

SPM Tutorial (Block Design Data)

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Introduction:

In this tutorial, we will guide you through a step-by-step procedure for analyzing a fMRI data set using SPM. Before starting this tutorial, ensure that you have at least 500MB of free disk space available.

Data location:

The data that you will be working with in this tutorial are located in the following directory:

```
/afs/cabi.rfmh.org/usr/ardekani/dv02/VisHemifield/bob_64_31sl/sur
```

To list the data files, type in the following command line:

```
$ cd /afs/cabi.rfmh.org/usr/ardekani/dv02/VisHemifield/bob_64_31sl/sur ; ls
```

There are 3503 image files in this directory. The images are in SMIS format. There are 31 slices in each volume of this data. Therefore, there are $3503/31=113$ volumes contained in the data set. The total data size is about 42MB.

Conversion to the ANALYZE format:

The first step is to convert the data into the ANALYZE format. To do so, make a new directory where you want to store the ANALYZE data and change to this directory. Lets assume that this directory is called avw. You can type the following UNIX command to create the avw directory in your home directory and change to it:

```
$ cd ; mkdir avw ; cd avw
```

The program for converting SMIS images to ANALYZE format is called "convert2analyze". Start this program by typing its name at the UNIX prompt.

```
$ convert2analyze
```

A file selector will be displayed. On the file selector, go to the sur directory where the original SMIS images are located. Note that because of the large number of data files in this directory, changing to it using the file selector can be very slow. Have patience! It is best to just wait and not to manipulate the GUI while you are waiting.

After changing to the sur directory, select all the *.SUR files in this directory and press OK. After you press OK, again because of the large number of files, it may take a little while for the next window to appear. The next window that appears is the "Image Dimensions Popup" window. You will see that the program has determined that there are 31 slices per volume and the image size is 64 x 64. The voxels in this data set are cubic with dimensions 3.5mm x 3.5mm x 3.5mm. Type in an ANALYZE filename prefix. Since the prefix of the SMIS images in this data set is "t", let's just type "t" here.

One last thing to do is to reflect the images with respect to the horizontal axis (y-axis) and to reverse the slice order (reflect z-axis). These reflections will bring the SMIS images into the orientation assumed by SPM in the ANALYZE files. So, check the "Reflect y-axis" and "Reflect z-axis" boxes and the press OK.

The window disappears immediately and the program starts the conversion process. It will take less than a minute to convert all images.

Once the images are converted, they are stored in the avw directory, since this was the directory from where you started the convert2analyze program. Each volume is saved in a separate ANALYZE file. Actually, there is a ".img" and ".hdr" file associated with each volume. Since there are 113 volumes in the data set, you will find 226 files in the avw directory. The names of the 113 image files are t0001.img t0002.img ... t0113.img and the corresponding header files are t0001.hdr t0002.hdr ... t0113.hdr.

Removing the first few volumes:

The first ANALYZE volume (t0001.img and t0001.hdr) is actually a navigator volume (in the present data set) and must be removed. In this example, we will also throw away the first 4 volumes of the actual images in order to ensure that the net magnetization has reached a sufficiently steady state condition and also to account for other transient effects that may or may not occur when the experiment starts. So, to remove these volumes, use the following command:

```
$ rm -f t000[1-5].img t000[1-5].hdr
```

After removing these files, you will be left with 108 volumes: t0006.img ... t0113.img and t0006.hdr ... t0113.hdr. The following UNIX command will show you the number of images volumes that are left after you remove the first 5:

```
$ ls t*.img | wc -w
```

There should be exactly 108 volumes.

Starting SPM:

To start SPM, create a new directory and change to that directory. Let's assume that the new directory is called "spm" and you create it in your home directory. The following command can be used:

```
$ cd ; mkdir spm ; cd spm
```

While in this directory, type spm99.

```
$ spm99
```

This will set some initialization parameters and start matlab. At the matlab prompt, type spm.

```
>> spm
```

This will bring up the initial SPM99 window. Make sure that you are running the SPM99, *not* the beta version SPM99b. If you cannot bring up SPM99, contact your system administrator who will only be too happy to serve you. Click on the "fMRI time-series" button. We are now ready to start the analysis.

Realignment (Motion Correction):

The first step is to correct for subject motion between volume acquisitions. To perform this, follow the steps below:

- 1- Click on the "Realign" button
- 2- Set "number of subjects" to 1
- 3- Set "num sessions for subject 1" to 1 (after this, a file selector will appear)
- 4- Change directory to the avw directory. This may take longer than usual since we have so many files there. Please be patient.
- 5- Select the t*.img files by clicking once on the t00*.img and t01*.img list. They will turn blue once selected.
- 6- Click "Done"
- 7- From the "Which option?" menu, select "Coregister & Reslice"
- 8- From the "Reslice interpolation method?" menu, select "Sinc Interpolation"
- 9- From the "Create what?" menu, select "Mean Image Only"

At this point you have started the motion detection processes. This step takes about 30 minutes on my SUN Ultra 20 Workstation. Perhaps this is a good time to take a break, go have a nice cup of coffee courtesy of Dr. Robert Bilder. But don't forget to contribute to the coffee fund!

After the realignment step is complete, the detected rotational and translational motion is displayed in the SPM Graphics window and a number of new files are created by SPM. These files are as follows:

- 1- spm99.ps – This is a Postscript file created in the spm directory and contains the graphs of the detected rotational and translational motion.
- 2- t0006.mat ... t0113.mat – MATLAB ".mat" files created by the realignment routine in the avw directory. They contain 4x4 matrices necessary for the realignment. For example, t0012.mat is the 4x4 transformation matrix that when applied to t0012.img will produce the motion corrected image.
- 3- realignment_params_t0006.txt – ASCII file created in the avw directory. This file contains 113 sets of realignment parameters. These are the 3 rotational and 3 translational parameters necessary to realign each of the t*.img files to the first file, that is t0006.img, using a rigid body transformation.
- 4- meant0006.img and meant0006.hdr – An ANALYZE image representing the mean of the 113 images after rigid body realignment.
- 5- meant0006.mat – This file is created by the realignment routine in the avw directory. It contains a 4x4 matrix which appears to be a transformation from voxel coordinates to real world coordinates in mm.

Spatial Normalization:

The next step is to register the images to a reference image in the Talairach space. In SPM, this is referred to as spatial normalization. To perform this, follow these steps:

- 1- Ensure that you have at least 120MB free disk space in the avw directory.
- 2- Click "Normalize"
- 3- For the "Which option?" menu, select "Determine Parameters & Write Normalized"
- 4- Set "# Subjects" to 1
- 5- In the file selector that appears, select the "meant0006.img" file in the avw directory and click "Done". This is the file from which the nonlinear transformation parameters required for the normalization are derived. Since this is an average image and less noisy, we will select it.
- 6- A second file selector window appears and you are prompted to select the "Images to write normalized." These are the images to which the nonlinear transformation derived in step 4 will be applied. Select the "t*.img" images and click "Done". These are the 108 ANALYZE images that we are now trying to bring into Talairach coordinates. The "t*.mat" matrices detected at the motion detection (realignment) step are combined with the nonlinear warping parameters detected in this stage of the processing in order to transform the original image into the Talairach space.
- 7- A third file selector window appears which prompts you to input a "Template Image". This is the reference image to which the "meant0006.img" is matched. The templates are located in /usr/local/spm/spm99/templates. This should be the default directory in the file selector. Select "EPI.img" and click "Done".
- 8- In the "Interpolation Method?" menu, select "Since Interpolation".

The normalization process takes about 25 minutes. The following files are created as a result of the normalization process:

- 1- The spm99.ps file in the spm directory is appended to include the Spatial Normalization results shown in the SPM Graphics window. The left-hand side shows orthogonal views of the template. The right-hand side shows the orthogonal views of the meant0006.img after matching it to the template by the nonlinear transformations.
- 2- 108 (nt*.img, nt*.hdr) ANALYZE images are created in the avw directory. These are to the 108 (t*.img, t*.hdr) images after having been transformed to the Talairach space. Notice that the size of these files is larger than that of the original files. The reason is that these images are matched to the template which has 68 slices of size 79x95 with cubic voxels of size 2mm x 2mm x 2mm.
- 3- nmeant0006.img and nmeant0006.hdr – This is an ANALYZE image created in the avw directory corresponding to the meant0006.img after spatial normalization to the template.
- 4- meant0006_sn3d.mat – MATLAB ".mat" file created in the avw directory. Contains the transformations necessary for spatial normalization of the meant0006.img to the standard template in the Talairach space.

After the spatial normalization is complete. I usually do some house cleaning by issuing the following commands while I am in the avw directory:

```
$ tar cvf t.tar t*.mat t*.hdr t*.img ; rm -f t*.mat t*.hdr t*.img
$ gzip t.tar
```

These will tuck away the original raw ANALYZE data into the compressed tar archive t.tar.gz. If you have plenty of disk space and you don't mind seeing zillions of files in the avw directory, then you don't have to perform this step.

Smoothing:

Spatial smoothing of the realigned and normalized images nt*.img is the next step in the procedure. Apply the following steps for smoothing:

- 1- Ensure that you have at least 120MB of free disk space in the avw directory
- 2- Click "Smooth"
- 3- In the "smoothing {FWHM in mm}" text area, type 7.0 7.0 7.0. As a rule of thumb, I usually use twice the voxel size for these parameters. Since our voxel size in this experiment is 3.5 x 3.5 x 3.5, I recommend 7 mm FWHM in each direction.
- 4- In the file selector window that appears, change to the avw directory (use "Previous Directories .."), select the "nt0*.img" images, and click "Done".

This starts the smoothing process that takes about 10-15 minutes. The process of smoothing produces the realigned, spatially normalized, and smoothed image set snt*.img. Now we are ready to analyze the data.

At the end of the smoothing process, it is a good idea to tuck away the normalized images using the following commands:

```
$ tar cvf nt.tar nt*.hdr nt*.img ; rm -f nt*.hdr nt*.img ; gzip nt.tar
```

Specifying a statistical model:

At this point, we will design a statistical model for our data. Before doing so, however, this is a good time to explain the stimulation paradigm. The stimulation pattern is as follows:

4-10L-4-10R-4-10T-4-10B-4-10L-4-10R-4-10T-4-10B

This is read as follows: 4 scans rest, 10 scans left, 4 scans rest, 10 scans right, 4 scans rest, 10 scans top, 4 scans rest, 10 scans bottom, etc. Here, "4 scans rest" means that four volumes were acquired while the subject was seeing a blank black screen, "10 scans left" means that 10 volumes were acquired while the left visual hemi-field of the subject was being stimulated using a flashing checkerboard pattern, etc.

But remember that we threw away the first 4 rest scans, so our stimulation paradigm is really:

10L-4-10R-4-10T-4-10B-4-10L-4-10R-4-10T-4-10B

Notice that there are 108 volumes in this paradigm.

To setup the model, use the following procedure:

- 1- Click "fMRI models"
- 2- From the "What would you like to do?" menu select "Specify a model"
- 3- Enter 3 for the "interscan interval {secs}", this is the TR of the experiment. In this case, it was 3 seconds.
- 4- Enter 108 for the "scans per session"
- 5- Enter 5 for the "number of conditions". In this experiment, we have the conditions: rest, left, right, top, and bottom, where for example, "top" means that the top visual hemi-field of the subject was being stimulated using a flashing checkerboard pattern.
- 6- Enter "rest" as the "name for condition/trial 1", "left" as the "name for condition/trial 2", "right" for trial 3, "top" for trial 4 and "bottom" for trial 5.
- 7- Click "no" at the "stochastic design" option.
- 8- Click "variable" at the "SOA" option.
- 9- In the "vector of onsets (scans) – rest" text area, enter the following number: 10 24 38 52 66 80 94. These are the indices for the volumes where the rest scans start, assuming that the first scans has the index 0. In other words, the 10th, 24th, 38th, ... scans are the starting volumes for rest epochs.
- 10- Click "no" as the "variable durations" option.
- 11- In the "vector of onsets (scans) – left" text area, enter: 0 56. This means that in our experimental paradigm, there are two "left" epochs starting at the 0th and 56th scan.
- 12- Click "no" as the "variable durations" option.
- 13- In the "vector of onsets (scans) – right" text area, enter: 14 70.
- 14- Click "no" as the "variable durations" option.
- 15- In the "vector of onsets (scans) – top" text area, enter: 28 84.
- 16- Click "no" as the "variable durations" option.
- 17- In the "vector of onsets (scans) – bottom" text area, enter: 42 98.
- 18- Click "no" as the "variable durations" option.
- 19- Select "none" as the "parametric modulation" option.
- 20- Select "epochs" as the "are these trials" option.
- 21- In the "Select type of response" menu, select the "fixed response (Box-car)" option.
- 22- Select "yes" at the "convolve with hrf" option.
- 23- Select "no" at the "add temporal derivatives" option.
- 24- Enter 4 for the "epoch length {scans} for rest", since the length of our "rest" epochs is 4 volumes.
- 25- Enter 10 for the "epoch length {scans} for left/right/top/bottom" since for these epochs, we acquired 10 volumes.
- 26- Select "no" as the "interactions among trials" option.

27- Enter 0 at the number of "user specified regressors".

This concludes the procedure for designing the statistical model. The SPM Graphics window will show some information about the statistical model. Click the "Print" button to save this page in the spm99.ps file that already exists in your spm directory.

The information about the statistical model that you have just designed is saved in the "SPM_fMRIDesMtx.mat" file in your spm directory.

Unfortunately, because of the sequential design of the SPM99 graphical user interface, if you make a mistake in any of the above 27 steps, you will have to start from the beginning. Why didn't they put all the widgets, gadgets, and controls on one window? Your guess is as good as mine!

Estimating the model parameters:

This section outlines the procedure for estimating the model parameters of the model you designed in the previous section.

- 1- Click "fMRI models".
- 2- From the "What would you like to do?" menu, select "estimate a specified model."
- 3- In the file selector window that pops up, select the SPM_fMRIDesMtx.mat file and click "Done". Thus you have selected the previously designed model to be estimated.
- 4- Another file selector window pops up at this point, here change to the avw directory and select the snt0*.img files and click "Done". These are of course are image data that we have preprocessed.
- 5- Select "scale" as the "remove Global effects" option.
- 6- Select "none" as the "High-pass filter?" option.
- 7- Select "none" as the "Low-pass filter?" option.
- 8- Select "none" as the "Model intrinsic correlations?" option.
- 9- Select "no" as the "Setup trial-specific" option.
- 10- Select "now" as the "estimate?" option.

The estimation process takes about 10 minutes. It produces the following files in your spm directory:

- 1- RPV.hdr and RPV.img
- 2- ResMS.hdr and ResMS.img
- 3- SPM.mat
- 4- SPMcfg.mat
- 5- Y.mat
- 6- Yidx.mat
- 7- beta_*.hdr and beta_*.img
- 8- mask.hdr and mask.img
- 9- xCon.mat

At the end of the process, it may be a good idea to save the "Statistical analysis: Design" information that is shown in the SPM Graphics window in the spm99.ps files. Click "Print" to do this.

Note that the last two stages can be combined by selecting "specify a model and estimate" from the "What would you like to do?" menu.

Activation results:

To obtain the activation results, apply the following procedure:

- 1- Click "Results".
- 2- In the file selector, change directory to your spm directory, select the SPM.mat file, and click "Done".
- 3- On the "SPM contrast manager" window, click "Define new contrast".
- 4- Define a contrast as follows: enter "left-rest" as "name"; select "t-contrast" type; enter -1 1 0 0 0 as "contras"; and click "submit" followed by "OK".
- 5- Repeat steps 3 and 4 three times in order to define "right-rest" as contrast $-1\ 0\ 1\ 0\ 0$; "top-rest" as contrast $-1\ 0\ 0\ 1\ 0$; and "bottom-rest" as contrast $-1\ 0\ 0\ 0\ 1$.
- 6- Select one of the 4 t-contrasts that you have defined and click "Done" in the "SPM contrast manager" window.
- 7- Select the "no" option from "mask with other contrast(s)".
- 8- Enter "title for comparison" and wait until the computation is finished.
- 9- Select the "yes" option for "corrected height threshold".
- 10- Enter a desired p value. In this example, enter 0.001.
- 11- Enter 27 at the "& extent threshold {voxels}" prompt. This way you eliminate any cluster of "activated" voxels with less than 27 voxels.
- 12- To see the results of other contrasts, click "Results" and repeat steps 6-11.

The SPM Graphics window will show the projection of the activated regions into sagittal, coronal, and transaxial sections in Talairach coordinates. Click "Print" to save this page in the spm99.ps file. At the end of this procedure, the following files will be created in your spm directory:

- 1- con_*.hr and con_*.img
- 2- spmT_*.hdr and spmT_*.img

Further visualization of the results:

To project the activation on a standard surface rendering:

- 1- From the "visualization" option, select "overlays", "render".
- 2- Select a render file from the file selector.
- 3- Select "new" as the "style" option.
- 4- Select "lots" as the "Brighten blobs" option.

Experiment with other options of the "overlays" menu.