AFNI Preprocessing:
Outline, Recommendations, and New(ish) Stuff

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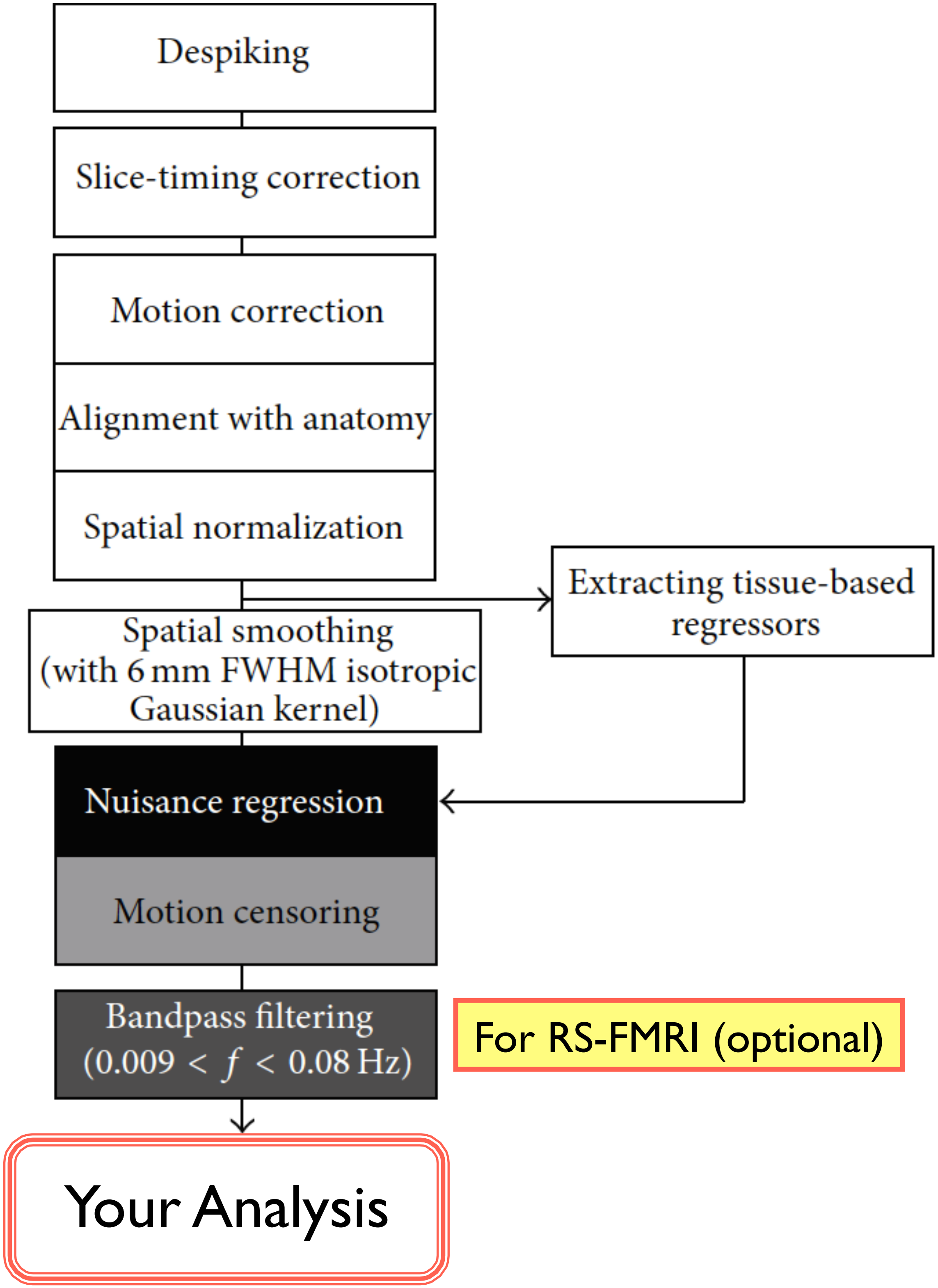


Despiking

Slice-timing correction

Motion correction

Alignment with anatomy

Spatial normalization

Spatial smoothing (with 6 mm FWHM isotropic Gaussian kernel)

Extracting tissue-based regressors

Nuisance regression

Motion censoring

Bandpass filtering ($0.009 < f < 0.08$ Hz)

For RS-FMRI (optional)

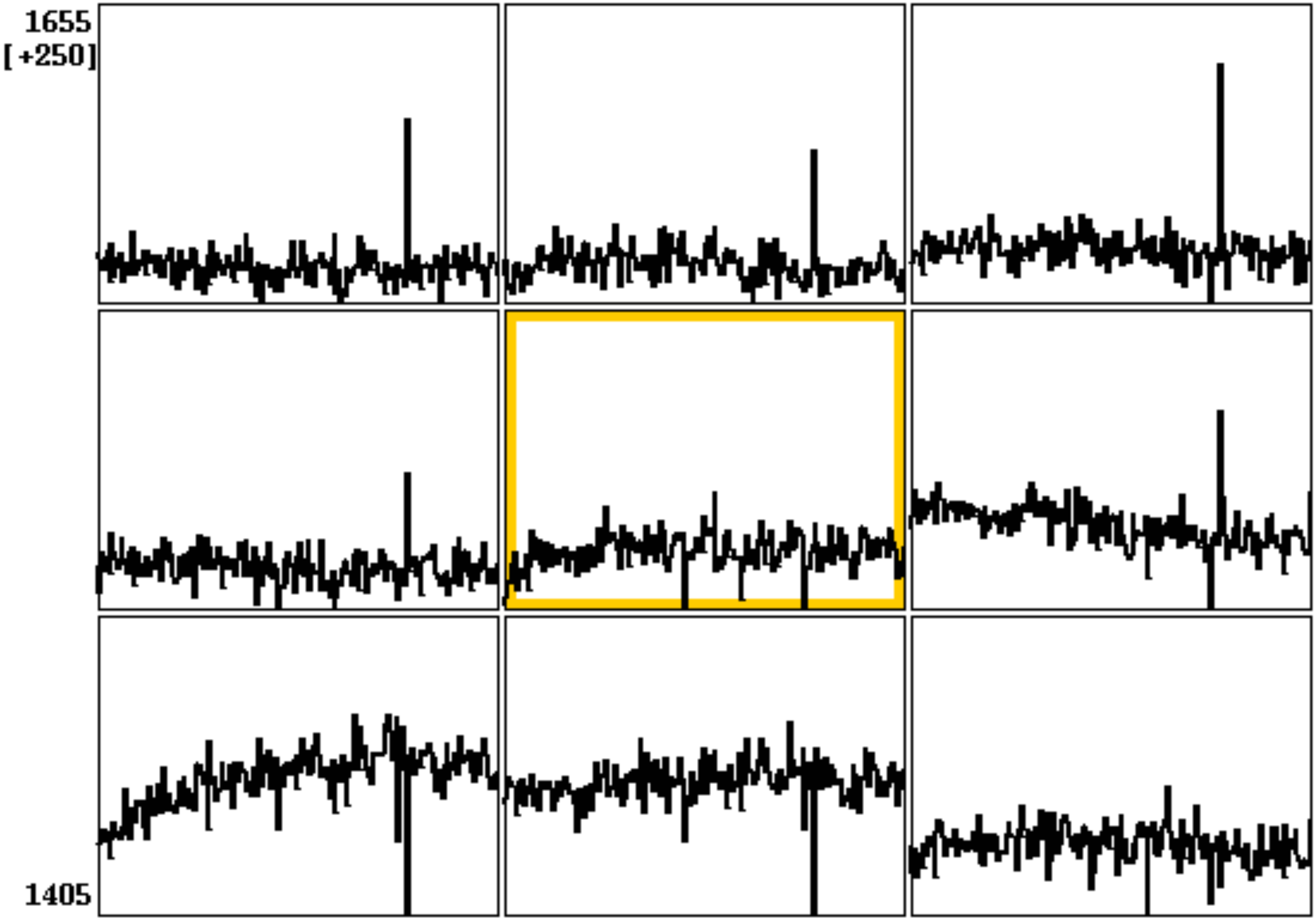
Your Analysis

AFNI's recommended pre-processing steps

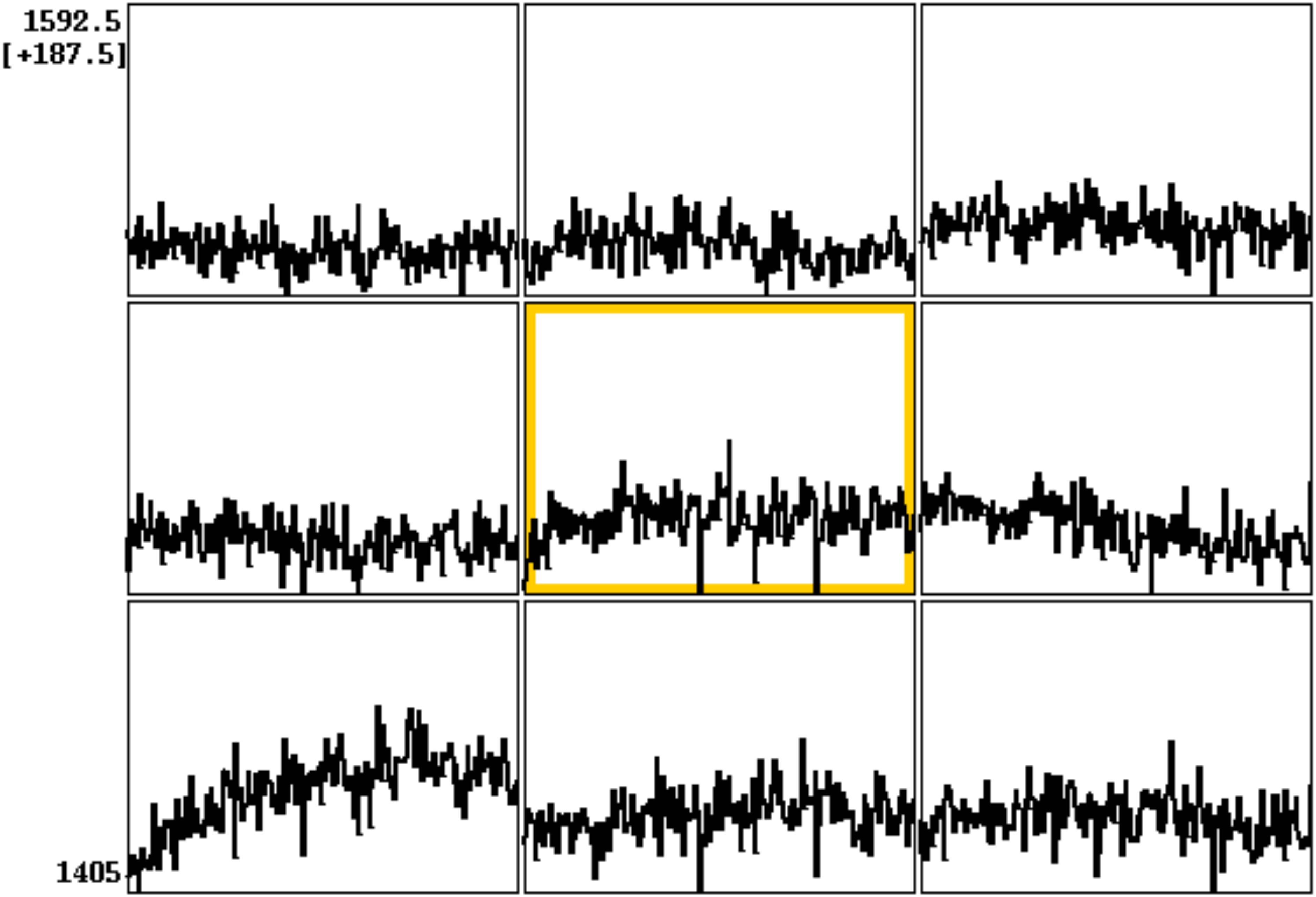
HJ Jo *et al*, 2010 and 2013

Carried out using **afni_proc.py**

Step 1 = Despiking (before)



Step 1 = Despiking (after)



More about Despiking

- **3dDespike** does each voxel time series *separately*
  - *Before* 3D time series registration
  - Empirically: we found this is better than *after* registration
- Why not rely on time series censoring later on?
  - Censoring *will* remove time points from analysis that are "bad" in a global (over the whole brain) sense
    - ❑ From head motion or from lots of coincident spikes
  - Censoring *will not* remove spikes that occur sporadically in space and time
    - ❑ Since censoring only works on an *all-or-nothing* basis for a particular 3D volume in the time series
- Important? Not always, but helps *sometimes.*

Step 2 = Slice Timing Correction

- 2D Slices acquired at different times within one 3D “volume” TR
- The same physiological BOLD effect in 2 different slices will show up differently due to being measured at different times
- And so will be less correlated (with each other or with task model) than they “should be”
- **3dTshift** interpolates in time to some common reference point before calculating correlations
  - Not ideal, because we are also interpolating noise

Lining Brains Up:
Step 3 = Motion Correct EPI
Step 4 = Align EPI with Anatomy
Step 5 = Spatial Normalization

- **Step 3**: Especially important for RS-FMRI, since the BOLD effect is smaller and more spatially diffused than in Task-FMRI, so correcting for subject head motion is crucial
- **Step 4**: Needed for step 5, and for identifying brain regions underneath the “blobs”
- **Step 5**: Needed for group studies and/or to use template-based atlases

More about Alignment - 1

- **Step 3**: Intra-EPI volumes are aligned with rigid-body matrix transformation (6 parameters) in program **3dvolreg**
  - Motion parameter estimates (angles and shifts) are saved for later uses (including Step 5)
  - Matrix for adjustment is saved for each time point
- **Step 4**: EPI-to-Anat alignment is with general matrix transformation (12 parameters) in program **3dAllineate**
  - Transformation matrix is saved for Step 5
  - Can include minor nonlinear warping for B_0 effects
    - [not currently in **afni_proc.py** but in **unWarpEPI.py**]

More about Alignment - 2

- **Step 5**: Anat-to-Template alignment can be done with nonlinear warping [**3dQwarp**] or with affine (general matrix) transformation [**3dAllineate**]
  - Nonlinear warping is slower, of course
  - This is where having a compute cluster helps
- **At the end**: the EPI-to-Template-space transformations – nonlinear warps and/or matrices – are catenated into one interpolation step (for each TR) in **3dNwarpApply** or **cat_matvec+3dAllineate**
  - Default interpolation is windowed sinc, but other options are available

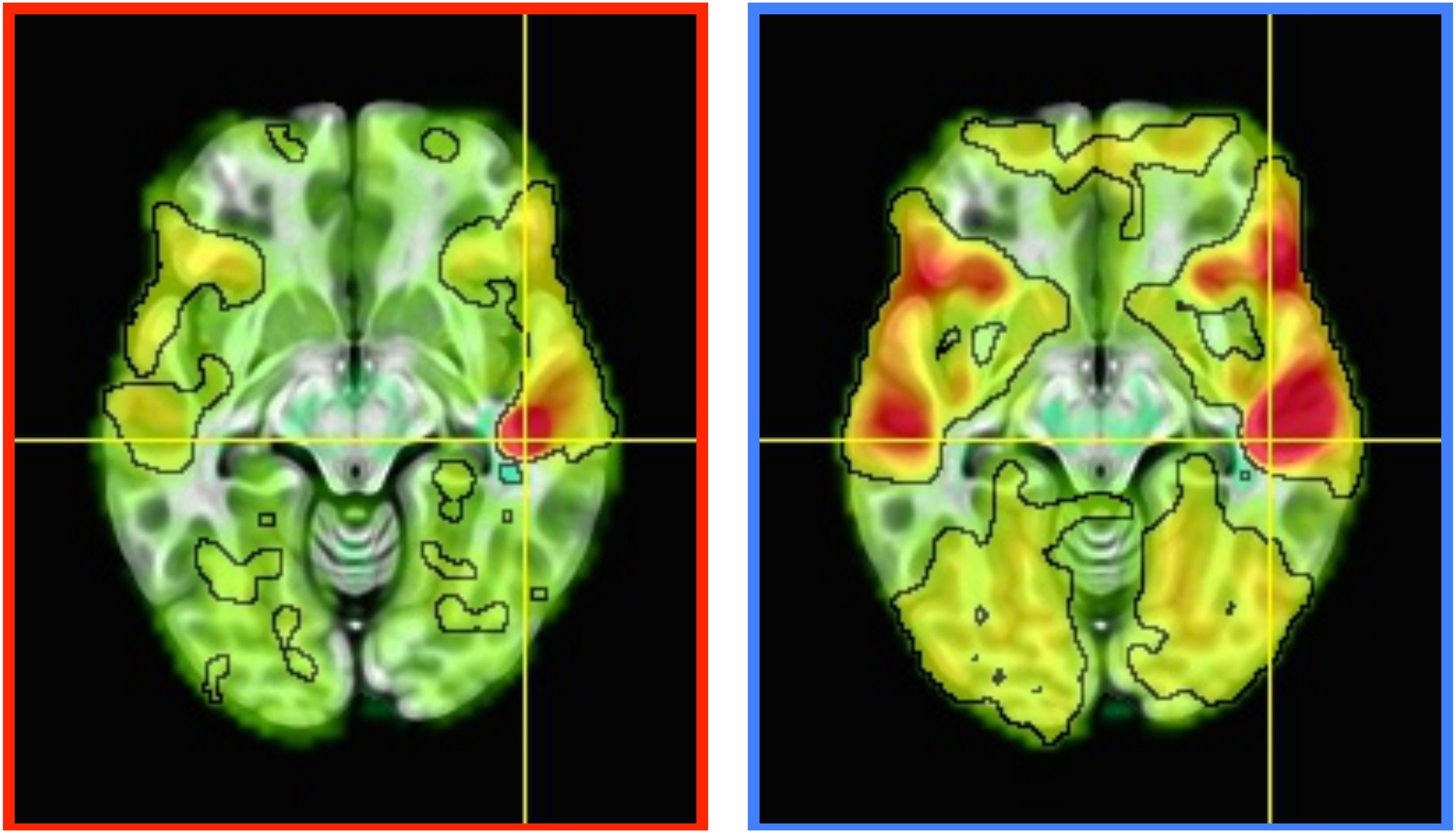
Alignment: **Affine** vs **Nonlinear**

Seed-Based Correlation (seed at crosshairs)

Average Across 36 Subjects (**InstaCorr**)

[**black** outlines = per-voxel $p \leq 10^{-4}$ in 1-sample t-test]

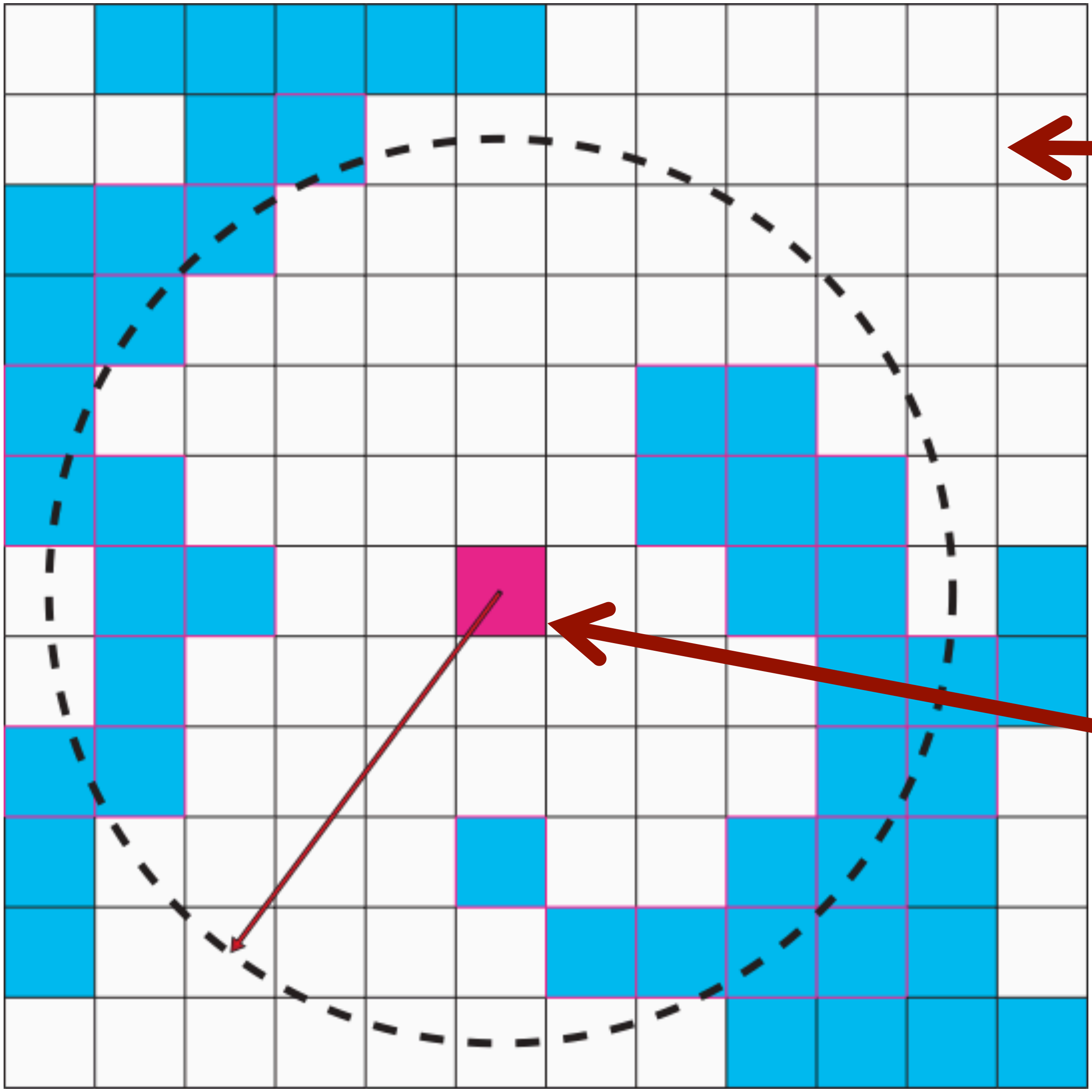
[**red** = mean correlation ≥ 0.3]



Step 6 = Extract Tissue Based Regressors

- The purpose of tissue based regressors is to extract fluctuations *in the data* that are ***not*** BOLD signal
- So we can regress them out of the data at Step 8
- Common choices include (options in **afni_proc.py**):
  - Average of all white matter (WM) signal time series
  - Several principal components of all WM time series (CompCor method)
  - Average global brain signal time series (GS) ☹
  - Average signal from CSF in ventricles
- Less common: **ANATicor** …

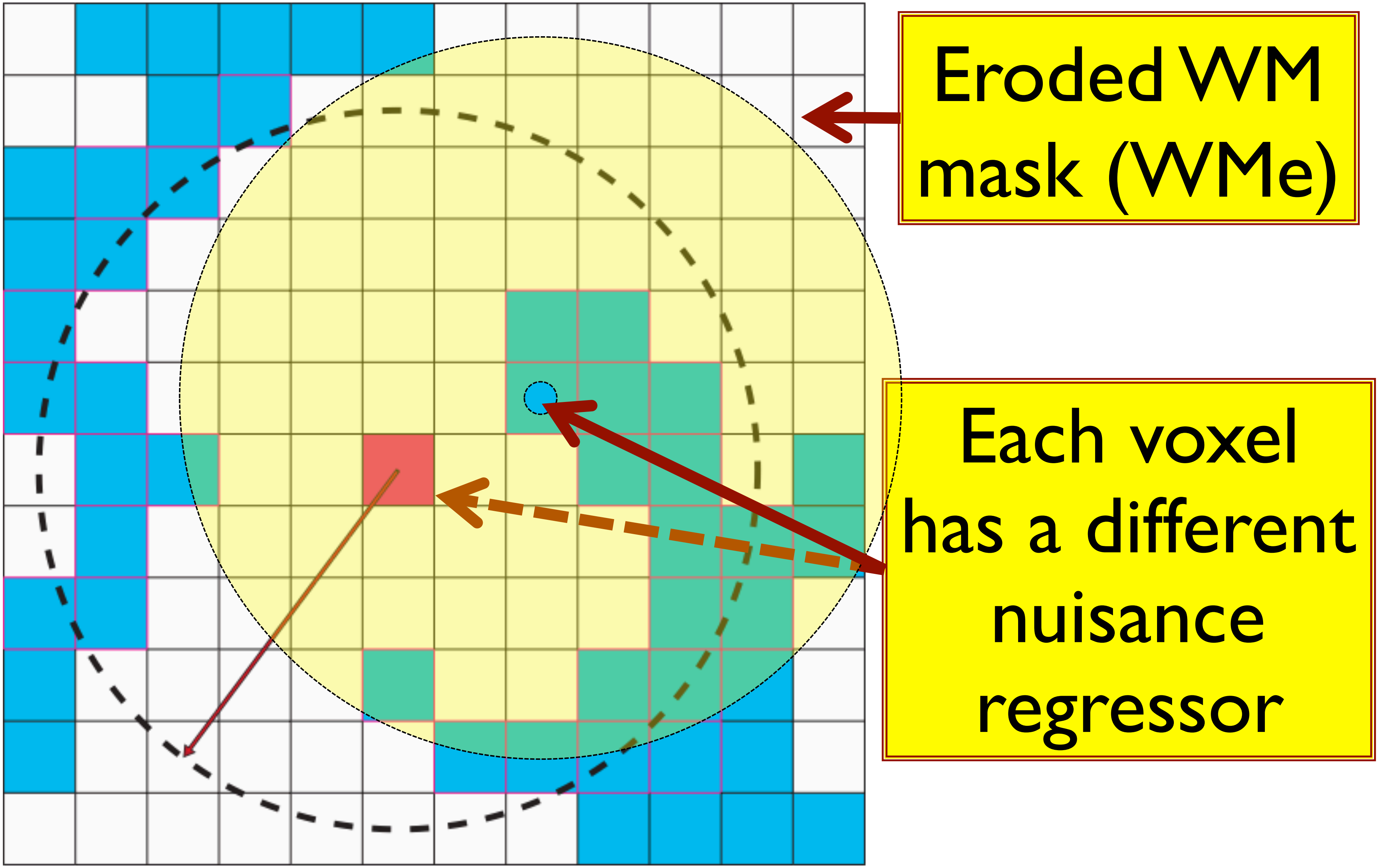
ANATicor – Tissue Based *per voxel*



Eroded WM mask (WMe)

Average over WMe voxels inside 20mm radius

ANATicor – Tissue Based *per voxel*



Eroded WM mask (WMe)

Each voxel has a different nuisance regressor

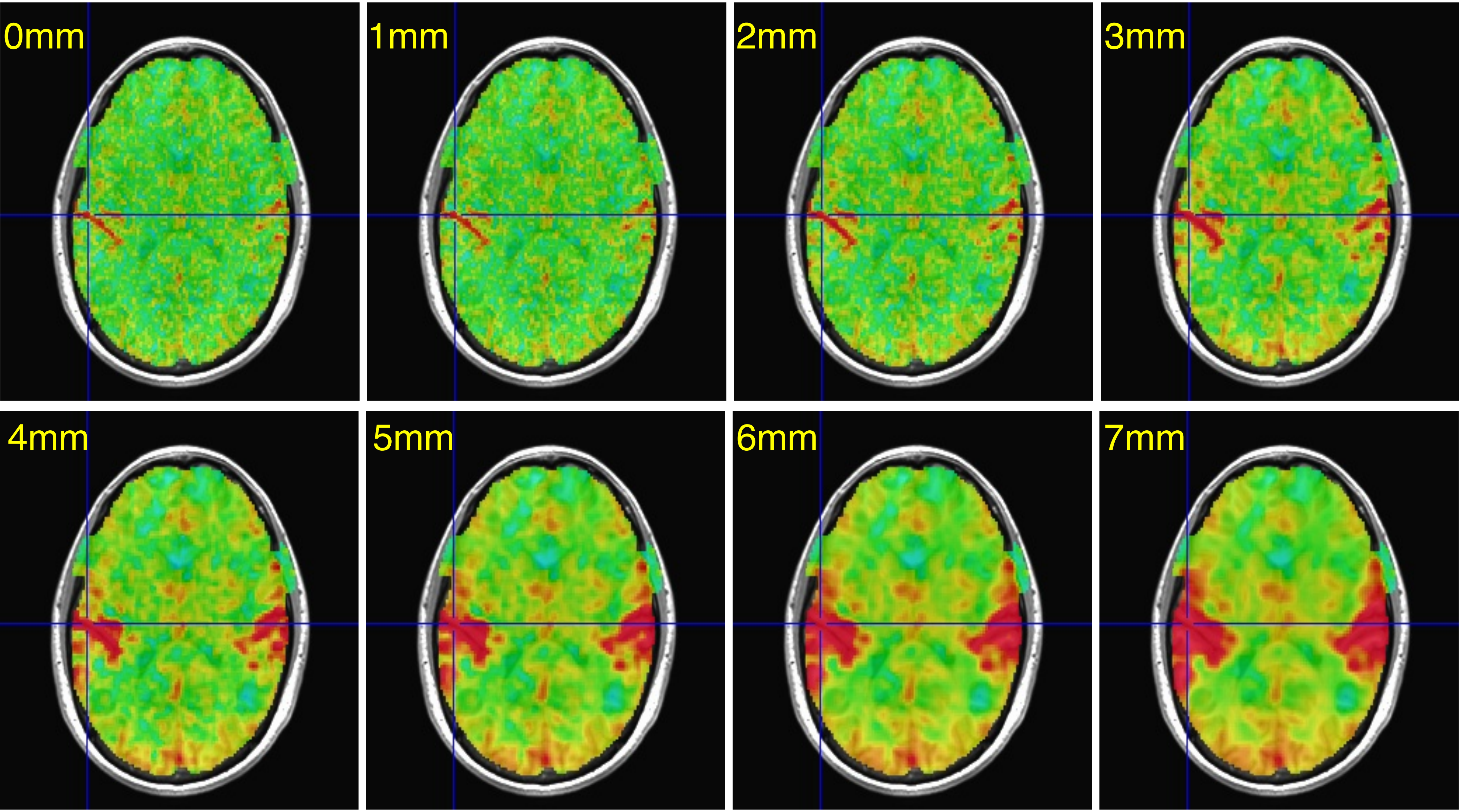
More about Nuisance Regressors

- Intended to model undesirable parts of the signal in time
- In **AFNI**:
  - Motion parameters and their derivatives
    - ☐ Too much motion in one TR, or too many outliers = Censor that time point
      - "Too much" = 0.3 mm (Task-FMRI), 0.2 mm (RS-FMRI)
  - **If** you have physiological measurements (respiration, pulse oximeter), **RetroTS.py** can create regressors that attempt to remove such effects
    - ☐ RETROICOR (Glover) + RVT (Birn)

Step 7 = Spatial Blurring

- Especially important for RS-FMRI, since the BOLD signal fluctuations are small
- Averaging locally will tend to cancel noise and add up coherent signals
- **Important**: blur *after* tissue based signal extraction
- *Otherwise*, will get unintended signals in WM and CSF extractions that were blurred in from nearby GM (gray matter)
- Blurring can be in entire volume [**3dmerge**] or restricted to the brain mask [**3dBlurInMask**]

Effects of Blurring on RS Correlation



- Vascular/cardiac effect being progressively smeared? Or real neural correlations seen via BOLD? Or some of both?

Step 8 = Nuisance Regression - 1

- In Task-FMRI, regression is to find the signal amplitudes (*betas*) of the task model components while at the same time removing the nuisance model components
  - **Nuisances**: motion parameters, *motion parameter time derivatives*, WM signals, respiration-derived signals, drifting baseline, *et cetera*
- In RS-FMRI, there are no task model components to estimate
  - All we want is to remove the nuisance components and compute the residuals – these residuals are the output, ready for correlations

Step 8 = Nuisance Regression - 2

- Another operation usually (but not always) used in RS-FMRI is called **bandpassing**
- It involves removing all frequency components from the data ***except*** those in a specific band
- Frequency: units are Hertz (Hz)
  - 1 Hz = 1 cycle per second
  - 0.01 Hz = 0.01 cycle per second = 1 cycle in 100 seconds
- In RS-FMRI, it is common to bandpass out all frequencies higher than 0.10 Hz (or 0.20 Hz) and smaller than 0.01 Hz
  - Keep only 10-100 (or 5-100) second cycles; faster or slower = **FILTERED OUT OF THE DATA**
- The idea is that these do not contain BOLD, just noise, so should be removed before correlation

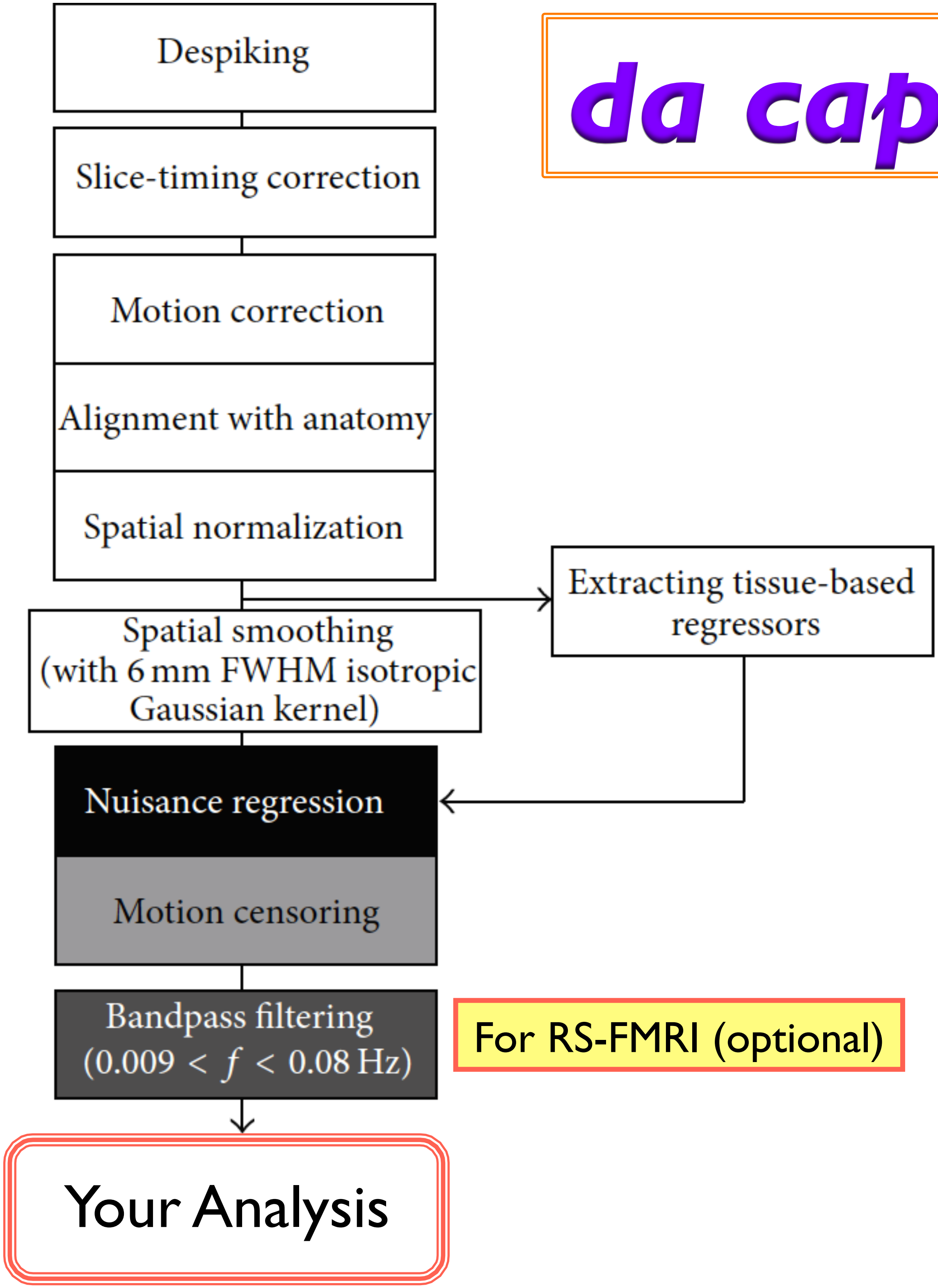
Step 8 = Nuisance Regression - 3

- It is also common to censor out "**bad**" time points, so they aren't used in the correlation
  - "Bad" = too much motion, or that volume has too many "outlier" data points
- In **AFNI**, nuisance regression, bandpassing, and censoring for RS-FMRI are all done in the same program: **3dTproject**
  - Which allows for voxel-specific regressors (**ANATicor**)
- For Task-FMRI, regression and single-subject statistics are done in program **3dREMLfit**
  - Also allows for voxel-specific nuisances and voxel-specific ARMA(1,1) temporal correlation modeling

Step 8 = Nuisance Regression - 4

- Bad old days: Some did these 2 steps in sequence:
  - Bandpass the data
  - Regress other nuisance components from the bandpassed data
- Doing these operations in 2 steps (instead of one) is not just ***bad***, it is ***WRONG*** (matrix multiplication is non-Abelian)
- Since the nuisance regressors will contain some of the unwanted frequency components, these unwanted components will "leak" back into the data at the second regression
  - If the nuisance regressors were bandpassed themselves, then the problem would not happen
- The same thing applies to bandpassing and censoring – they should be done together
- These reasons are why **3dTproject** was written

da capo



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RS-FMRI Preprocess via **afni_proc.py**

```
## Adapted from Example 9b in afni_proc.py -help
afni_proc.py -subj_id s620                              \
 -dsets s620_rest_r1.nii -copy_anat s620_anat.nii \
 -blocks despike tshift align tlrc volreg          \
         blur mask regress                         \
 -tcat_remove_first_trs 2                          \
 -volreg_align_e2a -tlrc_NL_warp                   \
 -blur_size 6 -blur_in_automask                    \
 -regress_anaticor_fast                            \
 -regress_censor_motion 0.2                        \
 -regress_censor_outliers 0.02                     \
 -regress_bandpass 0.01 0.20                       \
 -regress_apply_mot_types demean deriv             \
 -regress_run_clustsim no
```

```
motion limit              : 0.2
num TRs above mot limit   : 2
average motion (per TR)   : 0.0925227
average censored motion   : 0.0914375
max motion displacement   : 0.788964
max censored displacement : 0.788964
outlier limit             : 0.02
average outlier frac (TR) : 0.000486089
num TRs above out limit   : 0

num runs found            : 1
num TRs per run           : 225
num TRs per run (applied) : 221
num TRs per run (censored): 4
fraction censored per run : 0.0177778
TRs total (uncensored)    : 225
TRs total                 : 221
degrees of freedom used   : 12
degrees of freedom left   : 209

TRs censored              : 4
censor fraction           : 0.017778
num regs of interest      : 1
num TRs per stim (orig)   : 154
num TRs censored per stim : 2
fraction TRs censored     : 0.013
ave mot per sresp (orig)  : 0.090031
ave mot per sresp (cens)  : 0.089095
```

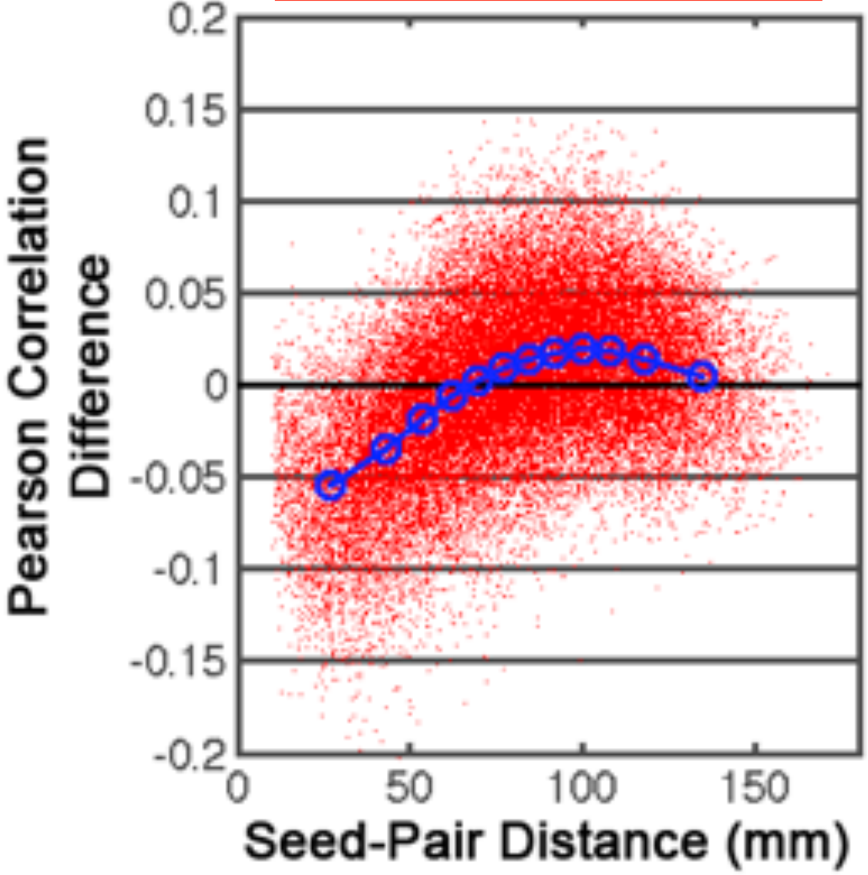
afni_proc.py outputs a summary of what was found during processing

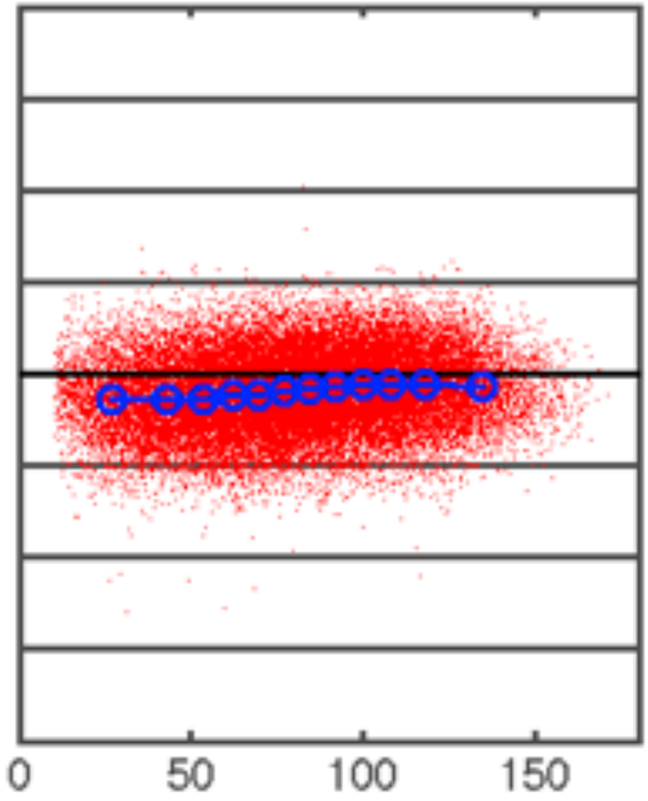
It also creates a review script to drive **AFNI** GUI to show you the data as it moves through the various processing blocks (intermediate datasets are saved for post mortems)

Example: Motion Artifacts

Effect of motion on RS-FMRI correlations depends on distance between regions (Power *et al* 2012)

Data generously made available by Power *et al*

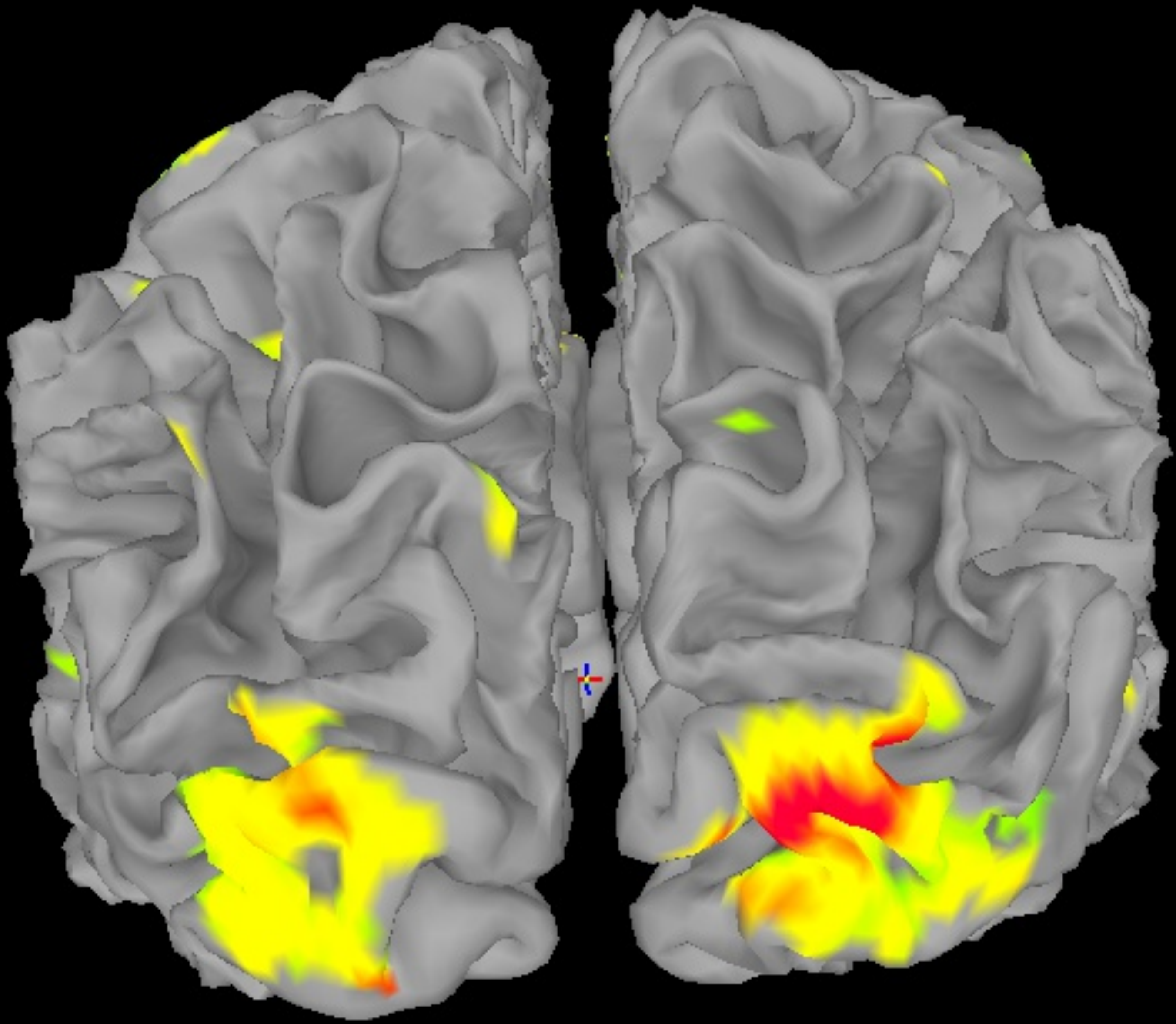

Original Method


Processed in AFNI

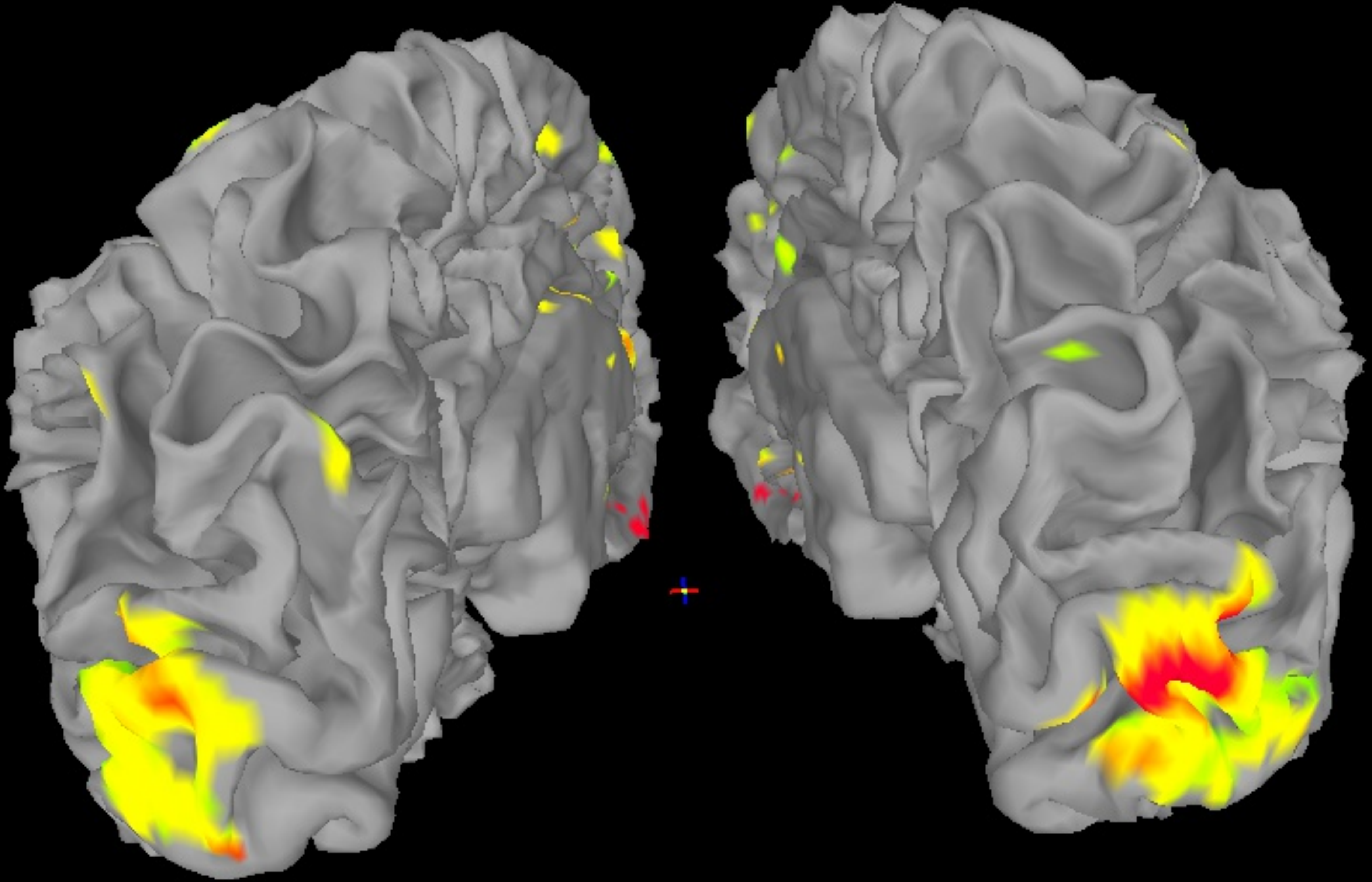
Final Note – Surface-Based Analysis

- Most of the processing steps can be carried out, with **afni_proc.py** *also* projecting the EPI data to surface models of the cortex
  - Surfaces typically come from FreeSurfer, but can be imported from other software
  - Surface models are standardized amongst subjects, to make group analyses simpler
- After 3D time series registration, EPI data get put onto the cortical surface nodes
- Processing proceeds on the surface from there
- Blurring is done on surface, not in volume

Task-FMRI Results on Surfaces



Opening up the Surfaces



Acknowledgments

Mike Beauchamp
Hang Joon Jo
Rick Reynolds
Ziad Saad
and a cast of thousands …

NIMH
National Institute
of Mental Health

NATIONAL INSTITUTE OF
NEUROLOGICAL
DISORDERS AND STROKE

Some AFNI Users