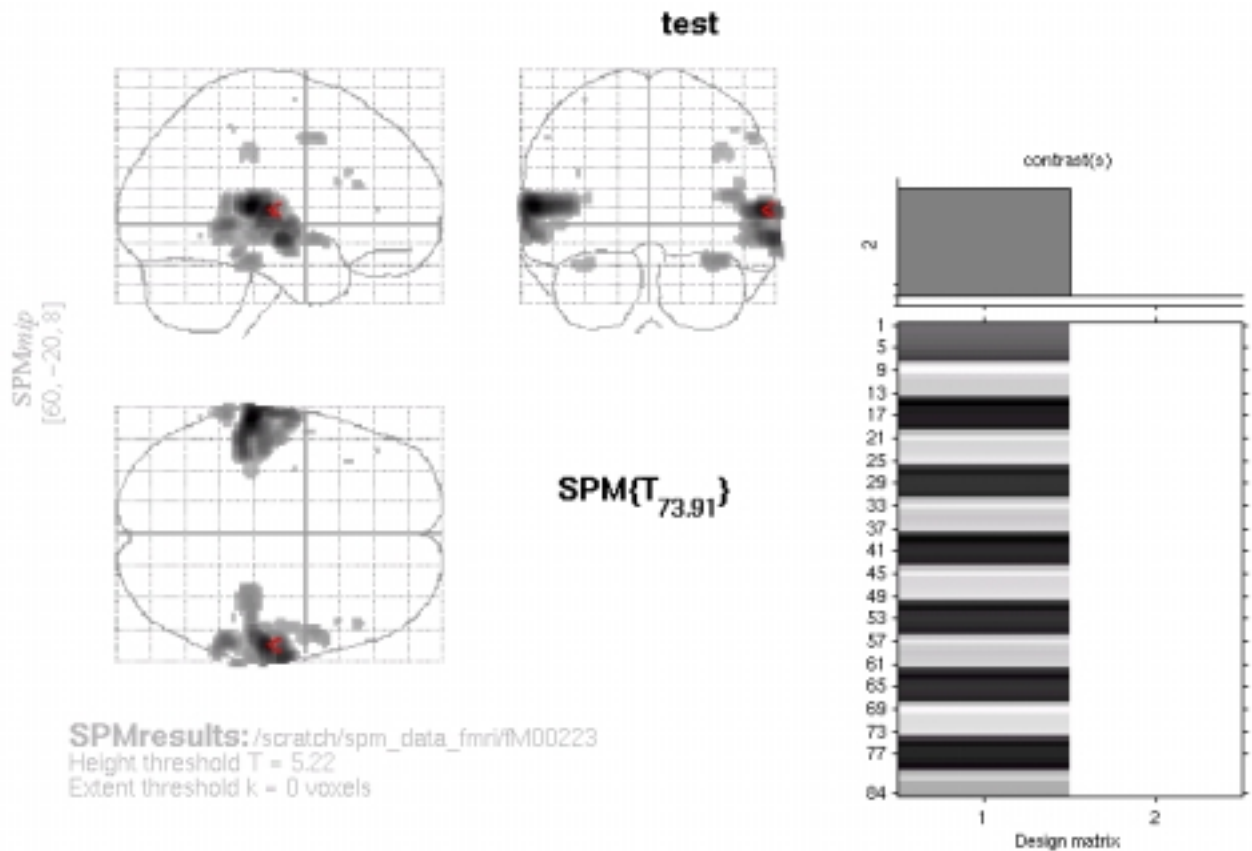
Select the newly defined 'activation' contrast & press "Done".

**Setting the threshold for this contrast:**

- Mask with other contrasts - "no"
- Title for comparison - 'activation'
- (short wait - progress displayed in MatLab command window)
- Corrected height threshold - "yes"
- Threshold - '0.05'
- Extent threshold - '0'

**Viewing results:**

The Maximum Intensity Projection (MIP) will appear in the graphics window. Drag the red cursor on the MIP to the auditory cortex. You can also move the cursor by typing values into the 'co-ordinates' boxes, by clicking on xyz co-ordinates in tables, and by right clicking on the MIP & choosing one of the "jump" options there.



This picture shows a typical “results” page from SPM99. The images to the left show a so-called “glass brain” with all results along a given summed and displayed on a single plane, similar to an X-ray view of the results. The red arrow indicates the current location relative to the anterior commissure (60, -20, 8).

The bar-graph on the right is a graphical representation of the contrast, or pattern of aural on-off signal. There is some variation within each dark or light bar due to the hemodynamic response function. The gray box at the upper right indicates that we are looking at this particular contrast. This is helpful if there is more than one contrast defined.



**Viewing results (cont.):**

Select "overlays.." to see the results superimposed on the reference MRI brain.

Select "sections"

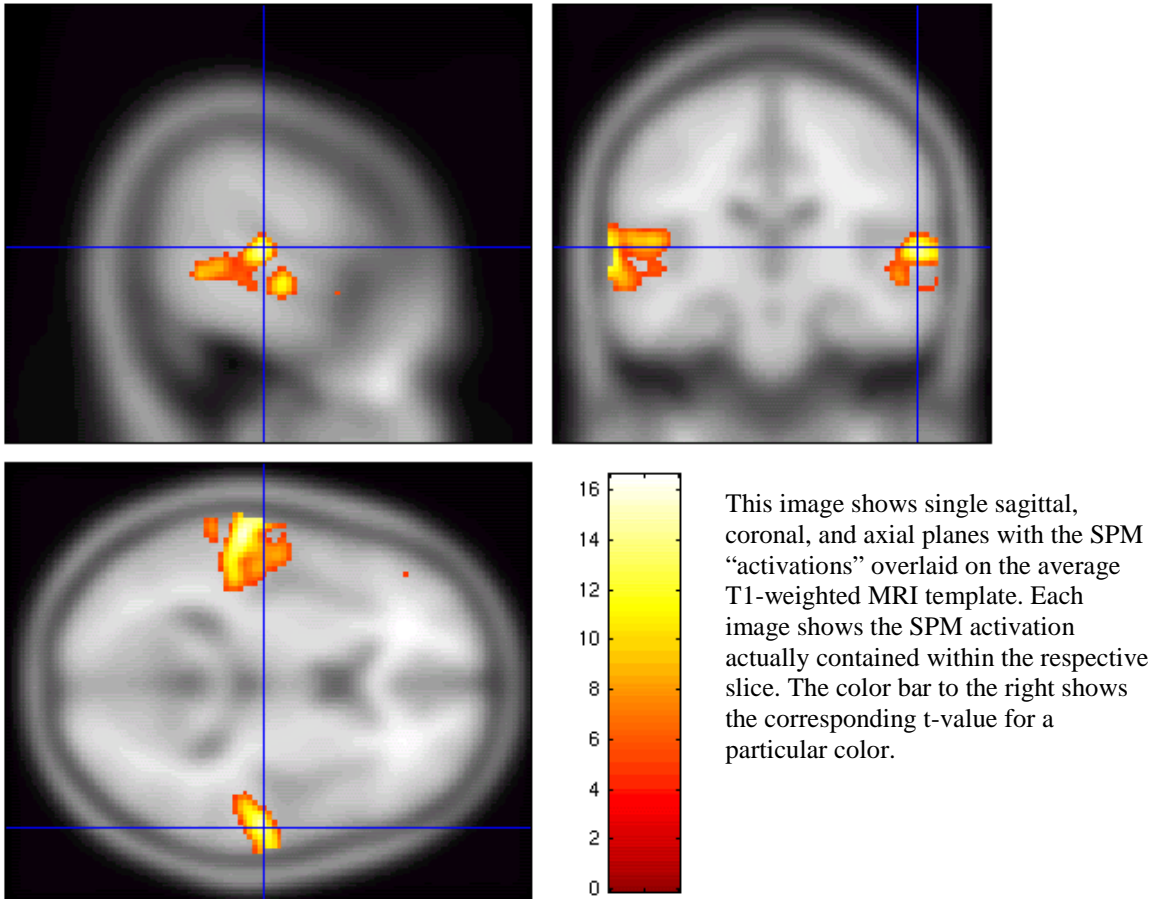
browse for one of:

/d8b/oakes/spm\_data\_fmri/fM00223/nmean\_fM00223\_004.img (mean fMRI image)

/d8b/oakes/spm\_data\_fmri/sM00223/sM00223\_002.img (anatomic image)

- Note that the coregistration for the anatomic image is a bit off.

click anywhere in the resulting display to move the crosshairs to that new location.



**Statistical Summaries:**

Press "volume" in the "p-values" panel. This displays a summary p-values for all local maxima.

Press "cluster" in the "p-values" panel. This displays p-values for local maximum in the current cluster.

**Plotting time-series (single pixel):**

Type in the co-ordinates (60 -20 8).

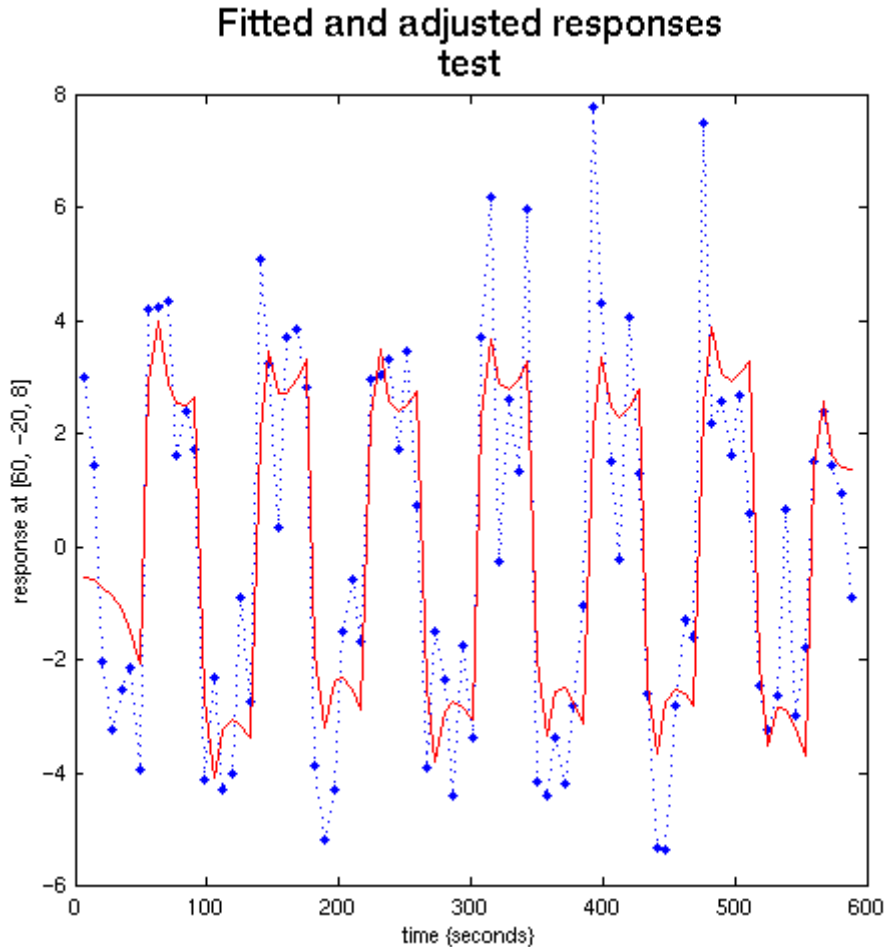
Press 'plot'

PullDown menu: Plot... - "Fitted and adjusted responses"

PullDown menu: Which contrast? - "activation"

PullDown menu: Plot against - "scan or time"

You should see a timecourse of the adjusted BOLD signal at this voxel, together with the fitted effect, as shown in the figure below.



**Plotting time-series (Volume-of-Interest):**

Select "V.O.I."

PullDown menu: Which contrast? - "activation"

name of region - "voi 4mm"

VOI radius (mm): - 4

You should now see a timecourse of the average BOLD signal for all of the supra-threshold voxels within a spherical region centered on the current voxel. As the region gets larger the plot will tend to get smoother.

### **Display Rendering of Activations on Brain Surface:**

Select "Render..."

PullDown menu: - Display  
Number of Sets: - 1  
Select SPM.mat: - browse for /d8b/oakes/spm\_data\_fmri/fM00223/anal/SPM.mat  
SPM contrast manager: - Select "003 {T} activation", then "Done"  
mask with other contrast(s)? - No  
title for comparison: - activation  
corrected height threshold - yes  
corrected p value - 0.05  
extent threshold (voxels) - 0  
Render file: - render\_single\_subj.mat  
Style - new  
Brighten blobs - more  
(short wait- see progress bar).

### **Check Coregistration (or compare several image volumes):**

If you want to check the validity of a coregistration in detail, you should use the "BrainSqueezer" program in SPAMALIZE. The "Check Coreg" facility in SPM99 does not provide contours and does not let you change a poor coregistration result. However, for a quick look, here is how to proceed.

Select "Check Coreg".

Select at least 2 images that are the same size (dimensions). If you select only spatially normalized images you will be fine.

The images should appear in the right-hand window.

Click on a location to place the crosshairs there for all images.