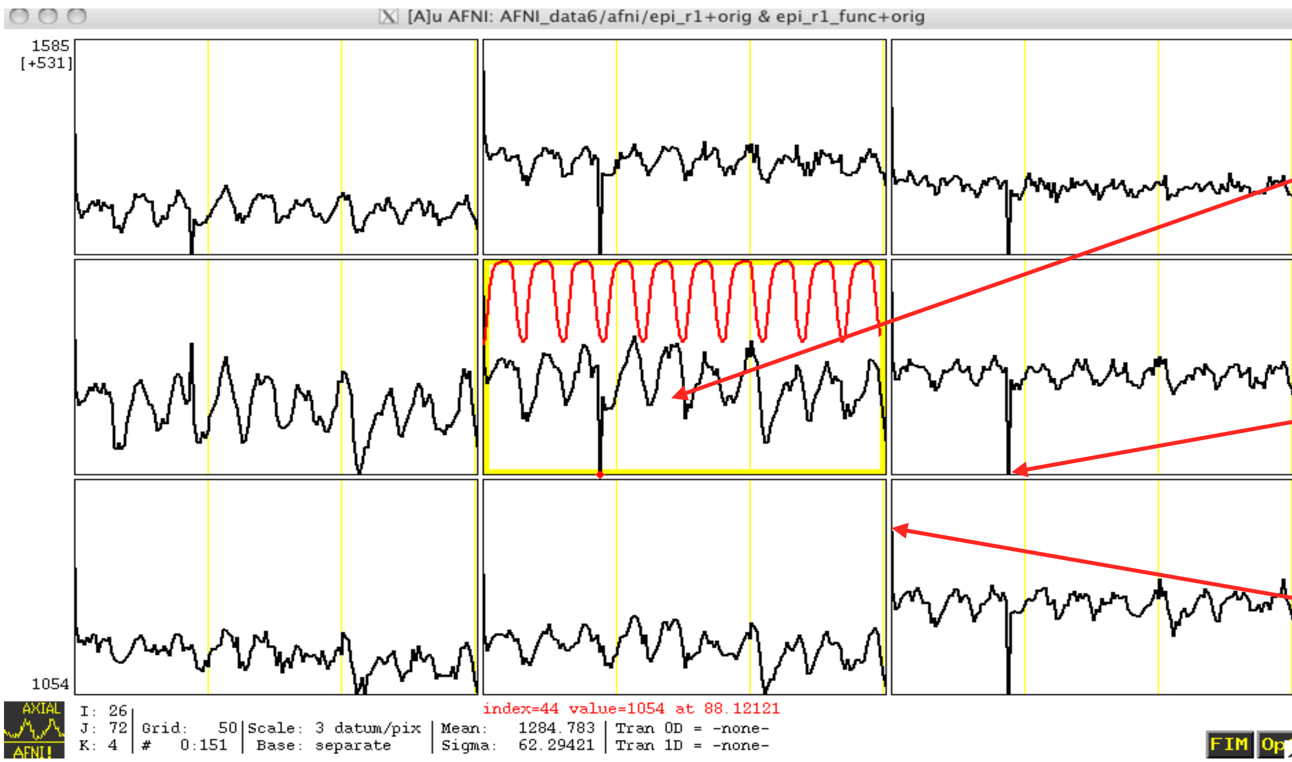
Hands-On Session: Regression Analysis

- What we have learned so far
  - Use data viewer 'afni' interactively
  - Model HRF with a shape-prefixed basis function
    - Assume the brain responds with the **same shape**
      - in any active regions
      - regardless stimulus types
    - Differ in magnitude: β is what we focus on
- What we will do in this session
  - Play with a case study
  - Spot check for the original data using GUI '**afni**'
  - Data pre-processing for time series regression analysis
  - Basic concepts of regressors, design matrix, and confounding effects
  - Statistical significance testing in regression analysis
  - Statistics thresholding with data viewer '**afni**' (two-sided vs. one-tailed with t)
  - Model performance (visual check of curve fitting and test via full F or R^2)

Data Quality Check

- To look at the data: type **`cd AFNI_data6/afni`**, then **`afni`**
- **`Switch Underlay`** to dataset **`epi_r1`**
  - Then **`Axial Image`** and **`Graph`**
  - **`FIM→Pick Ideal`** ; then click **`afni/epi_r1_ideal.1D`** ; then **`Set`**
  - Right-click in image, **`Jump to (ijk)`**, then **`26 72 4`**, then **`Set`**



- Data clearly has activity in sync with reference
  - 20s blocks
- Data also has a big spike at 89s
  - Head motion
- Spike at $t = 0$

Preparing Data for Analysis

- Eight preparatory steps are common:
  - Outliers: **3dToutcount** (or **3dTqual**), **3dDespike**
  - Temporal alignment or slice timing correction (sequential/interleaved): **3dTshift**
  - Image/volume registration (aka realignment, head motion correction): **3dvolreg**
  - Spatial normalization (standard space conversion): **adwarp, @auto_tlrc, anlign_epi_anat.py**
  - Blurring/smoothing: **3dmerge, 3dBlurToFWHM, 3dBlurInMask**
  - Masking: **3dAutomask**
  - Global mean scaling: **3dROIstats** (or **3dmaskave**) and **3dcalc**
  - Temporal mean scaling: **3dTstat** and **3dcalc**
- Not all steps are necessary or desirable in any given case

Data Analysis Script

- **In file epi_r1_regress:**

```
3dvolreg -base 3                \
         -verb                  \
         -prefix epi_r1_reg     \
         -1Dfile epi_r1_mot.1D  \
         epi_r1+orig
```

- 3dvolreg (3D image registration) will be covered in detail in a later presentation
- filename to get estimated motion parameters

- 3dDeconvolve = regression code

```
3dDeconvolve                            \
    -input epi_r1_reg+orig              \
    -nfirst      2                      \
    -num_stimts 1                       \
    -stim_times 1 epi_r1_times.1D       \
                  'BLOCK(20)'           \
    -stim_label 1 AllStim               \
    -tout                               \
    -bucket epi_r1_func                 \
    -fitts   epi_r1_fitts               \
    -xjpeg epi_r1_Xmat.jpg              \
    -x1D epi_r1_Xmat.x1D
```

- Name of input dataset (from 3dvolreg)
- Index of first sub-brick to process [skipping #0-1]
- Number of input model time series
- Name of input stimulus class timing file (τ's)
- and type of HRF model to fit
- Name for results in AFNI menus
- Indicates to output *t*-statistic for β weights
- Name of output "bucket" dataset (statistics)
- Name of output model fit dataset
- Name of image file to store **X** [AKA **R**] matrix
- Name of text file in which to store **X** matrix

- Type **tcsh epi_r1_regress**; then wait for programs to run

Screen Output of the `epi_r1_decon` script

- `3dvolreg` output

```
++ 3dvolreg: AFNI version=AFNI_2009_12_31_1431 (Mar 18 2010) [64-bit]
++ Reading input dataset ./epi_r1+orig.BRIK
++ Edging: x=4 y=4 z=2
++ Creating mask for -maxdisp
 + Automask has 66767 voxels
 + 8103 voxels left in -maxdisp mask after erosion
++ Initializing alignment base
++ Starting final pass on 152 sub-bricks: 0..1..2..3.. ***..150..151..
++ CPU time for realignment=7.25 s  [=0.0477 s/sub-brick]
++ Min : roll=-0.006  pitch=-2.057  yaw=-0.019  dS=-0.090  dL=-0.028  dP=-0.116
++ Mean: roll=+0.039  pitch=-0.127  yaw=+0.022  dS=+0.059  dL=+0.030  dP=+0.042
++ Max : roll=+0.119  pitch=+0.013  yaw=+0.076  dS=+0.209  dL=+0.087  dP=+0.272
++ Max displacement in automask = 2.46 (mm) at sub-brick 42
++ Wrote dataset to disk in ./epi_r1_reg+orig.BRIK          } Maximum movement estimate
```

- `3dDeconvolve` output

```
++ 3dDeconvolve: AFNI version=AFNI_2009_12_31_1431 (Mar 18 2010) [64-bit]
++ loading dataset epi_r1_reg+orig
*+ WARNING: Input polort=1; Longest run=304.0 s; Recommended minimum polort=3} Consider '-polort 3'
++ -stim_times using TR=2 s for stimulus timing conversion
++ Wrote matrix image to file epi_r1_Xmat.jpg       } Output file indicators
++ Wrote matrix values to file epi_r1_Xmat.x1D
++ ----- Signal+Baseline matrix condition [X] (150x3):  3.81681  ++ VERY GOOD ++
++ ----- Signal-only matrix condition [X] (150x1):  1  ++ VERY GOOD ++
++ ----- Baseline-only matrix condition [X] (150x2):  1.02336  ++ VERY GOOD ++   } Matrix Quality Assurance
++ ----- polort-only matrix condition [X] (150x2):  1.02336  ++ VERY GOOD ++
++ +++++ Matrix inverse average error = 1.47717e-15  ++ VERY GOOD ++
++ Calculations starting; elapsed time=1.553
++ voxel loop:0123456789.0123456789.0123456789.0123456789.0123456789. } Progress meter / pacifier
++ Calculations finished; elapsed time=4.979
++ Wrote bucket dataset into ./epi_r1_func+orig.BRIK
 + created 2 FDR curves in bucket header                  } Output file indicators
++ Wrote 3D+time dataset into ./epi_r1_fitts+orig.BRIK
```

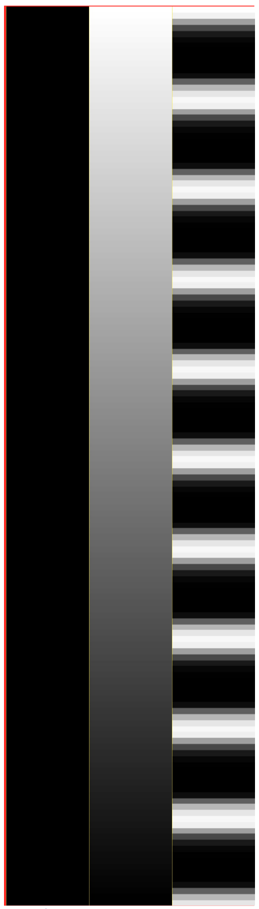
Modeling Serial Correlation in the Residuals

- Temporal correlation exists in the residuals of the time series regression model
  - ⋆ Caused by physiological (respiratory, cardiac, and vasomotor) effects
  - ⋆ First-order autocorrelation up to 0.4 in cortex
- Within-subject variability (or statistical value) would get deflated (or inflated) if temporal correlation is not accounted for in the model
- Should correct for the temporal correlation if bringing both effect size (β) and within- subject variability to group analysis
  - ⋆ Doesn't matter much if effect size is taken for group analysis
- ARMA(1, 1) assumed in 3dREMLfit
- Script automatically generated by **3dDeconvolve** (may use –x1D_stop)
  - ⋆ File **epi_r1_func.REML_cmd** under **AFNI_data6/afni**
  - ⋆ Run it by typing **tcsh –x rall_func.REML_cmd**

```
3dREMLfit -matrix epi_r1_Xmat.x1D -input epi_r1_reg+orig  \
  -tout -Rbuck epi_r1_func_REML -Rvar epi_r1_func_REMLvar \
  -Rfitts epi_r1_fitts_REML -verb
```

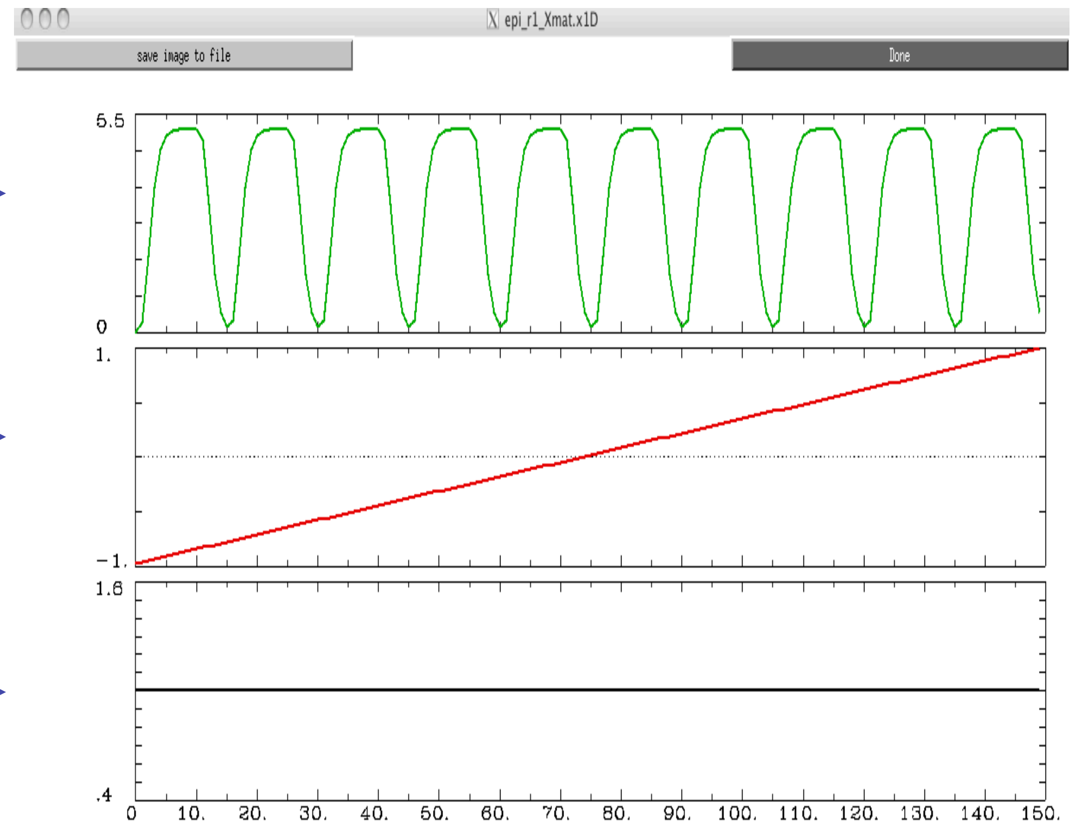
Stimulus Timing: Input and Visualization

epi_r1_times.txt = `4 34 64 94 124 154 184 214 244 274`
= times of *start* of each **`BLOCK(20)`** HRF copy



X matrix columns

- HRF⊗timing
- Linear in ***t***
- All ones



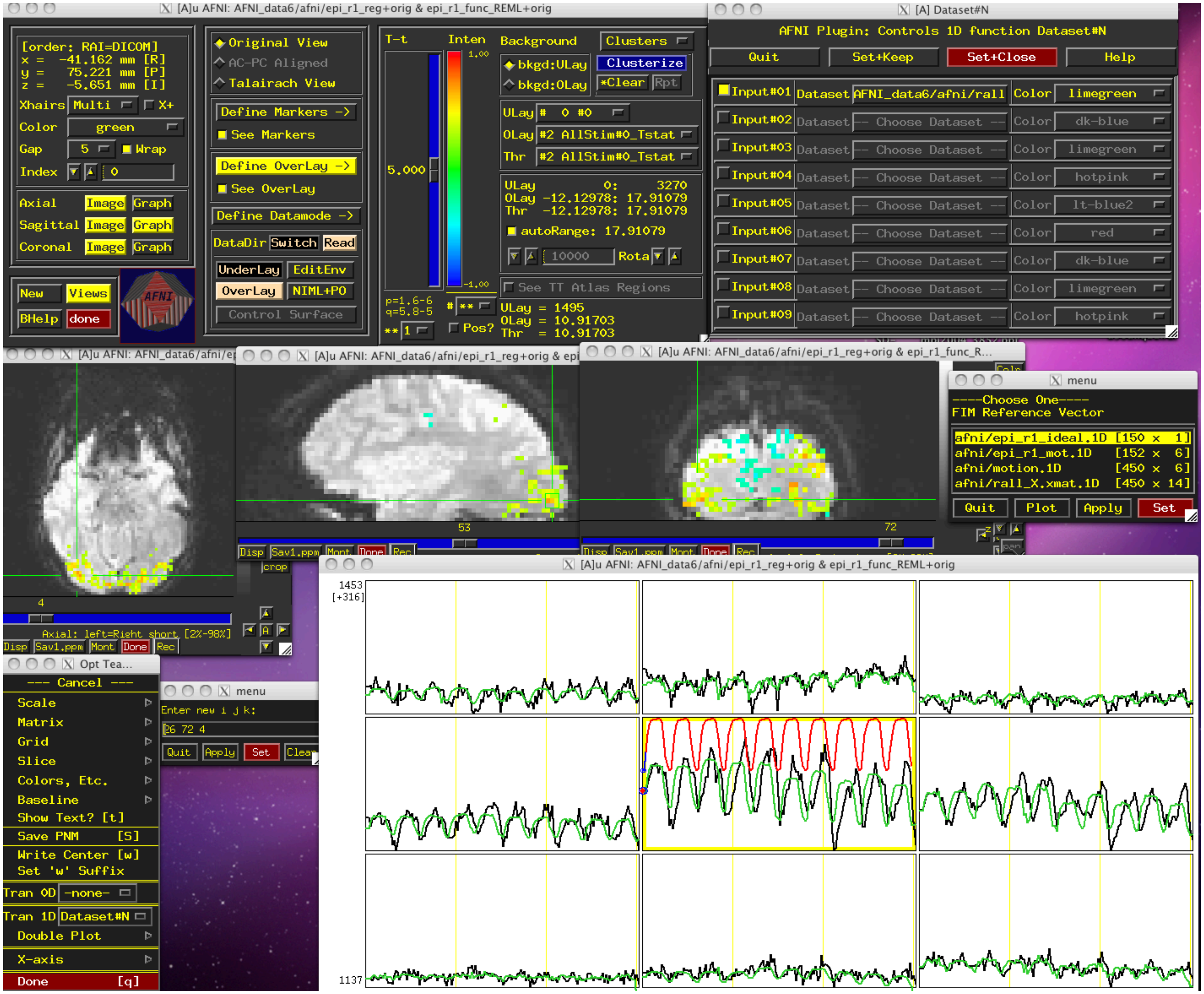
`aiv epi_r1_Xmat.jpg`

`1dplot -sepscl epi_r1_Xmat.x1D`

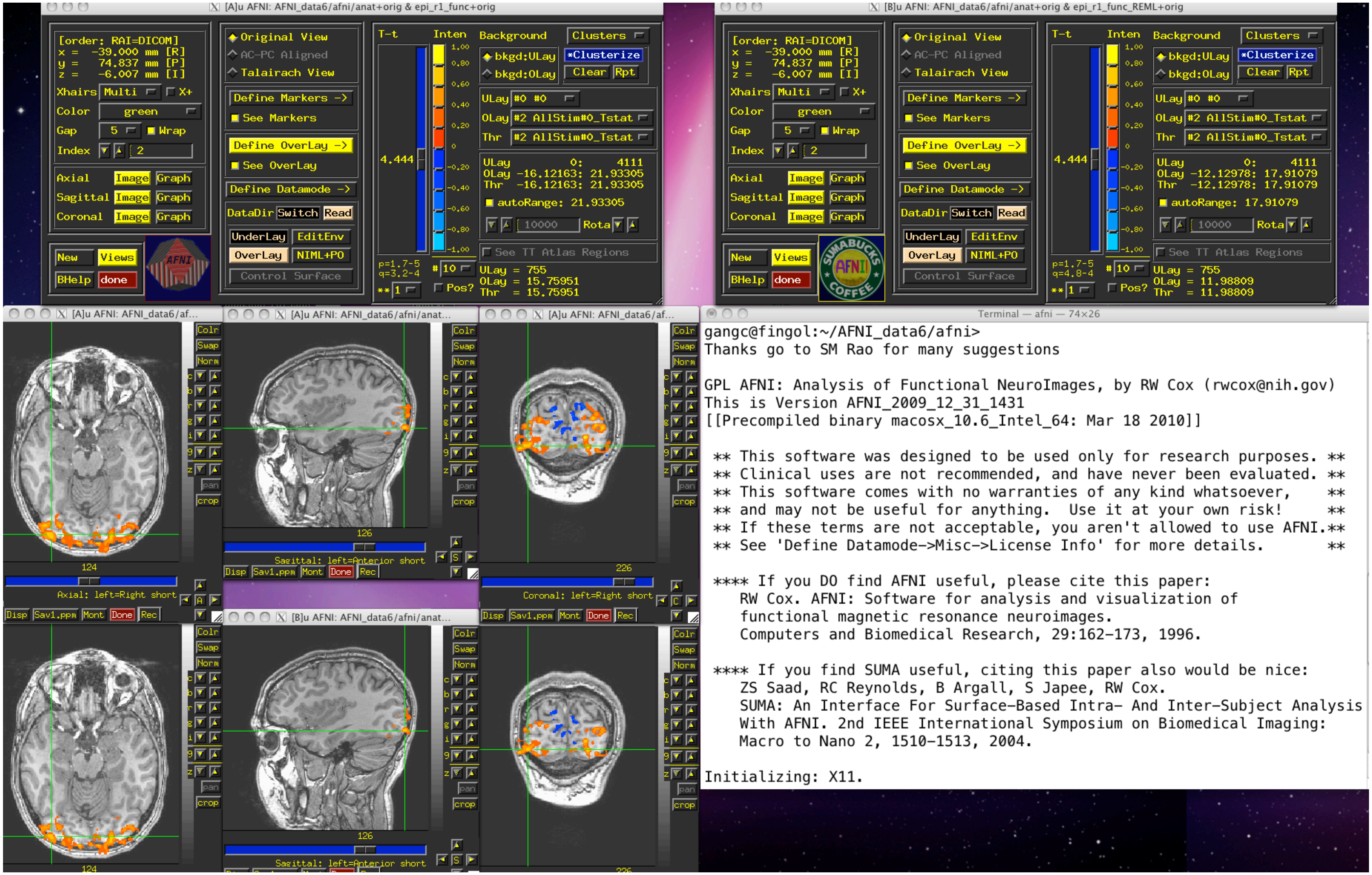
Look at the Activation Map

- Run **afni** to view what we've got (N.B.: a weak test with only 1 run)
  - **Switch Underlay** to `epi_r1_reg` (background: input for **3dDeconvolve**)
  - **Switch Overlay** to `epi_r1_func` (statistics: output from **3dDeconvolve**)
  - **Sagittal Image** and **Graph** viewers (time series at a few voxels)
  - **FIM→Ignore→2** to have graph viewer not plot 1st time point
  - **FIM→Pick Ideal**; pick `epi_r1_ideal.1D` (HRF: output from **–x1D**)
- **Define Overlay** to set up functional coloring
  - **Olay→Allstim#0_Coef** (sets coloring to be from β: color spectrum)
  - **Thr→Allstim#0_Tstat** (sets threshold to be t-statistic: slider bar)
  - **See Overlay** (otherwise won't see the function!) – should be on automatically
  - Play with threshold slider to get a meaningful activation map (e.g., $t(61)=3$ is a decent threshold): what's the difference between one- and two-sided? Which should be adopted? How to get one-side significance level on afni?
  - Again, use **Jump to (i j k)** to jump to index coordinates **26 72 4**

Check Model Performance



Compare 3dDeconvolve and 3dREMLfit



Group Analysis: will be carried out on β or `GLT` coef (+*t*-value) from single-subject analysis

Visually check model performance

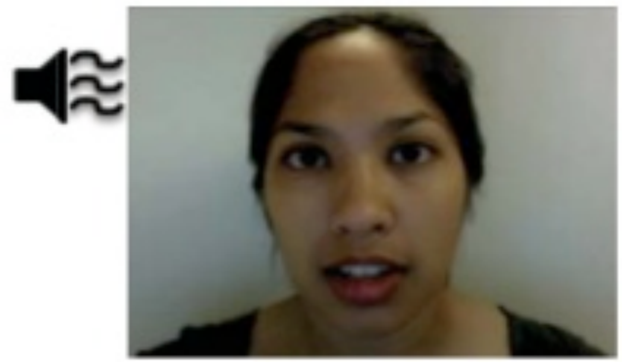
- Graph viewer: **Opt→Tran 1D→Dataset #N** to plot the model fit dataset output by **3dDeconvolve**
  - Will open the control panel for the **Dataset #N** plugin
  - Click first **Input** line to be 'on'; then choose **Dataset epi_r1_reg+orig**
  - Also choose **Color dk-blue** to get a pleasing plot
  - Click 2nd **Input** on; then choose **Dataset epi_r1_fitts+orig**
  - Also choose **Color limegreen** to get a pleasing plot
  - Then click on **Set+Close** (to close the plugin's control panel)
  - This tool lets you visualize how the model performs

A Case Study

- Speech Perception Task: Subjects were presented with audiovisual speech that was presented in a predominantly auditory or predominantly visual modality.
- A digital video system was used to capture auditory and visual speech from a female speaker.
- There were 2 types of stimulus conditions:

(1) **Auditory-Reliable**

Example: Subjects can clearly *hear* the word "cat," but the video of a woman mouthing the word is degraded.

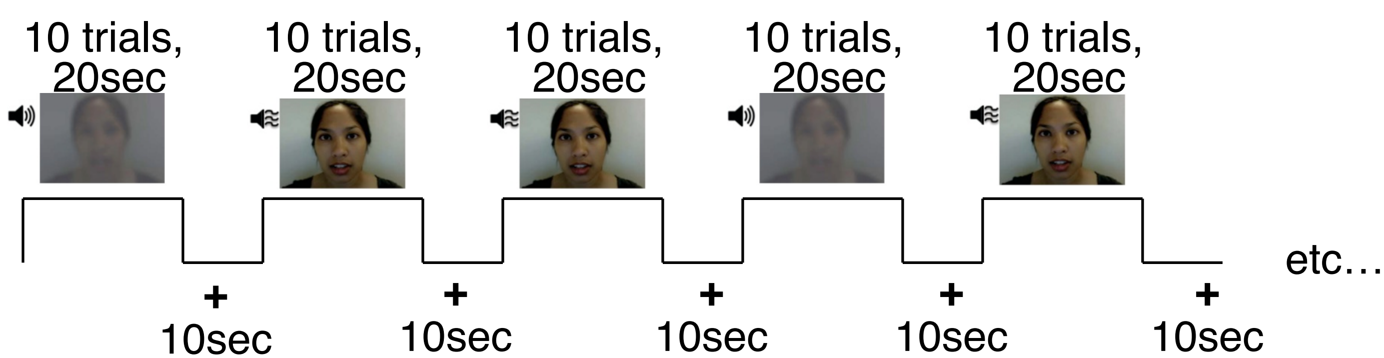


(2) **Visual-Reliable**

Example: Subjects can clearly *see* the video of a woman mouthing the word "cat," but the audio of the word is degraded.

Experiment Design

- 3 runs in a scanning session.
- Each run consisted of 10 blocked trials:
  - 5 blocks contained Auditory-Reliable (*Arel*) stimuli, and
  - 5 blocks contained Visual-Reliable (*Vrel*) stimuli.
- Each block contained 10 trials of *Arel* OR *Vrel* stimuli.
  - Each block lasted for 20s (1s for stimulus presentation, followed by a 1s inter-stimulus interval).
- Each baseline block consisted of a 10s fixation point.



Data Collected

- 2 anatomical datasets for each subject, collected from a 3T scanner
  - 124 axial slices
  - voxel dimensions = 0.938 x 0.938 x 1.2 mm

- 3 time series (EPI) datasets for each subject
  - 33 axial slices x 152 volumes (TRs) per run
  - TR = 2s; voxel dimensions = 2.75 x 2.75 x 3.0 mm

- Sample size, n = 10 (all right-handed subjects)

Regression Analysis

•Run script by typing **tcsh rall_regress** (takes a few minutes)

```
3dDeconvolve -input rall_vr+orig                                         \
    -concat '1D: 0 150 300'                                              \
    -num_stimts 8                                                        \
    -stim_times 1 stim_AV1_vis.txt 'BLOCK(20,1)' -stim_label 1 Vrel     \
    -stim_times 2 stim_AV2_aud.txt 'BLOCK(20,1)' -stim_label 2 Arel     \
    -stim_file 3 motion.1D'[0]' -stim_base 3 -stim_label 3 roll         \
    -stim_file 4 motion.1D'[1]' -stim_base 4 -stim_label 4 pitch        \
    -stim_file 5 motion.1D'[2]' -stim_base 5 -stim_label 5 yaw          \
    -stim_file 6 motion.1D'[3]' -stim_base 6 -stim_label 6 dS           \
    -stim_file 7 motion.1D'[4]' -stim_base 7 -stim_label 7 dL           \
    -stim_file 8 motion.1D'[5]' -stim_base 8 -stim_label 8 dP           \
    -gltsym 'SYM: Vrel -Arel' -glt_label 1 V-A                           \
    -tout -x1D rall_X.xmat.1D -xjpeg rall_X.jpg                          \
    -fitts rall_fitts -bucket rall_func                                  \
    -jobs 2
```

•2 audiovisual stimulus classes were given using `-stim_times`

•**Important to include motion parameters as regressors**?

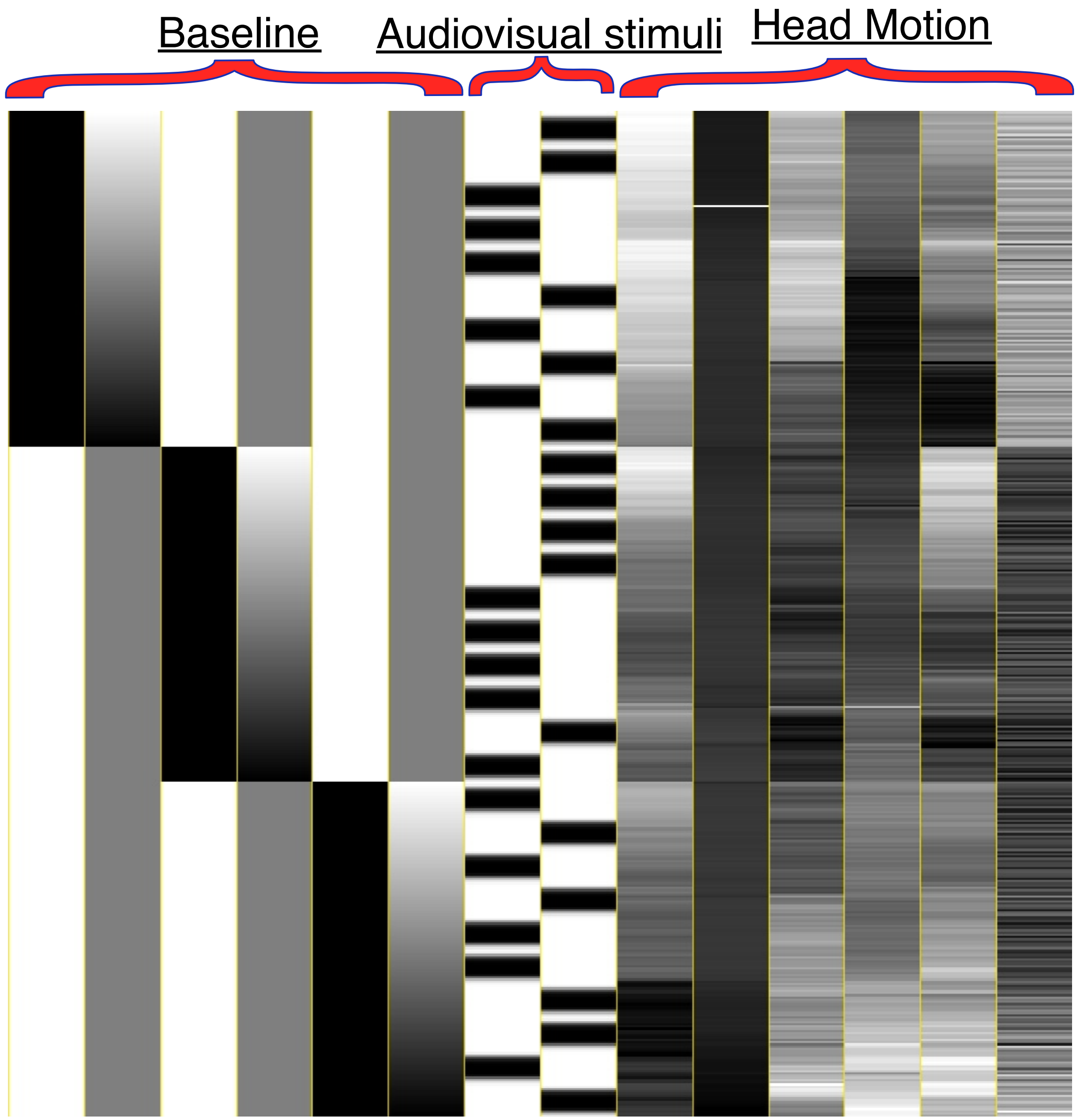
- May remove the confounding effects due to motion artifacts
- 6 motion parameters as covariates via `-stim_file` + `-stim_base`
- `motion.1D` generated from `3dvolreg` with the `-1Dfile` option
- Test the significance of head motion parameters
    - Switch from `-stim_base` to `-stim_label roll` …
    - Use `-gltsym 'SYM: roll \ pitch \yaw \dS \dL \dP'`

Modeling Serial Correlation in the Residuals

- Temporal correlation exists in the residuals of the time series regression model
- Within-subject variability (or statistical value) would get deflated (or inflated) if temporal correlation is not accounted for in the model
- Better correct for the temporal correlation if bringing both effect size and within-subject variability to group analysis
- ARMA(1, 1) assumed in 3dREMLfit
- Script automatically generated by 3dDeconvolve (may use –x1D_stop)
  - ⋆ File rall_func.REML_cmd under AFNI_data6/afni
  - ⋆ Run it by typing **tcsh –x rall_func.REML_cmd**

```
3dREMLfit -matrix rall_X.xmat.1D -input rall_vr+orig \
 -tout -Rbuck rall_func_REML -Rvar rall_func_REMLvar \
 -Rfitts rall_fitts_REML -verb
```

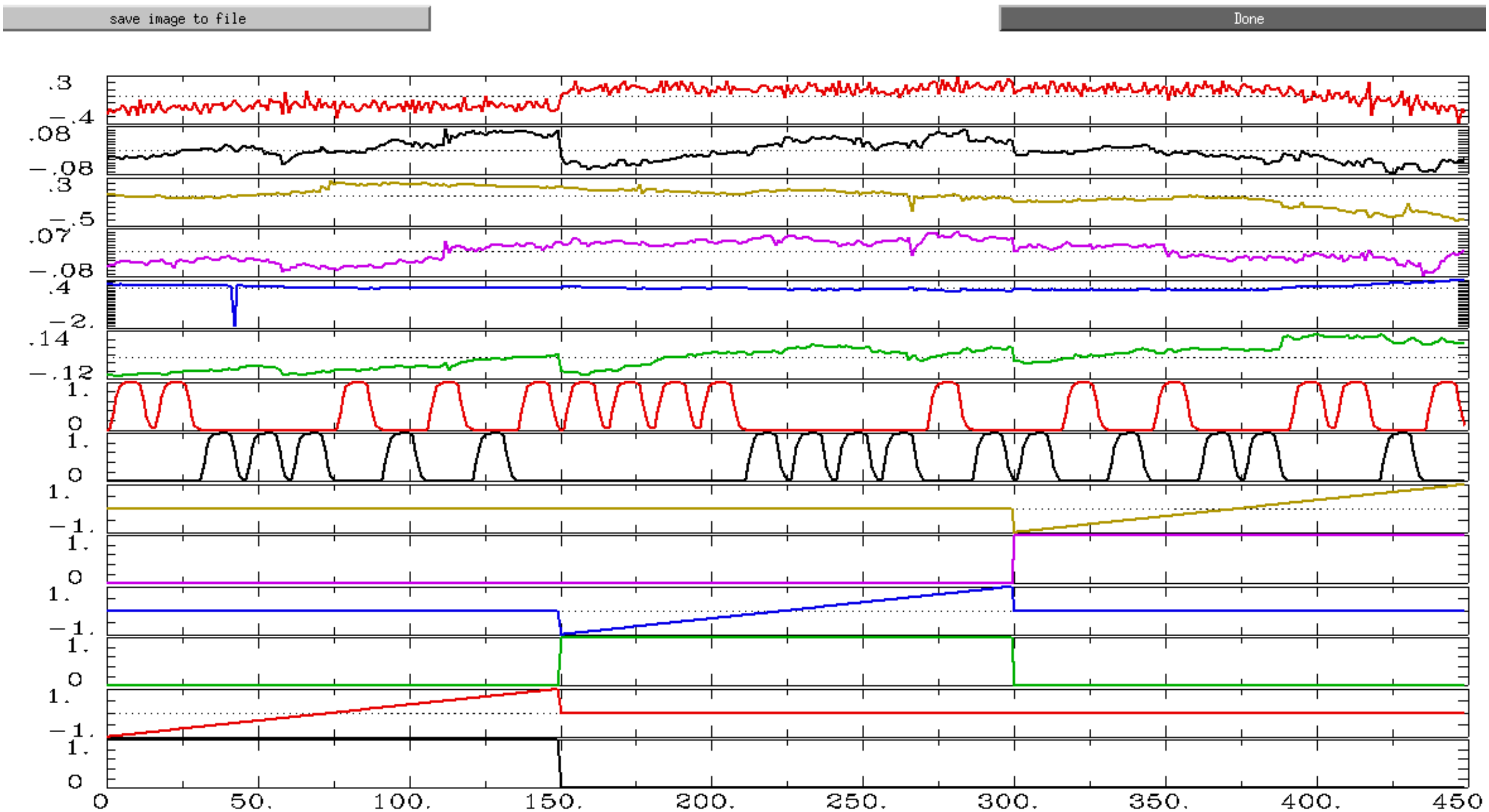
Regressor Matrix for This Script (via -xjpeg)



`aiv rall_xmat.jpg`

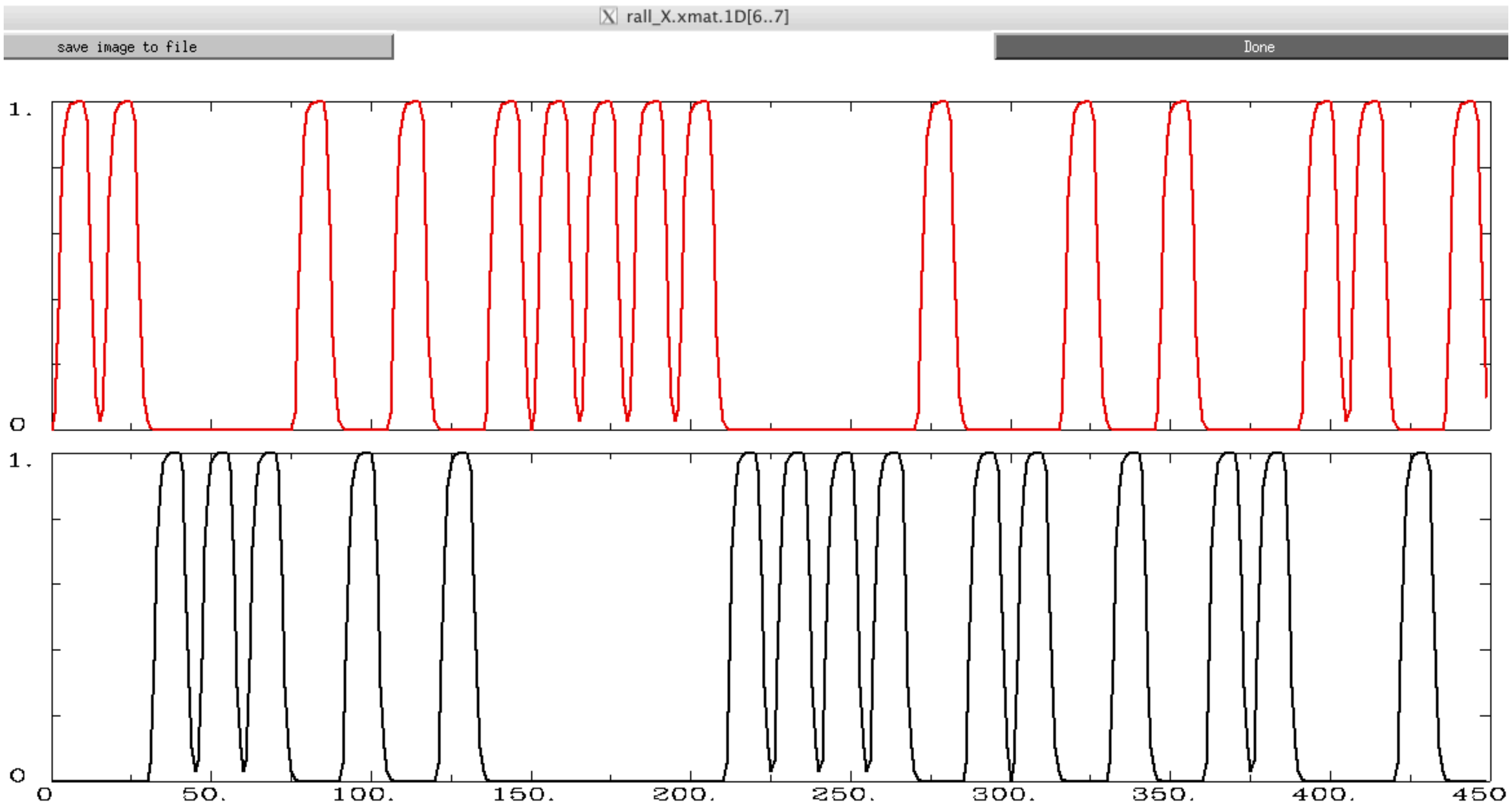
- 6 drift effect regressors
  - linear baseline
  - 3 runs times 2 params/run
- 2 regressors of interest
  - 3×3 design
- 6 head motion regressors
  - 3 rotations and 3 shifts

Showing All Regressors (via -x1D)



All regressors: **1dplot -sepscl rall_X.mat.1D**

Showing Regressors of Interest



Regressors of Interest: **1dplot rall_X.mat.1D'[6..7]'**

Options in 3dDeconvolve - 1

`-concat '1D: 0 150 300'`

- "File" that indicates where distinct imaging runs start inside the input file
  - Numbers are the time (TR) indexes inside the dataset file for start of runs
  - In this case, a text format .1D file put directly on the command line
    - Could also be a filename, if you want to store that data externally

`-num_stimts 8`

- 2 audiovisual stimuli (+6 motion), thus 2 **`-stim_times`** below
- Times given in the **`-stim_times`** files are *local* to the start of each run

`-stim_times 1 stim_AV1_vis.txt 'BLOCK(20,1)' -stim_label 1 Vrel`

- Content of **`stim_AV1_vis.txt`**

```
60 90 120 180 240
120 150 180 210 270
0 60 120 150 240
```

- Each of 3 lines specifies start time in seconds for stimuli within the run

Options in 3dDeconvolve - 2

`-gltsym 'SYM: Vrel -Arel' -glt_label 1 V-A`

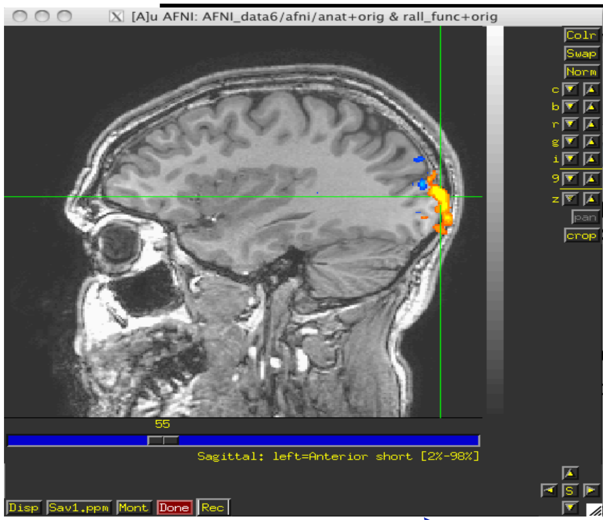
- **GLT**s: **G**eneral **L**inear **T**ests
- **3dDeconvolve** provides test statistics for each regressor separately, but if you want to test combinations or contrasts of the β weights in each voxel, you need the `-gltsym` option
- Example above tests the difference between the β weights for the `Virual-reliable` and the `Audio-reliable` responses
  - `SYM:` means symbolic input is on command line
    - Otherwise inputs will be read from a file
  - Symbolic names for each regressor taken from `-stim_label` options
  - Stimulus label can be preceded by `+` or `-` to indicate sign to use in combination of β weights
  - Leave space after each label!
- Goal is to test a linear combination of the β weights
  - Null hypothesis $\beta_{\mathrm{Vrel}} = \beta_{\mathrm{Arel}}$
  - e.g., does `Vrel` get different response from `Arel`?
- What do `'SYM: 0.5*Vrel +0.5*Arel'` and `'SYM: Vrel \ Arel'` test?

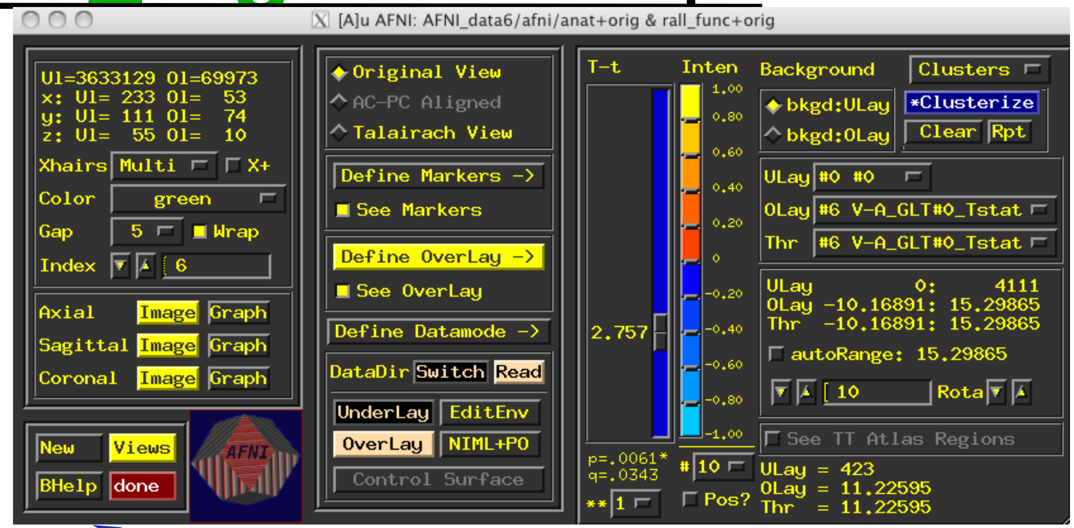
Options in 3dDeconvolve - 4

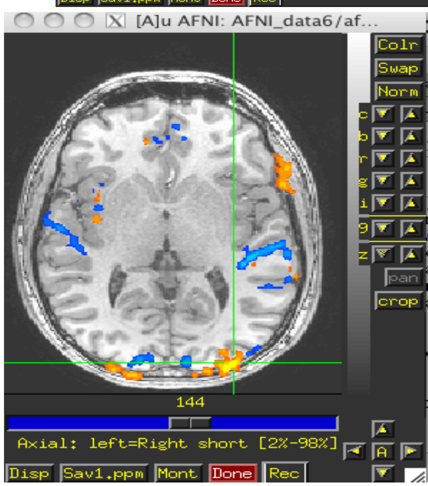
`-fout -tout` = output both *F*- and *t*-statistics for each stimulus class (**`-fout`**) and stimulus coefficient (**`-tout`**) — but not for the baseline coefficients (use **`-bout`** for baseline)

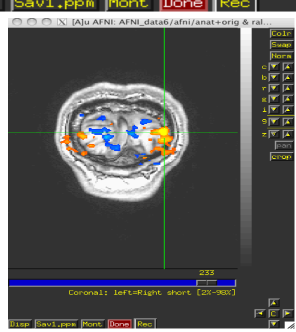
- The full model statistic is an *F*-statistic that shows how well all the regressors of interest explain the variability in the voxel time series data
  - Compared to how well *just* the baseline model time series fit the data times (in this example, have 24 baseline regressor columns in the matrix — 6 for the linear drift, plus 6 for motion regressors)
  - $F = [SSE(r) - SSE(f)]/df(\mathrm{n}) \div [SSE(f)/df(\mathrm{d})]$
- The individual stimulus classes also will get individual *F*- (if –fout added) and/or *t*-statistics indicating the significance of their individual *incremental* contributions to the data time series fit
  - If DF=1 (e.g., F for a single regressor), ***t*** is equivalent to *F*: $t(n) = F^2(1, n)$

Results of **rall_regress** Script



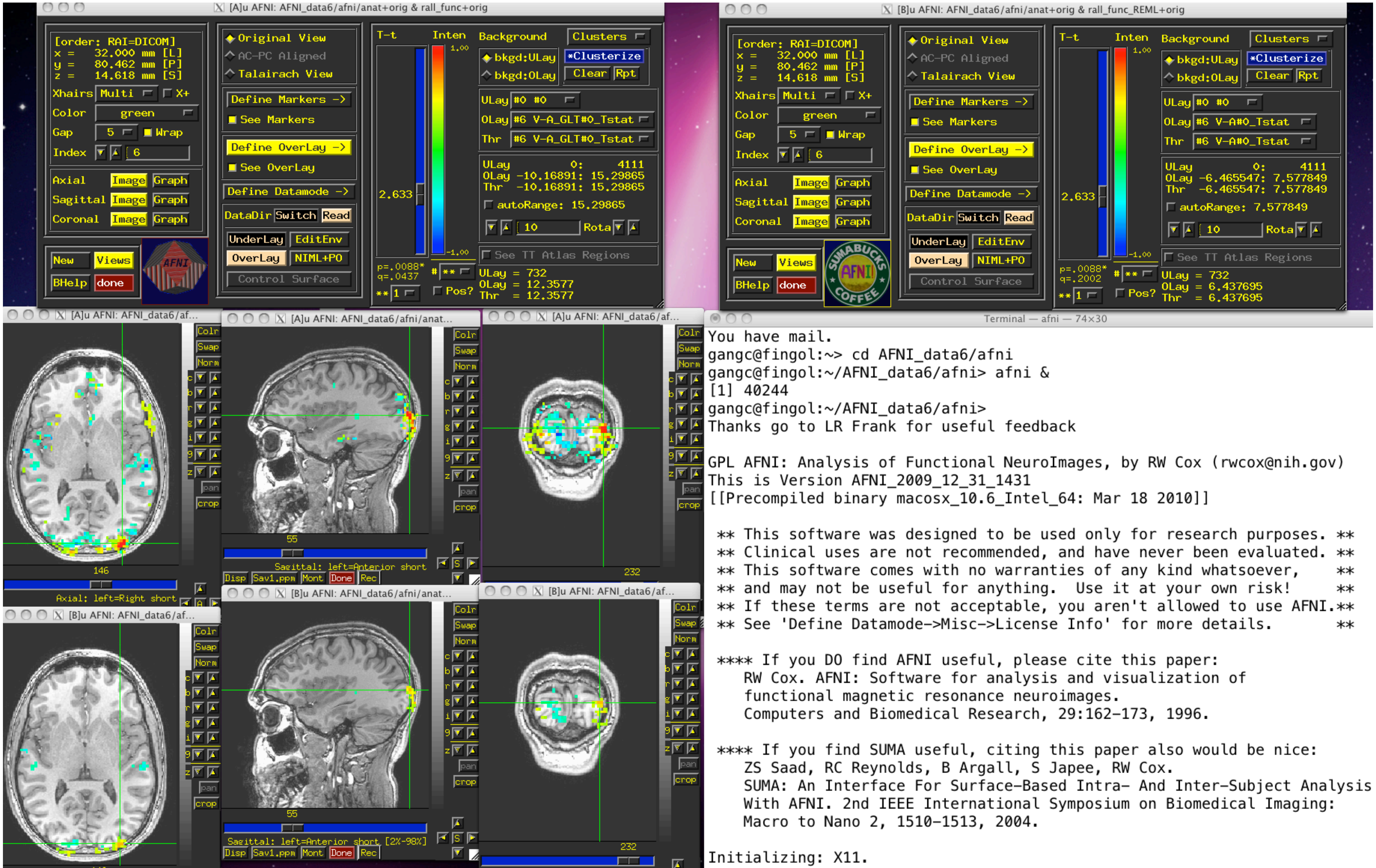






- Images showing results from third GLT contrast: **VrelvsArel**
- Menu showing labels from **3dDeconvolve**
- Play with these results yourself*!*

Compare 3dDeconvolve and 3dREMLfit



Group Analysis: will be carried out on β or `GLT` coef (+t-value) from single-subject analysis