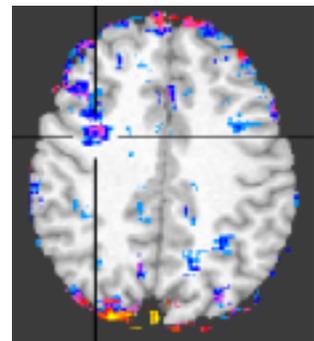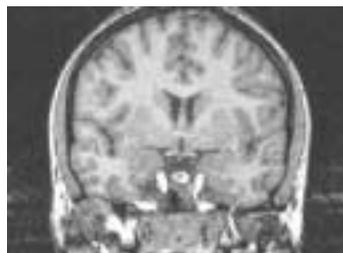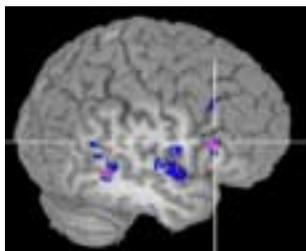# AFNI 101

This is a brief introduction to getting started using afni.

The main topics covered:

1. The basics of AFNI – Interface and Command-Line Programs.

2. Getting your file into an afni-friendly format with "to3d"

3. Navigating the afni interface

4. Creating a correlation map as a quick way to determine areas of activation

The Very Basics

• AFNI has two main facets: the interface (GUI) and the auxiliary command-line programs

• Interface

The Interface

- primarily used to visualize data and run simple, first-pass analyses

- contains a wide array of options to display anatomical and timeseries data

- has a 3D Volume Rendering option

- has "plugins" (under the "Define Datamode" menu) for some command-line programs
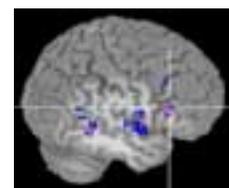
An Example of 3D Rendering

• Command-line programs

- very useful for multi-scan or multi-subject batch processing

- wide array of processing and analysis tools

- a few examples: motion correction, slice-timing correction, deconvolution, correlations, cluster analysis, t-tests, analysis-of-variance, multiple-testing correction

- to find out the function and options for a program, type:

    "program name" –help

- typical command-line structure:

    "program name"   –"option name" "selection"   –"another option" "selection" … "dataset name+orig"

    - note: you may or may not have options, and not all options have a selection after

    Some simple examples …

    3dinfo –verb run_1+orig   … This will print out all the information stored in the header of a dataset

    3dmerge –prefix masked_run1 –2clip 0 500 –doall run1+orig   … This will mask out (remove) all voxels with values below 500 and write a new dataset called "masked_run1+orig"

Page 2

Getting your File into AFNI format

AFNI File format

- each dataset has two files:

  ~.BRIK - contains the actual data

  ~.HEAD - a header file, it contains information about where the dataset is in space, what type of data it is, etc.

  for example, "run1+orig.BRIK" and "run1+orig.HEAD"

  - the "+orig" means that the data is in its "original" space, as opposed to the standardized Talairach "+tlrc" space.

- you must have a .BRIK and .HEAD file *with the same prefix* ("run1" in the above example) for afni to recognize the dataset

- in afni, there are two basic kinds of files – "anatomies" and "functions"

  - "anatomies" : include both high-resolution brains (e.g. SPGR) and BOLD timeseries data (this can be confusing since we tend to think of BOLD data as showing "function", but you want your BOLD data as underlay, and want to calculate activation maps based on it)

  - "functions" : these are typically the activation maps. They are derived or calculated from anatomies, and include correlations and other activation maps as well as masks and t-test results

- datasets from the same subject or of the same type should be stored in the same directory, called a "session" is afni

- to get a file into afni format, use the command "to3d"

to3d Examples

1. Timeseries data – output of reconstruction

- A typical output of reconstruction is a ".sdt" file – one large file containing only data (no header). Note: reconstruction can also output a series of I.### files – one for every timepoint. To use these, specify "I.*" as the input

- Command-line example:

  to3d –epan –prefix run1 –time:zt 33 300 2000 alt+z

  –xFOV 120R-L -yFOV 120A-P –zSLAB 70I-50S   –2swap

  –session ./   run1.sdt

  Input File

  options:

  "-epan" : Specifies the type of data, in this case, its epi. See "to3d" documentation for the tags for other file types.

  "-prefix" : what to call the new file, in this case "run1"

  "-time:zt" : specifies that this is timeseries data, and it stored in slices, then timepoints. The numbers after this option specify the **# of slices, # of timepoints**, and **TR** . The "**alt+z**" indicates the slices were interleaved in the +z direction, i.e. they were collected 1,3,5 … 33,2,4,6…. 32   **Important**: Slice-timing information is stored in the .HEAD file – but no slice-timing correction per se is applied unless you call the program "3dTshift"

  "-xFOV", "-yFOV", "-zSLAB": These are the field-of-view dimensions, i.e. the extent in x, y and z, in reference to the 0,0,0 point set by the scanner. The "FOV" indicates that you are specifying from the middle of the first slice to the middle of the last; the "SLAB" means you are specifying the outer edges of each slice. In most cases, you will use FOV for the x and y, and SLAB for z. See page 6 for more information on how to find the field-of-view dimensions.

  "-2swap" : byte-swap the input file

  "-session" : which directory to write to

Page 4

2. High-resolution "Anatomical" Scan

- Often, these files are written as I.### – one for every slice.

- Command-line example:

  to3d –spgr –prefix T1_highres –xFOV 120R-L -yFOV 120A-P –zSLAB 70I-50S –2swap –session ./ I.*

  - This will read up all files of the form I.*

  - Note that there is no "time" option here – there is only one "timepoint" so you don't need that option

  options:

  "-spgr" : Specifies the type of data, in this case, its epi. See "to3d" documentation for the tags for other file types.

  "-prefix" : what to call the new file, in this case "T1_highres"

  "-xFOV", "-yFOV", "-zSLAB": These are the field-of-view dimensions, i.e. the extent in x, y and z, in reference to the 0,0,0 point set by the scanner. See page 6 for more information on how to find the field-of-view dimensions.

  "-2swap" : byte-swap the input files

  "-session" : which directory to write to

General Notes with to3d:

- If an interface pops up after you have entered the command, then you are missing a piece of information afni needs.

- If the commandline is incompatible (e.g. you have entered more slices or timepoints than are in your input file), afni will crash and give you a somewhat-helpful error message as to why.

- If you don't byte-swap and you need to, afni will warn you, but will not apply the byte-swap for you.

Finding the FOV Dimensions from the Scanlog

When the data is read into afni, it needs to know both the orientation and location of the brain in space. So you have to input the numerical extent of the slices (actually, the bounding box of what was scanned, not just the brain), as well as which axes are which. Just to be confusing, what afni refers to as x,y, and z, ARE NOT the same x, y, and z planes with respect to the scanner.

There are two steps to finding the FOV dimensions

A. To find which axes are which

- The x-dimension refers to: as you look at a slice (as it was collected, so a coronal slice if you are collecting coronally), what is RIGHT to LEFT across the slice.

- The y-dimension refers to: as you look at a slice, what is TOP to BOTTOM down the slice.

- The z-dimension refers to: what is the dimension as you go from the FIRST slice to the LAST slice.

So for a sagittal acquisition, X is Anterior to Posterior OR Posterior to Anterior; Y is Superior to Inferior OR Inferior to Superior; Z is Left to Right OR Right to Left. The direction (i.e. A-P or P-A is determined by the prescription and is on the scanlog).

B. To find the numerical extent in X and in Y…

1.   Find the FOV from the **scanlog** and divide it by 2

2.   Find the dimension on the scanlog, and check to see if there is an offset. This is often listed as "S/I center" or whatever dimensions apply.

- If there is none – or if it says the offset is 0 – your FOV dimension is the number from a in each direction – as an example, if the field-of-view on the scanlog is 240, the files are collected sagittally, and there is no offset in X or Y, the X and Y are: 120A-120P (sometimes written 120A-P) and 120S-I.

- If there is an offset – add that number to what you got in #1 for the dimension the offset is in, and subtract it from the other dimension – if there was an offset of "2.2I" listed for the above example, the new FOV dimensions would be: 120A-P and 117.8S-122.2I. **Note that the two dimensions always add to the field of view listed on the scanlog**!

C. To find the numerical extent in Z …

- This is directly from the scanlog – it should be listed in the last blank in the section. It may have some direction about the way it is listed, i.e. "From A to P", and then should have **two** numbers corresponding to the coordinates of the first and last slice. So, for the sagittal acquisition example, it could say 60 L and 80 R, and if you are collecting from left to right, the Z FOV will be simple 60L-80R.

Reading Information from the Pfile

If you are unsure of the direction data was collected, or if you want to know more information about a Pfile, you can print out the scanning parameters as stored in the header of the Pfile.

➢ cd to the directory containing the Pfiles and type "ppi P######" or "rrh P#######" (the former stands for print p-file information, the latter stands for read raw header). Here is an example of the output from ppi

> P-FILE HEADER INFORMATION
>
> Subject Name: ANX010   ID: 010
>
> Date and time: Wed Feb 21 15:16:22 2001   Exam #: 1795
>
> Series number: 6   Series description: Cor EPI - No AS
>
> Pulse sequence: /usr/g/M/rjd/epibold
>
> TR: 2999.996 ms   TE: 50.000 ms Flip Angle: 90.0 degrees
>
> Variable Bandwidth: 62.00 Hz
>
> **Image center (1st slice):** (R/L(-))   0.00   (A/P(-)) 84.90 (S/I(-))   9.40
>
> Aquisition/Reconstruction Parameters
>
> X-FOV = 240.00 mm   X-res. = 64   X-dir. voxel size = 3.750 mm
>
> Y-FOV = 240.00 mm   Y-res. = 64   Y-dir. voxel size = 3.750 mm
>
> Z-dir. slice thickness = 7.0 mm   Scan Plane: Coronal
>
> Number of slices: 23   Number of functional images   323.0
>
> Gap between slices: 1.00 mm

➢ **Important:** The subject's name (full or initials) can be stored in the Pfile header – depending on what the MR tech types in. Remember, this is confidential information!

➢ Information concerning the direction and extent that the data was acquired is in the line starting with **"Image center (1st slice):".** From the scanlog, you should know which axes correspond to x, y, and z. The numbers for the x and y axes correspond to the offsets of the extent (see page 6 for how to use offsets). The sign of the number corresponds to the **direction** of the offset – a negative number after L/R corresponds to left offset, a positive is to the right, a negative number after S/I is an inferior offset, a positive is superior. In the z-direction, which is A/P in this example, there is no offset possible. The number corresponds to the coordinate of the first slice – so it is the first number in the extent of the z-direction. So from the sign of the number, you know which way slices were acquired; here we know that slices were collected from Anterior to Posterior, since the number in the z-direction is positive.

Final Note: Now that you know how to find the FOV dimensions, I'll admit that there is a shorter way.   Simply cd to the directory containing the Pfile, and type "read_FOV_Pfile all P#####.7" – and the FOV dimensions will print to the screen . The "all" specifies that you want all 3 dimensions; you may also enter "x", "y" or "z" to get just one dimension. **Caveat Emptor:** This program may not work correctly for certain oblique and reverse-spiral prescriptions.

The Basic AFNI Interface

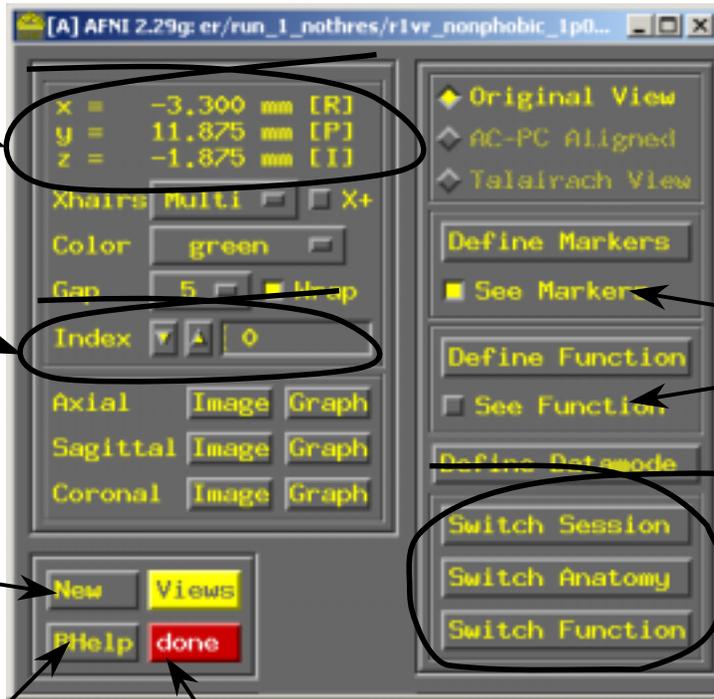Location of the cursor in space

Timepoint at the cursor location

Opens a new afni window, with the same sessions as the current afni

Turns on pop-up help boxes

Turns on the markers and functional overlay

Switch on a different session, anatomy or functional overlay

Hit twice to quit

Controls the color and gap between the crosshairs, and what planes you can see them

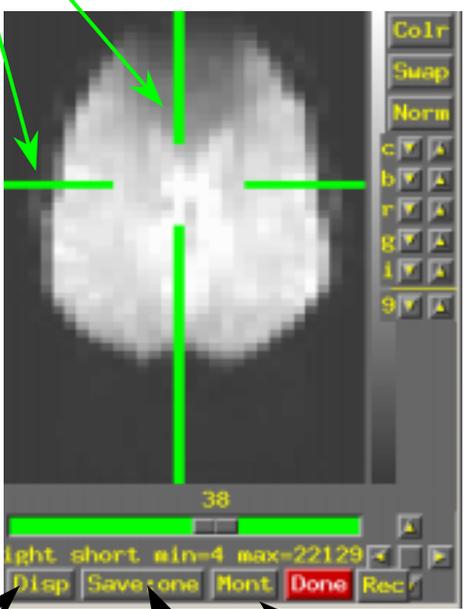These buttons open up an image for each plane, and the graphs

See Page 9 for more on these windows

Switches between the original, AC-PC and Talairach views (if the transforms have been done)

Opens up 3 other panels with options for Talairach markers, displaying the function, and misc. data options. See page # for more on these
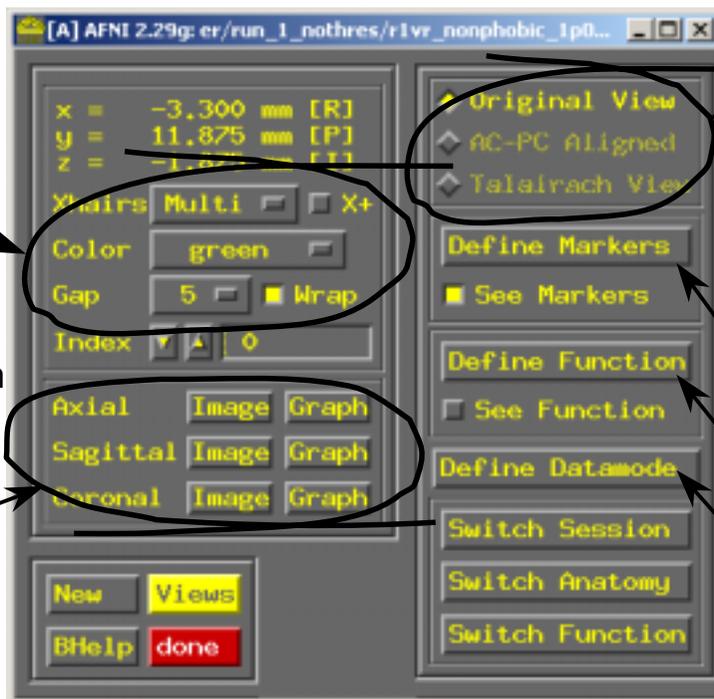
The 3 Image Windows

Crosshairs

Switch from black and white to color display
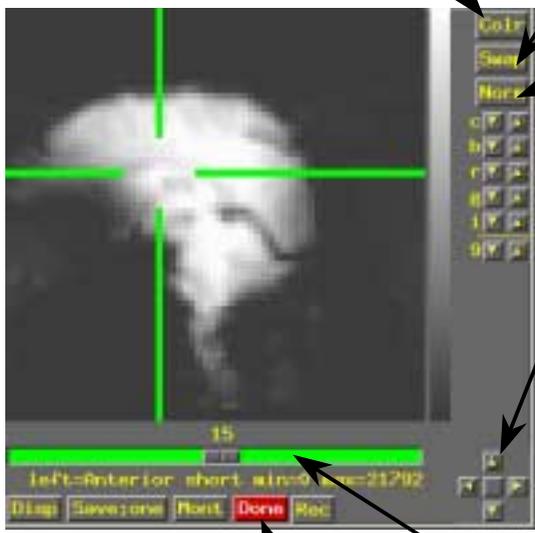
Swap black and white

Restore Defaults

Moves the cursor in space – the middle button closes the gap between the crosshairs

38

right short min=4 max=22129

Disp Save.one Mont Done Rec

15

left=Anterior short min=0 max=21792

Disp Save.one Mont Done Rec

Opens up a pop-up menu with more display options

Save this image to a file

Create a montage (a series of slices in a row)

Closes the window

Slice selector bar

Contrast

Brightness

Rotate the greyscale

Gamma

Size of the image in the window

Opacity of the color overlay

Orientation – left on the screen is the person's right

[A] AFNI 2.29g: demo_data/depall_data/s_negative_avgd+orig

Colr
Swap
Norm
c
b
r
g
i
9

11

left=Right float min=0 max=23467.8

Disp Save.one Mont Done Rec

Data storage type

Min and max values in this slice

Graph Windows

The 9 voxels displayed in the graph correspond to the 9 voxels in the image window. The center voxel in the image window is the center graph – highlighted in yellow
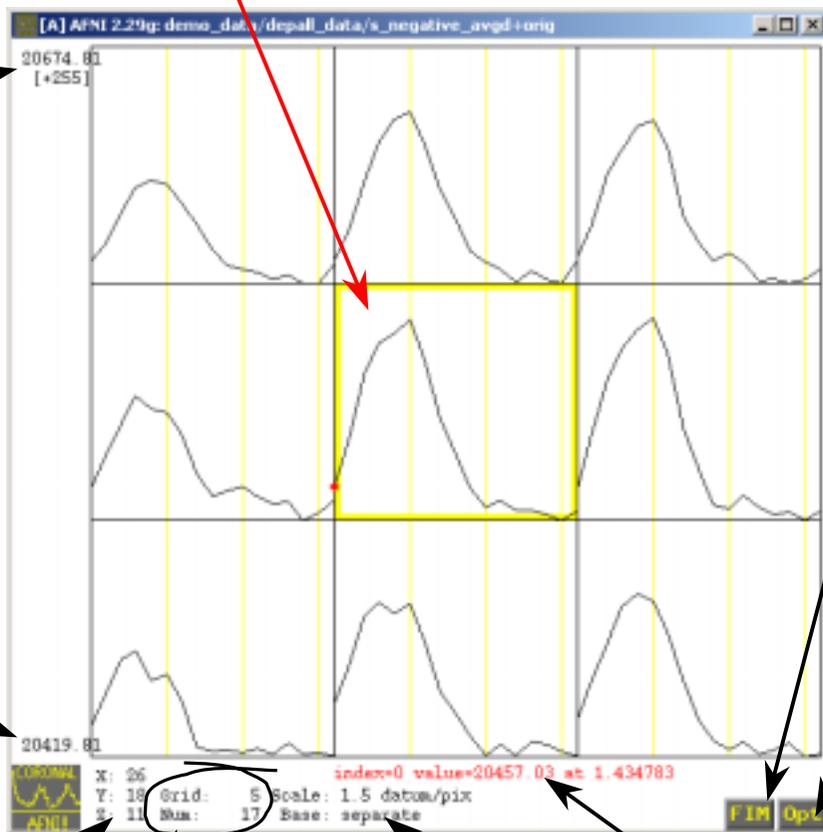
Top value in the scaled range. The range from high-low is in brackets – 255 in this case.

Lowest value in the scaled range for the graph display.

Pop-up menus for calculating correlations, etc. and more graphing options

The voxel coordinates for the center graph.

Spacing between gridlines (yellow lines) and number of timepoints per graph

Calculate the baseline separately for each voxel or use a common baseline

Timepoint and value at the cursor

A Note about scaling: The range will adjust as you click around the brain, but to alter the scale (zoom in or out) you need to either change the scale under "Opt", or hit Shift "+" to increase the scale, Shift "-" to decrease, or "a" to let afni automatically scale the graphs

"Opt" Pop-up Menu

Adjust the scale

The number of graphs displayed

Gridline spacing

Which slice is displayed

Cosmetic options

Calculate the baseline separately per graph or not

Write the center graph to a 1D file

Plot two graphs on each voxel (Tran 1D selects the 2nd timeseries to plot)

Close the window

Each selection with an arrow pops up another menu ...

Colors, etc: A whole bunch more display options for the graph
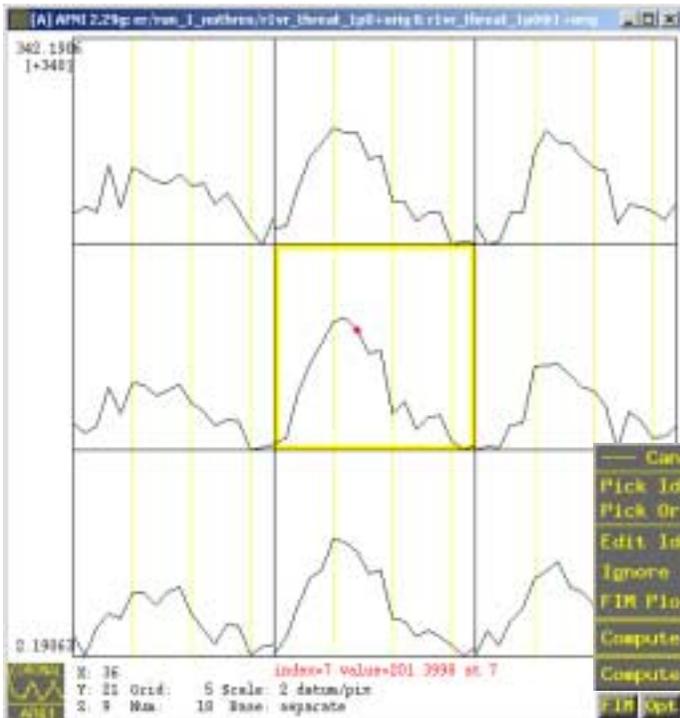
"Fim" Pop-up Menu
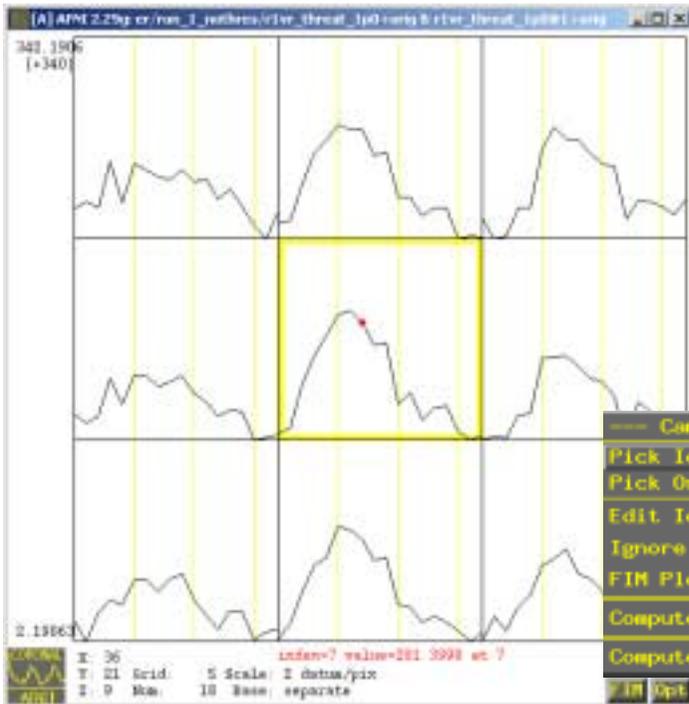
Close this menu

Select an ideal timeseries

Select an orthogonal

Edit the ideal selected

Timepoints to ignore (not displayed)

Display options

Compute FIM (correlation) and FIM+ (more options an functions available, writes a multi-brick function) – based on the selected ideal

"Edit Ideal" Menu

Select the center timeseres

Smooth the ideal

Allow for multiple time delays of the ideal

Clear the ideal from the display

1 = Linear trend ok
0 = no linear trend

Read in, write out the ideal

"Define Markers" Pop-up Menu

Allow editing of the markers – must be pressed to set the markers!

Colors of markers that are "primary" (P) and "set" (S)

Different markers to set
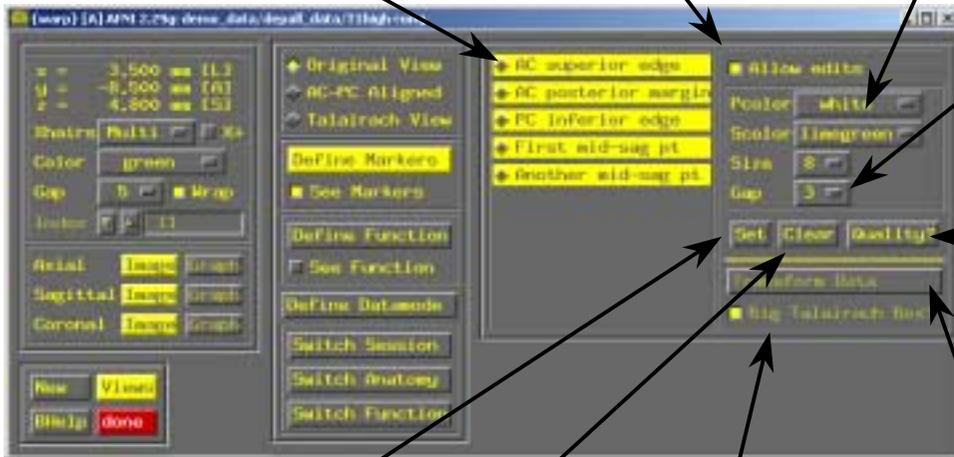
Size of markers and gap within

Perform quality control – must be done before dataset is written

Set a marker

Clear all markers

Big Talairach box includes the cerebellum – other box can cut it off

Do the transformation and write out the .HEAD file for that space

"Define Function" Pop-up Menu

Important: these options control the display of the function only - there is no actual editing of your dataset as you change these options.

Use the anatomy or the function as underlay

Use the function or the threshold as the overlay [1]

Color for portions of the range. Double-click to edit. Note that the gray-white pattern indicates NO COLOR

Max and min values for the current anatomy, function, and threshold datasets

Threshold value

P-value for this threshold, if applicable

Multiple of 10 for the threshold bar – 0 to threshold from 0 to 1, 1 for 0 to 10, etc.

Positive values only?

Number of colors

Rotates the color bar

Default is to use the max intensity as the top number in the range. De-select the "autoRange" and enter a new value to change this

[1] Correlations, t-tests and some other functional datasets have 2 bricks – one is the intensity, and one is a "hidden" threshold. For a t-test, the intensity is the average difference, and the threshold is the actual t-statistic. The default is to see the "intensity" as the overlay, and use the threshold to screen voxels (green slider-bar).

"Define Datamode" Pop-up Menu
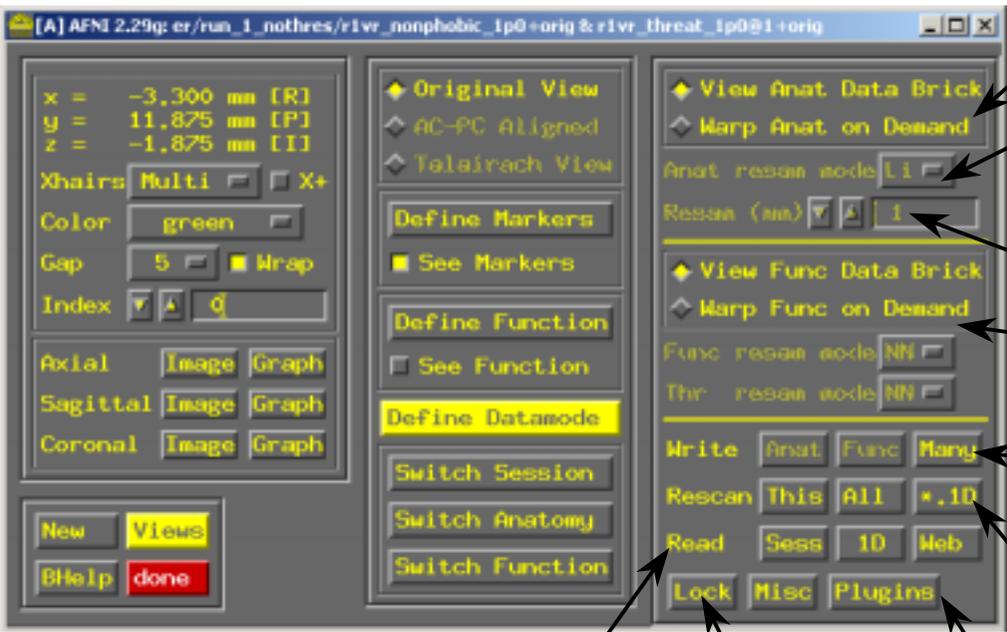
Allow warping (resampling)

Type of resample – Nearest Neighbor, Linear, Cubic, Blocky

resampled voxel size

Analogous options for the functional brick

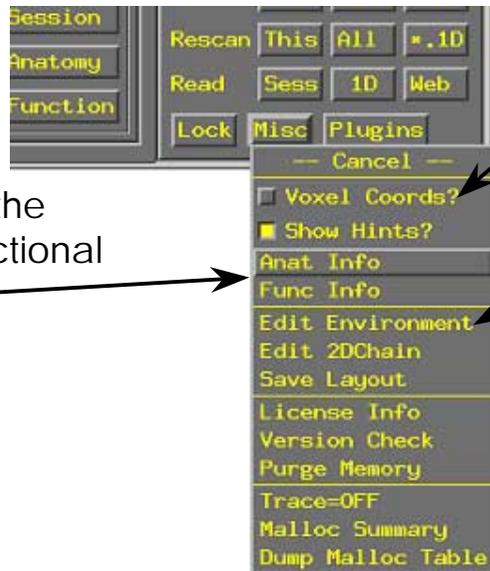Write out a resampled dataset

Rescan the current session, all sessions, or the 1D's

Read-in a new session or 1D-file

Spatial lock on afni interfaces (those created with the "new" button)

Plugins menu – interface versions of command-line programs

"Misc" Menu

Use voxel coordinates instead of mm

Edit environment variables

Print info about the anatomy or functional dataset

Purge Memory – very useful if you have been working with datasets for a long time- prevents crashing
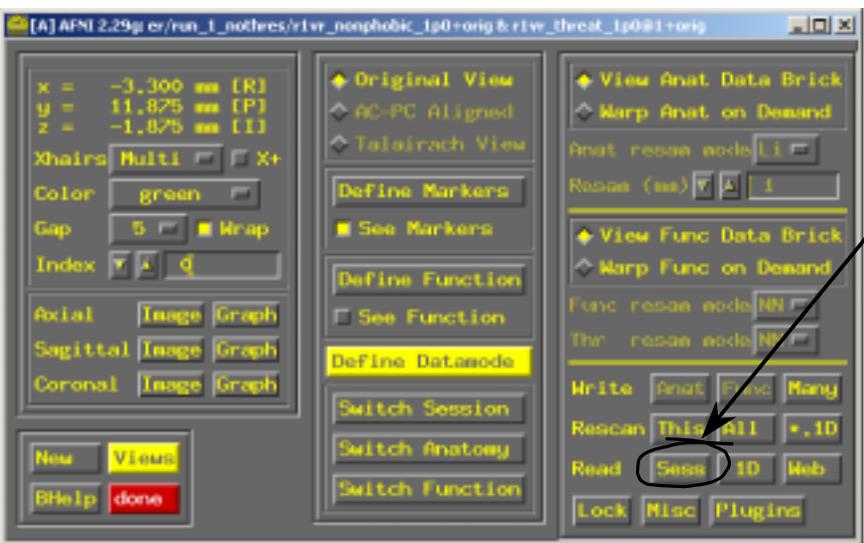
Page 15
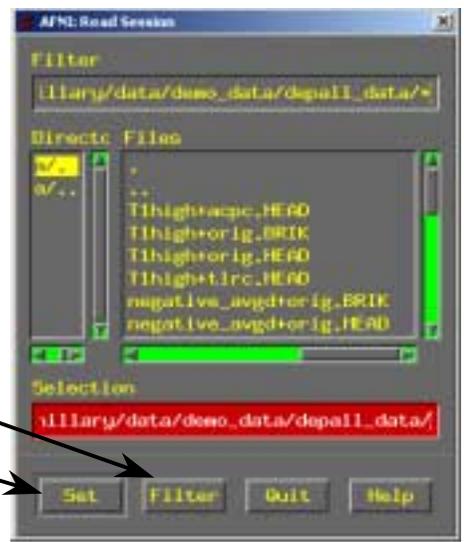
Changing Files and Sessions (directories)

The "Switch Anatomy" Menu- allows you to switch on any of the anatomies in the selected session. Hit "Apply" to make the change and keep the menu open, or "Set" to make the change and close the menu
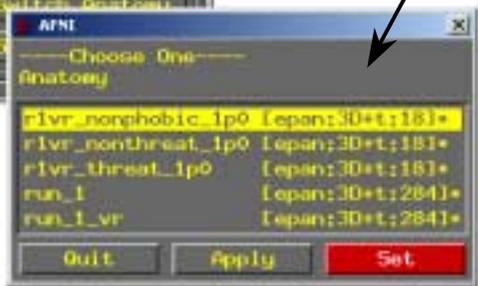
Analogous menus appear for the "Switch Function" and "Switch Session" buttons

To read in another session, hit Read – Session

Use the "filter" button to locate the directory, then hit "set" to read the datasets into a new session. You must switch on this new session (see above) to see the new files

# Quick and Easy Way to Find Areas of Activation

You can get an idea of what areas of the brain are activated by generating an correlation map.


1. Find a voxel that has a good, high hemodynamic response- the visual cortex is a good place to look for most studies. The smoother the graph the better- its best if it doesn't have any major noise bumps or dips. You also want the timecourse of the curve, e.g. the delay to onset of response and the length of the response, to be similar to other voxels.

2. Make sure the selected voxel is in the middle of the graph window. Click on the "Opt" button at the bottom of the graph window, then click on the "Write center [w]" button in the pop-up menu. This will write a file that contains information about this voxel, called a "1D" file, to the directory you started afni in.

3. Go to the main afni window, and click on the "Define Datamode" button, then on the "1D" button next to the word "Read". This will pop up a new window- select the 1D-file you just created. The numbers in the filename correspond to the coordinates of the voxel you selected. If there is no file there, try repeating the above step.

4. Go back to the graph window. Click on the "FIM" button, and then on "Pick ideal" in the pop-up menu. Select the 1D-file. A graph of this voxel should appear in red (or another color) above the middle graph in the graph window.

Page 17
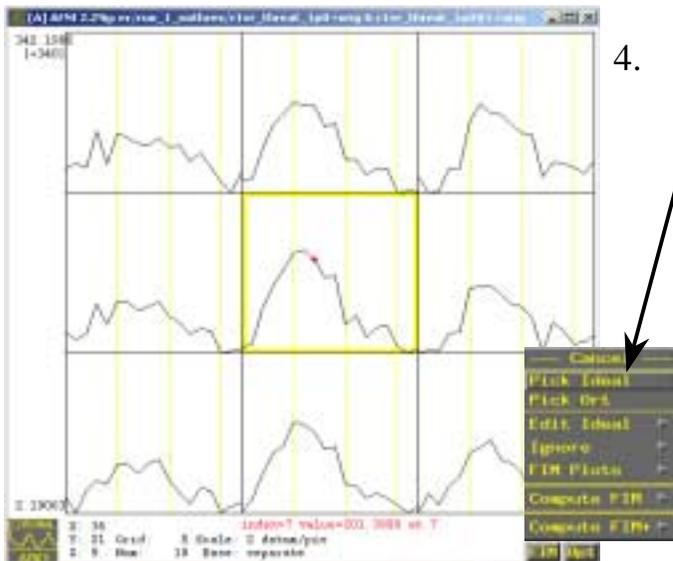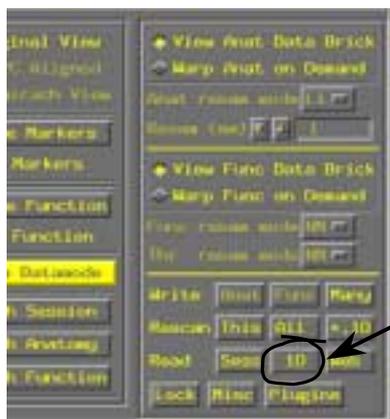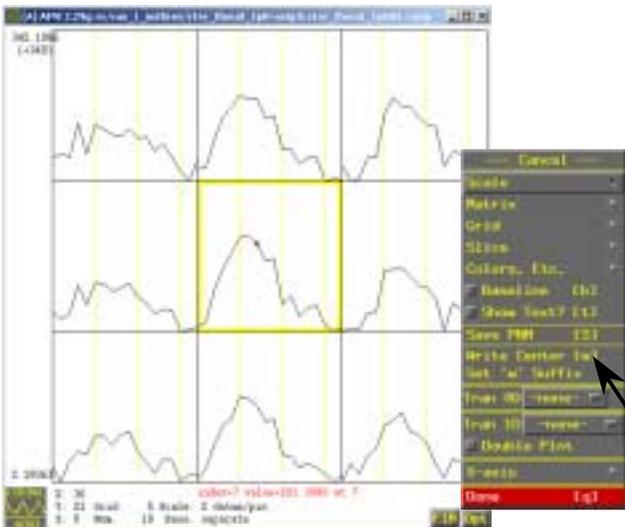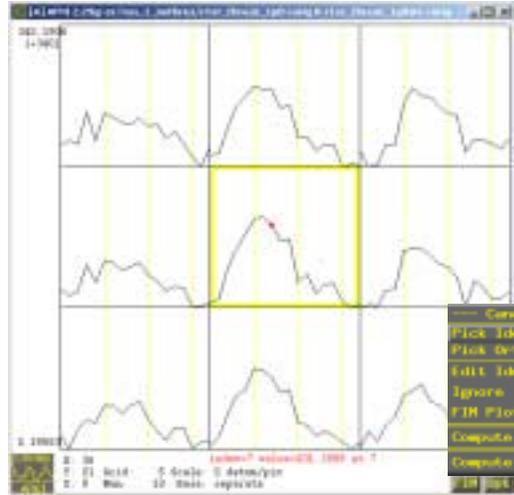
5. Click on the "FIM" button again, and then on "Edit ideal" in the pop-up menu, and then on "Smooth ideal" in the next pop-up menu. This will smooth the graph of the 1D-file. Repeat this step a few times until the ideal is relatively smooth.
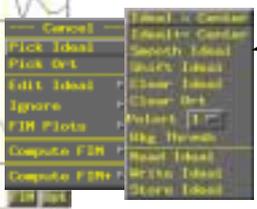
6. Click on the "FIM" button again, and then on "Compute FIM" in the pop-up menu, and then on "fico" in the next pop-up menu. Afni will compute a correlation of how similar each voxel in the dataset is to the ideal. A new file will be created; it is located under the "Switch Function" button in the main afni window. It is named as follows: "original filename"@1+orig, i.e. run_1_1@1+orig. If you repeat this step on the same file (anatomy), the new filename will be "original filename"@2. If you do create more than one function, be sure to write down which 1D-file, and which voxel you used for each file- once you create the function you can't easily tell which 1D-file you based it on.

7. Click the "Switch Function" button in the main afni window, and select it. Then click on the "Define Function" button in the main afni window. A new window will pop up; here you see what the colors of the function mean. Note that yellow indicates the highest positive correlation, light blue indicates the highest negative correlation, and no color means a correlation of zero. You want to consider only positively correlated areas of the brain.
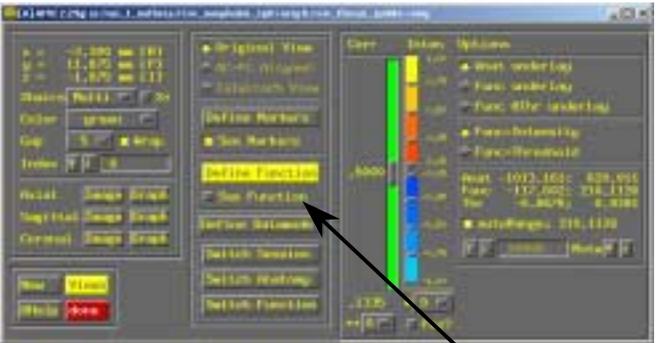
To see the function, make sure the "See Function" box is clicked in.