

# **3dDeconvolve**

**Advanced Features  
Et cetera**

**Just in case you weren't  
confused enough already**

## Other Features of 3dDeconvolve - 1

- **-input1D** = used to process a single time series, rather than a dataset full of time series
  - ★ e.g., test out a stimulus timing sequence on sample data
  - ★ **-nodata** option can be used to check **X** matrix for collinearity
- ★ **-censor** = used to turn off processing for some time points
  - ★ for time points that are “bad” (e.g., too much movement; scanner problem)
  - ★ **-CENSORTR 2:37** = newer way to specify omissions (e.g., run #2, index #37)
- **-sresp** = output standard deviation of HRF ( $\beta$ ) estimates
  - ★ can then plot error bands around HRF in AFNI graph viewer
- **-errts** = output residuals (difference between fitted model and data)
  - ★ for statistical analysis of time series noise
- **-TR\_times dt** = calculate **-iresp** and **-sresp** HRF results with time step **dt** (instead of input dataset TR)
  - ★ Can be used to make HRF graphs look better
- ★ **-jobs N** = run with independent threads — **N** of them
  - ★ extra speed, if you have a dual-CPU system (or more)!

## Other Features - 2

<http://afni.nimh.nih.gov/pub/dist/doc/misc/Decon/DeconSummer2004.html>

<http://afni.nimh.nih.gov/pub/dist/doc/misc/Decon/DeconSpring2007.html>

---

- Equation solver: Program computes **condition number** for **X** matrix (measures of how sensitive regression results are to changes in **X**)
  - ★ If the condition number is “bad” (too big), then the program will not actually proceed to compute the results
  - ★ You can use the **-GOFORIT** option on the command line to force the program to run despite **X** matrix warnings
    - But you should strive to understand why you are getting these warnings!!
- Other matrix checks:
  - ★ Duplicate stimulus filenames, duplicate regression matrix columns, all zero matrix columns
- ★ Check the screen output for **WARNINGS** and **ERRORS** ★
  - ★ Such messages also saved into file `3dDeconvolve.err`

## Other Features - 3

- ★ All-zero regressors *are* allowed (via `-allzero_OK` or `-GOFORIT`)
  - ★ Will get zero weight in the solution
  - ★ Example: task where subject makes a choice for each stimulus (e.g., male or female face?)
    - You want to analyze correct and incorrect trials as separate cases
    - What if some subject makes no mistakes? Hmmmm...
      - ➔ Can keep the all-zero regressor (e.g., all `-stim_times = *`)
      - ➔ Input files and output datasets for error-making and perfect-performing subjects will be organized the same way

---

- **3dDeconvolve\_f** program can be used to compute linear regression results in single precision (7 decimal places) rather than double precision (16 places)
  - ★ For better speed, but with lower numerical accuracy
  - ★ Best to do at least one run **both** ways to check if results differ significantly (Equation solver *should* be safe, but ...)

## Other Features - 4

- ★ Default output format is 16-bit short integers, with a scaling factor for each sub-brick to convert it to floating point values
  - ★ `-float` option can be used to get 32-bit floating point format output — more precision, and more disk space

---

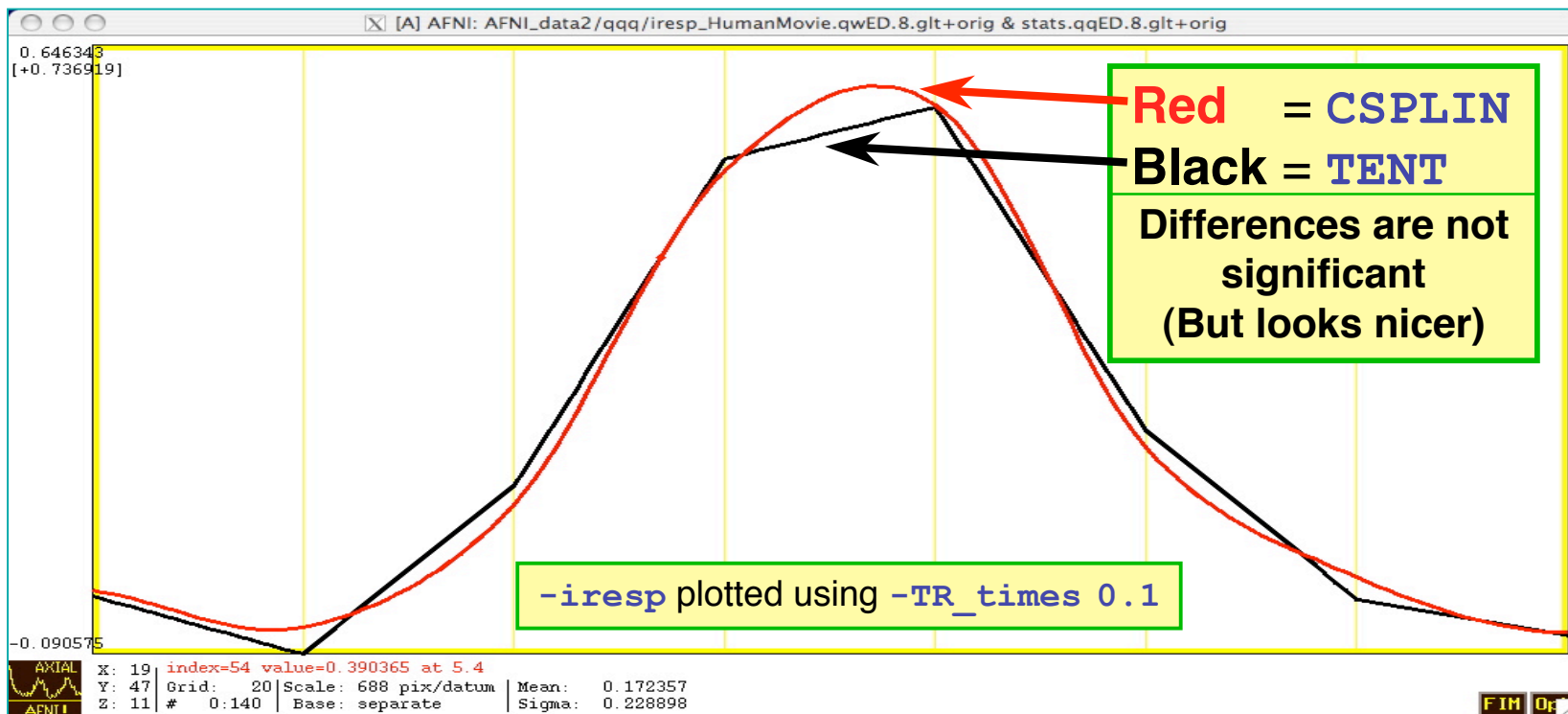
- `3dDeconvolve` recommends a `-polort` value, and prints that out as well as the value you chose (or defaulted to)
  - ★ `-polort A` can be used to let the program set the detrending (AKA “high pass filtering”, since detrending removes low frequency content from data) level automatically

---

- `-stim_file` is used to input a column directly into **X** matrix
  - ★ Motion parameters (as in previous examples)
  - ★ If you create a stimulus+response model outside `3dDeconvolve` (e.g., using program `waver`)

# Other Features - 5

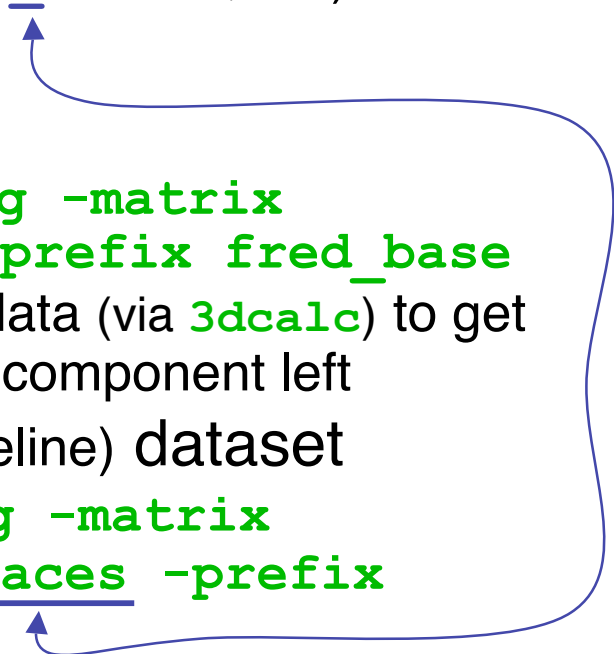
- `-stim_times` has some other basis function options for the HRF model besides **BLOCK** and **TENT**
  - ★ **CSPLIN** = cubic spline instead of **TENT** = linear spline
    - Same parameters: (`start, stop, number of regressors`)
    - Can be used as a “drop in” replacement for **TENT**



## Other Features - 6

- `-fitts` option is used to create a synthetic dataset
    - ★ each voxel time series is full (signal+baseline) model as fitted to the data time series in the corresponding voxel location

---

  - `3dSynthesize` program can be used to create synthetic datasets from *subsets* of the full model
    - ★ Uses `-x1D` and `-cbucket` outputs from `3dDeconvolve`
      - `-cbucket` stores  $\beta$  coefficients for each `X` matrix column into dataset
      - `-x1D` stores the matrix columns (and `-stim_labels`, etc.)
    - ★ Potential uses:
      - Baseline only dataset
        - ↳ `3dSynthesize -cbucket fred+orig -matrix fred.xmat.1D -select baseline -prefix fred_base`
        - ↳ Could subtract this dataset from original data (via `3dcalc`) to get signal+noise dataset that has no baseline component left
      - Just one stimulus class model (+ baseline) dataset
        - ↳ `3dSynthesize -cbucket fred+orig -matrix fred.xmat.1D -select baseline Faces -prefix fred_Faces`
- 

## Other Recent Small Changes

- Defaults are changed:
  - ★ **-nobout** & **-full\_first** & **-bucket** & **-x1D** are always implied
  - ★ Names of statistics sub-bricks are slightly altered (to be more consistent)

---
- Checks if **-stim\_times** inputs are out of range (AKA: the PSFB syndrome)
  - ★ Prints **WARNING** message, but continues analysis

---
- When using **-nodata** with **-stim\_times**, it is important to give the number of time points and the TR, as in **-nodata 250 2.3**
  - ★ With **-input1D**, use **-TR\_1D 2.3** to specify TR



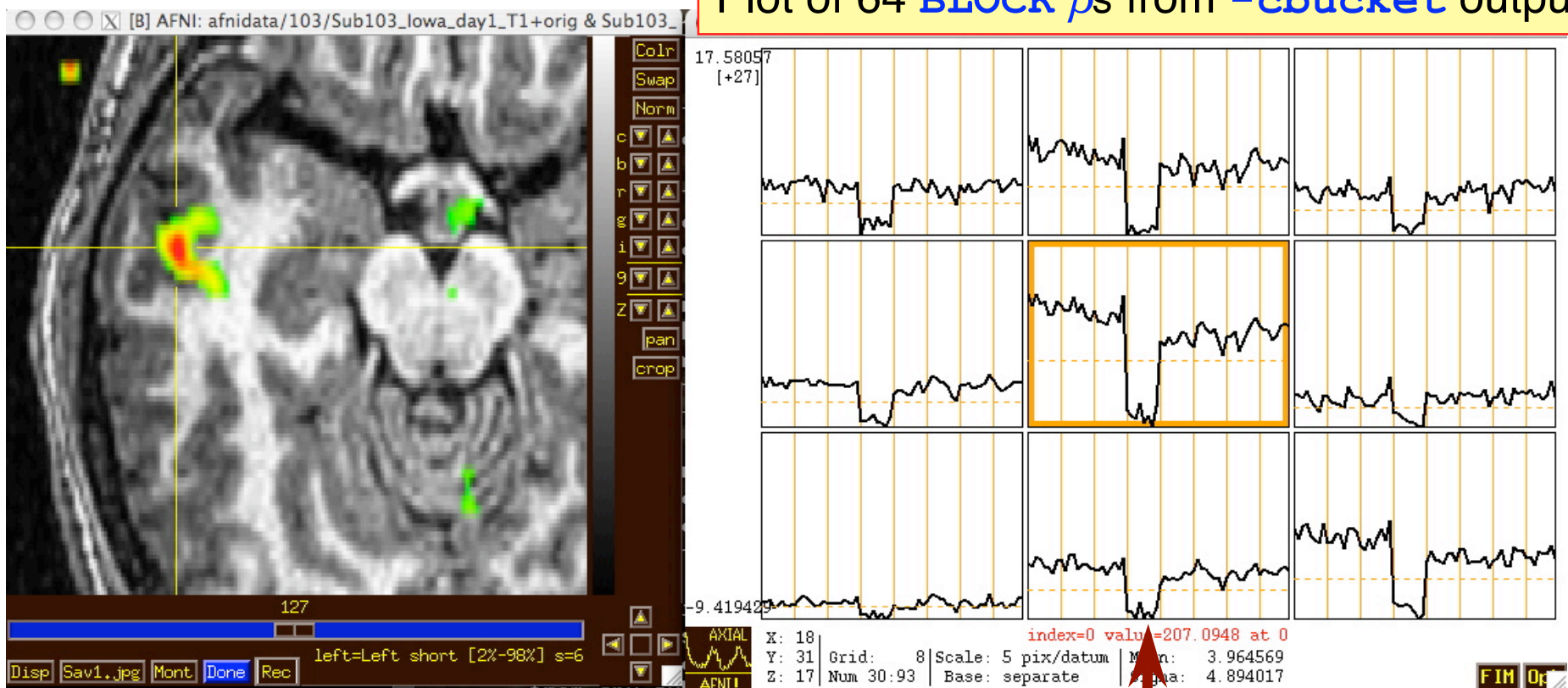
# IM Regression - 1

- **IM** = Individual **M**odulation
  - ★ Compute *separate* amplitude of response for each stimulus
    - Instead of computing average amplitude of responses to multiple stimuli in the same class
  - ★ Response amplitudes ( $\beta$ s) for each individual block/event will be highly noisy
    - Can't use individual activation map for much
    - Must pool the computed  $\beta$ s in some further statistical analysis (*t*-test via **3dttest**? inter-voxel correlations in the  $\beta$ s? correlate  $\beta$ s with something else?)
  - ★ Usage: **-stim\_times\_IM k tname model**
    - Like **-stim\_times**, but creates a separate regression matrix column for each time given

# IM Regression - 2

- Only application of IM thus far has been in checking some data we received from another institution
- Experiment: 64 blocks of sensorimotor task (8 runs each with 8 blocks)

Plot of 64 **BLOCK**  $\beta$ s from **-cbucket** output



N.B.: sign reversal in run #4 = stimulus timing error!

## IM Regression - 3

- IM works naturally with blocks, which only have 1 amplitude parameter per stimulus
- With event-related experiment and *deconvolution*, have multiple amplitude parameters per stimulus
  - ★ Difficulty: each event in same class won't get the same shaped HRF this way
  - ★ Desideratum: allow response shape to vary (that's deconvolution), but only allow amplitude to vary between responses in the same stimulus class
  - ★ Problem: get unknowns that multiply each other (shape parameters  $\times$  amplitude parameters) — and we step outside the realm of *linear* analysis
  - ★ Possible solution: **semi-linear** regression (nonlinear in global shape parameters, linear in local amplitude params)

# AM Regression - 1

- **AM** = **A**mplitude **M**odulated (or **M**odulation)
    - ★ Have some extra data measured about each response to a stimulus, and *maybe* the BOLD response amplitude is modulated by this
    - ★ Reaction time; Galvanic skin response; Pain level perception; Emotional valence (happy or sad or angry face?)
  - Want to see if some brain activations vary proportionally to this **ABI** (**A**uxiliary **B**ehaviorial **I**nformation)
- 

- Discrete levels (2 or maybe 3) of ABI:
    - ★ Separate the stimuli into sub-classes that are determined by the ABI (“on” and “off”, maybe?)
    - ★ Use a GLT to test if there is a difference between the fMRI responses in the sub-classes
- ```
3dDeconvolve ... \
-stim_times 1 regressor_on.1D 'BLOCK(2,1)' -stim_label 1 'On' \
-stim_times 2 regressor_off.1D 'BLOCK(2,1)' -stim_label 2 'Off' \
-gltsym 'SYM: +On | +Off' -glt_label 1 'On+Off' \
-gltsym 'SYM: +On -Off' -glt_label 2 'On-Off' ...
```
- “**On+Off**” tests for any activation in *either* the “on” or “off” conditions
  - “**On-Off**” tests for differences in activation *between* “on” and “off” conditions
  - Can use **3dcalc** to threshold on **both** statistics at once to find a **conjunction**

## AM Regression - 2

- Continuous (or several finely graded) ABI levels
  - ★ Want to find active voxels whose activation level also depends on ABI
  - ★ **3dDeconvolve** is a linear program, so must make the assumption that the change in fMRI signal as ABI changes is linearly proportional to the changes in the ABI values
- Need to make 2 separate regressors
  - ★ One to find the mean fMRI response (the usual `-stim_times` analysis)
  - ★ One to find the variations in the fMRI response as the ABI data varies
- The second regressor should have the form

$$r_{AM2}(t) = \sum_{k=1}^K h(t - \tau_k) \cdot (a_k - \bar{a})$$

- ★ Where  $a_k$  = value of  $k^{\text{th}}$  ABI value, and  $\bar{a}$  is the average ABI value
- Response ( $\beta$ ) for first regressor is standard activation map
- Statistics and  $\beta$  for second regressor make activation map of places whose BOLD response changes with changes in ABI
  - ★ Using 2 regressors allows separation of voxels that are active but are *not* detectably modulated by the ABI from voxels that *are* ABI-sensitive

# AM Regression - 3

- New feature of **3dDeconvolve**: `-stim_times_AM2`
- Use is very similar to standard `-stim_times`
  - ★ `-stim_times_AM2 1 times_ABI.1D 'BLOCK(2,1)'`
  - ★ The `times_ABI.1D` file has time entries that are “married” to ABI values:

|      |      |      |      |
|------|------|------|------|
| 10*5 | 23*4 | 27*2 | 39*5 |
| 17*2 | 32*5 |      |      |
| *    |      |      |      |
| 16*2 | 24*3 | 37*5 | 41*4 |
  - ★ Such files can be created from 2 standard ASCII .1D files using the new **1dMarry** program
    - The `-divorce` option can be used to split them up
- **3dDeconvolve** automatically creates the two regressors (unmodulated and amplitude modulated)
  - ★ Use `-fout` option to get statistics for activation of the pair of regressors (i.e., testing null hypothesis that *both*  $\beta$  weights are zero: that there is no ABI-independent *or* ABI-proportional signal change)
  - ★ Use `-tout` option to test each  $\beta$  weight separately
  - ★ Can **1dplot X** matrix columns to see each regressor

# AM Regression - 4

- The **AM** feature is new, and so needs more practical user experiences before it can be considered “standard practice”
  - ★ In particular: don’t know how much data or how many events are needed to get good ABI-dependent statistics
- If you want, `-stim_times_AM1` is also available
  - ★ It only builds the regressor proportional to ABI data directly, with no mean removed:
$$r_{AM1}(t) = \sum_{k=1}^K h(t - \tau_k) \cdot a_k$$
  - ★ Can’t imagine what value this option has, but you never know ... (if you can think of a good use, let me know)
- Future directions:
  - ★ Allow more than one amplitude to be married to each stimulus time (insert obligatory polygamy/polyandry joke here) – **this is done now**
    - How many ABI types at once is too many? I don’t know.
  - ★ How to deal with unknown nonlinearities in the BOLD response to ABI values? I don’t know. (Regress each event separately, then compute MI?)
  - ★ Deconvolution with amplitude modulation? Requires more thought.



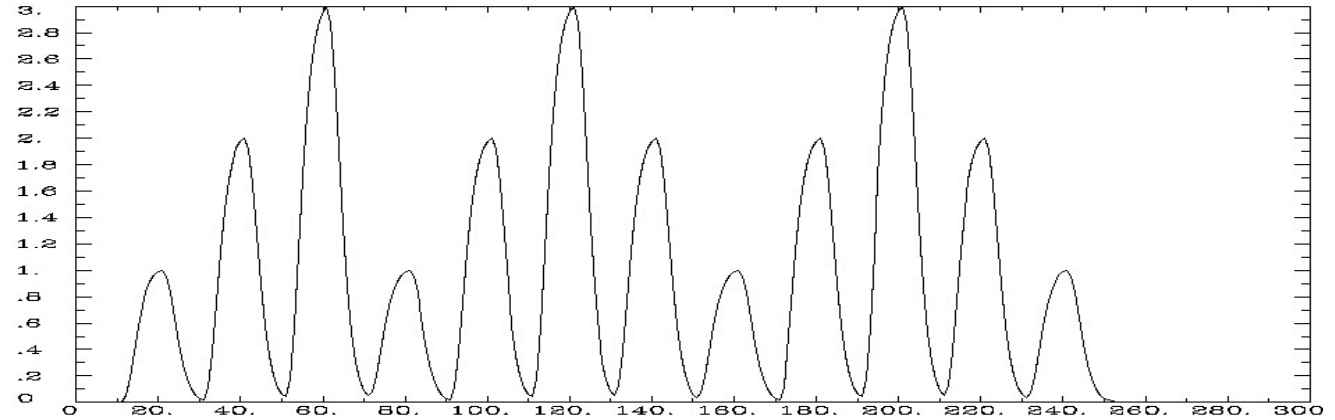
# AM Regression - 5

Timing: AM.1D = 10\*1 30\*2 50\*3 70\*1 90\*2 110\*3 130\*2 150\*1 170\*2 190\*3 210\*2 230\*1

- `3dDeconvolve -nodata 300 1.0 -num_stimts 1 \`  
`-stim_times_AM1 1 AM.1D 'BLOCK(10,1)' -x1D AM1.x1D`

- `1dplot AM1.x1D' [2]'`

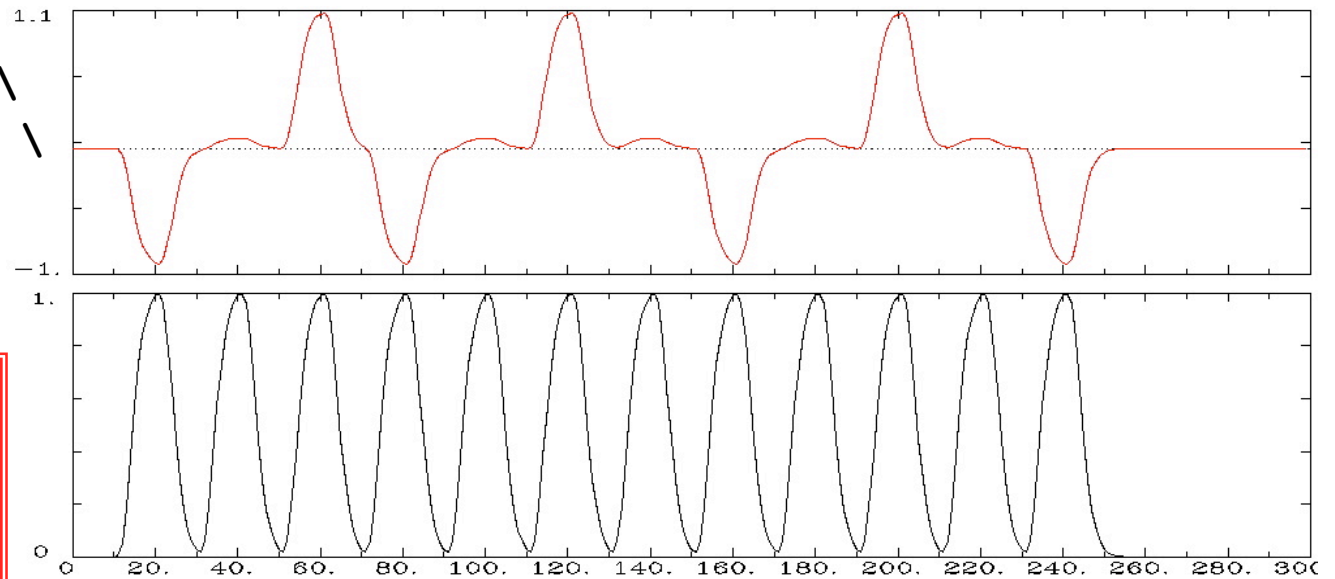
**AM1** model of signal  
(modulation = ABI)



- `3dDeconvolve -nodata 300 1.0 \`  
`-num_stimts 1 \`  
`-stim_times_AM2 1 \`  
`AM.1D 'BLOCK(10,1)' \`  
`-x1D AM2.x1D`

- `1dplot -sepscl \`  
`AM2.x1D' [2,3]'`

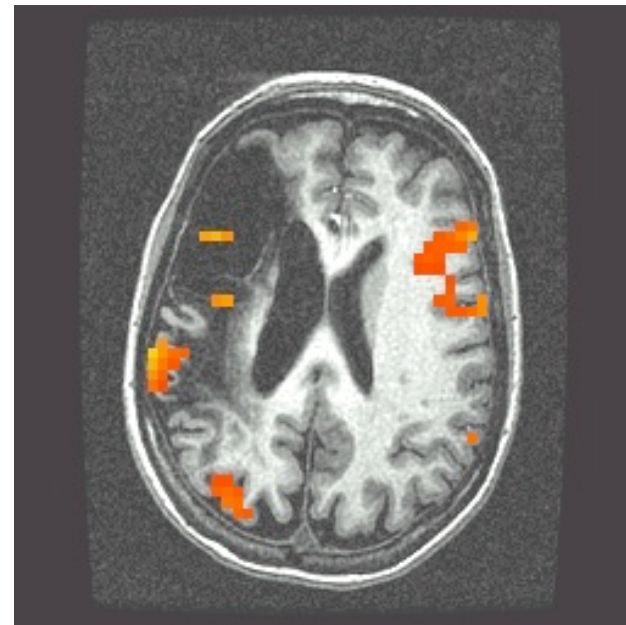
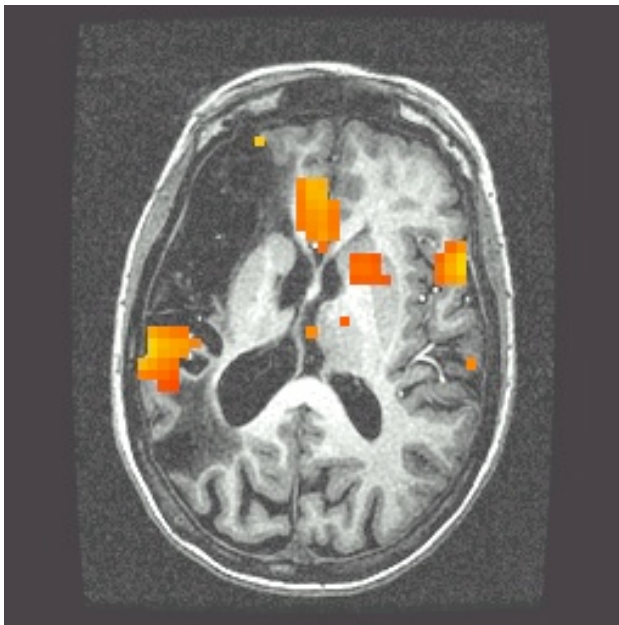
**AM2** model of signal:  
is 2D sub-space  
spanned by these 2  
time series





## AM Regression - 6

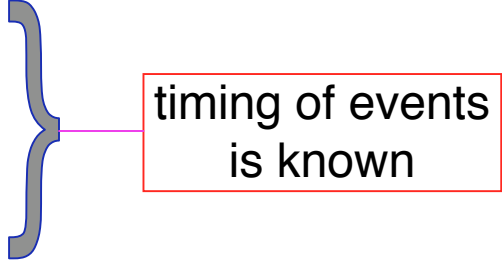
- First actual user: Whitney Postman (formerly NIDCD; PI=AI Braun)
- Picture naming task in aphasic stroke patient
- ABI data = number of alternative names for each image (e.g., “balcony” & “porch” & “veranda”, vs. “strawberry”), from 1 to 18
  - 8 imaging runs, 144 stimulus events
- 2 slices showing activation map for BOLD responses proportional to ABI ( $\beta_{AM2}$ )
  - What does this mean? Don't ask me!



## AM Regression - 7

- Alternative: use **IM** to get individual  $\beta$ s for each block/event and then do external regression statistics on those values
- Could do nonlinear fitting (to these  $\beta$ s) via **3dNLfim**, or inter-class contrasts via **3dtttest**, **3dLME**, **3dANOVA**, or intra-class correlations via **3dICC**, etc.
- What is better: **AM** or **IM**+*something more* ?
  - We don't know – experience with these options is limited thus far – you can always try both!
  - If **AM** doesn't fit your models/ideas, then **IM**+ is clearly the way to go
  - Probably need to consult with SSCC to get some hints/advice

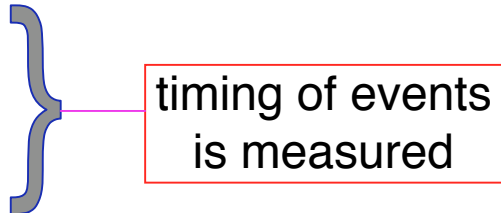
# Other Advanced Topics in Regression

- Can have activations with multiple phases that are not always in the same time relationship to each other; e.g.:
  - a) subject gets cue #1
  - b) variable waiting time (“hold”)
  - c) subject gets cue #2, emits response
    - ↳ which depends on both cue #1 and #2

timing of events is known
- ★ Cannot treat this as one event with one HRF, since the different waiting times will result in different overlaps in separate responses from cue #1 and cue #2
- ★ Solution is multiple HRFs: separate HRF (fixed shape or deconvolution) for cue #1 times and for cue #2 times
  - Must have significant variability in inter-cue waiting times, or will get a nearly-collinear model
    - ↳ impossible to tell tail end of HRF #1 from the start of HRF #2, if always locked together in same temporal relationship
  - How much variability is “significant”? Good question.

# Slightly More Complicated Case

- Solving a visually presented puzzle:
  - a) subject sees puzzle
  - b) subject cogitates a while
  - c) subject responds with solution
- The problem is that we expect some voxels to be significant in phase (b) as well as phases (a) and/or (c)
- Variable length of phase (b) means that shape for its response varies between trials
  - ★ Which is contrary to the whole idea of averaging trials together to get decent statistics (which is basically what linear regression for the  $\beta$  weights does, in an elaborate sort of way)
- Could assume response **amplitude** in phase (b) is constant across trials, and response **duration** varies directly with time between phases (a) and (c)
  - ★ Need three HRFs
  - ★ Can't generate (b) HRF in **3dDeconvolve**



Yes we can!  
-dmBLOCK model

# Noise Issues

- “Noise” in FMRI is caused by several factors, not completely characterized
  - ★ MR thermal noise (well understood, unremovable)
  - ★ Cardiac and respiratory cycles (partly understood)
    - In principle, could measure these sources of noise separately and then try to regress them out
      - ↳ RETROICOR program underway (Rasmus Birn of FIM/NIMH)
  - ★ Scanner fluctuations (e.g., thermal drift of hardware)
  - ★ Small subject head movements (10-100 mm)
  - ★ Very low frequency fluctuations (periods longer than 100 s)
- Data analysis should try to remove what can be removed and should allow for the statistical effects of what can't be removed
  - ★ “Serial correlation” in the noise time series affects the  $t$ - and  $F$ -statistics calculated by **3dDeconvolve**
  - ★ Next slides: new AFNI program for dealing with this issue

# Allowing for Serial Correlation

- $t$ - and  $F$ -statistics denominators: estimates of noise variance
    - ★ White noise estimate of variance:
      - $N$  = number of time points
      - $m$  = number of fit parameters
      - $N-m$  = degrees of freedom = how many equal-variance independent random values are left after the time series is fit with  $m$  regressors
- $$\hat{\sigma}^2 = \frac{1}{N-m} \sum_{i=0}^{N-1} [\text{data}_i - \text{fit}_i]^2$$
- **Problem:** if noise values at successive time points are correlated, this estimate of variance is biased to be too small, since there aren't really  $N-m$  independent random values left
    - ★ Denominator too small implies  $t$ - and  $F$ -statistics are too large!
    - ★ And number of degrees of freedom is also too large.
    - ★ So significance ( $p$ -value) of activations in individuals is overstated.
  - **Solution #1:** estimate correlation structure of noise and then adjust statistics (downwards) appropriately
  - **Solution #2:** estimate correlation structure of noise **and** also estimate  $\beta$  fit parameters using more efficient “generalized least squares”, using this correlation, all at once (REML method)

## New Program: 3dREMLfit

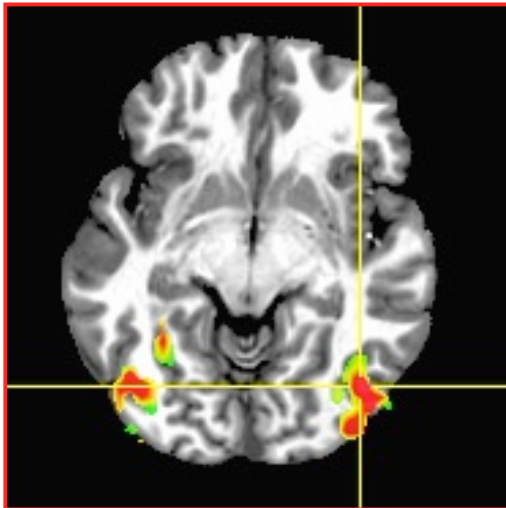
- Implements Solution #2
  - ★ REML is a method for simultaneously estimating variance + correlation parameters **and** estimating regression fit parameters ( $\beta$ s)
  - ★ Correlation structure of noise is ARMA(1,1)
    - 2 parameters **a** (AR) and **b** (MA) in each voxel
      - ➔ **a** describes how fast the noise de-correlates over time
      - ➔ **b** describes the short-range correlation in time (1 lag)
    - Unlike SPM and FSL, *each voxel* gets a separate estimate of its own correlation parameters
- Inputs to 3dREMLfit
  - ★ run 3dDeconvolve first to setup .xmat.1D matrix file and GLTs (don't have to let 3dDeconvolve finish analysis: `-x1D_stop`)
    - 3dDeconvolve also outputs a command line to run 3dREMLfit
  - ★ then, input matrix file and 3D+time dataset to 3dREMLfit
- Output datasets are similar to those in 3dDeconvolve



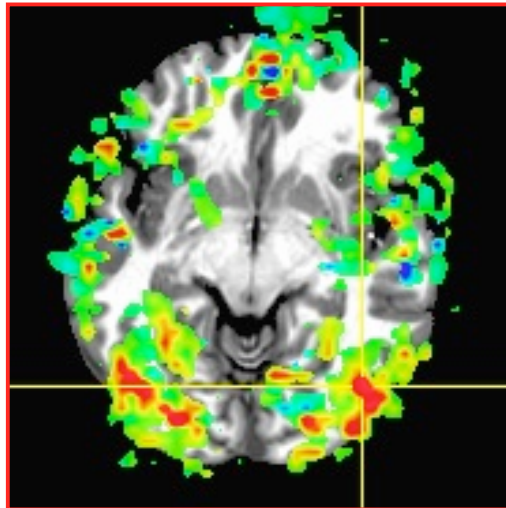
# Sample Outputs

- Compare with [AFNI\\_data3/afni/rall\\_regress](#) results
- `3dREMLfit -matrix rall_xmat.x1D -input rall_vr+orig -fout -tout \`  
`-Rvar rall_varR -Rbuck rall_funcR -Rfitts rall_fittsR \`  
`-Obuck rall_funcO -Ofitts rall_fittsO`

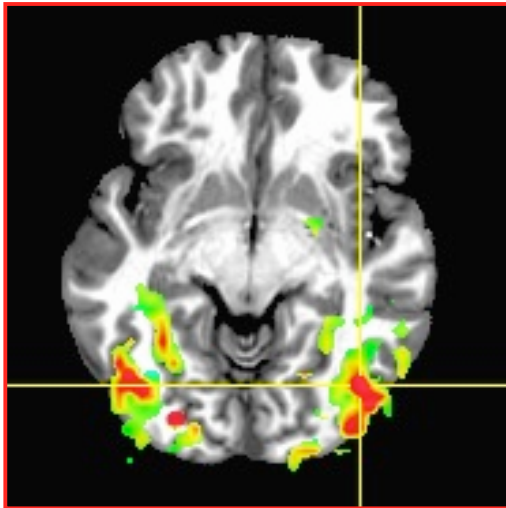
**REML**  
 $F=3.15$   
 $p=0.001$



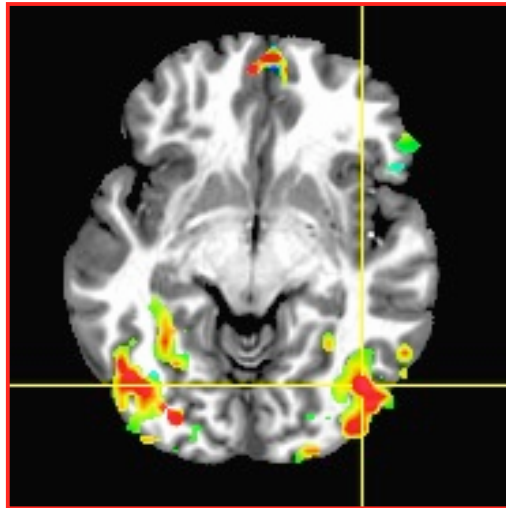
**OLSQ**  
 $F=3.15$   
 $p=0.001$



**REML**  
 $F=1.825$   
 $p=0.061$   
▪  $F$  = No activity outside brain!



**OLSQ**  
 $F=5.358$   
 $p=5e-7$   
▪  $F$  = No activity outside brain!



**O  
h  
M  
y  
G  
O  
D  
!?!**



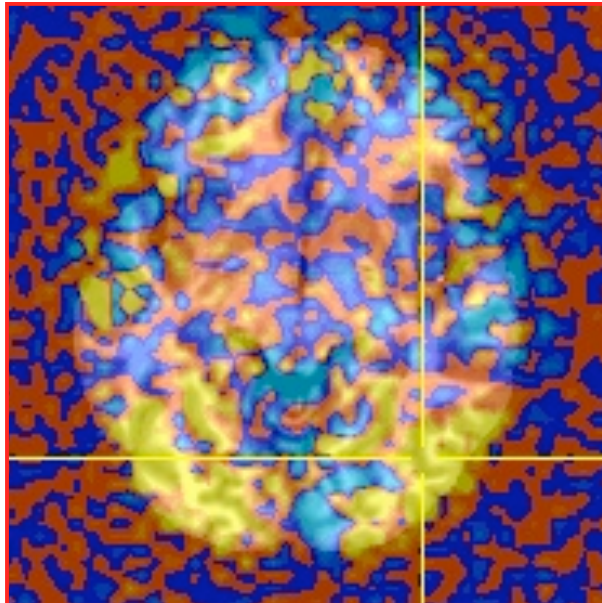
# It's Not So Bad: $\beta$ !

- For individual activation maps, **3dREMLfit**-ized  $t$ - and  $F$ -statistics are significantly different, and more accurate
- But ... There are at present very few applications for such individual FMRI activation maps
  - ★ pre-surgical planning; some longitudinal study?
- For standard group analysis, inputs are only  $\beta$  fit parameters
  - ★ Which don't change so much between REML and OLSQ

**Color Overlay =  $\beta$  weight from analysis on previous slide, no threshold**

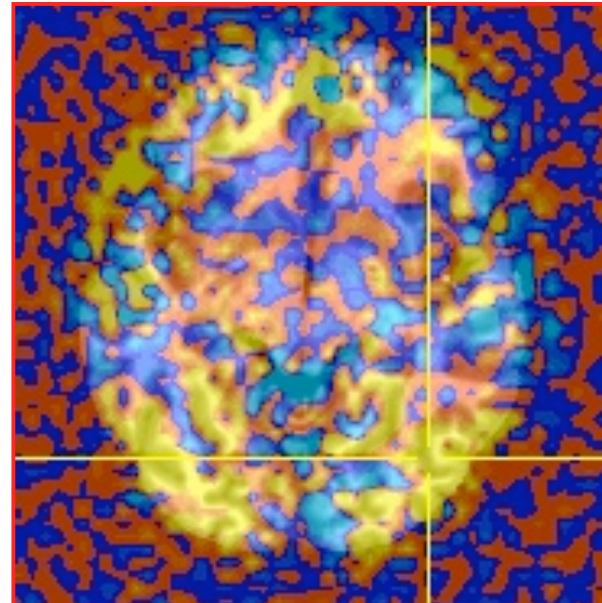
**REML**

**CPU  
500 s**



**OLSQ**

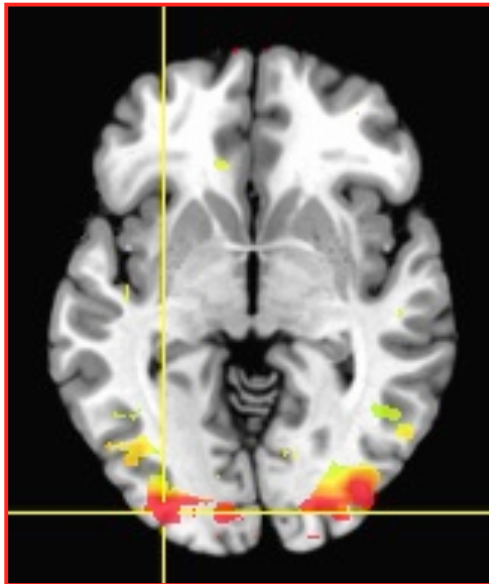
**CPU  
156 s**



# It's Not So Bad At All: Group Analysis!

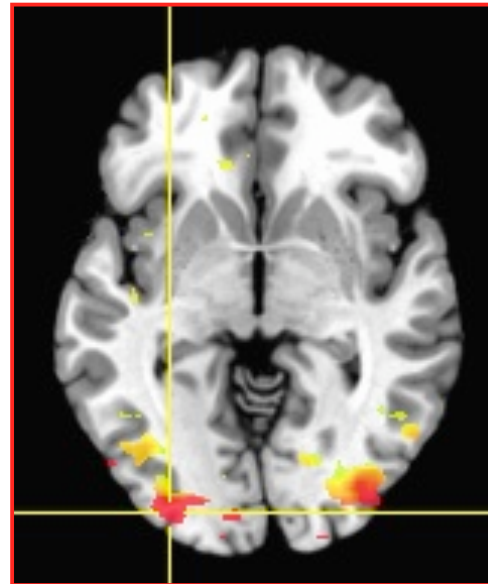
- Group analysis activation maps (**3dANOVA3**) from 16 subjects

**REML**



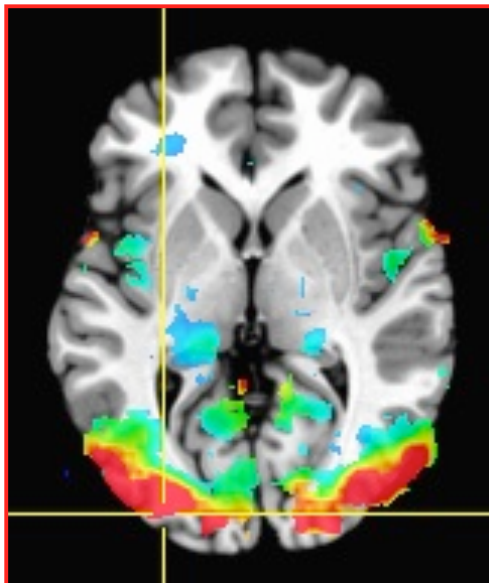
**F-test for  
Affect  
condition**

**OLSQ**

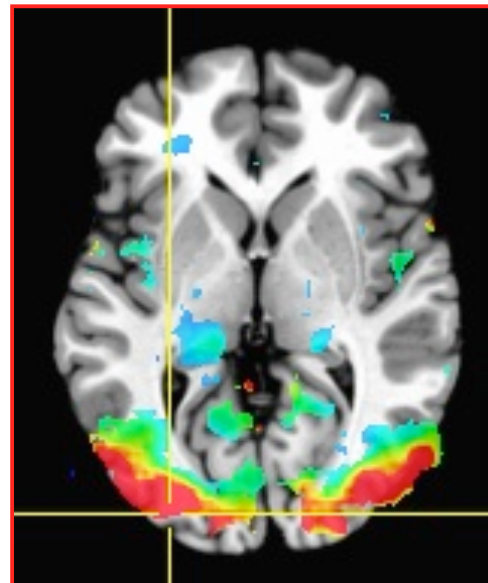


**F-test for  
Affect  
condition**

**F-test for  
Category  
condition**



**F-test for  
Category  
condition**



# Nonlinear Regression

- Linear models aren't the only possibility
  - ★ e.g., could try to fit HRF of the form  $h(t) = a \cdot t^b \cdot e^{-t/c}$
  - ★ Unknowns  $b$  and  $c$  appear nonlinearly in this formula
- Program **3dNLFim** can do nonlinear regression (including nonlinear deconvolution)
  - ★ User must provide a C function that computes the model time series, given a set of parameters (e.g.,  $a$ ,  $b$ ,  $c$ )
    - We could help you develop this C model function
    - Several sample model functions in the AFNI source code distribution
  - ★ Program then drives this C function repeatedly, searching for the set of parameters that best fit each voxel
  - ★ Has been used to fit pharmacological wash-in/wash-out models (difference of two exponentials) to fMRI data acquired during pharmacological challenges
    - e.g., injection of nicotine, cocaine, ethanol, etc.
    - these are difficult experiments to do **and** to analyze

## Deconvolution: The Other Direction

- Signal model:  $Z(t) = H(t)*A(t)$  + baseline model + noise
- $H(t)$  = HRF = response magnitude  $t$  seconds after activation
  - ★  $H(t)$  is **causal** = zero for  $t < 0$
  - ★ “\*” is symbol for convolution, not multiplication!
- **3dDeconvolve**: find out something about  $H(t)$  given  $A(t)$
- Sometimes (PPI) want to solve the problem in the other direction: assume a model for  $H(t)$  and find time series  $A(t)$ 
  - ★ Convolution is commutative:  $H(t)*A(t) = A(t)*H(t)$
  - ★ So the other direction looks to be the same problem
  - ★ But isn't, since  $H(t)$  is causal but  $A(t)$  is not
    - Also,  $H(t)*A(t)$  smooths out rough spots in  $A(t)$ , so undoing this deconvolution adds roughness to the data — including noise, which is already rough — which must be controlled or output  $A(t)$  will be junk
- Program **3dTfitter** solves this type of problem
  - ★ Also can allow for *per voxel* baseline model components

# Spatial Models of Activation

- Smooth data in space before analysis

---

- Average data across anatomically-selected regions of interest ROI (before or after analysis)
  - Labor intensive (*i.e.*, hire more students)

---

- Reject isolated small clusters of above-threshold voxels after analysis

# Spatial Smoothing of Data

- Reduces number of comparisons

---

- Reduces noise (by averaging)

---

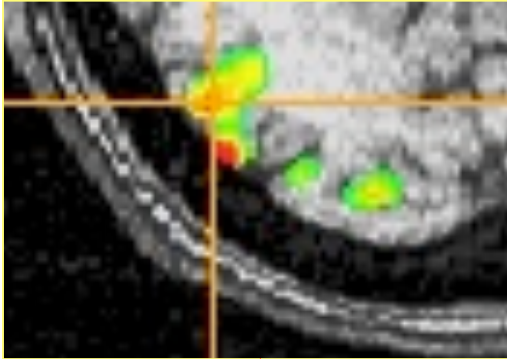
- Reduces spatial resolution
  - Blur it enough: Can make fMRI results look like low resolution (1990s) PET data

---

- Smart smoothing: average **only** over nearby brain or gray matter voxels
  - Uses resolution of fMRI cleverly
    - **3dBlurToFWHM** and **3dBlurInMask**
  - Or: average over selected ROIs
  - Or: cortical surface based smoothing



## 3dBlurToFWHM

- New program to smooth FMRI time series datasets to a specified smoothness (as estimated by FWHM of noise spatial correlation function)
  - ★ Don't just add smoothness (à la **3dmerge**) but control it (locally and globally)
  - ★ Goal: use datasets from diverse scanners
- Why blur FMRI time series?
  - ★ Averaging neighbors will reduce noise
  - ★ Activations are (usually) blob-ish (several voxels across) 
  - ★ Diminishes the multiple comparisons problem
- **3dBlurToFWHM** and **3dBlurInMask** blur only inside a mask region
  - ★ To avoid mixing air (noise-only) and brain voxels
  - ★ Partial Differential Equation (PDE) based blurring method
    - 2D (intra-slice) or 3D blurring

# Spatial Clustering

- Analyze data, create statistical map (e.g.,  $t$  statistic in each voxel)

---

- Threshold map at a low  $t$  value, in each voxel separately
  - Will have many false positives

---

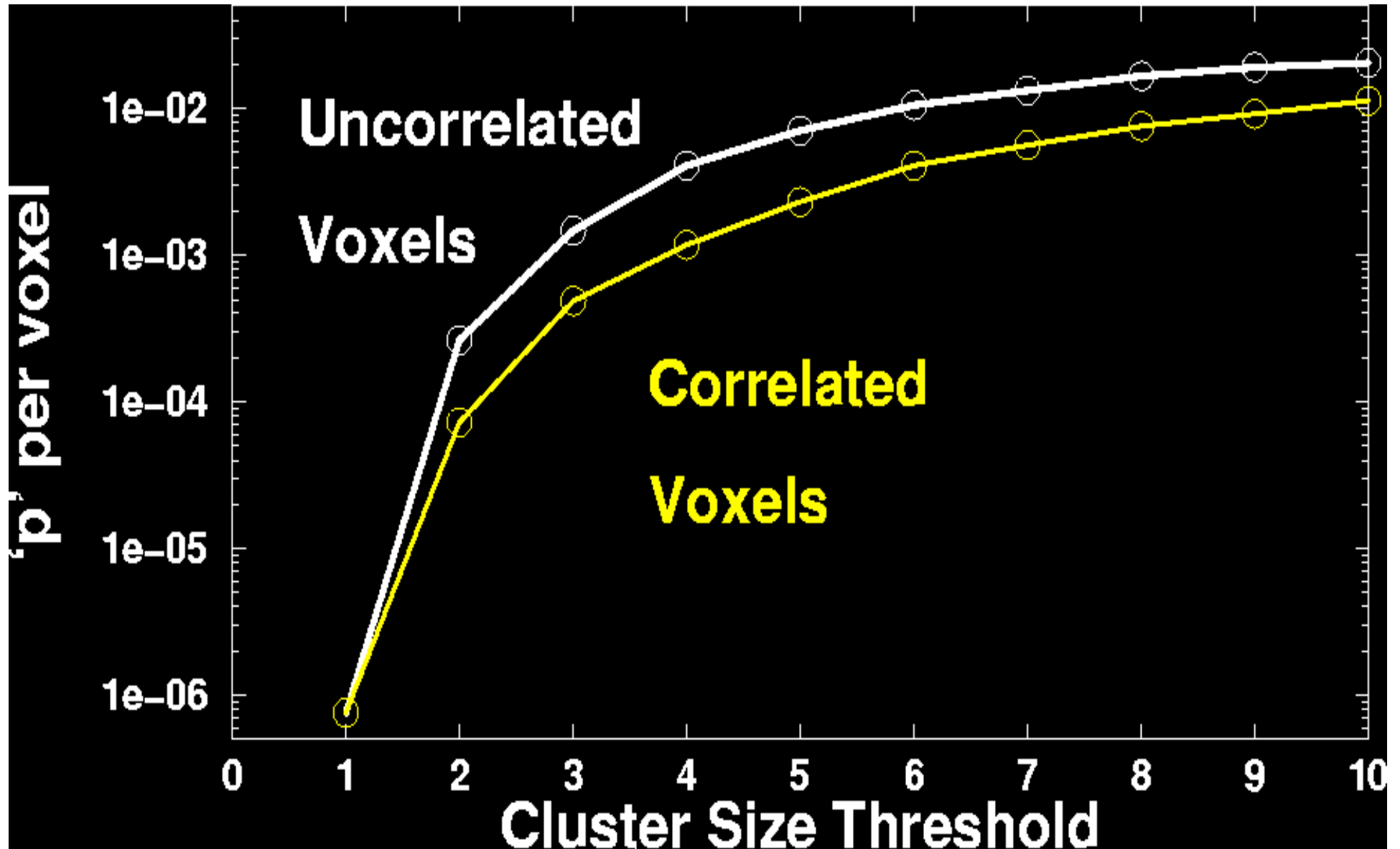
- Threshold map by rejecting clusters of voxels below a given size

---

- Can control false-positive rate by adjusting  $t$  threshold and cluster-size thresholds together



# Cluster-Based Detection






# What the World Needs Now

- Unified HRF/Deconvolution ⊕ Blob analysis
  - Time ⊕ Space patterns computed all at once, instead of arbitrary spatial smoothing
    - Increase statistical power by bringing data from multiple voxels together cleverly
  - Instead of time analysis followed by spatial analysis (described earlier)
  - Instead of component-style analyses (e.g., ICA) that do not use stimulus timing

---
- Difficulty: models for spatial blobs
  - Little information *à priori* ⇒ must be adaptive

## In the Thinking Stages

- “Area under curve” addition to `-gltsym` to allow testing of pieces of HRF models from `-stim_times`
- Slice- and/or voxel-dependent regressors
  - ★ For physiological noise cancellation, etc. 
  - ★ To save memory? (Could process each slice separately)
    - One slice-at-a-time regression can be done in a Unix script, using 3dZcutup and 3dZcat programs 
- Extend AM regression to allow for more than 1 piece of auxiliary information at each stimulus time 
- Interactive tool to examine `-x1D` matrix for problems
  - ★ and `3dDeconvolve` testing of GLT submatrices
- Semi-linear deconvolution program

# Multi-Voxel Statistics

Spatial Clustering  
&

False Discovery Rate:

“Correcting” the Significance

## Basic Problem

- Usually have 20-100K FMRI voxels in the brain
- Have to make at least one decision about each one:
  - ★ Is it “active”?
    - That is, does its time series match the temporal pattern of activity we expect?
  - ★ Is it differentially active?
    - That is, is the BOLD signal change in task #1 different from task #2?
- Statistical analysis is designed to control the error rate of these decisions
  - ★ Making *lots* of decisions: hard to get perfection in statistical testing

# Multiple Testing Corrections

• **Two types of errors**

- ★ **What is  $H_0$  in FMRI studies?**  $H_0$ : no effect (activation, difference, ...) at a voxel
- ★ Type I error = Prob(reject  $H_0$  when  $H_0$  is true) = false positive =  $p$  value
- Type II error = Prob(accept  $H_0$  when  $H_1$  is true) = false negative =  $\beta$
- power** =  $1-\beta$  = probability of detecting true activation
- ★ Strategy: controlling type I error while increasing power (decreasing type II errors)
- ★ Significance level  $\alpha$  (magic number 0.05) :  $p < \alpha$

## Justice System: Trial

Hidden Truth

|                                                                 | Defendant Innocent                              | Defendant Guilty                               |
|-----------------------------------------------------------------|-------------------------------------------------|------------------------------------------------|
| Reject<br>Presumption of Innocence<br>(Guilty Verdict)          | <b>Type I Error</b><br>(defendant very unhappy) | <b>Correct</b>                                 |
| Fail to Reject<br>Presumption of Innocence (Not Guilty Verdict) | <b>Correct</b>                                  | <b>Type II Error</b><br>(defendant very happy) |

## Statistics: Hypothesis Test

Hidden Truth

|                                                      | $H_0$ True<br>Not Activated             | $H_0$ False<br>Activated                 |
|------------------------------------------------------|-----------------------------------------|------------------------------------------|
| Reject $H_0$<br>(decide voxel is activated)          | <b>Type I Error</b><br>(false positive) | <b>Correct</b>                           |
| Don't Reject $H_0$<br>(decide voxel isn't activated) | <b>Correct</b>                          | <b>Type II Error</b><br>(false negative) |

- **Family-Wise Error (FWE)**

- ★ Multiple testing problem: voxel-wise statistical analysis

- With  $N$  voxels, what is the chance to make a false positive error (Type I) in one or more voxels?

**Family-Wise Error:**  $\alpha_{FW} = 1 - (1 - p)^N \rightarrow 1$  as  $N$  increases

- For  $N \cdot p$  small (compared to 1),  $\alpha_{FW} \approx N \cdot p$
- $N \approx 20,000+$  voxels in the brain
- To keep probability of even one false positive  $\alpha_{FW} < 0.05$  (the “corrected”  $p$ -value), need to have  $p < 0.05 / 2 \times 10^4 = 2.5 \times 10^{-6}$
- This constraint on the per-voxel (“uncorrected”)  $p$ -value is so stringent that we’ll end up rejecting a lot of true positives (Type II errors) also, just to be safe on the Type I error rate

- **Multiple testing problem in FMRI**

- ★ 3 occurrences of multiple tests: individual, group, and conjunction
- ★ Group analysis is the most severe situation (have the least data, considered as number of independent samples = subjects)

- **Two Approaches to the “Curse of Multiple Comparisons”**
  - ★ Control **FWE** to keep expected total number of false positives below 1
    - Overall significance:  $\alpha_{FW} = \text{Prob}(\geq \text{one false positive voxel in the whole brain})$
    - **Bonferroni correction**:  $\alpha_{FW} = 1 - (1-p)^N \approx Np$ , if  $p \ll N^{-1}$ 
      - Use  $p = \alpha/N$  as individual voxel significance level to achieve  $\alpha_{FW} = \alpha$
      - Too stringent and overly conservative:  $p = 10^{-8} \dots 10^{-6}$
    - Something to rescue us from this hell of statistical super-conservatism?
      - Correlation: Voxels in the brain are not independent
        - Especially after we smooth them together!
        - Means that Bonferroni correction is *way way* too stringent
      - Contiguity: Structures in the brain activation map
        - We are looking for activated “blobs”: the chance that pure noise ( $H_0$ ) will give a set of seemingly-activated voxels next to each other is lower than getting false positives that are scattered around far apart
        - Control FWE based on spatial correlation (smoothness of image noise) **and** minimum cluster size we are willing to accept

---

  - ★ Control false discovery rate (**FDR**)
    - FDR = expected proportion of false positive voxels among all **detected** voxels
      - Give up on the idea of having (almost) no false positives at all



# Cluster Analysis: **AlphaSim**

- **FWE control in AFNI**

- ★ Monte Carlo simulations with program **AlphaSim**

- Named for a place where primary attractions are randomization experiments
- Randomly generate some number (*e.g.*, 1000) of brain volumes with white noise (spatially uncorrelated)
  - That is, each “brain” volume is purely in  $H_0$  = no activation
  - Noise images can be blurred to mimic the smoothness of real data
- Count number of voxels that are false positives in each simulated volume
  - Including how many are false positives that are spatially together in clusters of various sizes (1, 2, 3, ...)
- Parameters to program
  - Size of dataset to simulate
  - Mask (*e.g.*, to consider only brain-shaped regions in the 3D brick)
  - Spatial correlation FWHM: from **3dBlurToFWHM** or **3dFWHMx**
  - Connectivity radius: how to identify voxels belonging to a cluster?
    - Default = NN connection = touching faces
  - Individual voxel significance level = uncorrected  $p$ -value
- Output
  - Simulated (estimated) **overall significance level** (corrected  $p$ -value)
  - Corresponding **minimum cluster size** at the input uncorrected  $p$ -value

• **Example:** `AlphaSim -nxyz 64 64 20 -dxyz 3 3 5 \`  
`-fwhm 5 -pthr 0.001 -iter 1000 -quiet -fast`

- Output is in 6 columns: focus on 1<sup>st</sup> and 6<sup>th</sup> columns (ignore others)
  - ★ 1<sup>st</sup> column: cluster size in voxels
  - ★ 6<sup>th</sup> column: alpha ( $\alpha$ ) = overall significance level = corrected  $p$ -value

| Cl | Size | Frequency | CumuProp | p/Voxel    | Max Freq | Alpha           |
|----|------|-----------|----------|------------|----------|-----------------|
|    | 1    | 47064     | 0.751113 | 0.00103719 | 0        | 1.000000        |
|    | 2    | 11161     | 0.929236 | 0.00046268 | 13       | 1.000000        |
|    | 3    | 2909      | 0.975662 | 0.00019020 | 209      | 0.987000        |
|    | 4    | 1054      | 0.992483 | 0.00008367 | 400      | 0.778000        |
|    | 5    | 297       | 0.997223 | 0.00003220 | 220      | 0.378000        |
|    | 6    | 111       | 0.998995 | 0.00001407 | 100      | 0.158000        |
|    | 7    | 32        | 0.999505 | 0.00000594 | 29       | 0.058000        |
|    | 8    | 20        | 0.999825 | 0.00000321 | 19       | <u>0.029000</u> |
|    | 9    | 8         | 0.999952 | 0.00000126 | 7        | 0.010000        |
|    | 10   | 2         | 0.999984 | 0.00000038 | 2        | 0.003000        |
|    | 11   | 1         | 1.000000 | 0.00000013 | 1        | 0.001000        |

- At this uncorrected  $p=0.001$ , in this size volume, with noise of this smoothness: the chance of a cluster of size 8 *or larger* occurring by chance alone is 0.029
- May have to run several times with different uncorrected  $p$ 
  - uncorrected (`-pthr`)  $p \uparrow \Leftrightarrow$  required minimum cluster size  $\uparrow$
- See detailed steps at <http://afni.nimh.nih.gov/sscc/gangc/mcc.html>

# Interactive Clustering

The screenshot displays the AFNI software interface with several key components:

- Control Panels:** Includes 'Original View' (AC-PC, Talairach), 'Define Markers', 'Define Overlay', 'Define Datanode', 'Switch Session', 'UnderLayer', 'EditEnv', 'OverLayer', 'NIML+PD', and 'Control Surface'.
- Cluster Edit Panel:** Features a color scale for correlation (0.3486 to 1.00), 'Clusterize' button, and parameters for background (bkgd:ULay), overlay (OLay), fit coefficient (Fit Coef), and threshold (Thr). It also shows 'autoRange: 3.76879' and 'Rota' options.
- Cluster Report Panel:** A table listing 7 clusters with their voxel counts and coordinates. A callout box points to this panel with the text: "Report on clusters of above threshold voxels".
- Timeseries Plot:** A line graph titled "Mean timeseries over cluster #2" showing signal intensity over 70 TR indices. The y-axis is labeled "Mean: Cluster #2 = 134 voxels".
- Clustering Parameters Panel:** A 'menu' window titled "Set Clusterize Parameters" with fields for 'rnn' (0) and 'vnul' (20), and buttons for 'Quit', 'Apply', and 'Set'. A callout box points to this panel with the text: "This panel controls the clustering operation".

## False Discovery Rate in



- Situation: making *many* statistical tests at once
  - e.g., Image voxels in FMRI; associating genes with disease
- Want to set threshold on statistic (e.g., *F*- or *t*-value) to control **false positive** error rate
- Traditionally: set threshold to control probability of making a **single** false positive detection
  - But if we are doing 1000s (or more) of tests at once, we have to be very stringent to keep this probability low
- **FDR**: accept the fact that there will be multiple erroneous detections when making lots of decisions
  - Control the **fraction** of positive detections that are wrong
    - Of course, no way to tell which individual detections are right!
  - Or at least: control the *expected value* of this fraction

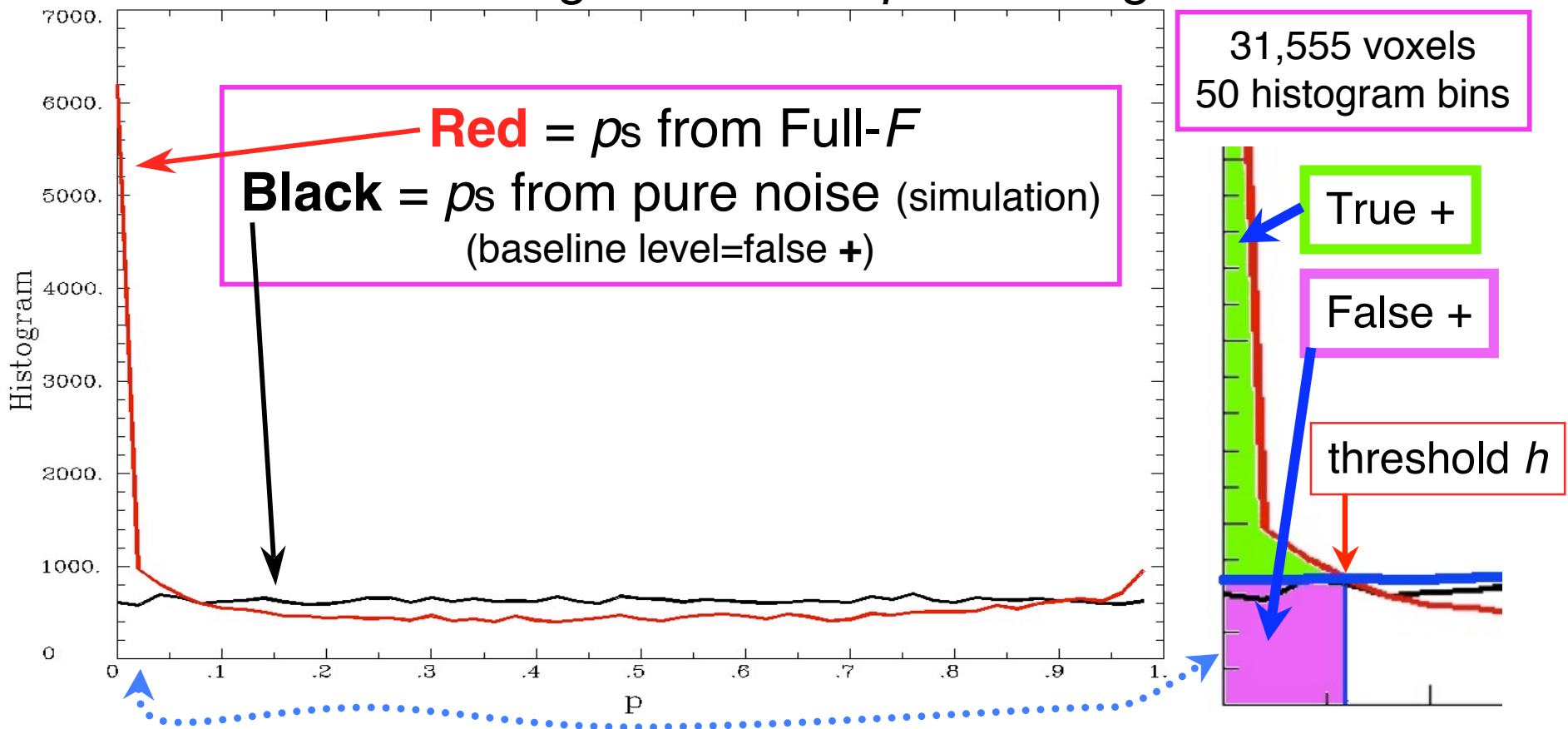
## FDR: $q$ [and $z(q)$ ]

- Given some collection of statistics (say,  $F$ -values from **3dDeconvolve**), set a threshold  $h$
- The **uncorrected  $p$ -value** of  $h$  is the probability that  $F > h$  when the null hypothesis is true (no activation)
  - “Uncorrected” means “per-voxel”
  - The “corrected”  $p$ -value is the probability that *any* voxel is above threshold in the case that they are all *unactivated*
  - If have  $N$  voxels to test,  $p_{\text{corrected}} = 1 - (1 - p)^N \approx Np$  (for small  $p$ )
    - o Bonferroni: to keep  $p_{\text{corrected}} < 0.05$ , need  $p < 0.05 / N$ , which is very tiny
- The FDR  **$q$ -value** of  $h$  is the fraction of false positives expected when we set the threshold to  $h$ 
  - Smaller  $q$  is “better” (more stringent = fewer false detections)
  - $z(q)$  = conversion of  $q$  to Gaussian  $z$ -score: e.g,  $z(0.05) \approx 1.95996$ 
    - o So that larger is “better” (in the same sense): e.g,  $z(0.01) \approx 2.57583$



# Basic Ideas Behind FDR $q$

- **If** all the null hypotheses are true, **then** the statistical distribution of the  $p$ -values will be uniform
  - Deviations from uniformity at low  $p$ -values  $\Rightarrow$  true positives
  - Baseline of uniformity indicates how many true negatives are hidden amongst in the low  $p$ -value region

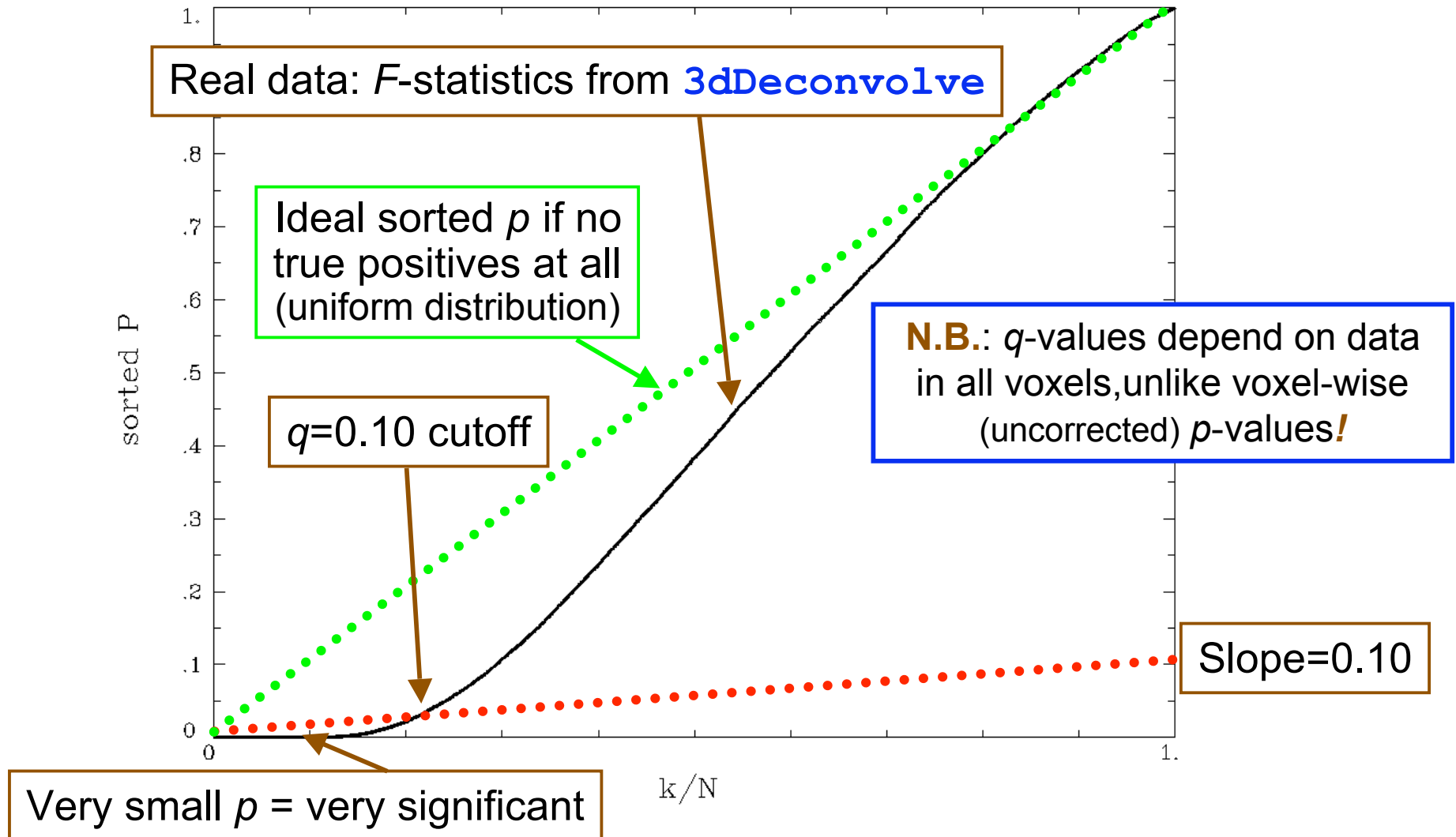


# How $q$ is Calculated from Data

- Compute  $p$ -values of each statistic:  $P_1, P_2, P_3, \dots, P_N$
- Sort these:  $P_{(1)} \leq P_{(2)} \leq P_{(3)} \leq \dots \leq P_{(N)}$  {subscript<sub>( )</sub>  $\equiv$  sorted}
- For  $k = 1..N$ ,  $q_{(k)} = \min_{m \geq k} [ N \cdot P_{(m)} / m ]$ 
  - Easily computed from sorted  $p$ -values by looping downwards from  $k = N$  to  $k = 1$
- By keeping track of voxel each  $P_{(k)}$  came from: can put  $q$ -values (or  $z(q)$  values) back into image
  - This is exactly how program **3dFDR** works
- By keeping track of statistic value ( $t$  or  $F$ ) each  $P_{(k)}$  came from: can create curve of threshold  $h$  vs.  $z(q)$
- **N.B.:**  $q$ -values depend on the data in ***all*** voxels, unlike these voxel-wise (uncorrected)  $p$ -values!
  - Which is why it's important to mask brain properly

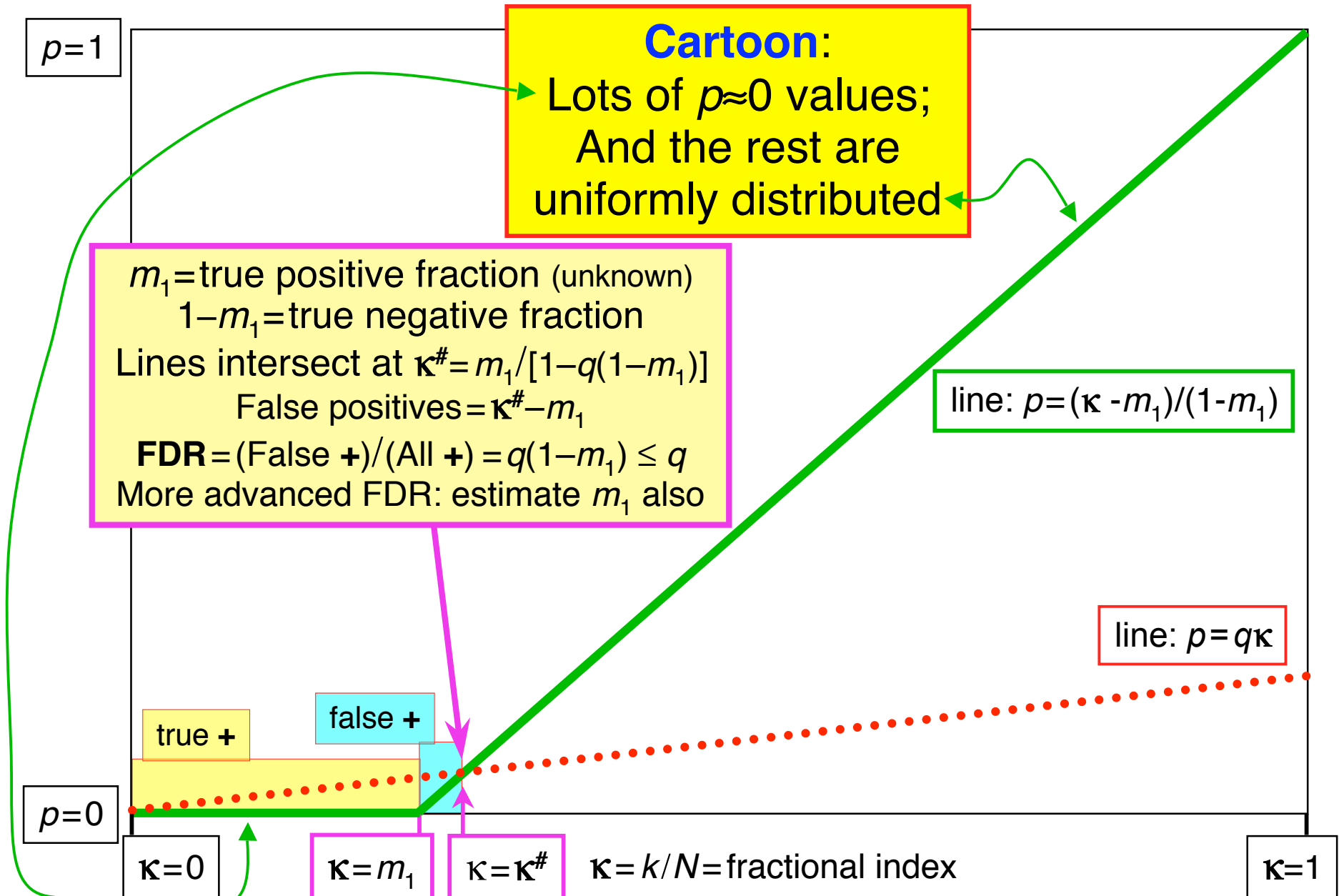
# Graphical Calculation of $q$

- Graph sorted  $p$ -values of voxel # $k$  vs.  $\kappa=k/N$  (the cumulative histogram of  $p$ , flipped sideways) and draw some lines from origin

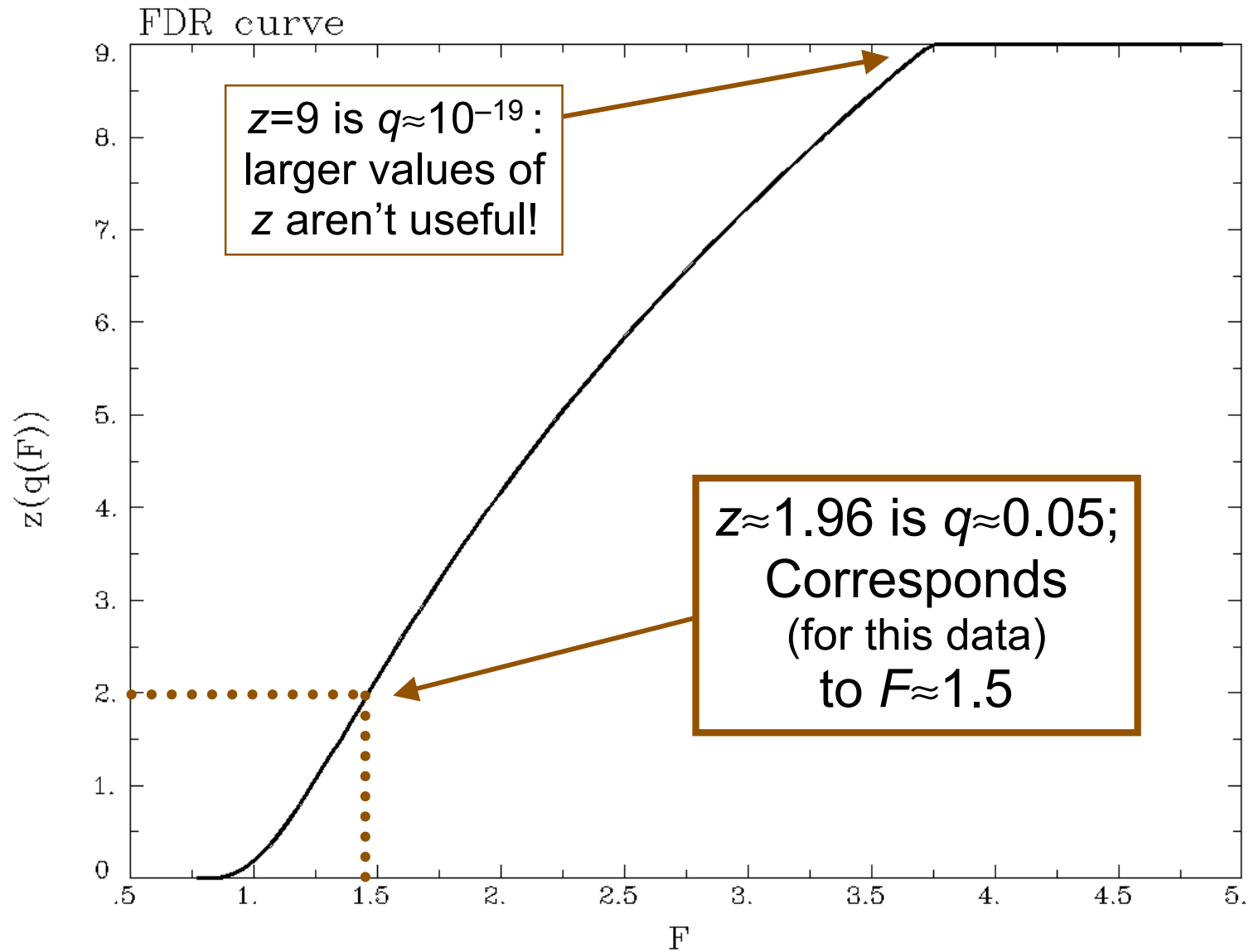




# Why This Line-Drawing Works



# Same Data: threshold $F$ vs. $z(q)$

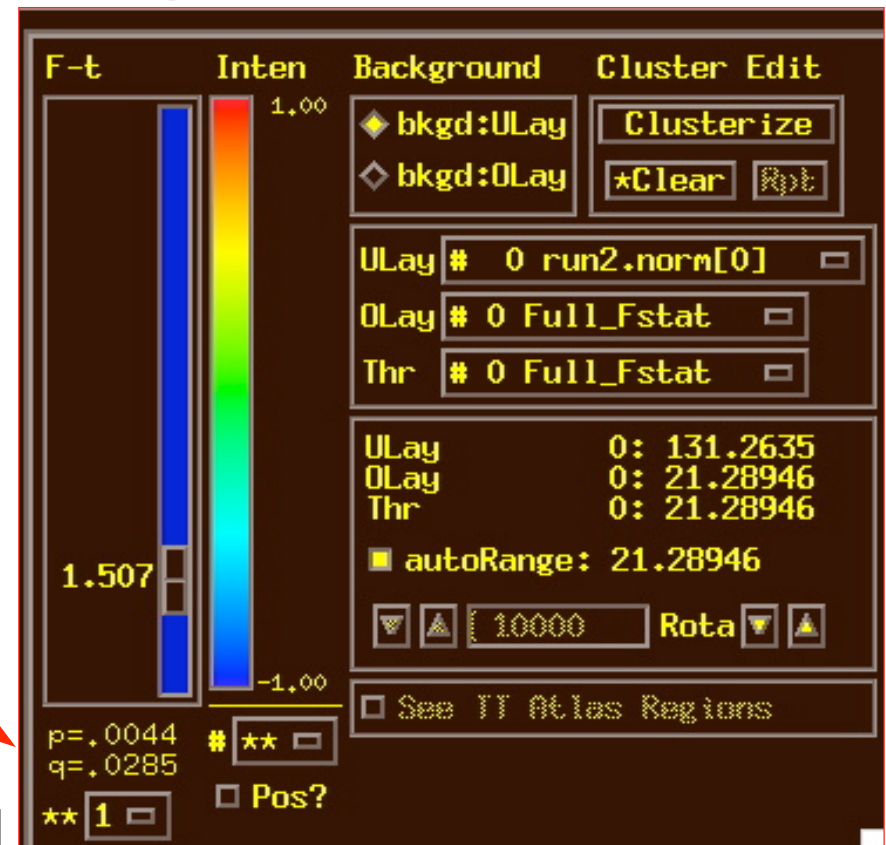


## Recent Changes to 3dFDR

- Don't include voxels with  $p=1$  (e.g.,  $F=0$ ), even if they are in the **-mask** supplied on the command line
  - This change decreases  $N$ , which will decrease  $q$  and so increase  $z(q)$ : recall that  $q_{(k)} = \min_{m \geq k} [N \cdot P_{(m)} / m]$
- Sort with Quicksort algorithm
  - Faster than the bin-based sorting in the original code
  - Makes a big speed difference on large 1 mm<sup>3</sup> datasets
    - Not much speed difference on small 3 mm<sup>3</sup> grids, since there aren't so many voxels to sort
- Default mode of operation is '**-new**' method
  - Prints a warning message to let user know things have changed from the olden days
  - User can use '**-old**' method if desired

# FDR curves: $h$ vs. $z(q)$

- **3dDeconvolve**, **3dANOVAX**, **3dttest**, and **3dNLfim** now compute FDR curves for all statistical sub-bricks and store them in output header
  - **3drefit -addFDR** does same for other datasets
    - **3drefit -unFDR** can be used to delete such info
  - **AFNI** now shows  $p$ - and  $q$ -values below the threshold slider bar
    - Interpolates FDR curve from header (threshold  $\rightarrow z \rightarrow q$ )
      - Can be used to adjust threshold by “eyeball”
- $q = \text{N/A}$  means it's not available
- MDF hint = “missed detection fraction”



# FDR Statistical Issues

- FDR is conservative ( $q$ -values are too large) when voxels are positively correlated (e.g., from spatially smoothing)
  - Correcting for this is not so easy, since  $q$  depends on data (including true positives), so a simulation like **AlphaSim** is hard to conceptualize
  - At present, FDR is an alternative way of controlling false positives, vs. **AlphaSim** (clustering)
    - Thinking about how to combine FDR and clustering
- Accuracy of FDR calculation depends on  $p$ -values being uniformly distributed under the null hypothesis
  - Statistic-to- $p$  conversion should be accurate, which means that null  $F$ -distribution (say) should be correctly estimated
  - Serial correlation in FMRI time series means that **3dDeconvolve** denominator DOF is too large
  - $\Rightarrow$   $p$ -values will be too small, so  $q$ -values will be too small
    - **3dREMLfit** can ride to the rescue!

# FWE or FDR?

- These 2 methods control Type I error in different sense
  - ★ FWE:  $\alpha_{FW} = \text{Prob} (\geq \text{one false positive voxel/cluster in the whole brain})$ 
    - Frequentist's perspective: Probability among **many** hypothetical activation maps gathered under identical conditions
    - Advantage: can directly incorporate smoothness into estimate of  $\alpha_{FW}$
  - ★ FDR = expected fraction of false positive voxels among all detected voxels
    - Focus: controlling false positives among detected voxels in **one** activation map, as given by the experiment at hand
    - Advantage: not afraid of making a few Type I errors in a large field of true positives
  - ★ Concrete example
    - Individual voxel  $p = 0.001$  for a brain of 25,000 EPI voxels
    - Uncorrected  $\rightarrow \approx 25$  false positive voxels in the brain
    - FWE: corrected  $p = 0.05 \rightarrow \approx 5\%$  of the time would expect one or more false positive clusters in the entire volume of interest
    - FDR:  $q = 0.05 \rightarrow \approx 5\%$  of voxels among those **positively** labeled ones are false positive
- What if your favorite blob fails to survive correction?
  - ★ Tricks (don't tell anyone we told you about these)
    - One-tail  $t$ -test?
    - ROI-based statistics – e.g., grey matter mask, or whatever regions you focus on
  - ★ Analysis on surface; or, Use better group analysis tool (3dLME, 3dMEMA, etc.)

# Conjunction Analysis

- **Conjunction**

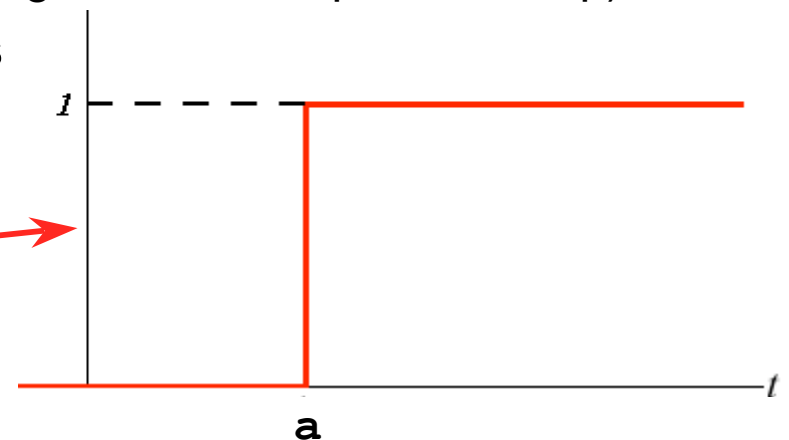
- ★ Dictionary: “a compound proposition that is true if and only if all of its component propositions are true”
- ★ FMRI: areas that are active under 2 or more conditions (**AND** logic)
  - e.g, in a visual language task and in an auditory language task
- ★ Can also be used to mean analysis to find areas that are exclusively activated in one task but not another (**XOR** logic) or areas that are active in either task (non-exclusive **OR** logic)
- ★ If have  $n$  different tasks, have  $2^n$  possible combinations of activation overlaps in each voxel (ranging from nothing there to complete overlap)

- ★ Tool: **3dcalc** applied to statistical maps

- Heaviside **step function** defines a *On/Off* logic

- $\text{step}(t-a) = 0$  if  $t < a$   
           $= 1$  if  $t > a$

- Can be used to apply more than one threshold at a time



- Example of forming all possible conjunctions

- ★ 3 contrasts/tasks A, B, and C, each with a *t*-stat from **3dDeconvolve**

- ★ Assign each a number, based on binary positional notation:

- A:  $001_2 = 2^0 = \mathbf{1}$  ; B:  $010_2 = 2^1 = \mathbf{2}$  ; C:  $100_2 = 2^2 = \mathbf{4}$

- ★ Create a mask using 3 sub-bricks of *t* (e.g., threshold = 4.2)

```
3dcalc -a ContrA+tlrc -b ContrB+tlrc -c ContrC+tlrc \  
-expr '1*step(a-4.2)+2*step(b-4.2)+4*step(c-4.2)' \  
-prefix ConjAna
```

- ★ Interpret output, which has 8 possible ( $=2^3$ ) scenarios:

- 000<sub>2</sub> = **0**: none are active at this voxel

- 001<sub>2</sub> = **1**: A is active, but no others

- 010<sub>2</sub> = **2**: B, but no others

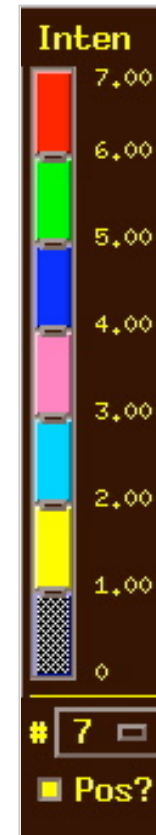
- 011<sub>2</sub> = **3**: A and B, but not C

- 100<sub>2</sub> = **4**: C but no others

- 101<sub>2</sub> = **5**: A and C, but not B

- 110<sub>2</sub> = **6**: B and C, but not A

- 111<sub>2</sub> = **7**: A, B, and C are all active at this voxel



Can display each combination with a different color and so make pretty pictures that *might* even mean something!



- **Multiple testing correction issue**

- ★ How to calculate the  $p$ -value for the conjunction map?
- ★ No problem, *if* each entity was corrected (e.g., cluster-size thresholded at  $t=4.2$ ) before conjunction analysis, via **AlphaSim**
- ★ But that may be too stringent (conservative) and over-corrected
- ★ With 2 or 3 entities, analytical calculation of conjunction  $p_{\text{conj}}$  is possible
  - Each individual test can have different uncorrected (per-voxel)  $p$
  - Double or triple integral of tails of non-spherical (correlated) Gaussian distributions — not available in simple analytical formulae
- ★ With more than 3 entities, may have to resort to simulations
  - Monte Carlo simulations? (AKA: Buy a fast computer)
  - Will Gang Chen write such a program? Only time will tell!