

Make the ROI in AFNI:

1. Follow the guideline outlined in [Making AFNI Masks](http://www.usask.ca/psychology/sarty/fMRI/afni.html). A local guide written by Ben Norris on the website <http://www.usask.ca/psychology/sarty/fMRI/afni.html>.
2. In the Dataset Copy popup window, do the following:
 1. Within each subject folder, choose your input dataset to be the axial_seT1_00003+orig [epan] file. (This is the high resolution MRI brain scan)
 2. Within each subject folder, for the output prefix name it as you wish. *Eg. brocasROI or something, this is the name of the ROI mask you are going to draw.*
 3. Datafill = Zero [One]
 4. Dataset = fim
 5. Leave Datum blank
 6. Then click COPY+CLOSE.
3. Load up the axial_seT1_00003+orig [epan] file as your Underlay and the ROI masks file you created as the Overlay.
4. Choose Define Datamode --> Plugins --> Draw Dataset. In the popup window, do the following:
 1. Click Choose Dataset on which to draw. Select the output ROI mask file name you created in Step 2-2. Set value = 1 and have Mode = filled curve.
 2. Leave this window open, and go back to the main afni window.
5. Click Axial ---> Image to see the axial slice underlay.
6. To draw on the Underlay, hold down the 3rd (rolling wheel) mouse button, draw desired ROI region and release mouse button. Your ROI you draw will fill in with RED. When finished drawing the ROIs, go back to the DRAW DATASET window and click SAVE.
7. Close up AFNI.

The ROI mask that you have just drawn, is simply a data file that gives a value of 1 to all the pixels and a 0 to everything else. This ROI mask can be multiplied by the task stimulus files in order to reduce down the huge task stimulus files to just contain the stimulus voxels in the desired ROI. This saves tonnes of time when running Vasily's smoothed_boldfold program, I've written a script to do this.

Running the ROIreduction.sh script:

In the Subject directory use gedit to open ROIreduction.sh

The script contains 4 task variables, one for reg, ex, nw and ph, 4 number variables and a ROIname variable. The 4 number variables must be set to have the correct value corresponding to the stimulus files in the subject directory. An example:

Subject folder 261, has the following stimulus files:

- reg_00007+orig.BRIK
- ex_00006+orig.BRIK
- nw_00005+orig.BRIK
- ph_00008+orig.BRIK

When you open up the ROIreduction.sh script you will see:

```
#!/bin/tcsh
set task1 = reg
set task2 = ex
set task3 = nw
set task4 = ph
set var1 =
set var2 =
set var3 =
```

```
set var4 =  
set ROIname =
```

so, referring to the stimulus files in 261, for reg_00007+orig.BRIK, we would set task1 = reg and var1 = 7. For ex_00006_orig.BRIK, we would set task2 = ex and var2 = 6, and so on. This just makes sure that proper stimulus files are used for the script. Finally, set ROIname = the ROI mask file name you created in Afni (like *brocasROI*).

When this script is run in each subject folder, it creates 4 directories (one for each task) and they are named as follows: reg_{your ROI mask name}analysis. eg. /reg_brocasROIanalysis

Inside each of these folders is two files of your reduced task files, one ending with .BRIK and one .HEAD. As an example, for the regular words task, the two files would be:

```
/borowsky/Basic_Reading/261/reg_brocasROIanalysis/reg_00007_reduced+orig.BRIK  
reg_00007_reduced+orig.HEAD
```

Vasily's program only reads stimulate files and not afni files, so the .BRIK file (the file that actually contains the desired data, the .HEAD files just has some extra stuff needed for afni) needs to be change into the stimulate file format, .sdt

At the command prompt, type ln -s {file name}.BRIK {same file name}.sdt

```
eg. ln -s reg_00007_reduced+orig.BRIK reg_00007_reduced+orig.sdt
```

This creates the stimulate file format .sdt in the same folder.

Just like the .HEAD files for Afni, stimulate requires a .spr file that contains some needed parameters. At the command prompt type: gedit reg_00007_reduced+orig.spr, to create this file.

Once gedit opens, the file will be blank, then click open file in gedit. In that window go back one directory (to the 261 main folder), and double click on reg_00007.spr. This is the .spr parameter file for the original task data and these parameters will be the same for your newly created reduced data set. This will open up in gedit, and you simply copy the file contents into reg_00007_reduced+orig.spr, save and close gedit. Be sure not to delete anything from reg_00007.spr because this is for the raw data that we don't want to lose any of it.

Go back to the /261/reg_brocasanalysis folder, and you should see 4 files now:

```
reg_00007_reduced+orig.BRIK  
reg_00007_reduced+orig.HEAD  
reg_00007_reduced+orig.sdt  
reg_00007_reduced+orig.spr
```

You are now ready to run the smoothed boldfold program.

Running Smoothed boldfold and Gord's Bfold programs:

In the same directory as the four files, type smoothed_boldfold.

This is the same program as Vasily's original smoothed_boldfold_width program, but I've altered it so that it outputs more values than just the width of the boldfold width. The source code is in the directory /fMRI/Jeff/boldwidth/

The program requires to input the name of the sdt/spr files to run. Type reg_00007_reduced+orig

It also requires several parameters to run. For the Basic_Reading data files the parameters to enter are:

Cutoff for correlation coefficient = 0.50
Number of periods = 5
Length of period = 9
Distance between periods = 0
Initial skip = 3
Number of basis functions = 5 (I used this because it gave the best looking curves).
Intensity cutoff level = 200

The program will then run all the calculations and it will spit out a bunch of files (takes about 15 minutes):

```
reg_00007_reduced+orig_BF_D0_eta_maps.sdt/.spr
    _D0.sdt/.spr (the smoothed curve)
    _D1.sdt/.spr (the derivative of the smoothed curve)
    _D1_inital.sdt/.spr (the derivative at t=0)
    _fmax.sdt/.spr (the intensity)
    _tmax.sdt/.spr (the time at which the curve peaks)
    _width.sdt/.spr (the Full width at half max)
```

These files give values for every voxel within the defined ROI that you have made, so you need to also run Gord's bfold program on the same reduced task .sdt file (eg. reg_00007_reduced+orig.sdt/.spr) at an eta = 0.65 in order to find the specific voxels that are activated within the ROI. Stimulate can then be used to overlay the bfold output files at eta = 0.65 on top of the smoothed boldfold files, so that only the desired activated voxel at eta=0.65 are used in computing the average values from Vasily's program.

In the same folder that you ran Vasily's program type: bfold.

The program will ask for the same reduced task .sdt file. Type in reg_00007_reduced+orig for example. It also requires the following parameters to be defined:

Select cluster type: (1) unclustered
Eta threshold options for unclustered maps: (1) Specify an eta cutoff
Enter eta threshold for unclustered magnitude map: 0.65
Enter first volume of first period: 3
Enter length, in volumes, of period: 19
Enter number of volumes between periods: 0
Enter number periods: 5
Enter number of active volumes at start of period: 11
Enter noise cutoff: 200
Motion correction option: (1) OFF
MRI/analysis computer combos: (4) Data from INTEL, this computer INTEL

It will do its thing and also spit out a whole bunch of files. It will also give a warning about time series outliers, but you can ignore these. Of all the files bfold outputs the one of importance in this analysis is {your reduced file name}_BF_magn_map.sdt

eg. reg_00007_reduced+orig_BF_magn_map.sdt

Putting it all together in Stimulate to get averaged values computed by smoothed boldfold:

Once you have the bfold files and the smoothed_boldfold files in the directory, you can then run Stimulate. In Stimulate, first load the Stim file --> select one of the smoothed_boldfold files (eg. the width calculation file). Then load a map file --> select the bfold magn_map file. Make Stimulate show

8 windows, and you should just see voxel that corresponding to the desired ROI.

Select chg: POS in stimulate in order to show the voxels that pass the $\eta = 0.65$ cutoff. Now, make an ROI in stimulate that covers **all** voxels that are shown. Some voxel will be white (not active) and some will be yellow (active). Select all of them. (You can make the stimulate ROI the whole window).

Choose, time course full and then click plot.

The time course display will show up and the value at the top of graph display will be the value that you are looking for. The graph display will be blank because these maps the average of the time course, so there is only one value, and therefore the top value of the graph display is the value you wish to obtain. Also, at the bottom of the graph display is a value called Hotpoints and it tells how many of voxels in the stimulate ROI are active and this is the value I used for volume in our analysis.

Repeat this procedure for all the desired average values, such as initial derivative, intensity, and time to peak, and repeat for each subject.