Instant Analyses in **AFNI** and **SUMA**: Clusters and Correlations

Data for this presentation:
AFNI_data5/ directory

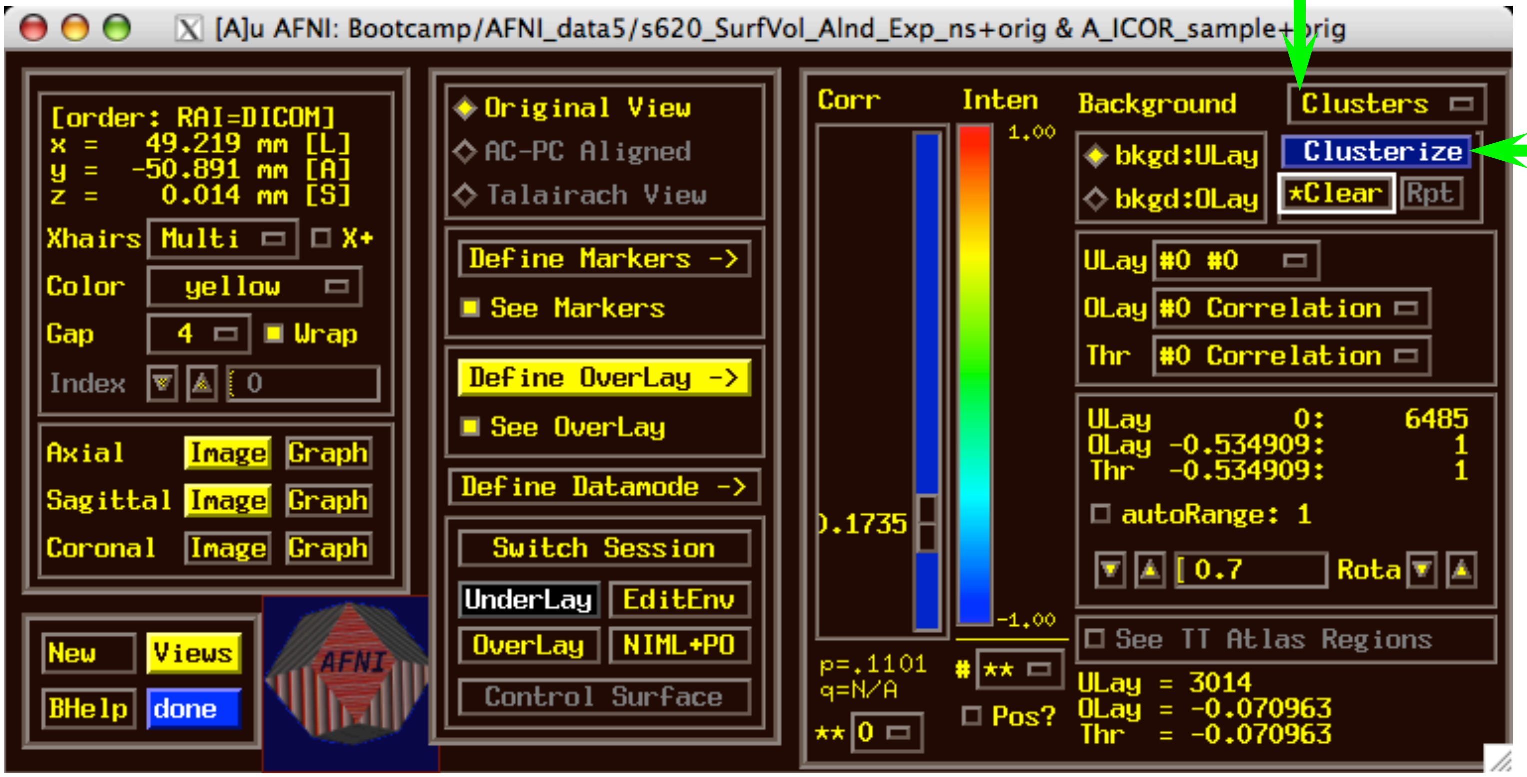
All data herein from Alex Martin, *et al*. [NIMH IRP]

AFNI! "Insta" Functions

- 3 new capabilities added to the interactive AFNI
- Each one: compute new dataset volumes instantly to replace the Overlay volume for image viewing
- **Clusters** = interactive clustering
  - remove clusters below a user-chosen size
  - display a table of clusters
- **InstaCorr** = interactive exploration of inter-voxel time series correlation
  - choose a seed voxel and see correlation map
  - SUMA version also exists
- **InstaCalc** = interactive version of **`3dcalc`**
  - e.g., display ratio of 2 datasets

AFNI! Clusters: Setup

- Open **Define Overlay**, choose **Clusters** from menu in top right corner



[A]u AFNI: Bootcamp/AFNI_data5/s620_SurfVol_Alnd_Exp_ns+orig & A_ICOR_sample+orig

```
[order: RAI=DICOM]
x =    49.219 mm [L]
y =   -50.891 mm [A]
z =     0.014 mm [S]
```

```
ULay            0:     6485
OLay  -0.534909:          1
Thr   -0.534909:          1
```

```
ULay  = 3014
OLay  = -0.070963
Thr   = -0.070963
```

- Then press **Clusterize** to get the clusters control menu

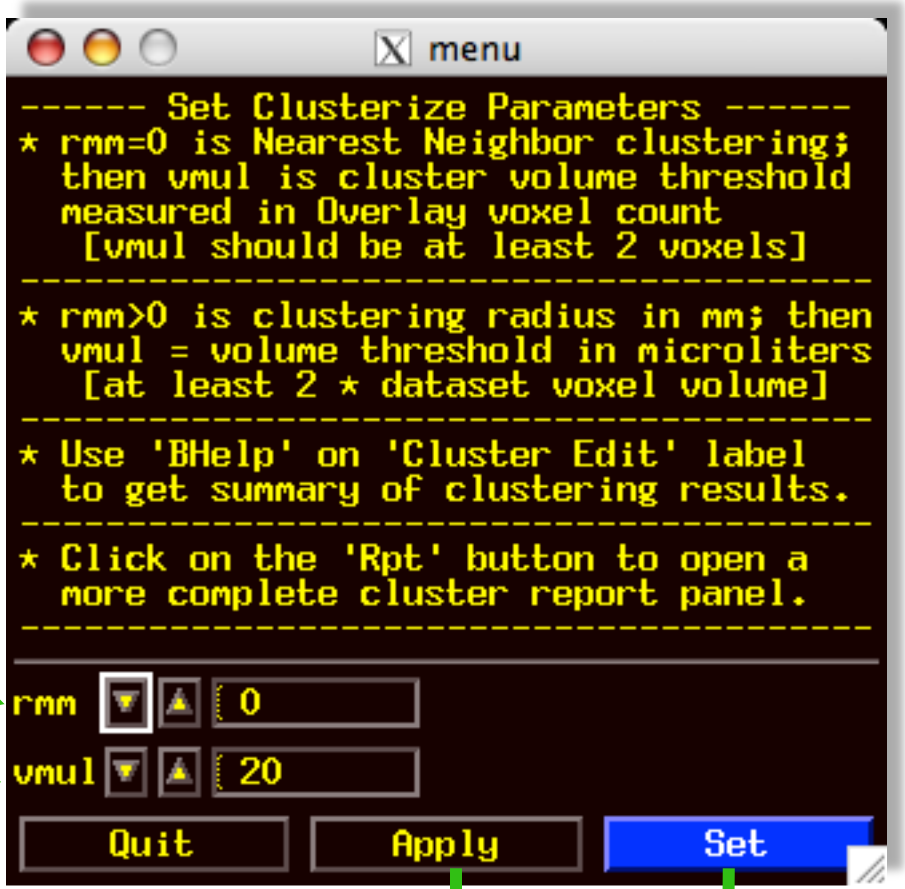
Clusters Control Menu

Operates on user’s chosen Overlay dataset at the user’s threshold;
Next slide example:
AFNI_ICOR_sample

Default: NN clustering

Default: 20 voxel minimum cluster size

Clustering is done in 3D



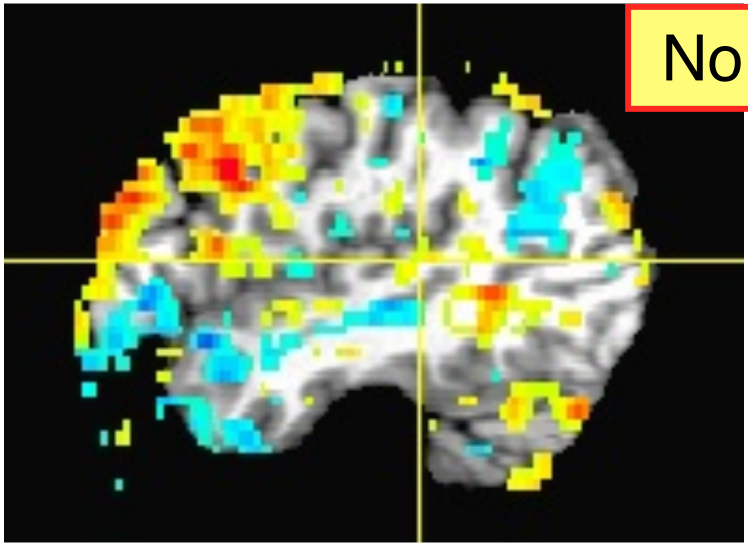
```
------ Set Clusterize Parameters ------
* rmm=0 is Nearest Neighbor clustering;
  then vmul is cluster volume threshold
  measured in Overlay voxel count
   [vmul should be at least 2 voxels]
-----------------------------------------
* rmm>0 is clustering radius in mm; then
  vmul = volume threshold in microliters
   [at least 2 * dataset voxel volume]
-----------------------------------------
* Use 'BHelp' on 'Cluster Edit' label
  to get summary of clustering results.
-----------------------------------------
* Click on the 'Rpt' button to open a
  more complete cluster report panel.
-----------------------------------------
```

rmm 0

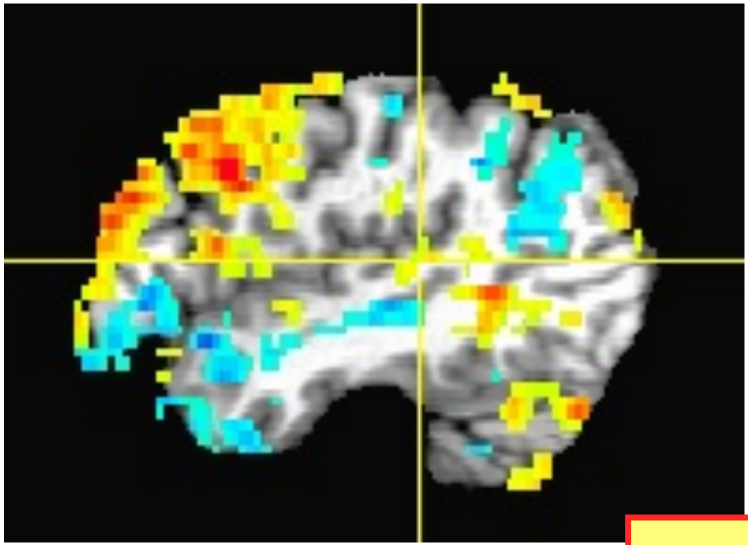
vmul 20

Press one of these buttons to create clusterized volume for display as new Overlay

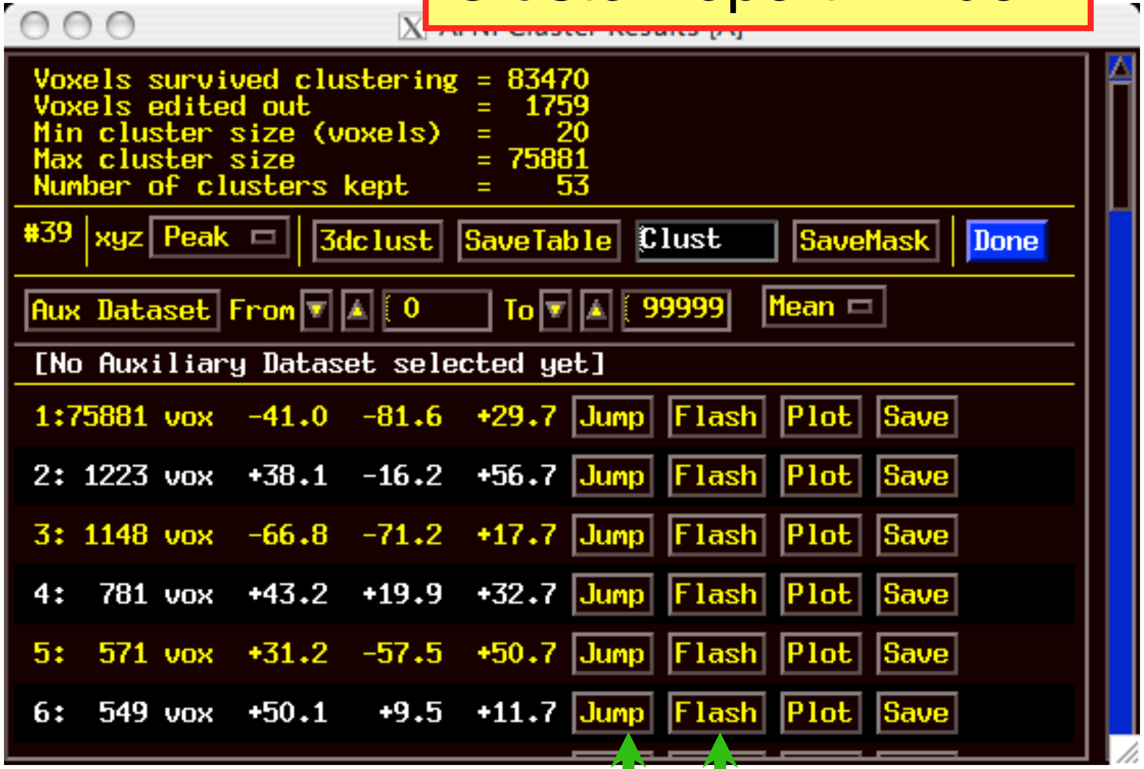
Clusters Results



No clustering



With clustering



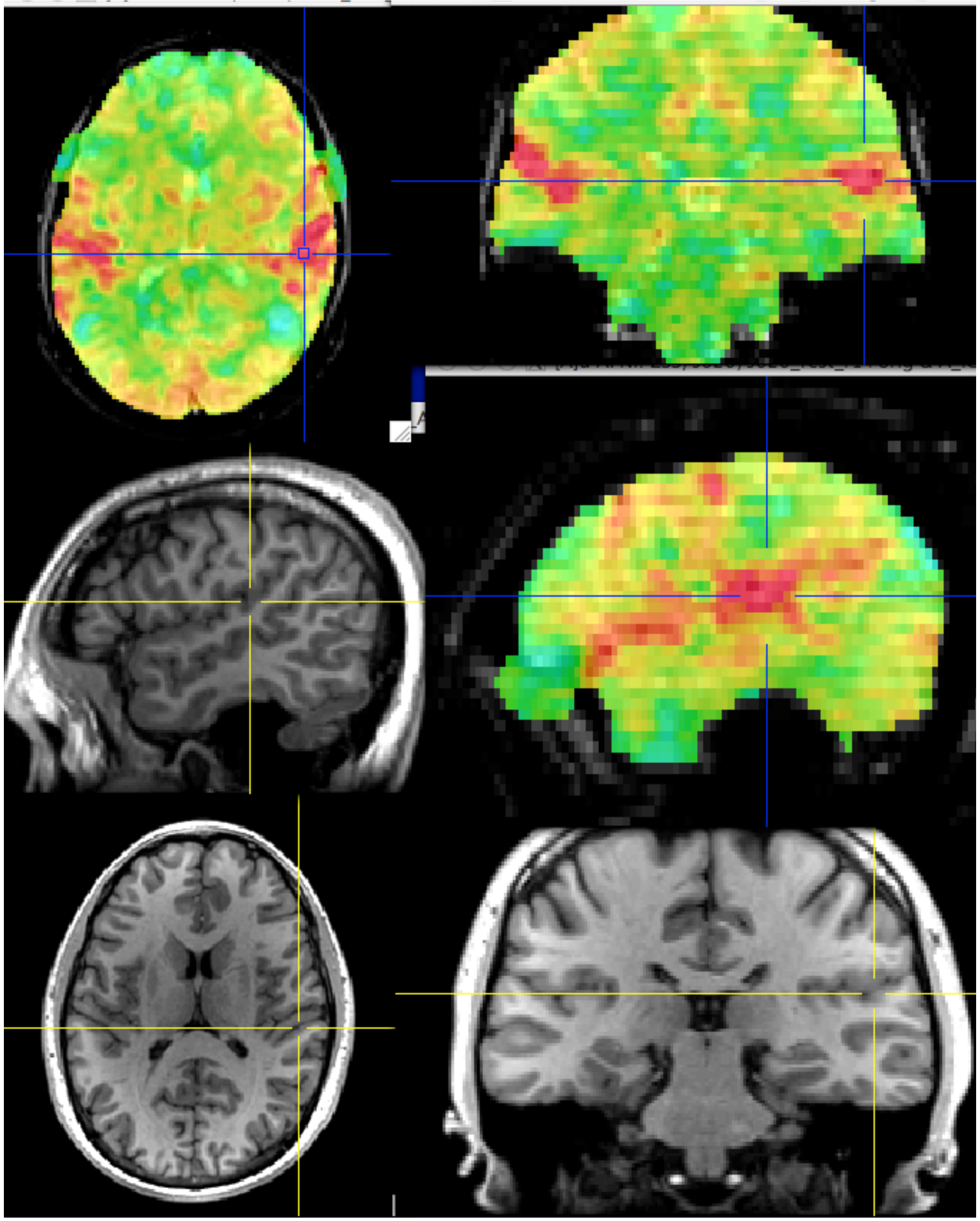
Cluster report window

```
Voxels survived clustering = 83470
Voxels edited out          =  1759
Min cluster size (voxels)  =    20
Max cluster size           = 75881
Number of clusters kept    =    53
```

```
[No Auxiliary Dataset selected yet]
1:75881 vox  -41.0  -81.6  +29.7
2: 1223 vox  +38.1  -16.2  +56.7
3: 1148 vox  -66.8  -71.2  +17.7
4:  781 vox  +43.2  +19.9  +32.7
5:  571 vox  +31.2  -57.5  +50.7
6:  549 vox  +50.1   +9.5  +11.7
```

`Jump`: crosshairs move
`Flash`: colors on & off

AFNI! InstaCorr



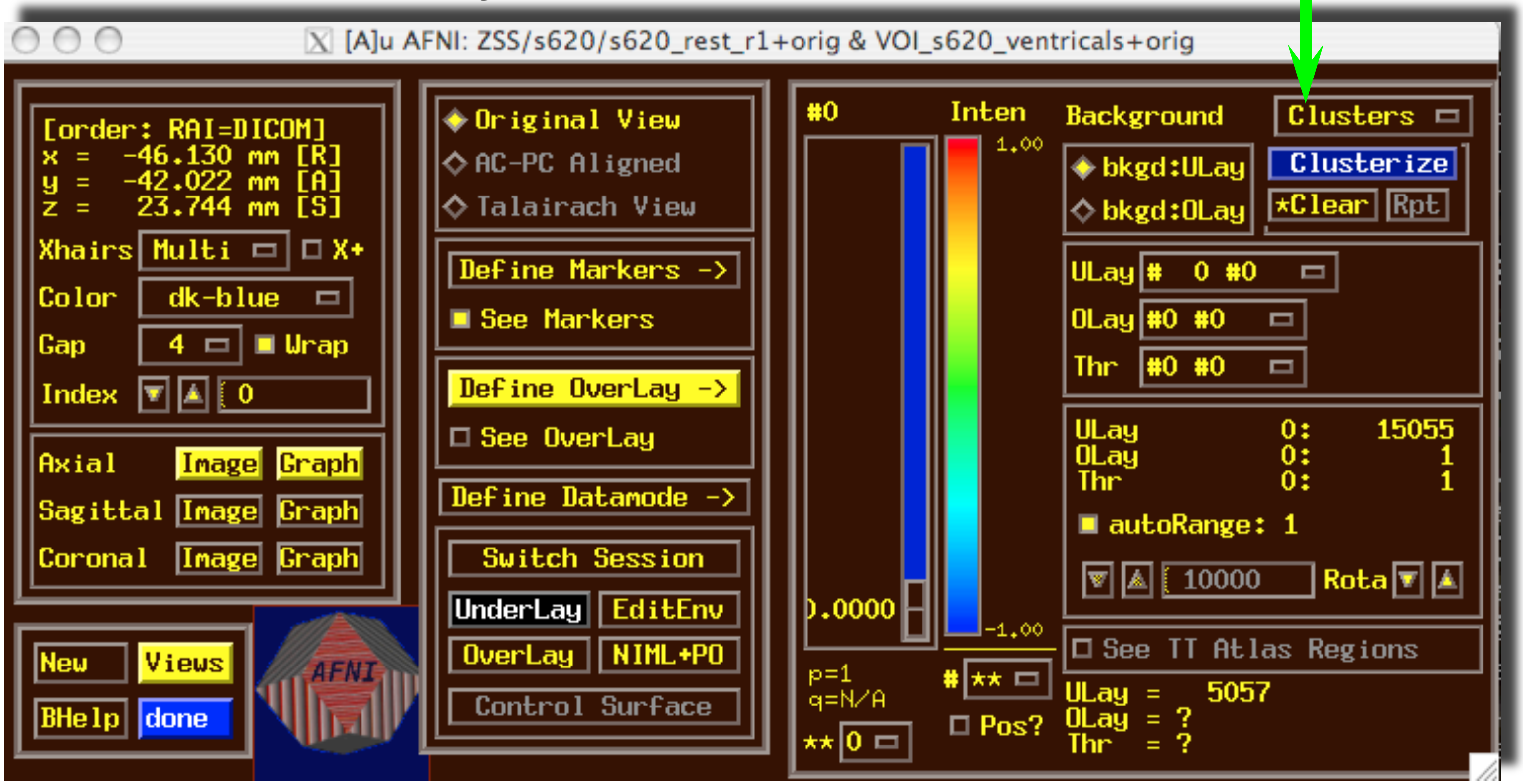
- On-the-fly **insta**ntaneous **corr**elation map of resting state data with interactively selected seed voxel
- **Setup phase**: prepares data for correlations (several-to-10+ seconds)
- **Correlation phase**: you select seed voxel, correlation map appears by *magic*

InstaCorr: Outline of 2 Phases

- **Setup phase**:
  - Masking: user-selected *or* Automask
  - Bandpass and other filtering of voxel time series
  - Blurring inside mask = the slowest part
- **Correlation phase**:
  - Correlate selected seed voxel time series with all other prepared voxel time series
  - Make new dataset, if needed, to store results
  - Save seed time series for graphing
  - Redisplay color overlay
  - Optional: compute FDR curve for correlations
    - Calculation is slow, so FDR is not turned on by default

InstaCorr: Setup

- Open **Define Overlay**, choose **InstaCorr** from menu in top right corner



[A]u AFNI: ZSS/s620/s620_rest_r1+orig & VOI_s620_ventricals+orig

```
[order: RAI=DICOM]
x =  -46.130 mm [R]
y =  -42.022 mm [A]
z =   23.744 mm [S]
```

Xhairs Multi; Color dk-blue; Gap 4; Index 0

ULay # 0 #0; OLay #0 #0; Thr #0 #0

```
ULay     0:   15055
OLay     0:       1
Thr      0:       1
```

■ autoRange: 1

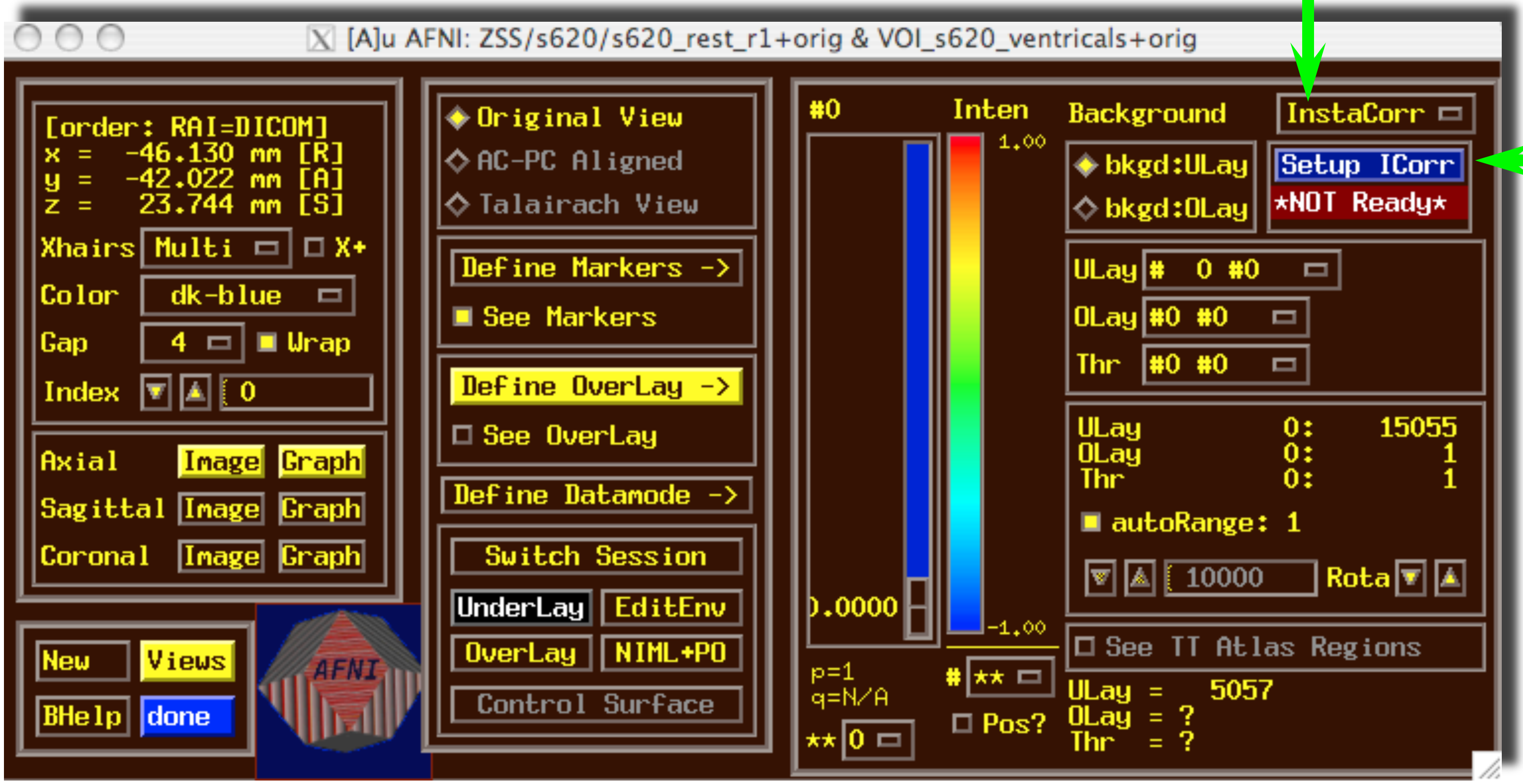
10000 Rota

p=1
q=N/A

ULay = 5057
OLay = ?
Thr = ?

InstaCorr: Setup

- Open **Define Overlay**, choose **InstaCorr** from menu in top right corner



[A]u AFNI: ZSS/s620/s620_rest_r1+orig & VOI_s620_ventricals+orig

```
[order: RAI=DICOM]
x =   -46.130 mm [R]
y =   -42.022 mm [A]
z =    23.744 mm [S]
```

Xhairs Multi, Color dk-blue, Gap 4, ■ Wrap, Index 0

ULay # 0 #0
OLay #0 #0
Thr #0 #0

```
ULay        0:    15055
OLay        0:        1
Thr         0:        1
```

■ autoRange: 1

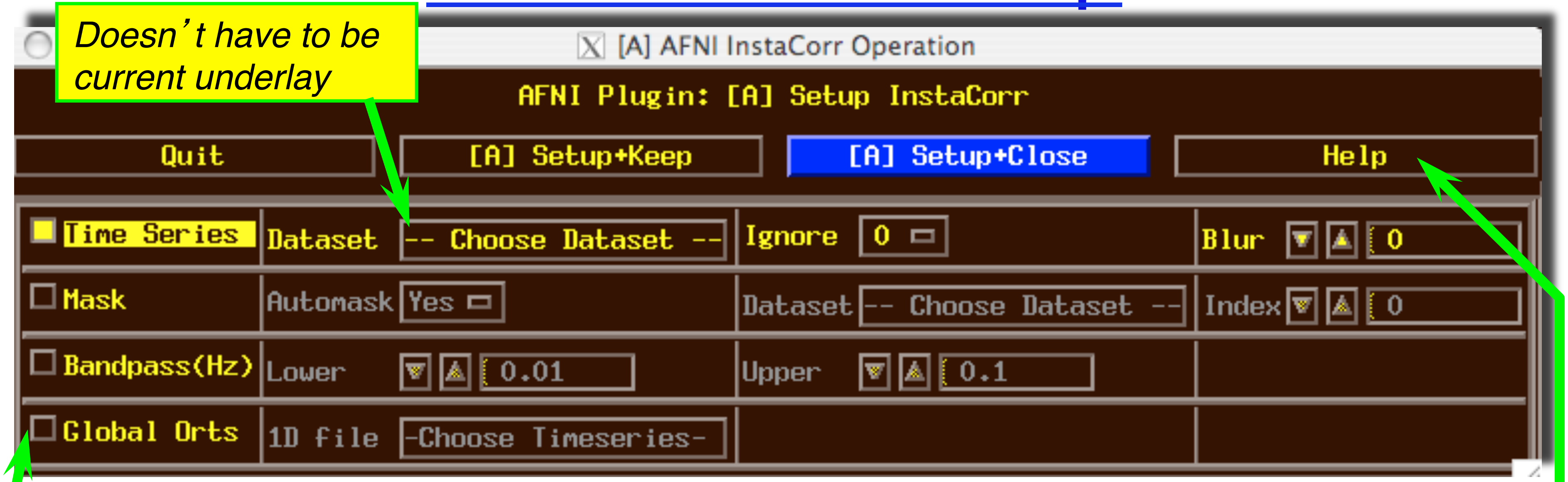
10000

ULay = 5057
OLay = ?
Thr = ?

p=1
q=N/A

- Then press **Setup ICorr** button to get control panel

InstaCorr: Setup


[A] AFNI InstaCorr Operation
AFNI Plugin: [A] Setup InstaCorr
Quit
[A] Setup+Keep
[A] Setup+Close
Help

Doesn't have to be current underlay

■ Time Series | Dataset -- Choose Dataset -- | Ignore 0 | Blur 0

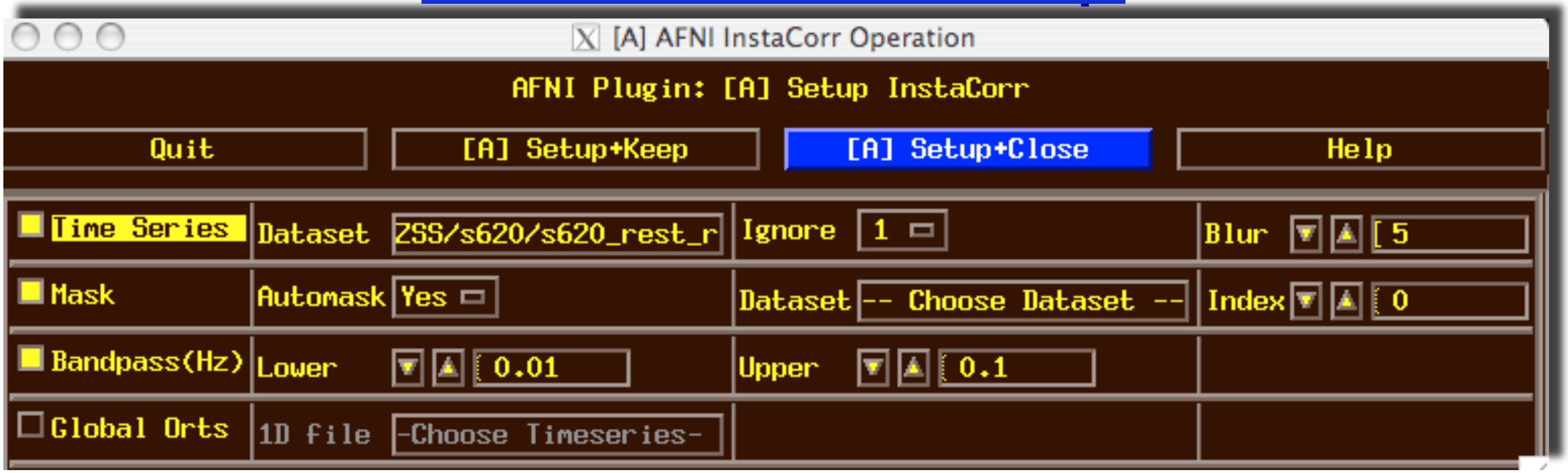
□ Mask | Automask Yes | Dataset -- Choose Dataset -- | Index 0

□ Bandpass(Hz) | Lower 0.01 | Upper 0.1

□ Global Orts | 1D file -Choose Timeseries-

- Mostly self-explanatory (I hope) — *cf.* **Help**
- **Global Orts** = extra time series to be projected out of dataset before correlation
  - All columns in selected 1D file
  - *e.g.*, movement parameters
  - The first **Ignore** rows (time points) will be skipped
- When ready, press one of the **Setup** buttons

InstaCorr: Setup


[A] AFNI InstaCorr Operation
AFNI Plugin: [A] Setup InstaCorr
Quit
[A] Setup+Keep
[A] Setup+Close
Help

■ Time Series — Dataset ZSS/s620/s620_rest_r — Ignore 1 — Blur 5
■ Mask — Automask Yes — Dataset -- Choose Dataset -- — Index 0
■ Bandpass(Hz) — Lower 0.01 — Upper 0.1
□ Global Orts — 1D file -Choose Timeseries-

- Text output to shell window details the setup procedures:

```
++ InstaCorr preparations:
 + Automask from '/Users/rwcox/data/Resting/ZSS/s620/
 s620_rest_r1+orig.BRIK' has 197234 voxels
 + Extracting dataset time series
 + Filtering 197234 dataset time series
 + bandpass: ntime=139 nFFT=160 dt=3.5 dFreq=0.00178571
 Nyquist=0.142857 passband indexes=6..56
 + Spatially blurring 139 dataset volumes
 + Normalizing dataset time series
++ InstaCorr setup: 197234 voxels ready for work: 15.43 sec
```

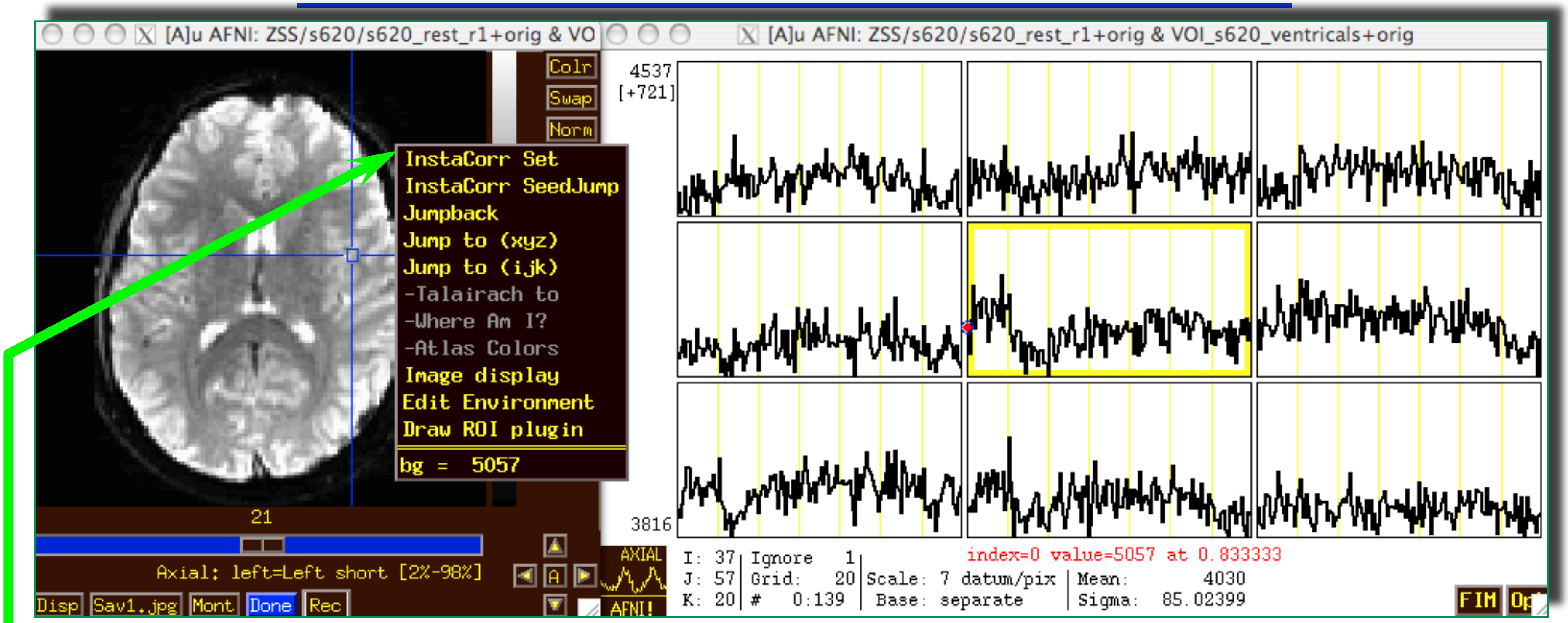
Dataset being analyzed

Most of the CPU time:
Uses `BlurInMask`

Preprocess via afni_proc.py

```
## Adapted from Example 9b in afni_proc.py -help
## Eliminated tlrc block; add blur_size of 5mm
afni_proc.py -subj_id s620                            \
 -dsets s620_rest_r1+orig.HEAD                        \
 -copy_anat s620_t1_al2epi+orig.HEAD                  \
 -blocks despike tshift align volreg blur mask regress \
 -tcat_remove_first_trs 2                             \
 -volreg_align_e2a                                    \
 -blur_size 5                                         \
 -regress_anaticor_fast                               \
 -regress_censor_motion 0.2                           \
 -regress_censor_outliers 0.1                         \
 -regress_bandpass 0.01 0.2                           \
 -regress_apply_mot_types demean deriv                \
 -regress_run_clustsim no  -regress_est_blur_errts
```

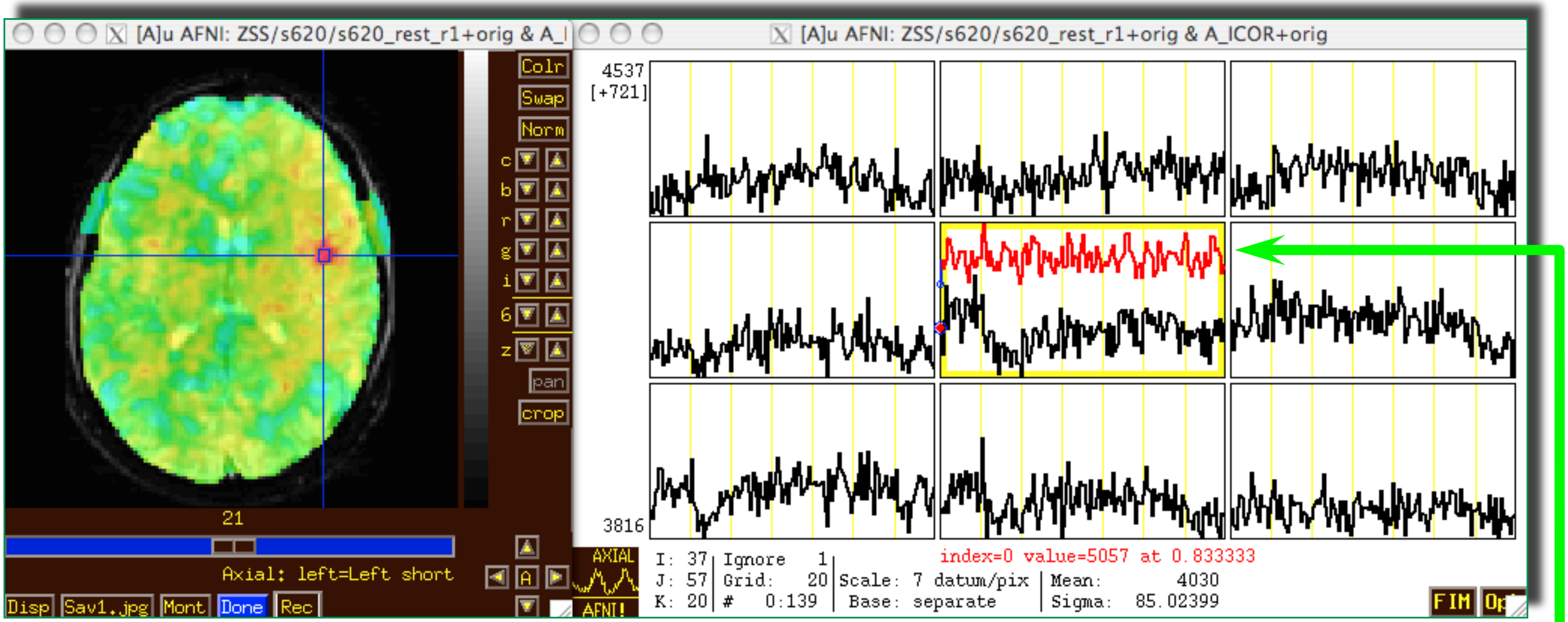
InstaCorr: The Fun Part



```
I: 37 Ignore   1            index=0 value=5057 at 0.833333
J: 57 Grid:   20 Scale: 7 datum/pix   Mean:       4030
K: 20 #    0:139  Base: separate      Sigma:  85.02399
```

- In image viewer, set crosshairs to desired seed voxel
- **Right-click** popup menu **InstaCorr Set**
  - Creates new dataset **A_ICOR** for Overlay
  - ***Shortcut***: **Shift+Ctrl+Left-click** sets new crosshair location, then does **InstaCorr Set**
    - Can also hold down **Shift+Ctrl+Left-click** and drag seed around
- **InstaCorr SeedJump** jumps focus to current seed

InstaCorr: The Fun Part



[A]u AFNI: ZSS/s620/s620_rest_r1+orig & A_I

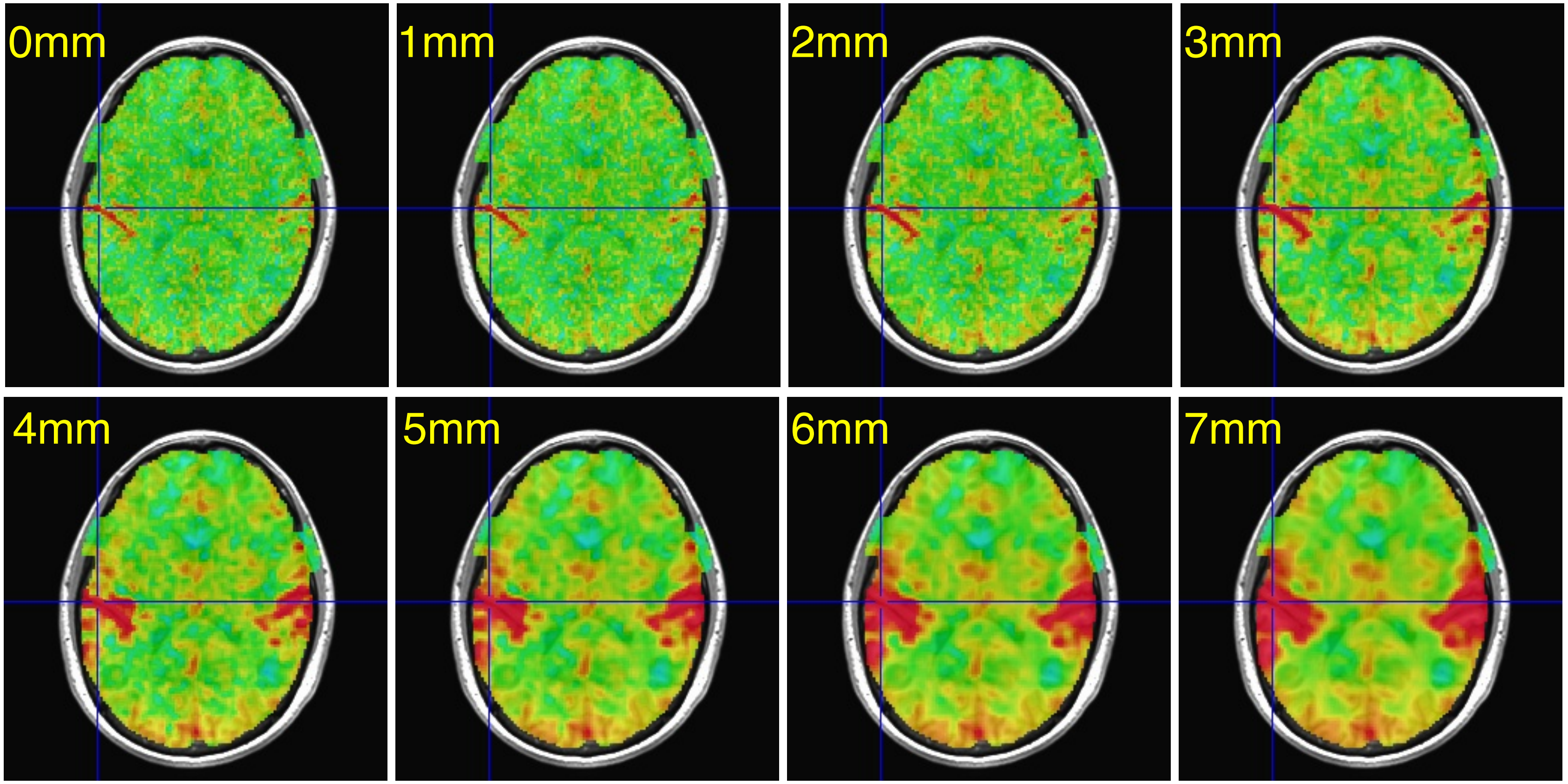
[A]u AFNI: ZSS/s620/s620_rest_r1+orig & A_ICOR+orig

Axial: left=Left short

```
                                       index=0 value=5057 at 0.833333
I: 37 | Ignore    1 |
J: 57 | Grid:    20 | Scale: 7 datum/pix | Mean:       4030
K: 20 | #     0:139 |  Base: separate    | Sigma:  85.02399
```

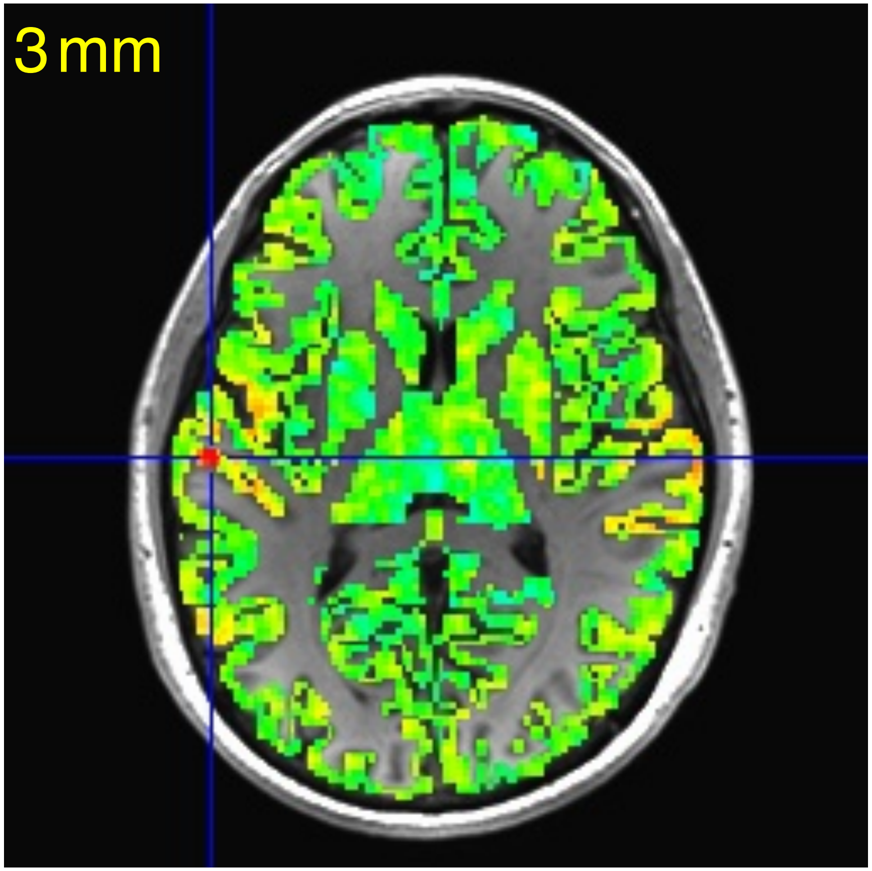
- In graph window:
  - Set `Ignore` with **FIM⊠Ignore** menu (or **I** key)
  - Set seed overlay with **FIM⊠Pick Ideal** menu
- When you change seed voxel, saved overlay time series will change (but you have to refresh graph to see it)

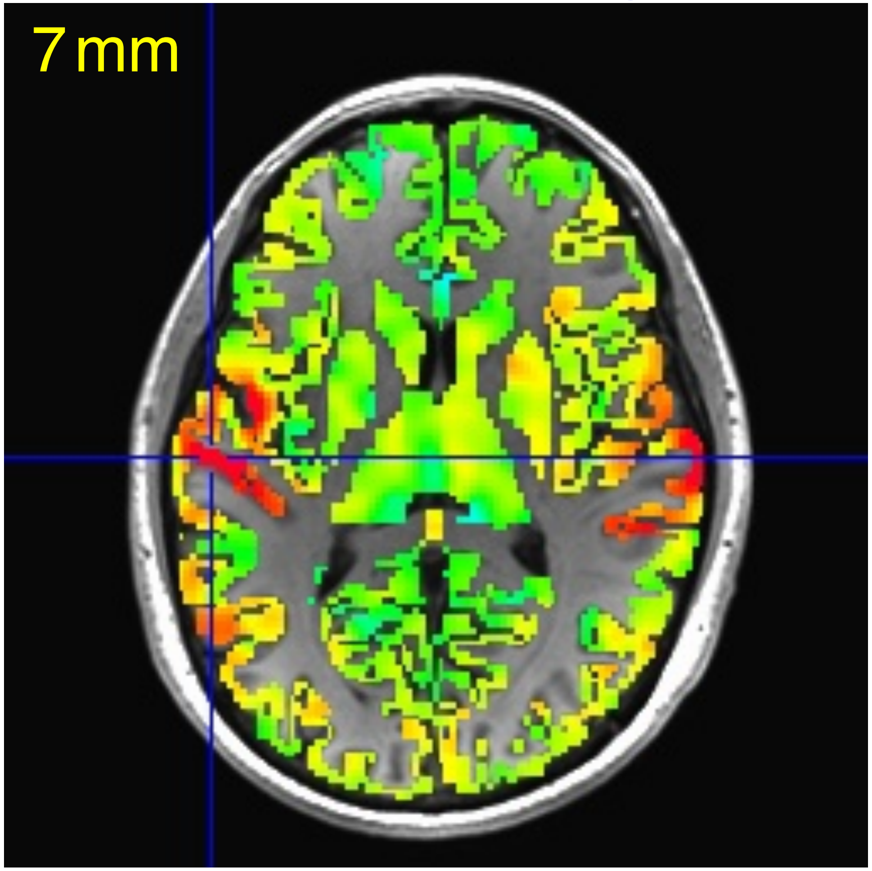
InstaCorr: Effects of Blurring



- Is this a pure vascular/cardiac effect being progressively smeared? Or real neural correlations seen via BOLD? Or some of both? *Venograms*?
  - Dataset was RETROICOR-ized; mask is whole brain

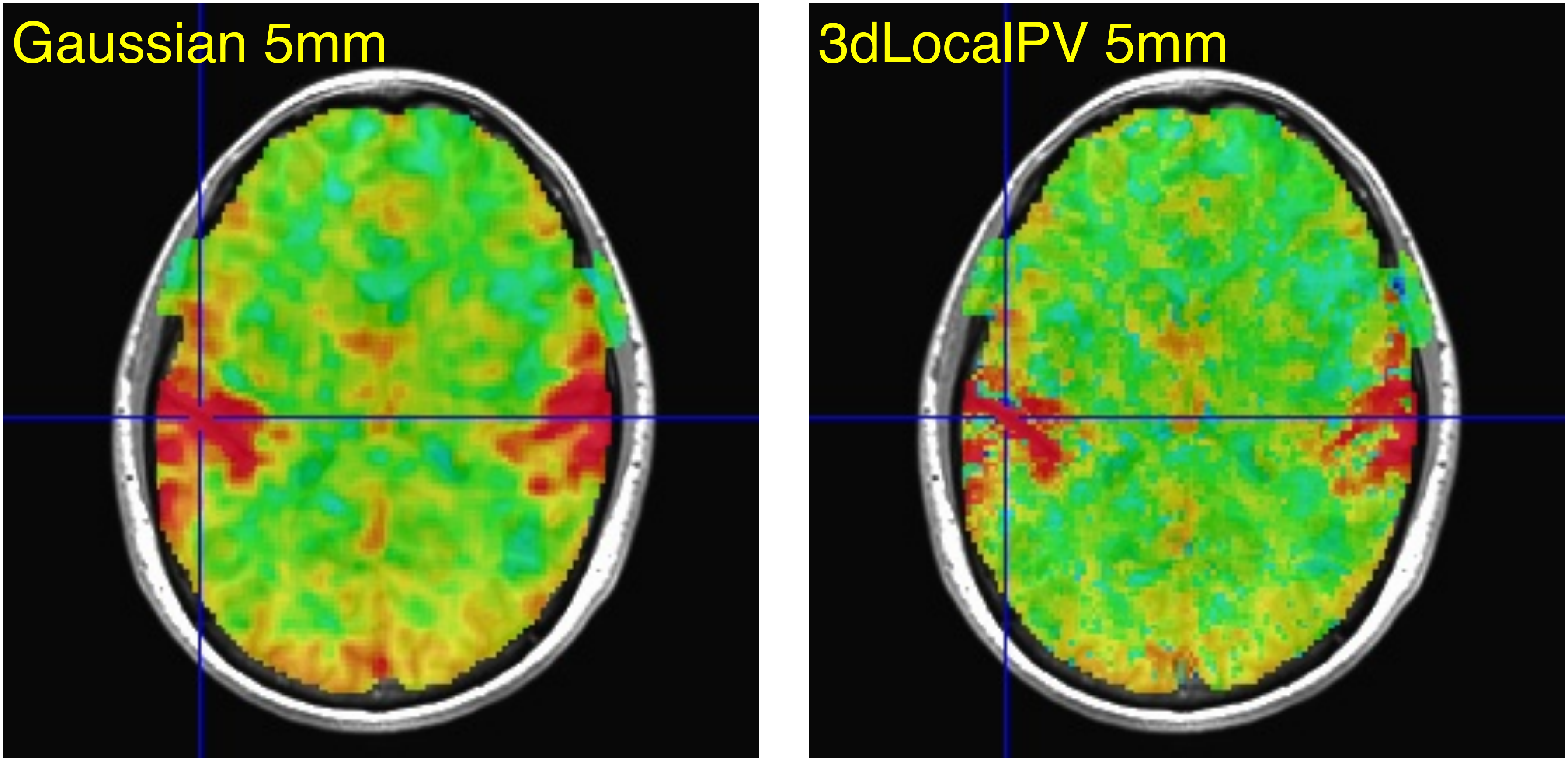
InstaCorr: Effects of Blurring





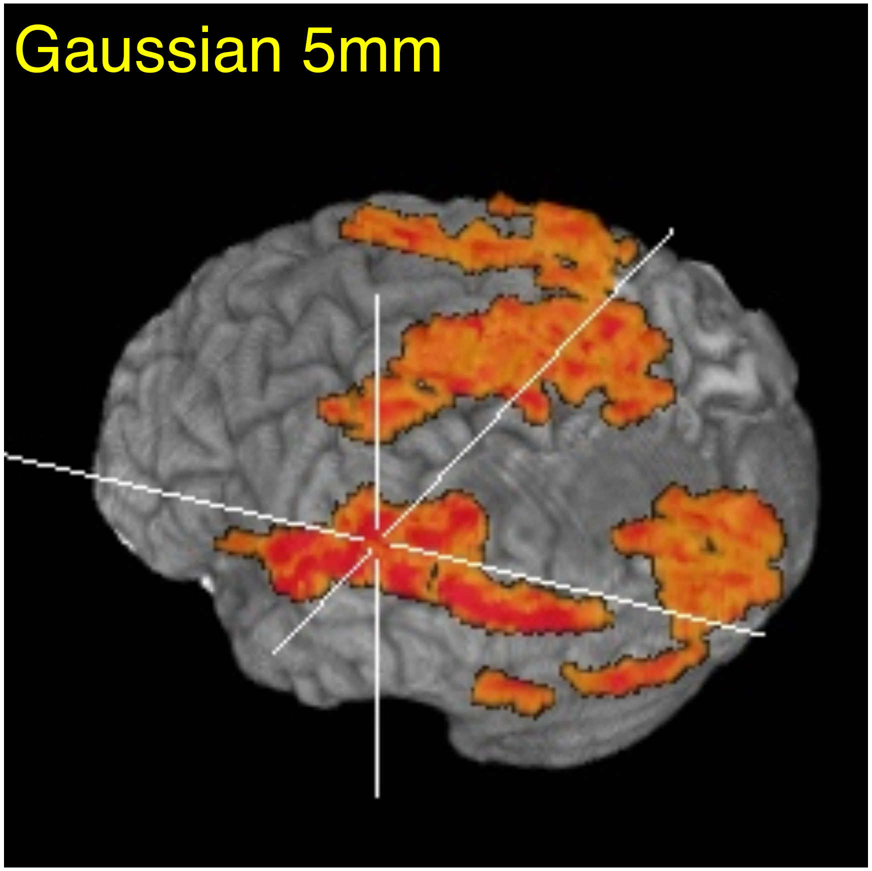
- Similar calculations, but with FreeSurfer-generated gray matter mask instead of Automask from EPI data
  - Blurring is done only inside the mask (`3dBlurInMask`)
    - Using a discrete PDE-based iterative approach

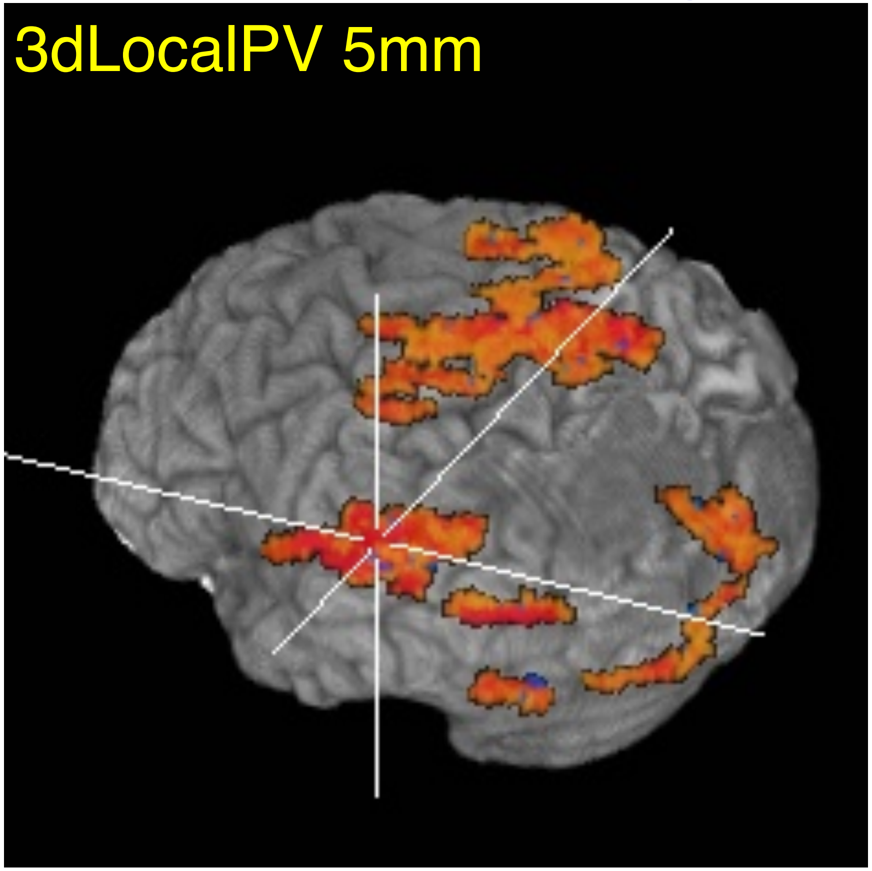
InstaCorr: SVD-based “Blurring”



- Similar calculations, with Automask from EPI data, using `3dLocalPV` over 5 mm radius sphere (67 voxels)
  - Project each vector onto 2-dim principal subspace
  - Far too slow to calculate interactively (at this time)

InstaCorr: SVD-based "Blurring"





- Volume rendering of InstaCorr maps (threshold at r=0.5)
  - Renderer updates automatically if **`DynaDraw`** is on
- SVD smoothing has cleaner spatial structure?
  - Or has it lost some information? *I don't know.*

InstaCorr: Options and Plans

- Underlay doesn’t have to be EPI data; could be anat
  - Can use InstaCorr in multiple AFNI controllers
- FDR: `setenv AFNI_INSTACORR_FDR YES`
  - Will slow things down by a significant factor
- Saving **`A_ICOR`** dataset: overwrites previous copies
- Future Possibilities:
  - Select ROI-based Orts to be detrended?
    - Based on ROIs from FreeSurfer or atlases?
  - Or multiple seeds (partial + multiple correlations)?
  - Interactive local SVD “smoothing”? (needs speedup)
  - → Group analysis InstaCorr (in standardized space)
    - Not quite “Insta” any more; □0.1□#Subjects sec per seed
    - External script to do subject setups
  - Use time series subsets? (*e.g.*, for block design data)

Group InstaCorr

- If you have a robust enough system (multiple CPUs, several gigabytes of RAM), you can explore the *group* analysis of resting state seed-based correlations
- **Setup Phase**:
  - Unlike individual InstaCorr, the setup is done outside the AFNI GUI with command line programs
  - Step 1: pre-process all datasets (including into template space) by using **`afni_proc.py`**
    - We recommend something like Example 9b in the **`afni_proc.py`** help output
  - Step 2: collect groups of time series datasets into one big file = **`3dSetupGroupInCorr`**
- **Interactive Phase**: point-and-click to set seed voxel

3dGroupInCorr: Setup #2

- **3dSetupGroupInCorr** reads all filtered & blurred resting state EPI datasets, masks & normalizes them, and writes them to one *big* file for **3dGroupInCorr**
  - Sample tcsh script fragment below: 2 groups of subjects

```
set AAA = ( s601 s604 … s644 s646 )
set BBB = ( s611 s612 … s652 s654 )
set ggg = ( )
foreach fred ( $AAA )
  set ggg = ( $ggg errts.${fred}.fanaticor+tlrc.HEAD )
end
3dSetupGroupInCorr -mask ALL_am50+tlrc -prefix AAA $ggg
set ggg = ( )
foreach fred ( $BBB )
  set ggg = ( $ggg errts.${fred}.fanaticor+tlrc.HEAD )
end
3dSetupGroupInCorr -mask ALL_am50+tlrc -prefix BBB $ggg
```

3dGroupInCorr: Interactive Phase

- Start server program (2-sample *t*-test here):

```
3dGroupInCorr -setA AAA.grpincorr.niml \
              -setB BBB.grpincorr.niml
```

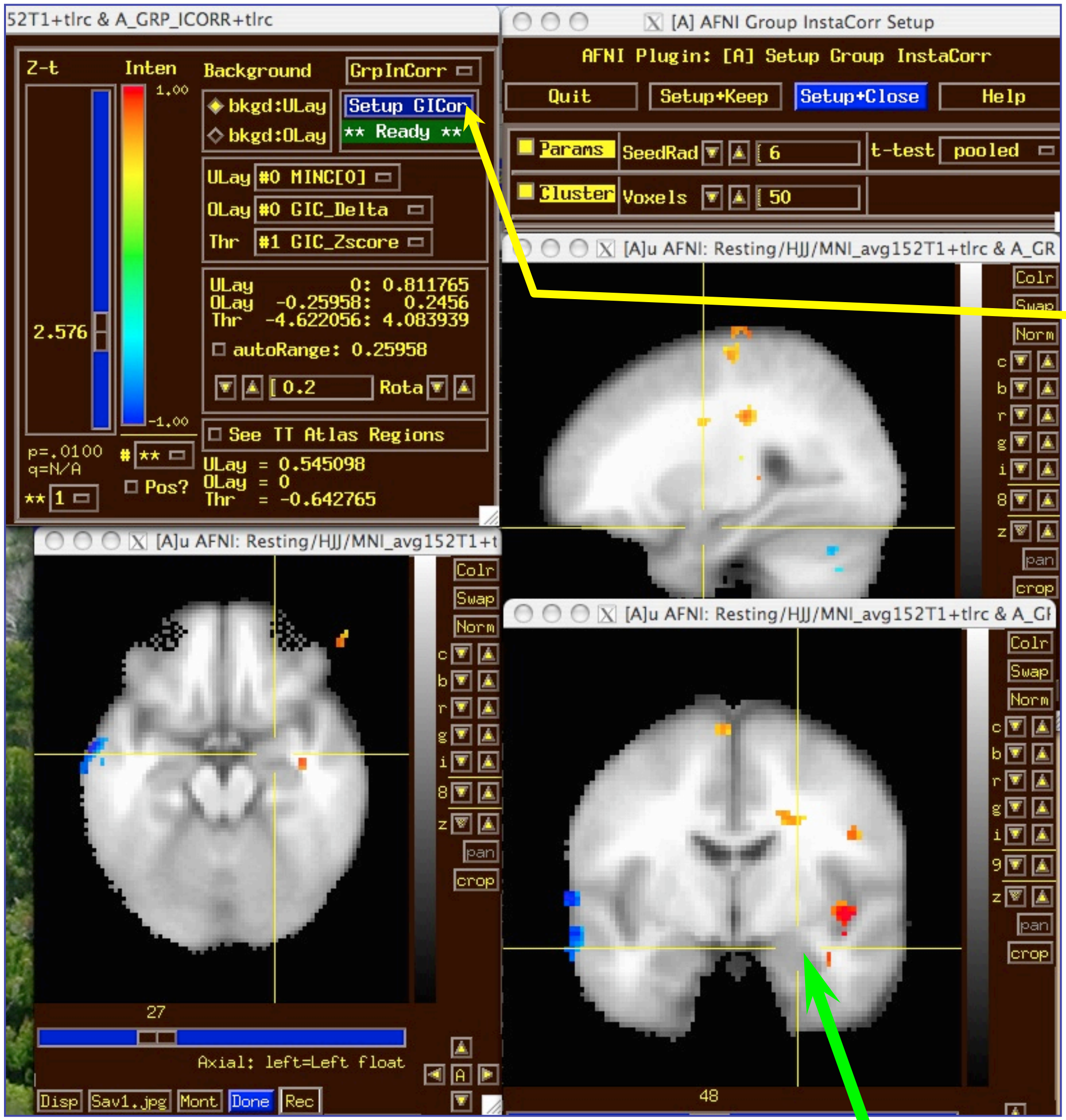
  - o Startup takes a little while, as all data must be read into RAM (in this example, 3.2 Gbytes)
  - o After data is read, connects to AFNI using a NIML socket
  - o Server will use multiple CPUs if compiled with OpenMP (currently on Mac OS X 10.5 and 10.6)

- In a separate terminal window, start AFNI:

```
afni -niml ~/abin/MNI_avg152T1+tlrc.HEAD
```

  - o Then open the **`Define Overlay`** control panel
  - o Select **`GrpInCorr`** from the **`Clusters`** menu

3dGrpInCorr: Interactive Results



[A] AFNI Group InstaCorr Setup

AFNI Plugin: [A] Setup Group InstaCorr

SeedRad 6
t-test pooled
Voxels 50

◆ bkgd:ULay
◇ bkgd:OLay

ULay #0 MINC[0]
OLay #0 GIC_Delta
Thr #1 GIC_Zscore

```
ULay        0: 0.811765
OLay -0.25958:   0.2456
Thr -4.622056: 4.083939
□ autoRange: 0.25958
[0.2   Rota
```

2.576 p=.0100 q=N/A # ** □ Pos? ** 1

```
ULay = 0.545098
OLay = 0
Thr  = -0.642765
```

[A]u AFNI: Resting/HJJ/MNI_avg152T1+tlrc & A_GR

Seed voxel

- Use same buttons as individual subject InstaCorr to set seed
- Use **Setup GICor** panel to set the few options available interactively
  - **SeedRad** = extra smoothing radius for seed voxel time series (flat average)
  - **Cluster** = min number of voxels to keep above thresh

3dGrpInCorr: What It Computes

- Step 1: Extract seed time series from each dataset; correlate with all voxel time series in that dataset
- Step 2: Group analysis: *t*-test among correlation bricks
- 1-sample *t*-test (**`-setA`** only) gives 2 sub-bricks:
  - mean of $\tanh^{-1}$(correlation with seed)
  - *Z*-score of *t*-statistic of this mean
- 2-sample test (**`-setA`** *and* **`-setB`**) gives 6 sub-bricks:
  - difference of means (**A−B**) of $\tanh^{-1}$(correlation)
  - *Z*-score of *t*-statistic of this difference
    - Pooled or unpooled variance, or paired *t*-test (your option)
  - Plus 1-sample results for **`-setA`** and **`-setB`** separately
    - View these in AFNI **`[B]`** and **`[C]`** controllers, to see it all*!*

3dGrpInCorr: To Do It By Hand?

- After preprocessing all datasets, you would have to do the following steps on each resting state dataset:
  - Extract seed time series from each dataset [**`3dmaskave`**]
  - Correlate seed time series with all voxels from its dataset [**`3dDeconvolve`** or **`3dfim`**]
  - Convert to $\tanh^{-1}(\text{correlation})$ [**`3dcalc`**]
- Then do the following on the results from the above
  - Compute the *t*-test [**`3dttest`**]
  - Convert to *Z*-score [**`3dcalc`**]
  - Read into AFNI for display
- Even with a script, this would be annoying to do a lot
  - Just ask Daniel Handwerker*!*

Group InstaCorr: Final Notes

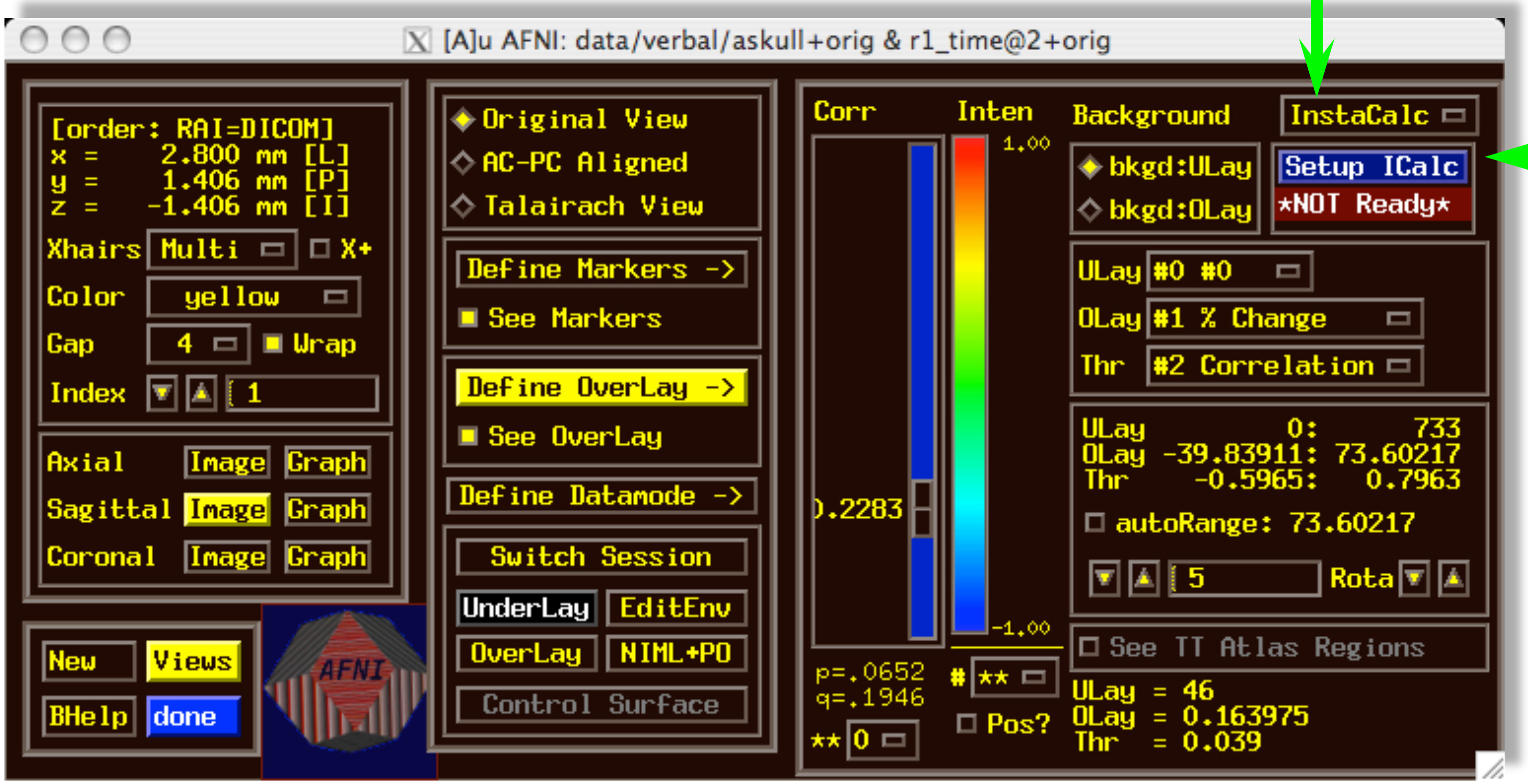
- Time series datasets can have different lengths
  - But all must have the same spatial grid and use the same mask!
- **Fun Stuff**: volume render results with **`DynaDraw`**
- Sometimes AFNI drops the shared memory connection to **`3dGroupInCorr`**
  - Due to unknown bugs somewhere in AFNI
  - Program tries to reconnect when this happens
  - If this gets bad, use the **`–NOshm`** option to **`3dGroupInCorr`** to force it to use TCP/IP only
    - Slower data transfer, but more reliable

Group InstaCorr: Finalest Notes

- Shift+Ctrl+Click+Drag method for dynamically setting the seed voxel also works with Group InstaCorr
  - But speed of interaction can be ***slow***
- Can also include subject-level covariates (e.g., IQ, age) in the analysis *at the group step*
  - To regress them out (nuisance variables), and/or to test the slope of $\tanh^{-1}(\text{correlation})$ vs. covariate
- Can also run in batch mode (no talking to AFNI)
- Further ideas (i.e., to dream the impossible dream):
  - Granger-ize: correlate with lag-0 ***and*** lag-1 of seed and test Granger causality
  - Allow user to set other seeds to be "partialed out" of the analysis

InstaCalc: Dataset Calculator

- Open **Define Overlay**, choose **InstaCalc** from menu in top right corner



[A]u AFNI: data/verbal/askull+orig & r1_time@2+orig

```
[order: RAI=DICOM]
x =    2.800 mm [L]
y =    1.406 mm [P]
z =   -1.406 mm [I]
```

ULay #0 #0
OLay #1 % Change
Thr #2 Correlation

```
ULay          0:       733
OLay -39.83911: 73.60217
Thr    -0.5965:   0.7963
```

□ autoRange: 73.60217

5

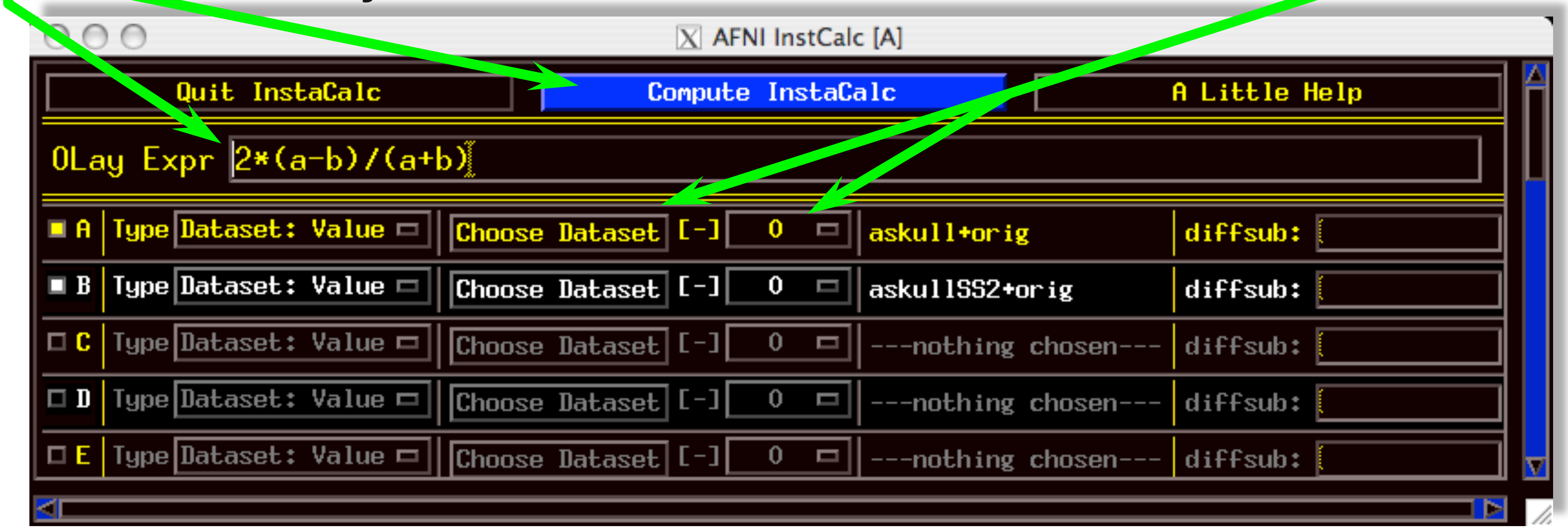
p=.0652
q=.1946

```
ULay = 46
OLay = 0.163975
Thr  = 0.039
```

- Then press **Setup ICalc** button to get control panel

InstaCalc: Setup

- Select datasets with **Choose Dataset** buttons
  - and sub-bricks with the **[-]** controls
- Enter symbolic expression
- Press **Compute InstaCalc**
- Creates new 1-brick dataset **A_ICALC** for Overlay
  - voxel-by-voxel calculations



OLay Expr 2*(a-b)/(a+b)

■ A	Type	Dataset: Value	Choose Dataset	[-]	0	askull+orig	diffsub:
■ B	Type	Dataset: Value	Choose Dataset	[-]	0	askullSS2+orig	diffsub:
□ C	Type	Dataset: Value	Choose Dataset	[-]	0	---nothing chosen---	diffsub:
□ D	Type	Dataset: Value	Choose Dataset	[-]	0	---nothing chosen---	diffsub:
□ E	Type	Dataset: Value	Choose Dataset	[-]	0	---nothing chosen---	diffsub:

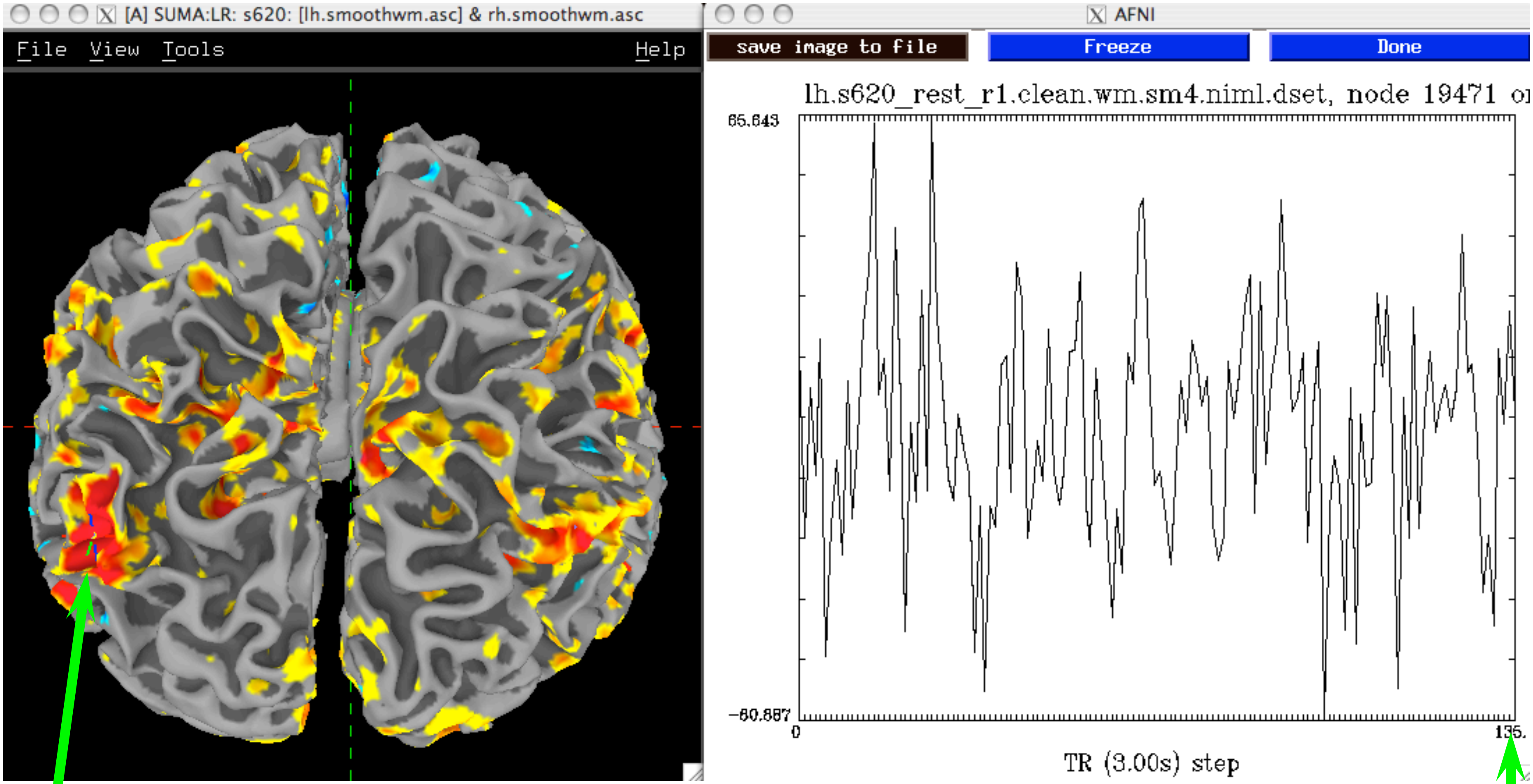
SUMA! InstaCorr

- Similar in concept to AFNI **InstaCorr** but requires external pre-processing of time series datasets
  - Removal of baseline, projection to surface, blurring
- In the **AFNI_data5/** directory, run the script

  tcsh ./@run_REST_demo

  - starts SUMA with 2 hemispheres
  - loads pre-processed datasets into SUMA
  - sets up SUMA's **InstaCorr**
- After all the setup is ready, right-clicking on the surface will do the **InstaCorr** calculations
- **`3dGroupInCorr`** also works with SUMA

InstaCorr: Sample


[A] SUMA:LR: s620: [lh.smoothwm.asc] & rh.smoothwm.asc
File
View
Tools
Help
AFNI
save image to file
Freeze
Done
lh.s620_rest_r1.clean.wm.sm4.niml.dset, node 19471 o
65.643
−60.887
0
135.
TR (3.00s) step

- Seed voxel and Seed voxel time series graph