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 (β) 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 $\mathbf{X}$ matrix (measures of how sensitive regression results are to changes in $\mathbf{X}$)
  - 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 $\mathbf{X}$ 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 **WARNING**s and **ERROR**s


  - 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? Hmmm…
      - 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

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- **`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 32-bit floating point numbers
  - **`-short`** option gives 16-bit short integers (with scaling factor for each sub-brick to convert it to floats) — less precision, and less disk space

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- **`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

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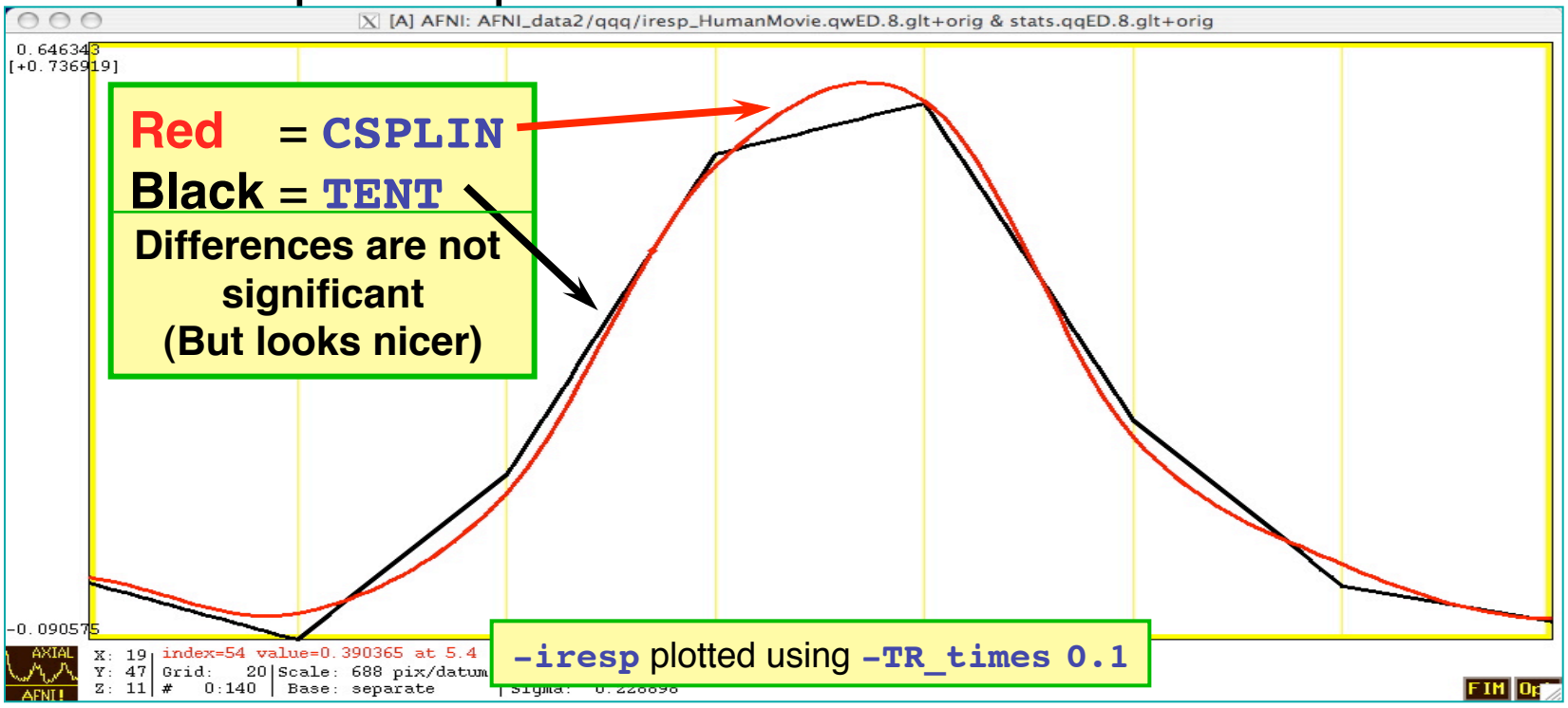
- **`-stim_file`** is used to input a column directly into **X** matrix
  - Motion parameters (as in previous examples)
  - If you create a stimulus+response model outside **`3dDeconvolve`** (e.g., using program **`waver`**)

Other Features - 5

-stim_times has other basis function options for HRF model besides **BLOCK** and **TENT**

- **CSPLIN** = cubic spline, instead of **TENT** = linear spline
  - Same parameters: **(start,stop,number of regressors)**
  - A "drop in" replacement for **TENT**



- **TENTzero** & **CSPLINzero** = force start & end of HRF = 0
- **MION** = model from Leite et al. (*NeuroImage* 2002)

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 β coefficients for each $\mathbf{X}$ matrix column into dataset
  - **-x1D** stores the matrix columns (and **-stim_label**s, 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

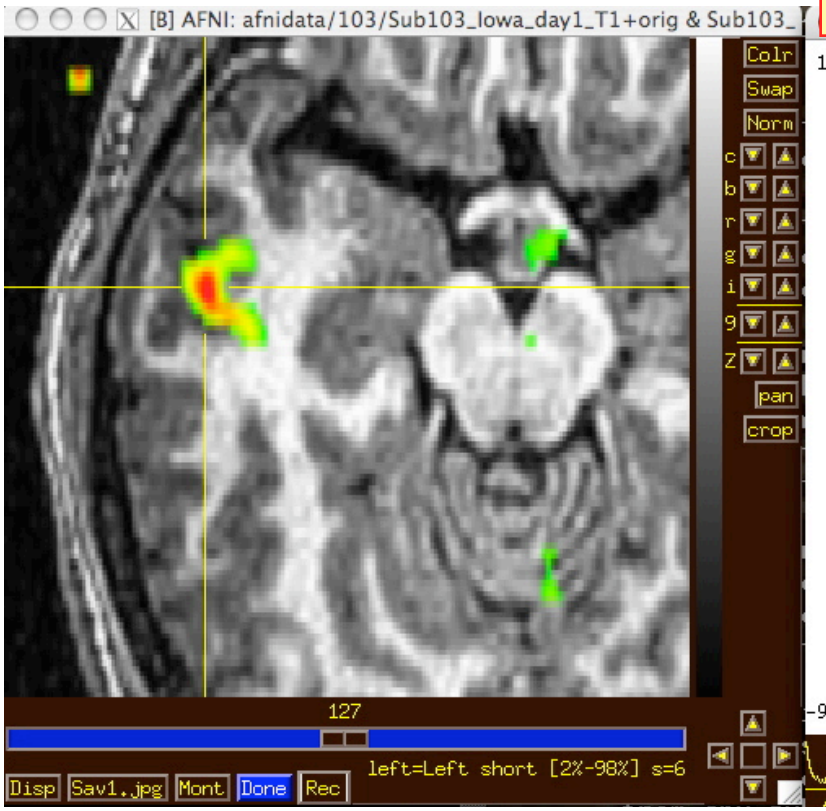
- When using **`-nodata`** with **`-stim_times`**, it is important to give the number of time points and the TR, as in **`-nodata 250 2.3`**
  - With **`-input1D`**, use **`-TR_1D 2.3`** to specify TR

IM Regression - 1

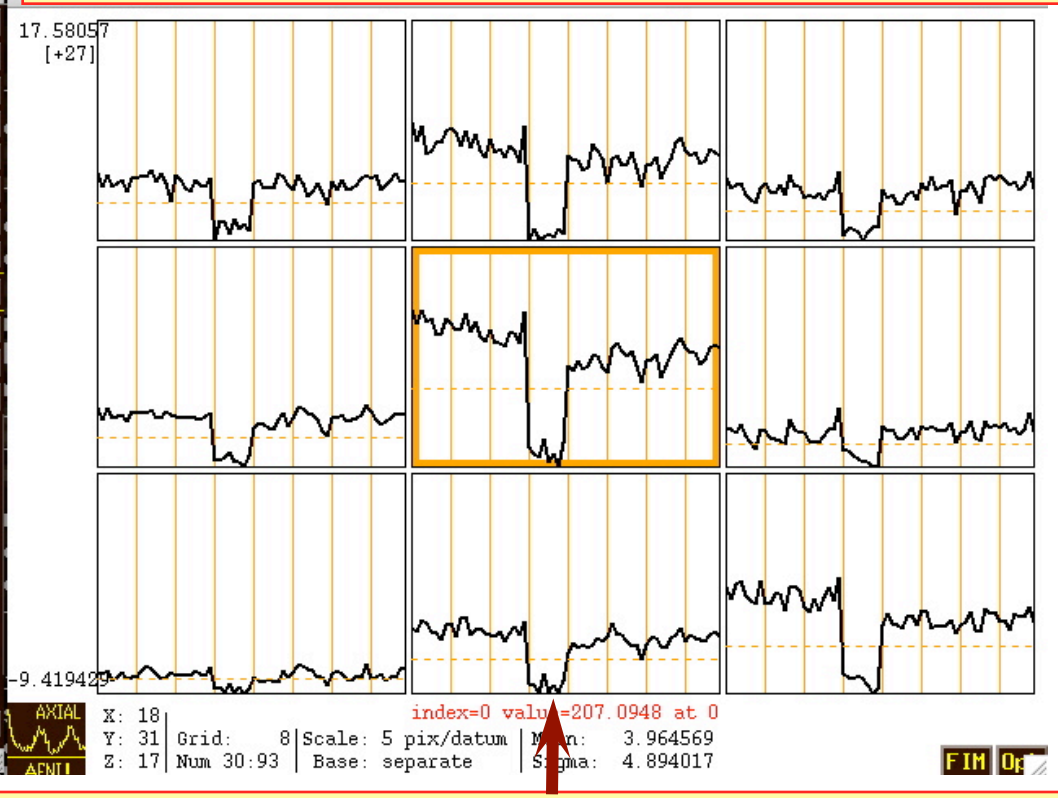
- **IM** = **I**ndividual **M**odulation
  - Compute *separate* amplitude of response for each stimulus
    - Instead of computing average amplitude of responses to multiple stimuli in the same class
  - Response amplitudes (βs) for each individual block/ event will be highly noisy
    - Can't use individual activation map for much
    - Must pool the computed βs in some further statistical analysis (*t*-test via `3dttest`? inter-voxel correlations in the βs? Correlate βs 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

- First application of IM was checking some data we received from another institution
- Experiment: 64 blocks of sensorimotor task (8 runs each with 8 blocks)



Plot of 64 **BLOCK** [*w*]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 × 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 Modulation)
  - 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 is $r_{\mathrm{AM2}}(t) = \sum_{k=1}^{K} h(t-\tau_k)\cdot(a_k - \bar{a})$
  - Where a_k =value of k^{th} ABI value, and a is the average ABI value
  - N.B.: If UNIX environment variable **AFNI_3Deconvolve_rawAM2** is set to **YES**, then mean of the a_k is not removed.
- Response (β) for first regressor is standard activation map
- Statistics and β 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 pair of regressors (i.e., testing null hypothesis that *both* β weights are zero: that there is no ABI-independent *or* ABI-proportional signal change)
  - Use **-tout** option to test each β weight separately
  - Can **1dplot** $\mathbf{X}$ 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:

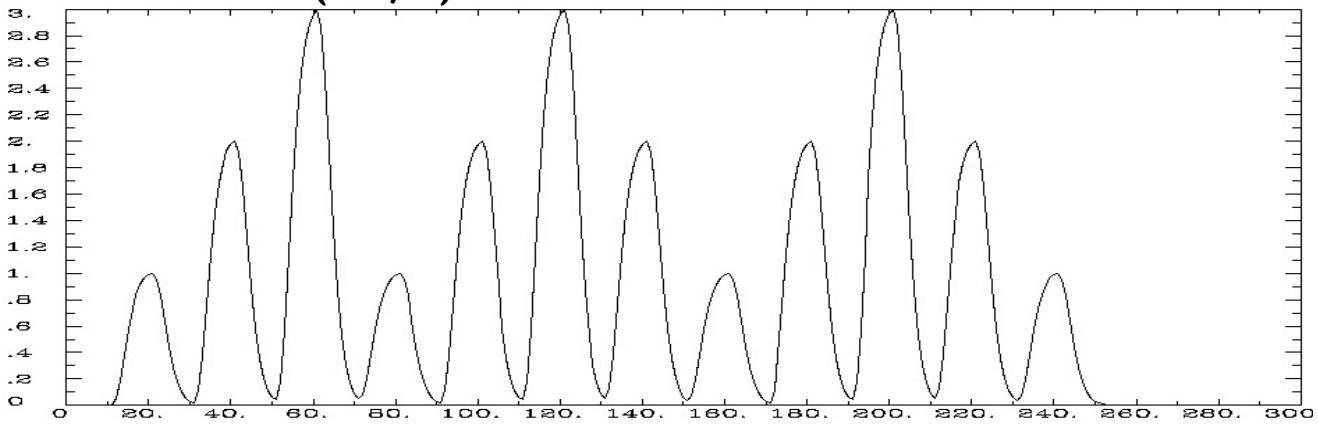
$$r_{\mathrm{AM1}}(t) = \sum_{k=1}^{K} h(t-\tau_k)\cdot a_k$$

  - 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

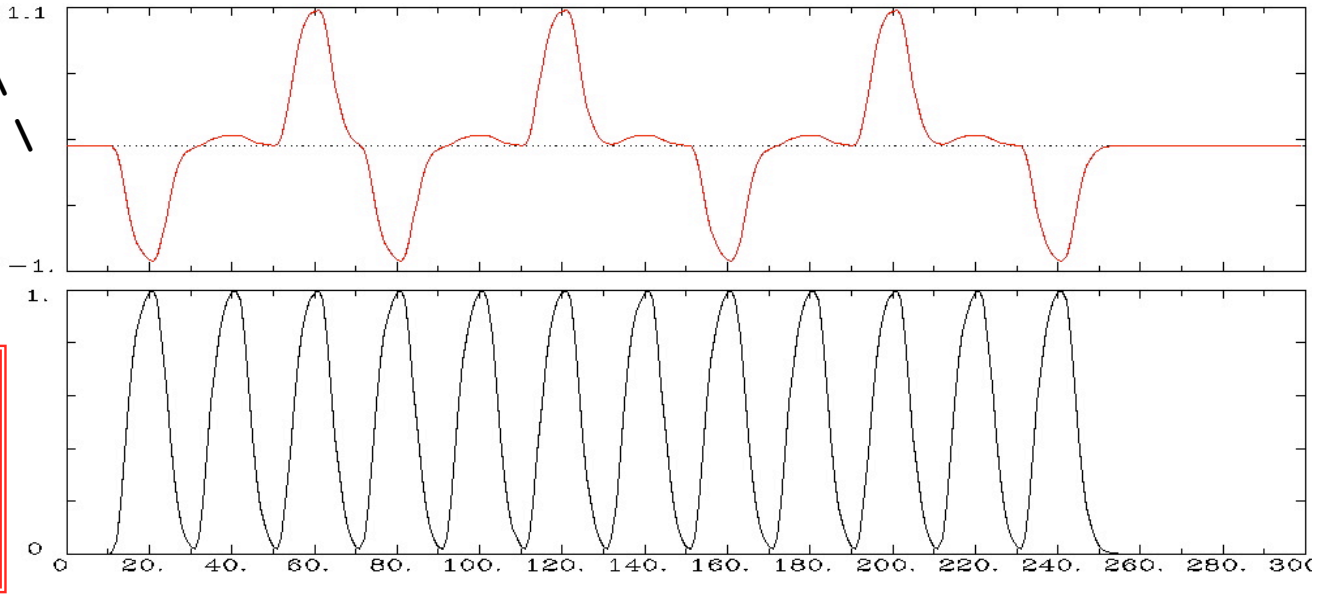
Timing: **AM.1D** = 10*1 30*2 50*3 70*1 90*2 110*3 130*2 150*1 170*2 190*3 210*2 230*1

- **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)

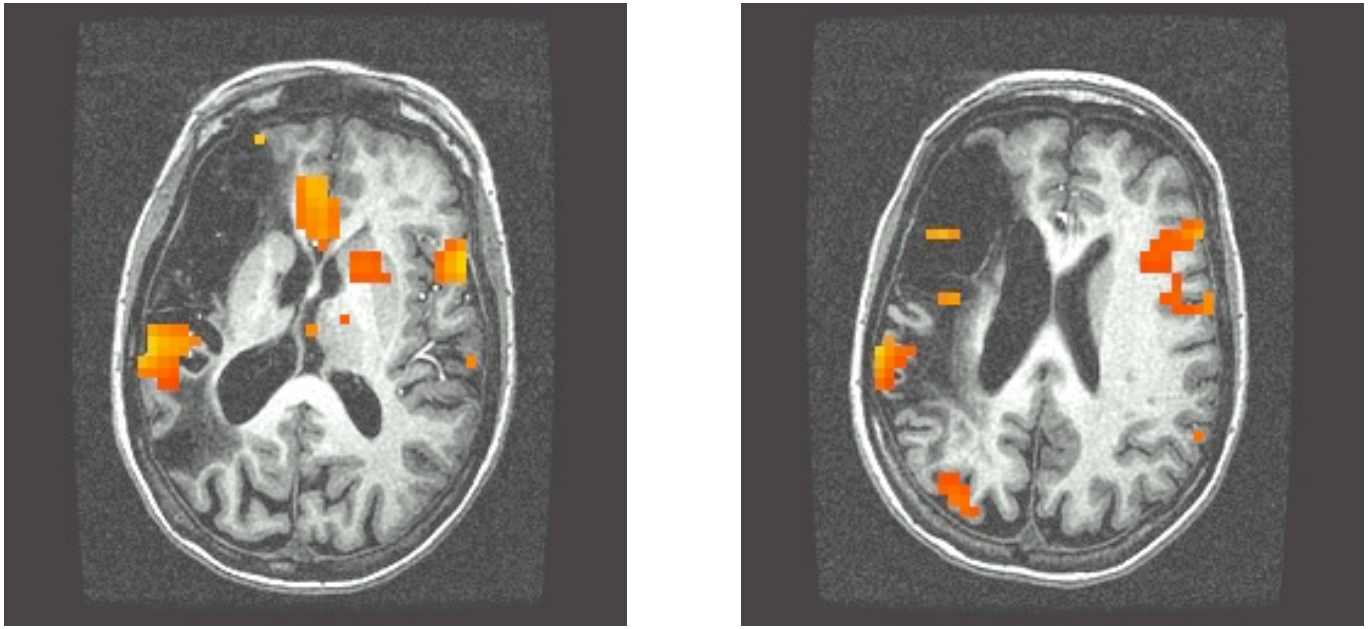
- **3dDeconvolve -nodata 300 1.0 **
  **-num_stimts 1 **
  **-stim_times_AM2 1 **
  **AM.1D 'BLOCK(10,1)' **
  -x1D AM2.x1D
- **1dplot -sepscl **
  AM2.x1D'[2,3]'



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=Al 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 (β_{AM2})
  - What does this mean? Don't ask me!

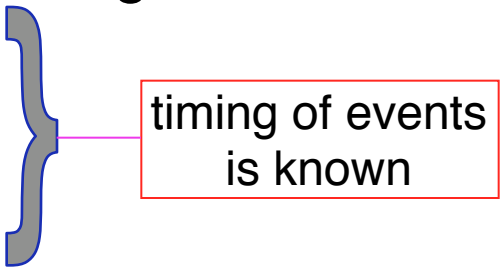


AM Regression - 7

- Alternative: use **IM** to get individual *β*s for each block/event and then do external regression statistics on those values
- Could do nonlinear fitting (to these *β*s) via **`3dNLfim`**, or inter-class contrasts via **`3dttest`**, **`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

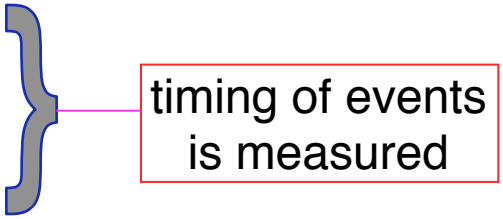
- Can have activations with multiple phases that are not always in the same time relationship to each other; e.g.:
  a) subject gets cue #1
  b) variable waiting time ("hold")
  c) subject gets cue #2, emits response
     - which depends on both cue #1 and #2



- 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.

More Complicated Experiment

- 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 β 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
  - Scanner fluctuations (e.g., thermal drift of hardware, timing errors)
  - Small subject head movements (10-100 μm)
  - 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 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 $\boldsymbol{\beta}$ fit parameters using more efficient "generalized least squares", using this correlation, all at once (REML method)

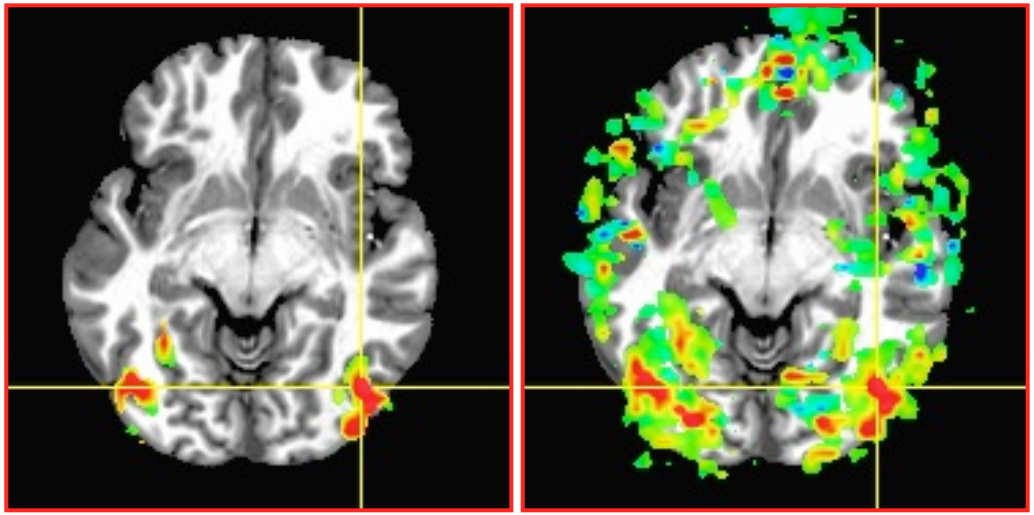
New Program: `3dREMLfit`

- Implements Solution #2
  - REML is a method for simultaneously estimating variance + correlation parameters ***and*** estimating regression fit parameters (***β***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

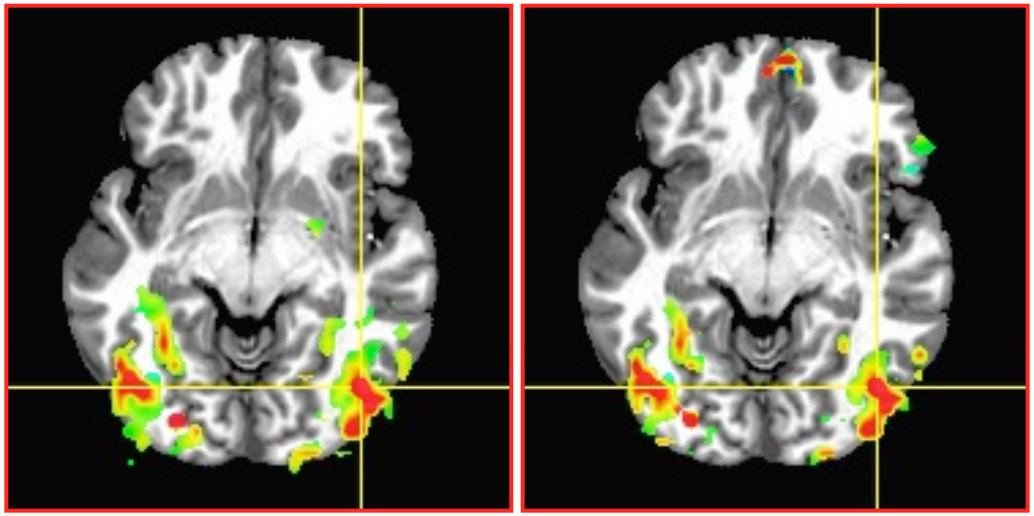
- Compare with **AFNI_data3/afni/rall_regress results**
- **3dREMLfit -matrix rall_xmat.x1D -input rall_vr+orig -fout -tout **
  **-Rvar rall_varR -Rbuck rall_funcR -Rfitts rall_fittsR **
  -Obuck rall_funcO -Ofitts rall_fittsO

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 *!?!*

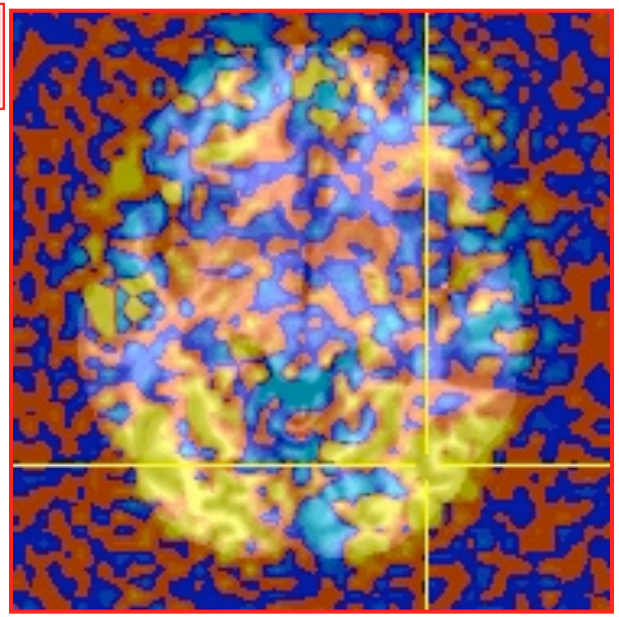
It's Not So Bad: β !

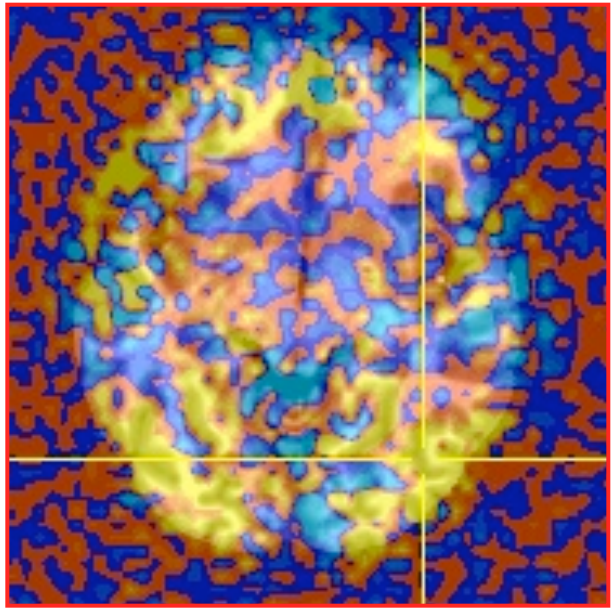
- For individual activation maps, `3dREMLfit`-ized *t*- and *F*-statistics are significantly different, and more accurate
- But … There are at present very few applications for such individual FMRI activation maps
  - pre-surgical planning; some longitudinal study?
- For standard group analysis, inputs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

Color Overlay = β weight from analysis on previous slide, no threshold

REML

CPU 500 s





OLSQ

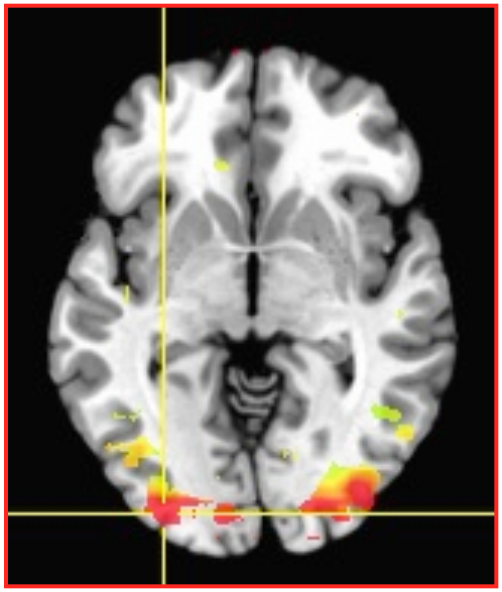
CPU 156 s

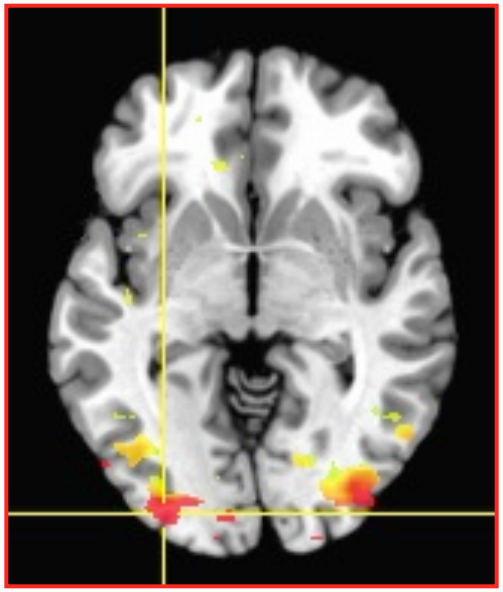
It's Not So Bad At All: Group Analysis!

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

REML

***F*-test for Affect condition**

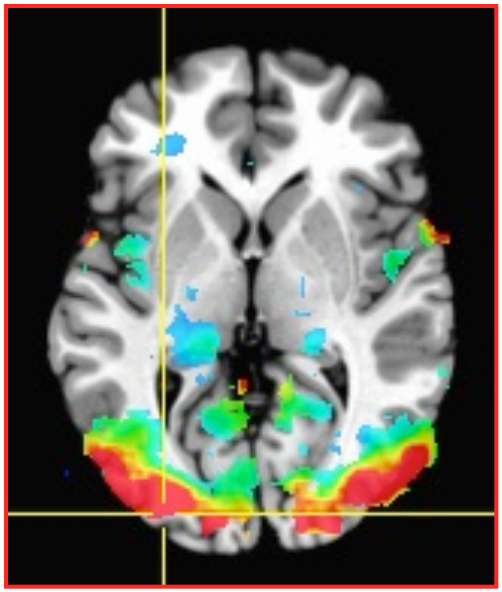


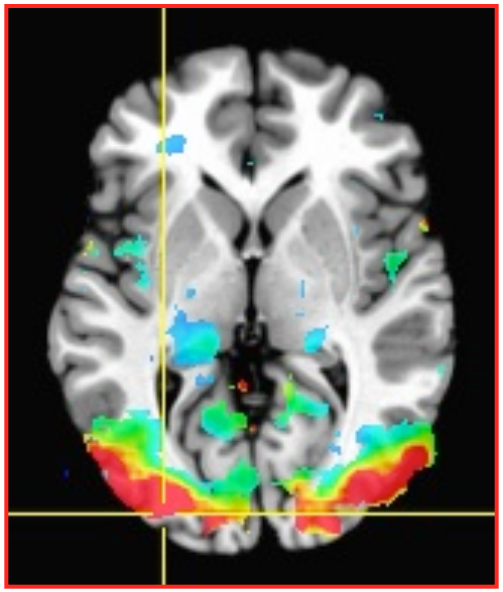


OLSQ

***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: $Z(t) = H(t) \star A(t)$ + 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: $H(t) \star A(t) = A(t) \star H(t)$
  - So the other direction looks to be the same problem
  - But isn't, since $H(t)$ is causal but $A(t)$ is not
    - Also, $H(t) \star A(t)$ smooths out rough spots in $A(t)$, so undoing this deconvolution adds roughness — 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)
  - *Or* could use ROIs from atlases, *or* from FreeSurfer per-subject parcellation

---

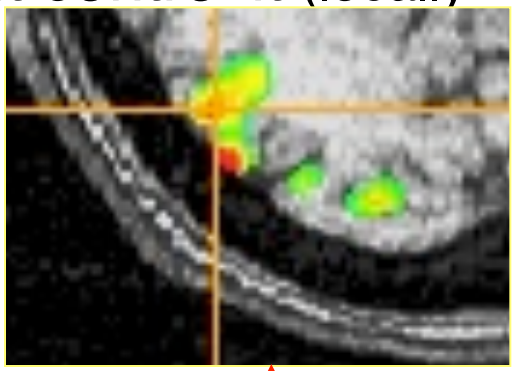
- Reject isolated small clusters of above-threshold voxels after analysis

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
- Estimate smoothness with **`3dFWHMx`**

3dBlurToFWHM

- 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

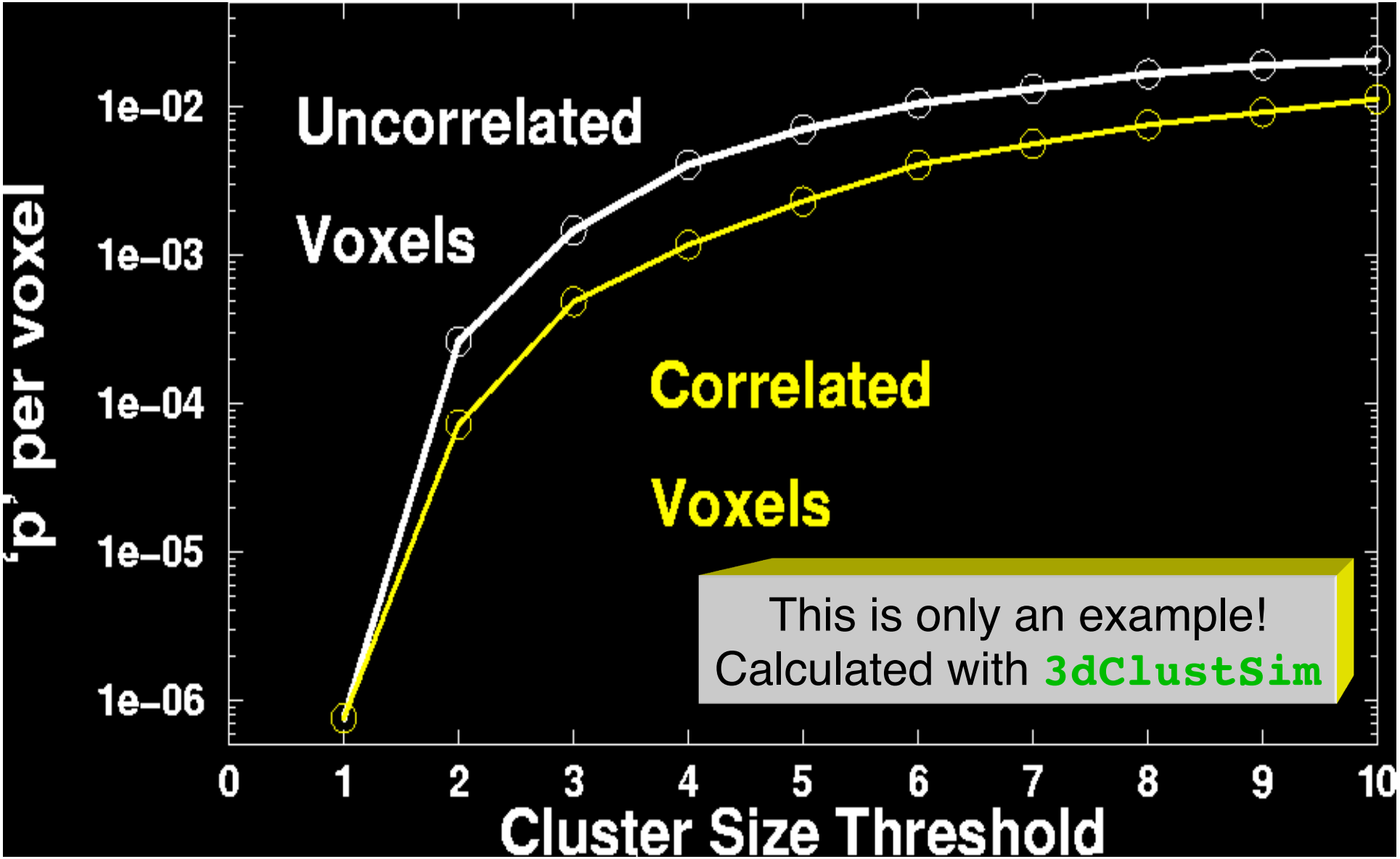


- Why blur FMRI time series?
  - Averaging neighbors will reduce noise
  - Activations are (usually) blob-ish (several voxels across)
  - Diminishes the multiple comparisons problem
- **3dBlurToFWHM** and **3dBlurInMask** blur only inside a mask region
  - To avoid mixing air (noise-only) and brain voxels
  - 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*** (or ***F***) threshold and cluster-size thresholds together: `3dClustSim`

Cluster-Based Detection



What the World Needs Now

- Unified HRF/Decon×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. ← Very close now
  - 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 Or 3dREMLfit
- Extend AM regression to allow for more than 1 piece of auxiliary information at each stimulus time ← Done!
- 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 50-200K 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 $\mathbf{H_0}$ in FMRI studies? $\mathbf{H_0}$ = 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 = β
    power = $1-\beta$ = probability of detecting true activation
  - Strategy: controlling type I error while increasing power (decreasing type II errors)
  - Significance level **α** (magic number 0.05) : $p < \alpha$

Justice System: Trial

Hidden Truth

	Defendant Innocent	Defendant Guilty
Reject Presumption of Innocence (Guilty Verdict)	**Type I Error** (defendant very unhappy)	**Correct**
Fail to Reject Presumption of Innocence (Not Guilty Verdict)	**Correct**	**Type II Error** (defendant very happy)

Statistics: Hypothesis Test

Hidden Truth

	H_0 True Not Activated	H_0 False Activated
Reject H_0 (decide voxel is activated)	**Type I Error** (false positive)	**Correct**
Don't Reject H_0 (decide voxel isn't activated)	**Correct**	**Type II Error** (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 Np small (compared to 1), $\alpha_{FW} \approx Np$
    - $N \approx 50{,}000+$ voxels in the brain
    - To keep probability of even one false positive $\alpha_{FW} < 0.05$ (the "corrected" p-value), need to have $p < 0.05 / 5\times10^4 = 10^{-6}$
    - This constraint on the per-voxel ("uncorrected") p-value is so stringent that we would 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: α_{FW} = Prob(≥ one false positive voxel in the whole brain)
    - **Bonferroni correction**: $\alpha_{FW} = 1-(1-p)^N \approx Np$, if $p << N^{-1}$
      - Use $p = \alpha/N$ as individual voxel significance level to achieve $\alpha_{FW} = \alpha$
      - Too stringent and overly conservative: $p = 10^{-8} \ldots 10^{-6}$
    - What can 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

---

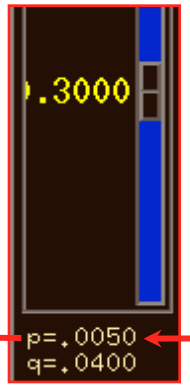
  - Control false discovery rate (FDR) — Much more on this a little later!
    - FDR = expected proportion of false positive voxels among all detected voxels
      - Give up on the idea of having (almost) no false positives at all

Cluster Analysis: 3dClustSim

- **FWE control in AFNI**
  - Monte Carlo simulations with program **3dClustSim** [supersedes **AlphaSim**]
    - Named for a place where primary attractions are randomization experiments
    - Randomly generate some number (*e.g.*, 10,000) of brain volumes with white noise (spatially uncorrelated)
      - That is, each "brain" volume is purely in $\mathbf{H_0}$ = 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 input to program
      - Size of dataset to simulate
      - Mask (e.g., to consider only brain-shaped regions in the simulated 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 = α)
      - Corresponding minimum cluster size at the input uncorrected *p*-value

- **Example:** `3dClustSim -nxyz 64 64 30 -dxyz 3 3 3 -fwhm 7`

```
# 3dClustSim -nxyz 64 64 30 -dxyz 3 3 3 -fwhm 7
# Grid: 64x64x30 3.00x3.00x3.00 mm^3 (122880 voxels)
# CLUSTER SIZE THRESHOLD(pthr,alpha) in Voxels
# -NN 1  | alpha = Prob(Cluster >= given size)
#  pthr  |  0.100  0.050  0.020  0.010
# ------ | ------ ------ ------ ------
 0.020000    89.4   99.9  114.0  123.0
 0.010000    56.1   62.1   70.5   76.6
 0.005000    38.4   43.3   49.4   53.6
 0.002000    25.6   28.8   33.3   37.0
 0.001000    19.7   22.2   26.0   28.6
 0.000500    15.5   17.6   20.5   22.9
 0.000200    11.5   13.2   16.0   17.7
 0.000100     9.3   10.9   13.0   14.8
```



p-value of threshold

At a per-voxel p=0.005, a cluster should have **44+** voxels to occur with $\alpha < 0.05$ from noise *only*

`3dClustSim` can be run by `afni_proc.py`: results get stored into statistics dataset, and then used in AFNI **Clusterize** GUI

• Interactive Clustering

False Discovery Rate in 

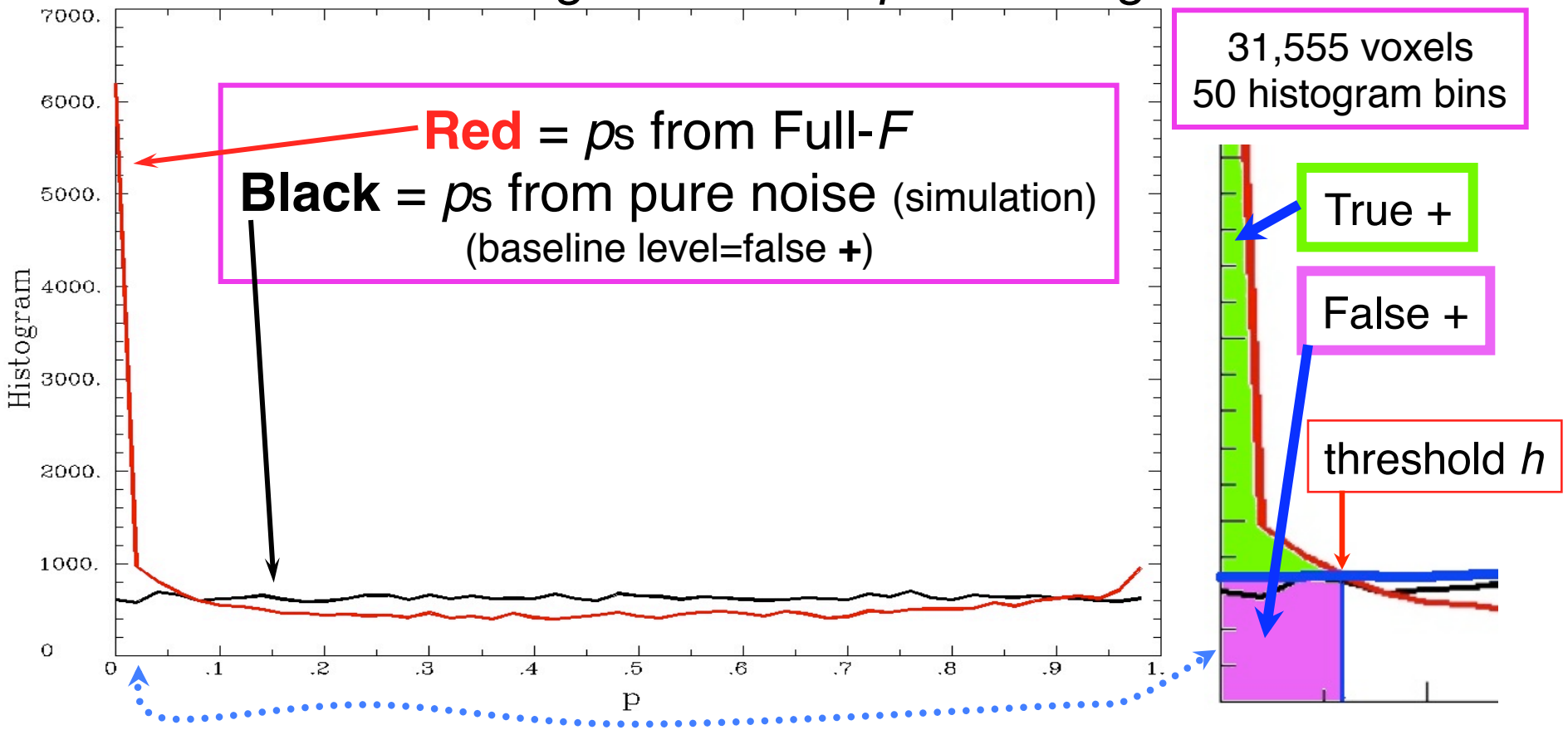
- Situation: making *many* statistical tests at once
  - e.g, Image voxels in FMRI; associating genes with disease
- Want to set threshold on statistic (e.g., *F*- or *t*-value) to control ***false positive*** error rate
- Traditionally: set threshold to control probability of making a ***single*** false 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 *un*activated
  - If have *N* voxels to test, $p_{\text{corrected}} = 1-(1-p)^N \approx Np$ (for small *p*)
    - 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*: e.g, *z*(0.05)≈1.95996
    - So that larger is "better" (in the same sense) e.g, *z*(0.01)≈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 ➔ true positives
  - Baseline of uniformity indicates how many true negatives are hidden amongst in the low *p*-value region

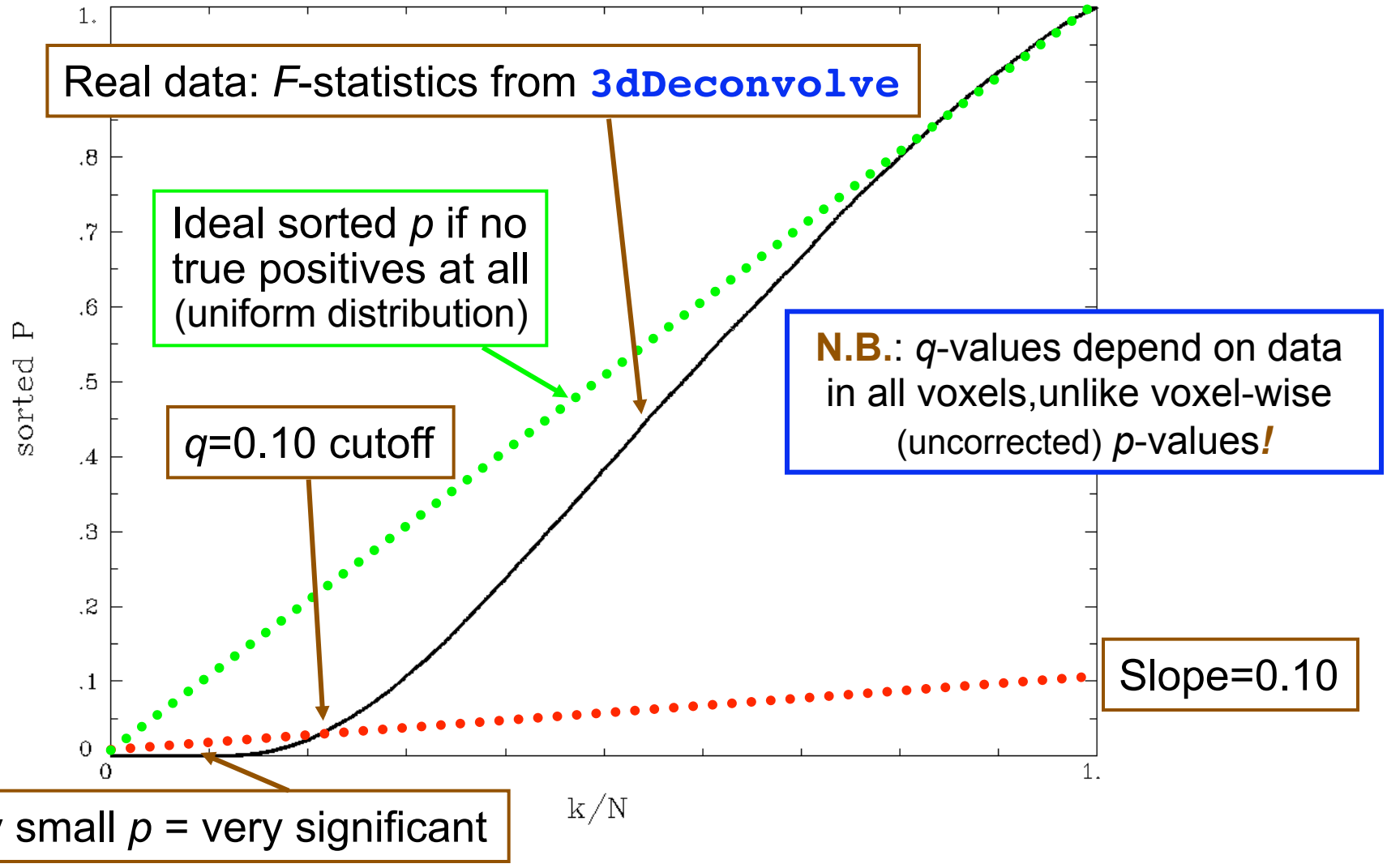


How *q* is Calculated from Data

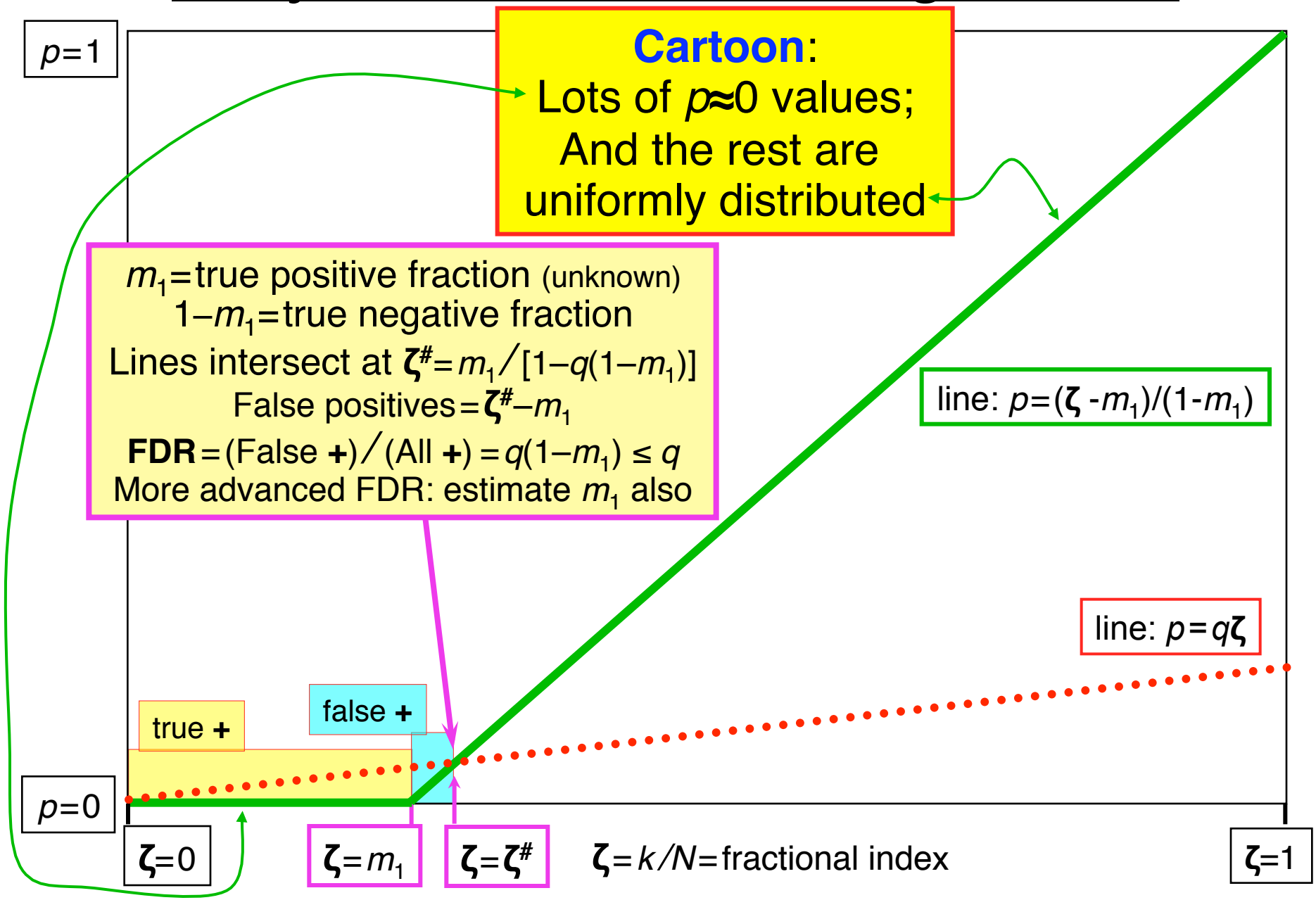
- Compute *p*-values of each statistic: $P_1, P_2, P_3, \ldots, P_N$
- Sort these: $P_{(1)} \leq P_{(2)} \leq P_{(3)} \leq \ldots \leq P_{(N)}$ {subscript$_{()}$ ➔sorted}
- For $k = 1..N$, $q_{(k)} = \min_{m \geq k} [N \bullet P_{(m)} / m]$
  - Easily computed from sorted *p*-values by looping downwards 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*

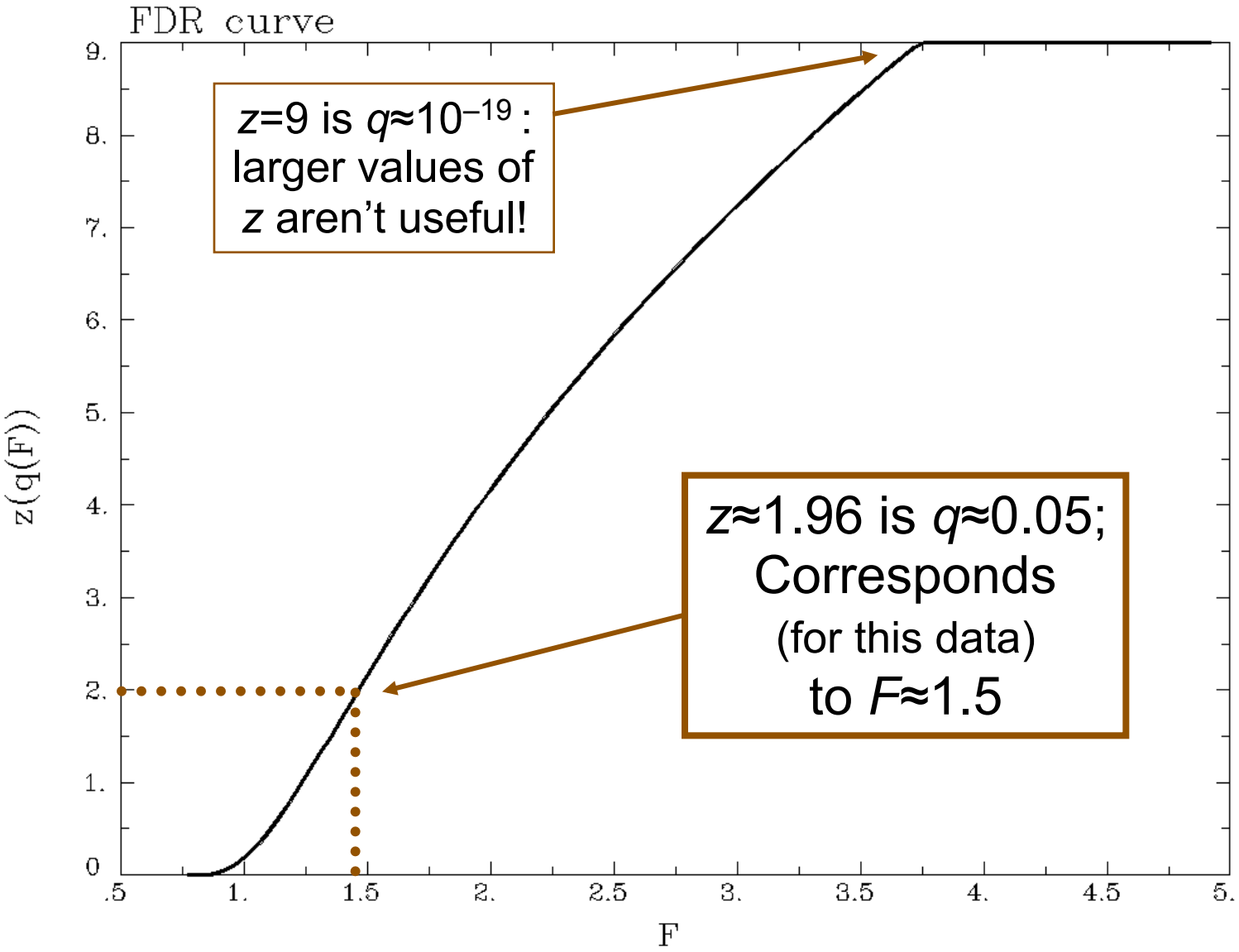
- Graph sorted *p*-values of voxel #*k* vs. $\zeta = k/N$ (the cumulative histogram of *p*, flipped sideways) and draw some lines from origin



Why This Line-Drawing Works



Same Data: threshold *F* vs. *z*(*q*)

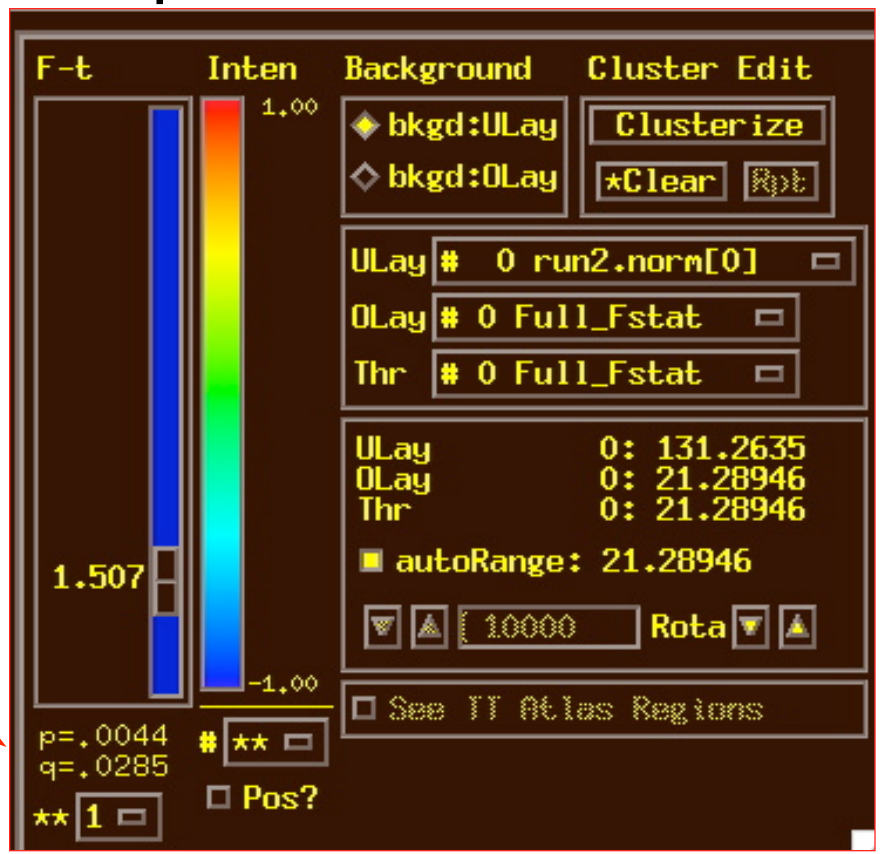


Recent Changes to **3dFDR**

- Don't include voxels with p=1 (e.g., F=0), even if they are in the **-mask** supplied on the command line
  - This changes decreases N, which will decrease q and so increase $z(q)$: recall that $q_{(k)} = \min_{m \geq k} [\, N \bullet P_{(m)} \,/\, m]$
- Sort with Quicksort algorithm
  - Faster than the bin-based sorting in the original code
  - Makes a big speed difference on large 1 mm^3 datasets
    - Not much speed difference on small 3 mm^3 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➔*z*➔*q*)
    - Can be used to adjust threshold by "eyeball"



q = N/A means it's not available

MDF hint = "missed detection fraction"

FDR Statistical Issues

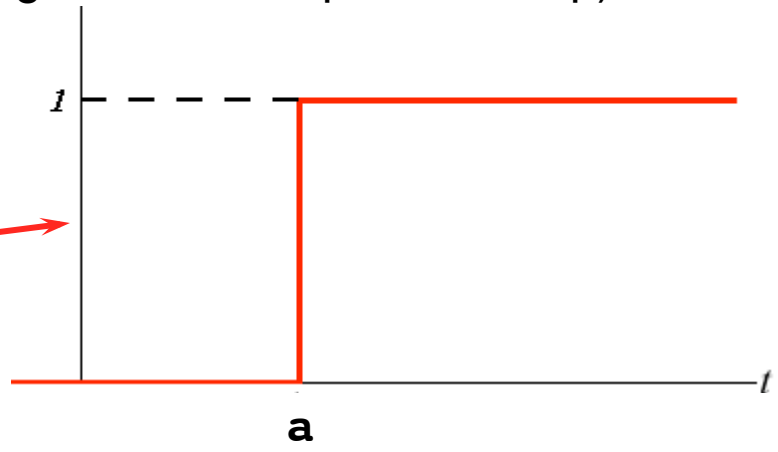
- FDR is conservative (*q*-values are too large) when voxels are positively correlated (e.g., from spatially smoothing)
  - Correcting for this is not so easy, since *q* depends on data (including true positives), so a simulation like **`3dClustSim`** is hard to conceptualize
  - At present, FDR is an alternative way of controlling false positives, vs. **`3dClustSim`** (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
  - ➔ *p*-values will be too small, so *q*-values will be too small
    - **`3dREMLfit`** rides to the rescue*!*

FWE or FDR?

- These 2 methods control Type I error in different senses
  - FWE: α_{FW} = Prob (≥ 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 α_{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 50,000 EPI voxels
    - Uncorrected → ≈50 false positive voxels in the brain
    - FWE: corrected $p = 0.05$ → ≈5% of the time would expect one or more purely false positive clusters in the entire volume of interest
    - FDR: $q = 0.05$ → ≈5% of voxels among those positively labeled ones are false positive
- What if your favorite blob (activation area) fails to survive correction?
  - Tricks (don't tell anyone we told you about these)
    - One-tail *t*-test? NN=3 clustering?
    - 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
  - In FMRI papers: Is 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
    - **step(t-a)** = 0 if **t** < **a**
      = 1 if **t** > **a**
    - Can be used to apply more than one threshold at a time

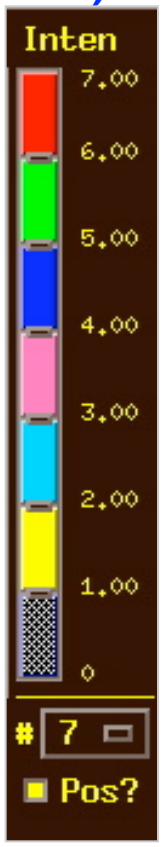


- Example of forming all possible conjunctions
  - 3 contrasts/tasks A, B, and C, each with a *t*-stat from **3dDeconvolve**
  - Assign each a number, based on binary positional notation:
    - A: $001_2 = 2^0 = \mathbf{1}$; B: $010_2 = 2^1 = \mathbf{2}$; C: $100_2 = 2^2 = \mathbf{4}$
  - Create a mask 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:

$000_2 = \mathbf{0}$: none are active at this voxel
$001_2 = \mathbf{1}$: A is active, but no others
$010_2 = \mathbf{2}$: B, but no others
$011_2 = \mathbf{3}$: A and B, but not C
$100_2 = \mathbf{4}$: C but no others
$101_2 = \mathbf{5}$: A and C, but not B
$110_2 = \mathbf{6}$: B and C, but not A
$111_2 = \mathbf{7}$: 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 **`3dClustSim`**
  - But that may be too stringent (conservative) and over-corrected
  - With 2 or 3 entities, analytical calculation of conjunction p_{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!