

## FSL Atlases and ROI Analyses

*The next several tasks are meant to help you learn how to extract data using masks and using the FSL atlases. Extraction of data using ROIs is invaluable for both statistics and display purposes (i.e. creating scatterplots/ bargraphs for your papers/ presentation). This assignment should also expose you to Unix and the fsl package in general*

Using the first level feat outputs for a single subject, we will:

- \*Create a separate ROI for the left and right motor cortex using an FSL atlas
- \*Register the ROIs to one single subject's native data
- \*Create a mask of supra-threshold voxels within the ROIs
- \*Extract data from the mask (mean intensity, variance, zstat, and timecourse)

### GETTING STARTED

- 1) Open X11 and Terminal (or, if you prefer to use X11, you may just use that.)
- 2) The path to all of today's data is: ~/fsl\_coursedata
- 3) You will be using the bash shell today
  
- 4) Often, it is easier to set a path than repeatedly type it, especially with long path names. In this case the path is short, but I suggest setting paths anyway. It is a good habit.

Eg (in bash) to create the variable TOP\_DIR:

```
> export TOP_DIR=~fsl_coursedata
```

Instead of typing ~/fsl\_coursedata you just type

```
${TOP_DIR}
```

eg

```
> cd ${TOP_DIR}
```

**I will refer to ~/fsl\_coursedata as \${TOP\_DIR} for my entire lab**

#### 4) Create an output directory

Eg

```
> mkdir ~/Bramen_Lab_Outputs
```

You may find setting an output path is often easier

Eg:

```
> export OUT_DIR=~/.Bramen_Lab_Outputs
```

**In all assignments, I will refer to the output directory as \$OUT\_DIR/<output> when an output is called for.**

### **USING THE ATLAS TOOLS IN FSL TO CEATE AN ROI**

assignment name = atlas

Each atlas is a single volume/mask, with several (or more) regions delineated by a different intensity. When you view the atlas, you will see each region is a different color, based on that region's intensity.

- Using fslview, open the 2mm MNI standard  
> fslview &  
-File -> Open Standard -> **/usr/local/fsl/current/data/standard/MNI152\_T1\_2mm\_brain**
- Add the MGH Probabilistic Cortical atlas  
-File -> Open Standard -> **/usr/local/fsl/current/data/atlas/HarvardOxford/HarvardOxford-cort-maxprob-thr0-2mm.nii.gz**

EXTRA INFO: The MNI atlas is probabilistic. There are therefore several to select from, depending on how certain you need to be of you ROI. This choice depends on how you plan to use the ROI. In this case, we can be liberal with the regions we want to select, and therefore. We will use the atlas with the lowest probabilities and largest regions.

- Find the Precentral Gyrus (aka - primary motor cortex (M1)), which is located just anterior of the central sulcus...
  - If your anatomy isn't very good, you can also use the atlas tools to help you with your anatomy:  
-Tools -> Toolbars -Atlas Tools
- Note the intensity of the region - you will need this value to extract it form the atlas.

EXTRA INFO: Cortical regions have the same intensity bilaterally, but subcortical regions (in the subcortical atlas) have different intensities in different hemispheres.

Therefore, extracting left and right ROI requires different methods for cortical and subcortical structures.

#### EXTRACT THE BILATERAL ROI (M1) FROM THE ATLAS USING FSLMATHS

- Type fslmaths to see usage.  
> fslmaths

- You will need to use the following options:

- 1) -thr : use following number to threshold current image (zero anything below the number)
- 2) -uthr : use following number to upper-threshold current image (zero anything above the number)

HINT: MAKE SURE YOU USE THE .nii.gz file extension for inputs when using fslmaths

- 3) Your input is:

**/usr/local/fsl/current/data/atlas/HarvardOxford/HarvardOxford-cort-maxprob-thr0-2mm.nii.gz**

- 4) Your output is: bilateral\_M1

- Open the standard in fslviw again (if it is not still open) and overlay your new ROI to make sure you did it correctly

> fslview &

-File -> Open Standard -> **/usr/local /fsl/current/data/standard/MNI152\_T1\_2mm\_brain**

- Add your bilateral\_M1

-File -> Add -> <open your file>

#### CREATE A LEFT AND A RIGHT M1 ROI

- Type fslmaths to see usage  
> fslmaths

- You will use the following options:

- 1) -mas

- I have created a mask for each hemisphere in :  $\${TOP\_DIR}$ /bramen-lab/atlas/

- 2) I hope your input is obvious ;0)

- 3) Your output will be: LM1 and RM1

- Again, inspect the results. Make sure you did in fact create a left M1 in the left hemisphere (right is left!) and vice versa.

HINT: If you still have your other masks open, they will make it hard to see your output. You can make them invisible by highlighting the filename, then unclicking the icon that looks like an eye.

## **EXTRACTING DATA**

assignment name = extract

*We will be using data from the fsl tutorial.*

**\$TOP\_DIR/fsl\_course\_data/fmri/ptt/ac/**

**export SUBJECT\_DIR \$TOP\_DIR/fmri/ptt/ac**

**I will from now on refer to this path as  $\${SUBJECT\_DIR}$**

*If you have not done the tutorial before, the task used was a basic hand motor task.*

*Condition 1 (cope/pe 1), the subject used only their index finger.*

*Condition 2 (cope/pe 2), the subject performed a sequential finger tapping task*

*Condition 3 (cope/pe 3), the subject performed a random finger tapping task*

REGISTER THE ROIS WE CREATED IN THE LAST ASSIGNMENT TO THE SUBJECT'S PREPROCESSED DATA

- Since feat was already run, we know the transformation between the BOLD data and the MNI brain:

$\${SUBJECT\_DIR}/ac\_left.feats/reg/example\_func2standard.mat$

- What we need is the inverse transformation matrix  
You can do this two ways. 1) use the flirt GUI or 2) (and this is not obvious from the flirt usage, so I will give you this one) you can use the following command:

**> convert\_xfm -inverse**

**$\${SUBJECT\_DIR}/ac\_left.feats/reg/example\_func2standard.mat -omat$   
 $\${OUT\_DIR}/Std2Example\_func.mat$**

APPLY THE TRANSFORMATION MATRIX **Std2Example\_func.mat** TO OUR 2x ROI

- Type flirt to see usage.  
> flirt
- You will use the following options:
  - 1) -ref
    - What is the reference/ target image? Pretend this is not a mask and we were registering the standard to .... What? (that is the reference)
  - 2) -in (I hope your inputs are obvious ;0)
  - 3) -applyxfm -init (you should know what the matrix is)
  - 4) Your outputs will be: LM12ac\_left AND RM12ac\_left
- Again, inspect the results using fslview. Make sure you did in fact create a left and right M1 in the subject's native space!

*Often we only want to extract data for display purposes. Therefore, it is common to use use only supra-threshold voxels.*

## CREATE ROIS (IN SUBJECT SPACE) THAT CONTAIN ONLY SUPA-THRESHOLD VOXELS

*First, since we plan to extract data from 2 of the 3 conditions, we want to be sure we include any voxles active during ANY of the two tasks. That means adding the 2 thresh\_zstat# files together using fslmaths*

- Type fslmaths to see usage  
> fslmaths

*Now we an mask the summed thresh\_stats from above using the ROIs we created*

- You will use the following options:
  - 1) -mas (use our ROIs, in subject space)
  - 2) Input is the mask we created above
  - 3) Your output will be: LM12ac\_left\_sig and RM12ac\_left\_sig
- Again, inspect the results in fslview. Make sure you did in fact create smaller ROI.

## USE THE ROI TO EXTRACT DATA

Let's simply extract the data from 2 of the conditions (Conditions 2 and 3)

- Type fslmaths to see usage  
> fslstats
- You will use the following options:
  - 1) -k (mask - use the output from the last exercise)
  - 2) -m (output the mean)
  - 3) Your inputs will be
    - magnitude (stats/cope2.nii.gz; stats/cope3.nii.gz)
      - (This is the most commonly extracted variable in my experience. You can use it to create scatter plots for your manuscripts – especially if you are looking at any correlations between signal and some factor. You can also use this to do a pure ROI analysis. EG - Does the size of the response to this task differ between the two tasks in the dominant hemisphere? What about the non-dominant hemisphere?)
    - variance (stats/varcope2.nii.gz; stats/varcope3.nii.gz)

- (Not used often enough in my opinion. You can ask many interesting research questions using variance/ stability of the HRF – especially in an event related design.– is there more variability in the HRF response in the non-dominant hemisphere? Does variance increase with increasing difficulty in the non-dominant hemisphere more than the dominant hemisphere? In a clinical population, does variance account for reduced apparent “activity” (i.e. statistical significance?) - This is a variable that can help you interpret findings every bit as much as magnitude, and this is not the same as looking at inter-subject variance, which is what is typically plotted)
- statistical significance (zstat1)
  - (why might you want to extract the zstats from individual subjects? Here are some examples from my research – I have been asked to use zstats from an ROI to perform structure-function analyses. EG – use mean zstat in an ROI and correlate it with cortical thickness...)

4) outputs are generally only numbers printed on a screen. BUT I have a

**TIP AND TRICK:** Save outputs destined for the screen in a text file using a “>”  
 EG : some command -followed by -lots of arguments > \${OUT\_DIR}/filename1.txt

Therefore, outputs will be  
 > \${OUT\_DIR}/RM1\_cope2.txt  
 > \${OUT\_DIR}/ LM1\_cope2.txt; ... and so on  
*If you have time to play with the data in matlab now (or want to later) you can now make some scatter plots*