Region of Interest (ROI) in AFNI - Workbook

- **PART 1: Why ROI and how to draw a ROI in AFNI**
- **PART 2: Statistics on ROI**

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- **PART 1: Why ROI and how to draw a ROI in AFNI**

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- **Why ROI?**

Usually A ROI analysis is done for the following purposes:

1. Average time series from a group of voxels(ROI);
3. Extract timeseries information from a group of voxels (ROI);
4. Mean percent change per entire ROI;

- **ROI** can be drawn for :
  A. an **anatomical region of interest** or
  B. a **cluster in an activation map**;

• A. ROI based on an anatomical region of interest

- **Procedure:**

Step 1. Structural-Functional overlay check.

Purpose: To decide if the structural volume can be used to draw to draw the ROI.

Outline of procedure:

> afni &
> Switch anatomy : 3dvol
> Axial Image; Sagittal Image ; Coronal Image;
> Warp anatomy on demand
> anat resam Mode: Li
> Resam(mm): 1

> afni – new
> Switch anatomy**: Rrun1+orig; (Motion corrected functional brick)**
> Warp func on demand
> Warp anatomy on demand
> anat resam Mode: Li
> Resam(mm): 1
> Define Datamode: -Lock – Set all

Define landmarks and check of the structural / functional landmarks overlays properly.

!!!! If the structural and the functional volume do not overlay properly, coregister the 2 modalities **or** use the functional volume to draw the ROI based on the brain activation map (output of 3dDeconvolve).

NOTE: Some difficulties arise when the mask BRIK and the 3d+tome BRIK have different co-ordinate spaces or voxel dimensions.

The anatomical brik is a high resolution image with the voxel size 1X1X1 (mm).
The functional briks are collected at a lower spatial resolution with the voxel size 3.125X3.125X5.0 (mm).

If you draw the mask on a high resolution anatomical dataset, because of differing voxel sizes and image volumes, the mask dataset cannot be directly applied to the 3d+time dataset(functional brik).

Solution: Resample the input mask dataset created at high resolution to the same resolution as the template dataset(from a fine grid to a coarse grid). Use 3dfractionize afni program. 3dfractionize was originally written for mask datasets that contained only a single ROI(new version –vote option to preserve the mask values).

3dfractionize –template allruns+orig –input ROImask+orig –clip 0.2 –vote –prefix resROI_mask

Step 2. Draw the histogram of the functional volume .

Outline of procedure:

> Define Datamode
> Plugins
> Histogram
> Source: Rrun1+orig
> Plot and Keep

Write down the top value in the brik (e.g: 2000)

Step 3. Create an empty mask dataset (fim) based on the top voxel value in the brik.

If top value=2000 =>

> 3dcalc –prefix ROImask –a 'Rrun1+orig[10]' –expr 'step(a-2000)'
> 3drefit –fim ROImask+orig
> chmod 775 ROImask+orig

Step 4. Draw the ROI

Many ROIs can be draw on the same ROImask, each ROI has to have a different colour and value.

If the ROImask has been created on a functional volume:

> afni &
> Switch anatomy : Rrun1+orig
> Switch Function: 3dDeconvolve_output+orig
> Display Image; Image; Image

> afni – new
> Switch anatomy**: Rrun1+orig;**
> Switch Function: ROImask+orig
> Display Image; Image; Image
> Define Datamode: -Lock – Set all
> Define Datamode -> Plugins -> Draw dataset;
> Draw Dataset Window:
Choose Dataset on Which to Draw: ROImask+orig;
Drawing value: 1;
IMPORTANT: For each ROI choose a different Drawing Value(next ROI: 3,4 etc);
Drawing color: yellow;
IMPORTANT: For each ROI choose a different Drawing Color;
Draw the outline of the ROI on each appropiate slice:
Drawing Mode: Points;
Save the ROI;

Step 5. Making correction to the mask;

Method 1: Use a drawing value of zero and redraw the mask;

Method 2: Delete the poor ROI from the mask;
e.g: remove a ROI with a functional mask of 2:

```
3dmerge -2uclip 1 3 -prefix newROI_mask ROI_mask+orig.BRIK
```

Step 6. (optional) Create a symmetrical mask:

If you are drawing on a +acpc or +tlrc dataset, so that the midline of the brain is set to x=0, then you could use the program 3dLRflip to flip a mask dataset, producing a new one that is the mirror image of the first, then combine them with 3dcalc, as in

```
3dLRflip -prefix sivle elvis+tlrc
3dcalc -a elvis+tlrc -b sivle+tlrc -expr 'a+b' -nscale -prefix elvis_cloned
/bin/rm -f sivle+tlrc*
```

B. Creating a ROI based on a cluster in an activation map;

Program 3dmerge can find voxel cluster in activation map and then convert each cluster in a ROI with a separate data value;

```
3dmerge –1clust_order 1 500 \
-1tindex 1 –1thresh 0.4 \
-prefix ROI_cluster stim_anl+orig
```

Command explanation:

- Treshold the dataset on subbrik 1 to value 0.4
- Cluster together all surviving nonzero voxels using a contiguity test of 1 mm and keeping only cluster at least 500 mm cube in volume;
- -All voxels in the largest cluster is assigned value 1, in the secind largest value 2 etc abd the result is written to the ROI_cluster brik;

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- **PART 2: Statistics on ROI:**

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- **Statistics on ROI:**

1.1 Compute the average of timeseries for a voxels, with the voxel selected from a mask dataset; (interactive version : ROI Average Plugin)

Purpose: Count the active voxels per ROI at a particular r-value treshold, for each particular condition and output the average value of BOLD signal over ROI;

General Format: 3dmaskave -mask <mask name> -mrange <ROI mask Value> <ROI mask Value> -dindex 1 -drange <r threshold> 1 <fico name> >> ROI<maskValue>count.txt

3dmaskave -mask ROImask**+**orig -mrange 2 2 -drange -1 1 stim_anl+orig[59,61,63] > avgROI2_59_61_63.txt

Commands explanations:

3dmaskave – program to compute the average ofvoxels from a dataset with the voxels selected from a mask dataset;
-mask : is the mask that contain ROIs;
-mrange a b means that only the mask values between a and b will be used ;
-drange a b means to only include voxels from the dataset whose values fall in the range ‘a’ to ‘b’ (inclusive). Otherwise all voxels are included;

1.2 Compute the average of timeseries for voxels from a dataset, using multiple regions selected by a single ROI dataset;

3dROIstats –mask ROI_mask+orig stim_anl+orig;

output: for each subbrick of the input dataset and for each functional mask value there will be displayed the average value over the ROI ;

You can load the 3dROIstats in Excel for further analysis (statistics over other subject’s data);

1.3 Extract timeseries information from a ROI;

Dump out all voxels in a dataset that match some values given in mask dataset;

Main application is to dump out functional activation values that match a ROI so they can be processed in other programs (Excel);

3dmaskdump –mask ROImask+orig mrange 2 2 -xyz data+orig

Commands explanations:
-xyz means to write the x,y,z, coordinates from the 1st input dataset at the start of each line output;

output: functional activation value aver the entire dataset;

1.4. Mean percent change per entire ROI

Bellow is an example of how to derive the mean percent change per ROI, for a particular condition, based on the active voxels of that condition, at a particular %change treshold level.

General Format :
3dmaskave –mask <mask name> -mrange <lowROIlimit> <high ROIlimit> -drange <inclusion range for percent change> -sigma –dump –indump <fbuc> >> meanpercentROI.txt

3dmaskave –mask ROImask+orig -mrange 1 1 -drange 0 10 -sigma –dump –indump stim_anl_PC+orig >> meanpercentROI_cond1.txt