

# 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]

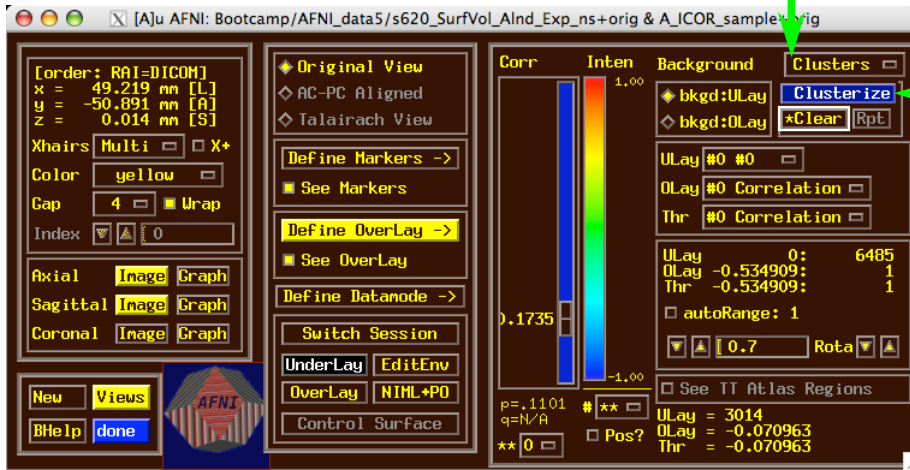


## **AFNI!** “**Insta**” Functions

- 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



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

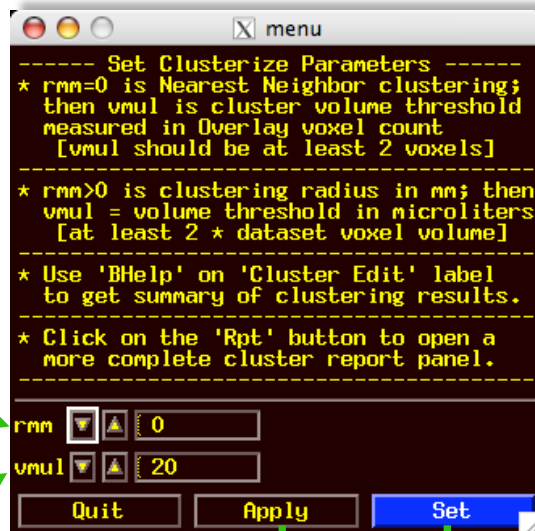
## Clusters Control Menu

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



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

# Clusters Results

No clustering

Cluster report window

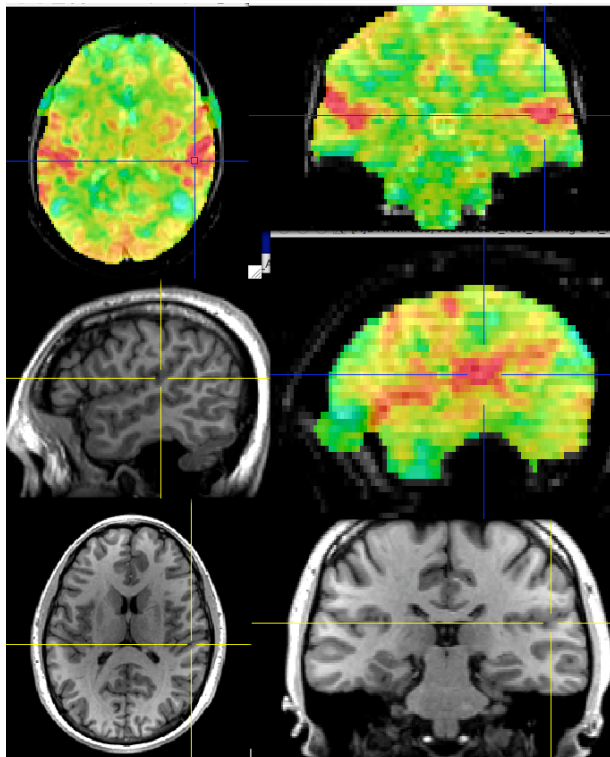
```

Voxels survived clustering = 83470
Voxels edited out = 1759
Min cluster size (voxels) = 20
Max cluster size = 75881
Number of clusters kept = 53
#39 xyz Peak 3dclust SaveTable Clust SaveMask Done
Aux Dataset From 0 To 99999 Mean
[No Auxiliary Dataset selected yet]
1: 75881 vox -41.0 -81.6 +29.7 Jump Flash Plot Save
2: 1223 vox +38.1 -16.2 +56.7 Jump Flash Plot Save
3: 1148 vox -66.8 -71.2 +17.7 Jump Flash Plot Save
4: 781 vox +43.2 +19.9 +32.7 Jump Flash Plot Save
5: 571 vox +31.2 -57.5 +50.7 Jump Flash Plot Save
6: 549 vox +50.1 +9.5 +11.7 Jump Flash Plot Save
  
```

Jump: crosshairs move  
Flash: colors on & off

With clustering

# AFNI! InstaCorr



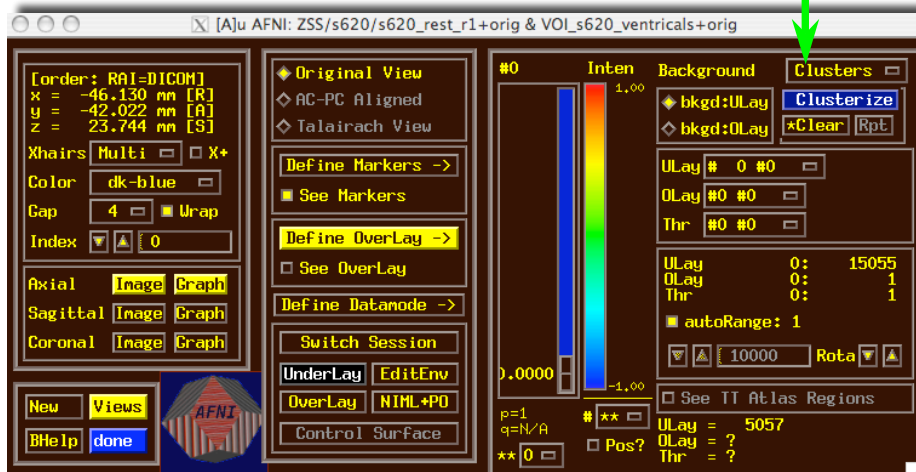
- On-the-fly **instantaneous correlation** map of resting state data with interactively selected seed voxel
- **Setup phase:** prepares data for correlations (several-to-10+ seconds)
- **Correlation phase:** you select seed voxel, correlation map appears by *magic*

## 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

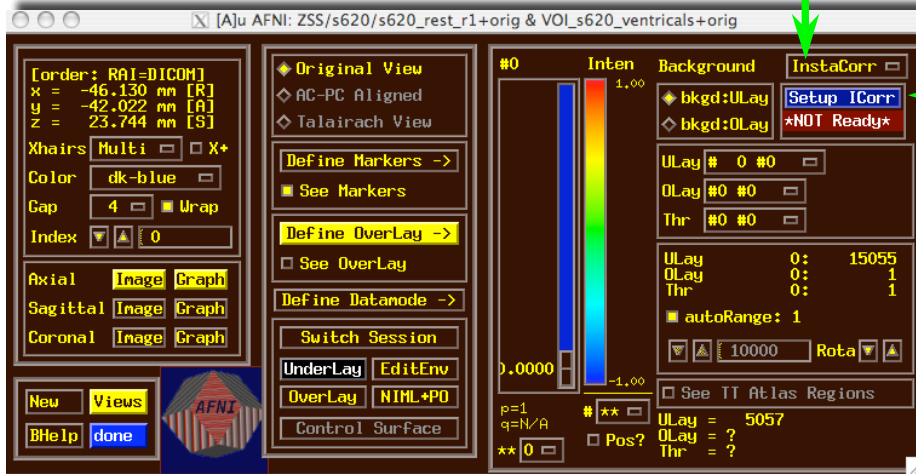
## InstaCorr: Setup

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



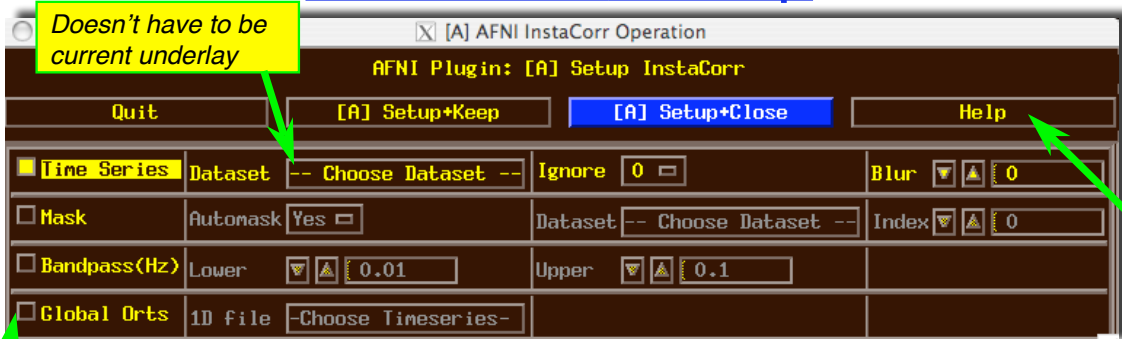
# InstaCorr: Setup

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



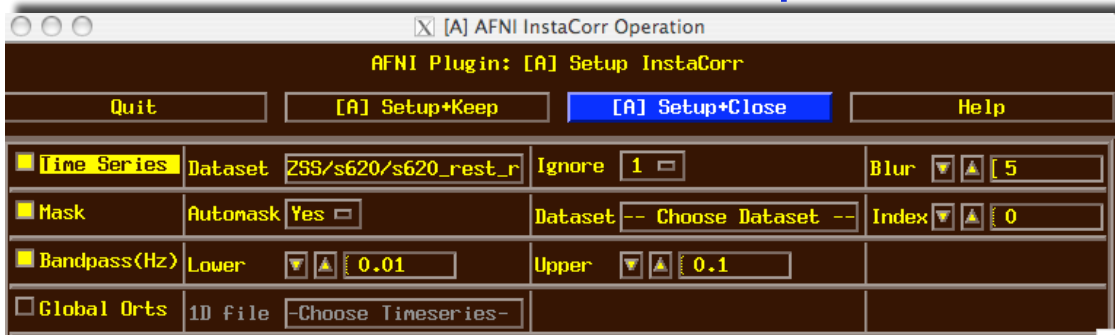
- Then press **Setup ICorr** button to get control panel

# InstaCorr: Setup



- 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



- Text output to shell window details the setup procedures:

```
++ InstaCorr preparations:
```

```
+ Automask from
```

```
'/Users/rwcox/data/Resting/ZSS/s620/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
```

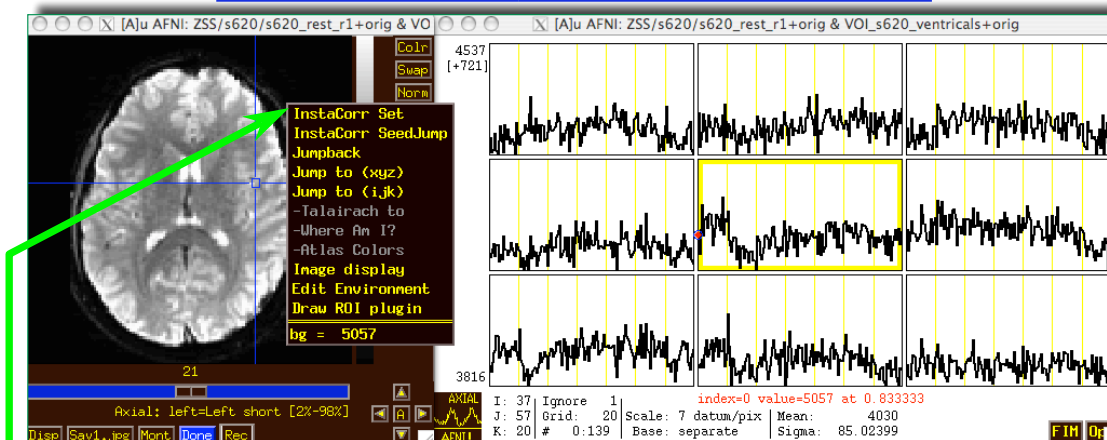
```
+ Normalizing dataset time series
```

```
++ InstaCorr setup: 197234 voxels ready for work: 15.43 sec
```

Dataset being analyzed

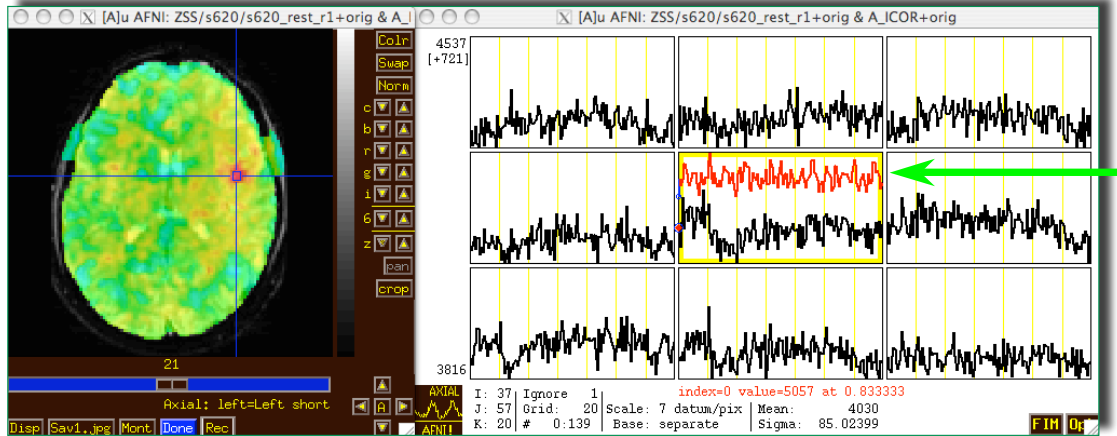
Most of the CPU time:  
Uses BlurInMask

## InstaCorr: The Fun Part



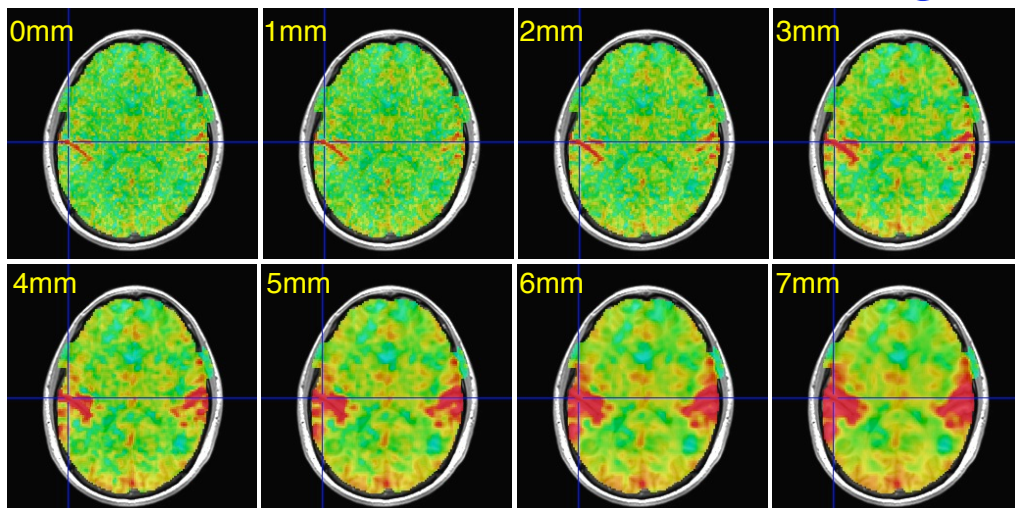
- In image viewer, set crosshairs to desired seed voxel
- **Right-click** popup menu → **InstaCorr Set**
  - Creates new dataset **A\_ICOR** for Overlay
  - **Shortcut: Shift+Ctrl+Left-click** sets new crosshair location, then does **InstaCorr Set**
    - Can also hold down **Shift+Ctrl+Left-click**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

# InstaCorr: The Fun Part



- 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)

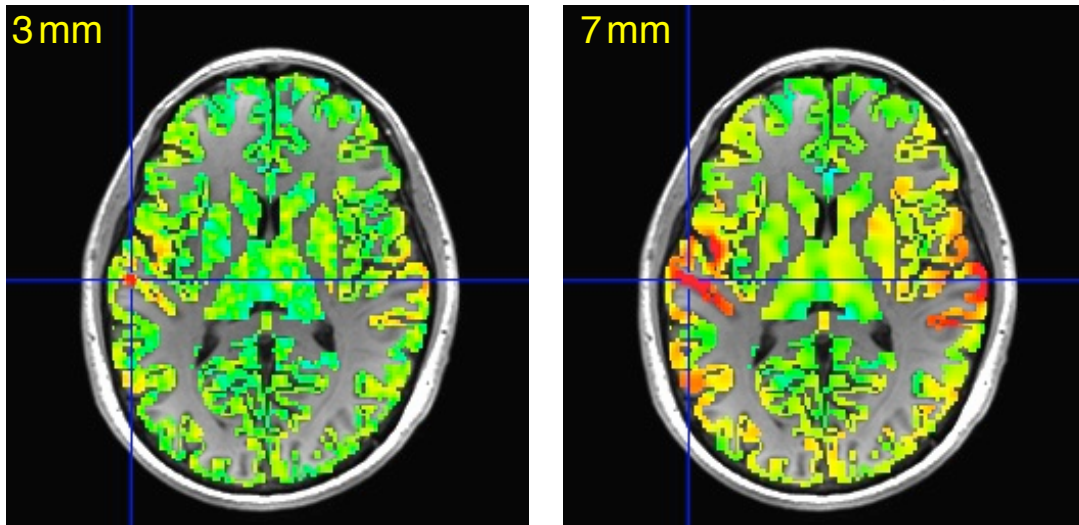
# InstaCorr: Effects of Blurring



- 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

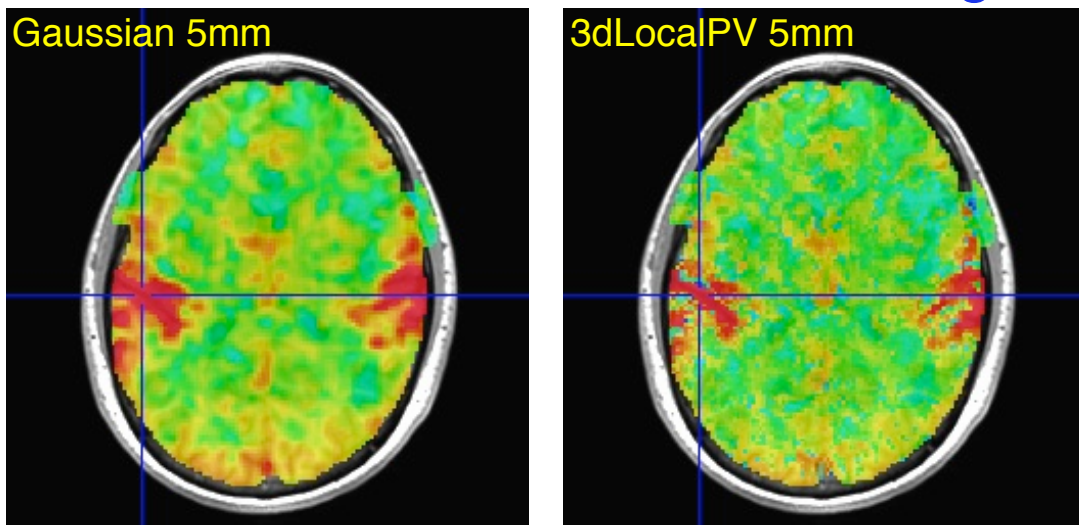


## InstaCorr: Effects of Blurring



- 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”

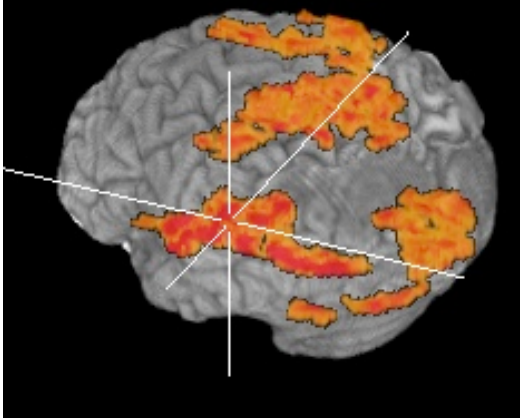


- Similar calculations, with Automask from EPI data, using **3dLocalPV** over 5 mm radius sphere (67 voxels)
  - Project each vector onto 2-dim principal subspace
  - Far too slow to calculate interactively (at this time)

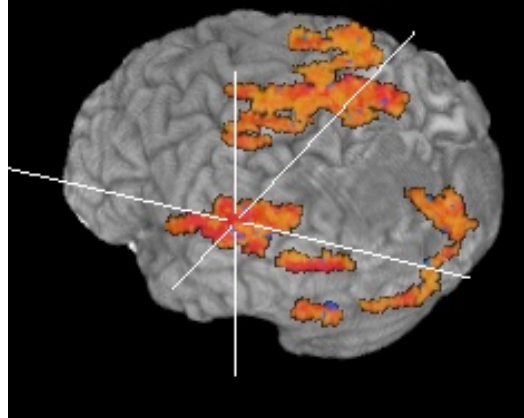


## InstaCorr: SVD-based “Blurring”

Gaussian 5mm



3dLocalPV 5mm



- 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;  $\approx 0.1 \times \#$ 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: transform all time series datasets to standard space = **@auto\_tlrc** and **adwarp**
  - Step 2: filter and blur all time series dataset = **3dBandpass**
  - Step 3: collect groups of time series datasets into one big file = **3dSetupGroupInCorr**
- **Interactive Phase:** point-and-click to set seed voxel

31 Dec 2009

## 3dGroupInCorr: Setup #1

- Assume datasets are named as follows:

- T1-weighted anatomical = **sXXX\_anat+orig**
- Resting state EPI = **sXXX\_rest+orig**

```
foreach aset ( s*_anat+orig.HEAD )
  set sub = `basename $aset _anat+orig.HEAD`
  # transform anat to MNI space
  @auto_tlrc -base ~/abin/MNI_avg152T1+tlrc.HEAD -input $aset
  # transform EPI to MNI as well (assume anat & EPI are aligned)
  adwarp -apar ${sub}_anat+tlrc.HEAD -dpar \
    ${sub}_rest+orig.HEAD -resam Cu -dxyz 2.0
  # make individual subject mask
  3dAutomask -prefix ${sub}_amask ${sub}_rest+tlrc.HEAD
end

# Combine individual EPI masks into group mask

3dMean -datum float -prefix ALL_am *_amask+tlrc.HEAD
3dcalc -datum byte -prefix ALL_am50 -a ALL_am+tlrc \
  -expr 'step(a-0.499)'
```

## 3dGroupInCorr: Setup #2

- Bandpass and blur each dataset inside mask
  - skip first 4 time points, and remove global signal
  - of course, you can choose your own options for filtering
    - Can also have 1 voxel-dependent time series to detrend, via `-dsort`

```

foreach rset ( s*_rest+tlrc.HEAD )
  set sub = `basename $rset _rest+tlrc.HEAD`
  # create global signal file for this dataset
  3dmaskave -mask ALL_am50+tlrc -quiet \
            $rset'[4..$]' > ${sub}_GS.1D
  # 3dBandpass does blurring, filtering, and detrending
  3dBandpass -mask ALL_am50+tlrc -blur 6.0 \
            -band 0.01 0.10 -prefix ${sub}_BP \
            -input $rset'[4..$]' -ort ${sub}_GS.1D
end
/bin/rm -f *_GS.1D *_amask+tlrc.*

```

## 3dGroupInCorr: Setup #3

- **3dSetupGroupInCorr** reads all filtered & blurred resting state EPI datasets, masks & normalizes them, and writes them to one *big* file for **3dGroupInCorr**
  - Sample 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 ${fred}_BP+tlrc.HEAD )
end
3dSetupGroupInCorr -mask ALL_am50+tlrc -prefix AAA $ggg
set ggg = ( )
foreach fred ( $BBB )
  set ggg = ( $ggg ${fred}_BP+tlrc.HEAD )
end
3dSetupGroupInCorr -mask ALL_am50+tlrc -prefix BBB $ggg

```

## 3dGroupInCorr: Interactive Phase

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

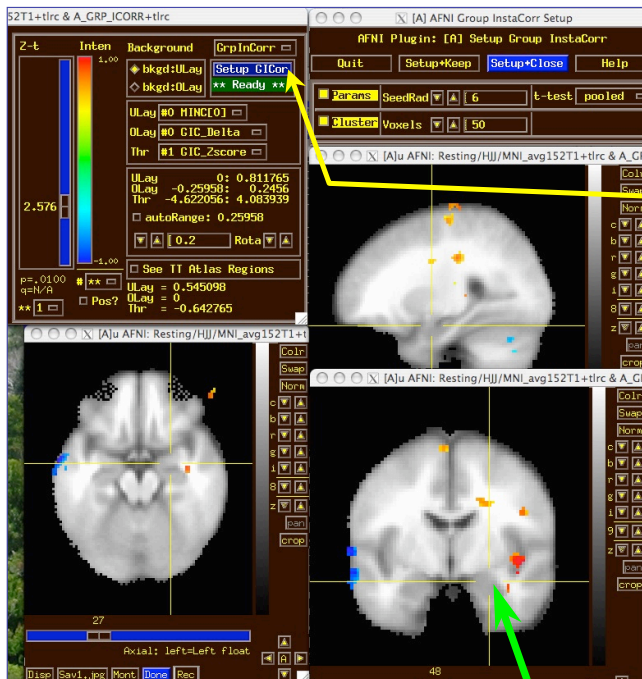
```
3dGroupInCorr -setA AAA.grpincorr.niml \
               -setB BBB.grpincorr.niml
```

  - Startup takes a little while, as all data must be read into RAM (in this example, 3.2 Gbytes)
  - After data is read, connects to AFNI using a NIML socket
  - 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
```

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

## 3dGrpInCorr: Interactive Results



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

- Extracts seed time series from each input dataset; correlates it with all voxel time series in that dataset
  - Group analysis: *t*-test between correlation datasets
- 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 **3dBandpass** of 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  $\tanh^{-1}$ (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
- Brand new software = still rough around the edges  
⇒ need *constructive* feedback

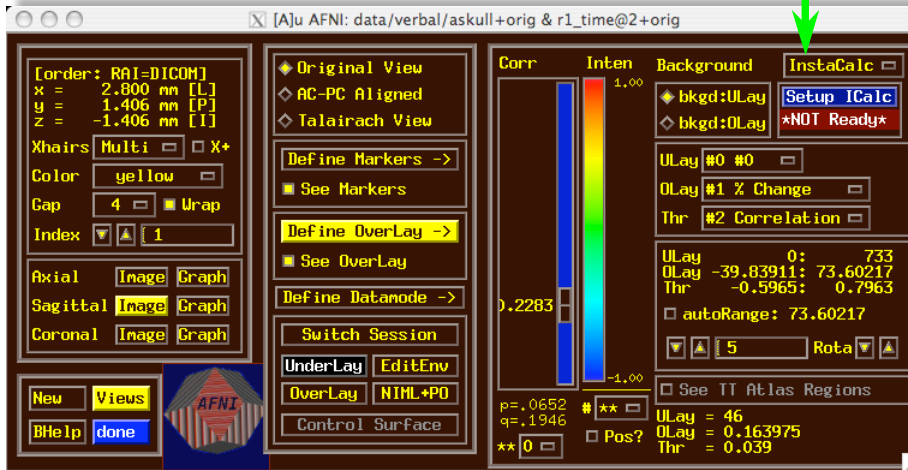
## Group InstaCorr: Finalest Notes

- Shift+Ctrl+Click+Drag method for dynamically setting the seed voxel also works with **Group InstaCorr**
  - But speed of interaction can be slow
- Can now [May 2010] 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 now [Jan 2011] run in batch mode
- Further ideas:
  - Granger-ize: correlate with lag-0 **and** lag-1 of seed and test Granger causality
  - Allow user to set other seeds to be "partialled out" of the analysis



# InstaCalc: Dataset Calculator

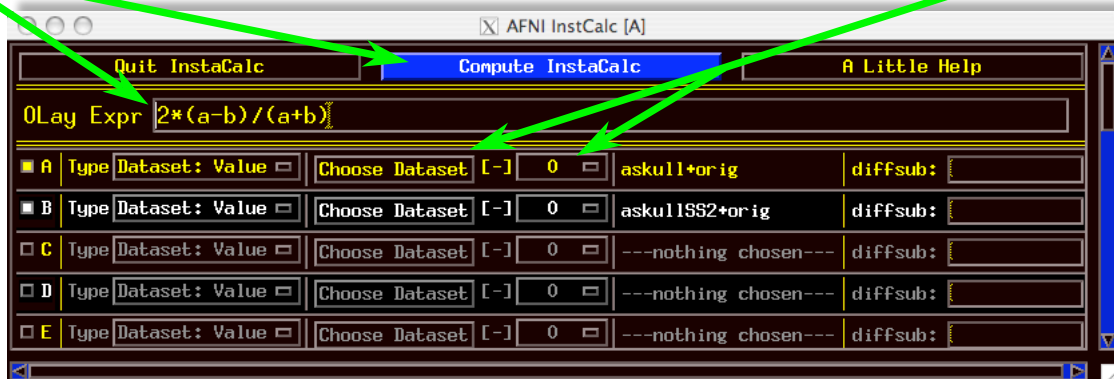
- Open **Define Overlay**, choose **InstaCalc** from menu in top right corner



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

# InstaCalc: Setup

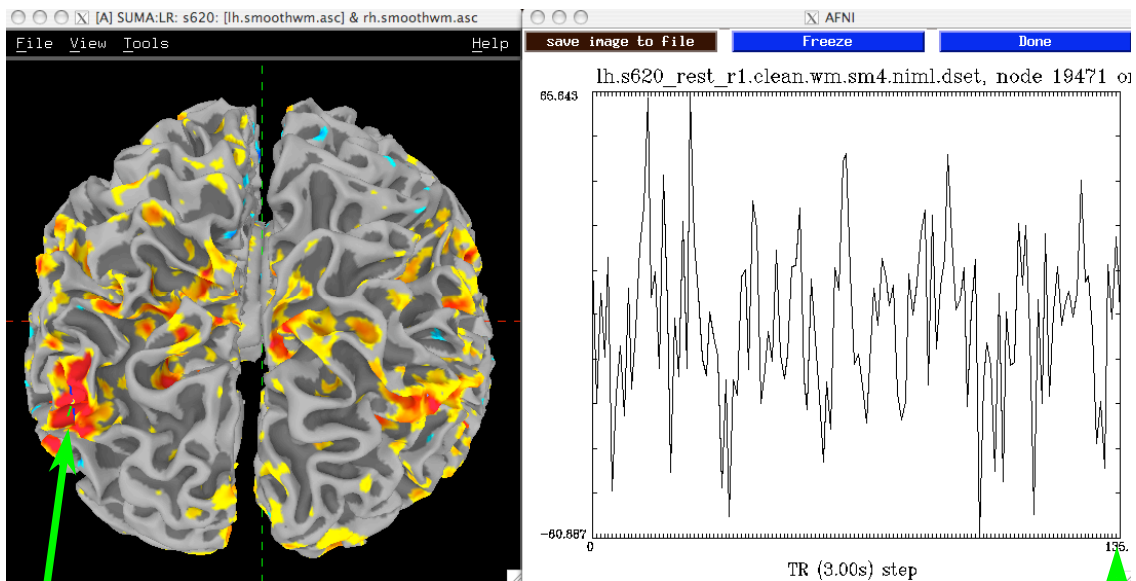
- Select datasets with **Choose Dataset** buttons
  - and sub-bricks with the [-] controls
- Enter symbolic expression
- Press **Compute InstaCalc**
- Creates new 1-brick dataset **A\_ICALC** for Overlay
  - voxel-by-voxel calculations



## 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

## SUMA! InstaCorr: Sample



• Seed voxel and Seed voxel time series graph