Deconvolution Signal Models

- Simple or Fixed-shape regression (previous talks):
  - ★ We fixed the shape of the HRF — amplitude varies
  - ★ Used `-stim_times` to generate the signal model (AKA the "ideal") from the stimulus timing
  - ★ Found the amplitude of the signal model in each voxel — solution to the set of linear equations = β weights
- Deconvolution or Variable-shape regression (now):
  - ★ We allow the shape of the HRF to vary in each voxel, for each stimulus class
  - ★ Appropriate when you don't want to over-constrain the solution by assuming an HRF shape
  - ★ ***Caveat***: need to have enough time points during the HRF in order to resolve its shape (e.g., TR $\leq$ 3 s)

Deconvolution: Pros & Cons (+ & –)

+ Letting HRF shape varies allows for subject and regional variability in hemodynamics
+ Can test HRF estimate for different shapes (e.g., are later time points more "active" than earlier?)
– Need to estimate more parameters for each stimulus class than a fixed-shape model (e.g., 4-15 *vs.* 1 parameter = amplitude of HRF)
– Which means you need more data to get the same statistical power (assuming that the fixed-shape model you would otherwise use was in fact "correct")
– Freedom to get any shape in HRF results can give weird shapes that are difficult to interpret

Expressing HRF via Regression Unknowns

- The tool for expressing an unknown function as a finite set of numbers that can be fit via linear regression is an **expansion in basis functions**

$$h(t) = \beta_0 \psi_0(t) + \beta_1 \psi_1(t) + \beta_2 \psi_2(t) + \cdots = \sum_{q=0}^{q=p} \beta_q \psi_q(t)$$

  - ★ The basis functions $\psi_q(t)$ & expansion order p are known
    - o Larger $p \Rightarrow$ more complex shapes & more parameters
  - ★ The unknowns to be found (in each voxel) comprises the set of weights β_q for each $\psi_q(t)$
- β weights appear only by multiplying known values, and HRF only appears in signal model by linear convolution (addition) with known stimulus timing
  - Resulting signal model still solvable by linear regression

3dDeconvolve with "Tent Functions"

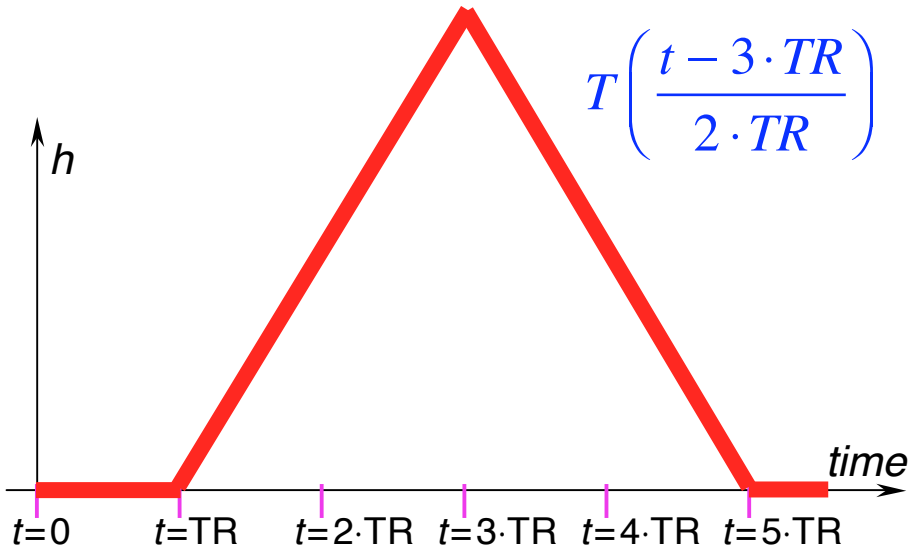
- Need to describe HRF shape and magnitude with a finite number of parameters
  - ★ And allow for calculation of $h(t)$ at any arbitrary point in time after the stimulus times:

$$r_n = \sum_{k=1}^{K} h(t_n - \tau_k) = \text{sum of HRF copies}$$

- Simplest set of such functions are **tent functions**
  - ★ Also known as "piecewise linear splines"

$$T(x) = \begin{cases} 1-|x| & \text{for } -1 < x < 1 \\ 0 & \text{for } |x| > 1 \end{cases}$$

N.B.: cubic splines are also available



Tent Functions = Linear Interpolation

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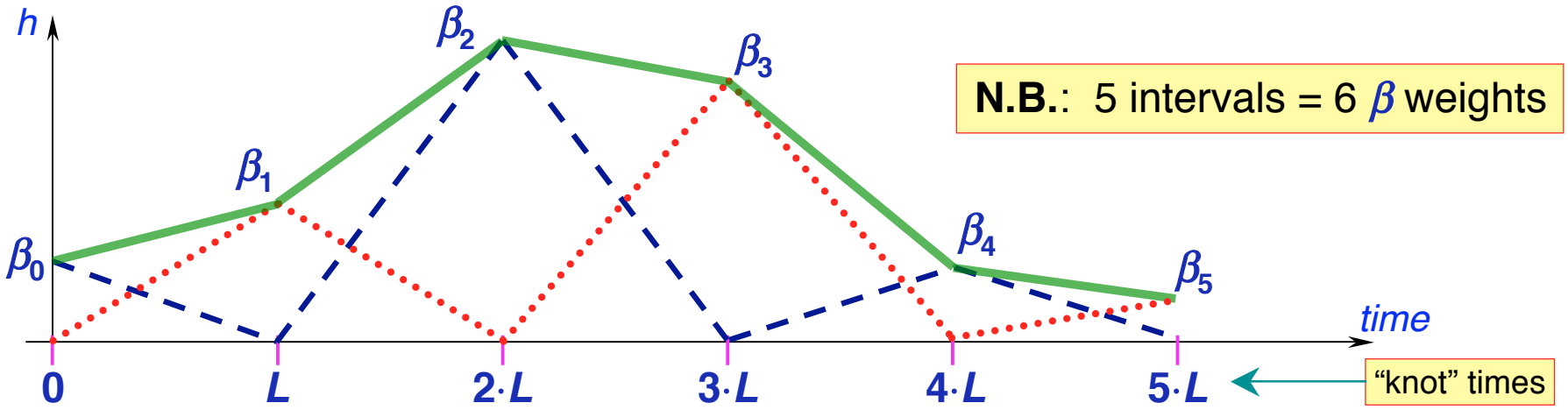
- Expansion of HRF in a set of spaced-apart tent functions is the same as linear interpolation between "knots"

$$h(t) = \beta_0 \cdot T\left(\frac{t}{L}\right) + \beta_1 \cdot T\left(\frac{t-L}{L}\right) + \beta_2 \cdot T\left(\frac{t-2\cdot L}{L}\right) + \beta_3 \cdot T\left(\frac{t-3\cdot L}{L}\right) + \cdots$$



- Tent function parameters are also easily interpreted as function values (e.g., β_2 = response at time $t = 2 \cdot L$ after stim)
- User must decide on relationship of tent function grid spacing L and time grid spacing TR (usually would choose $L \geq$ TR)
- In `3dDeconvolve`: specify duration of HRF and number of β parameters (details shown a few slides ahead)

Tent Functions: Average Signal Change

- For input to group analysis, usually want to compute average signal change
  - ★ Over entire duration of HRF (usual)
  - ★ Over a sub-interval of the HRF duration (sometimes)
- In previous slide, with 6 β weights, average signal change is

$$ {}^1\!/_2\,\beta_0 + \beta_1 + \beta_2 + \beta_3 + \beta_4 + {}^1\!/_2\,\beta_5 $$

- First and last β weights are scaled by half since they only affect half as much of the duration of the response
- In practice, may want to use $\mathbf{0}\cdot\beta_0$ since immediate post-stimulus response is not neuro-hemodynamically relevant
- All β weights (for each stimulus class) are output into the "bucket" dataset produced by `3dDeconvolve`
- Can then be combined into a single number using `3dcalc`

Deconvolution and Collinearity

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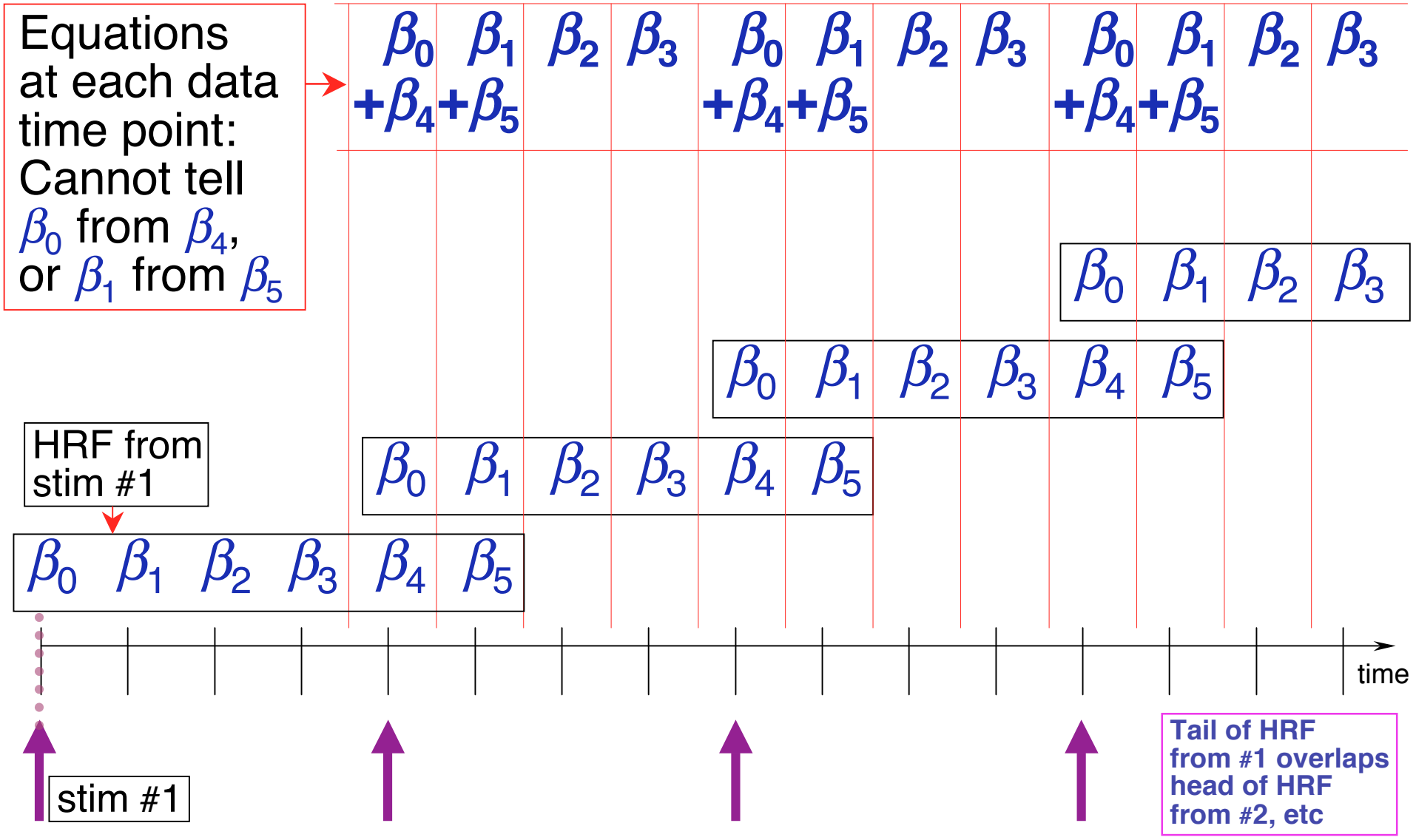
- Regular stimulus timing can lead to collinearity!



Deconvolution Example - The Data

- **`cd AFNI_data2`**
  - ★ data is in **`ED/`** subdirectory (10 runs of 136 images each; TR=2 s)
  - ★ script = **`s1.afni_proc_command`** (in **`AFNI_data2/`** directory)
    - o stimuli timing and GLT contrast files in **`misc_files/`**
  - ★ this script runs program **`afni_proc.py`** to generate a shell script with all AFNI commands for single-subject analysis
    - o Run by typing **`tcsh s1.afni_proc_command`** ; then copy/paste

    `tcsh -x proc.ED.8.glt |& tee output.proc.ED.8.glt`

- Event-related study from Mike Beauchamp
  - ★ 10 runs with four classes of stimuli (short videos)
    - o Tools moving (e.g., a hammer pounding) - **ToolMovie**
    - o People moving (e.g., jumping jacks) - **HumanMovie**
    - o Points outlining tools moving (no objects, just points) - **ToolPoint**
    - o Points outlining people moving - **HumanPoint**
  - ★ Goal: find brain area that distinguishes natural motions (**HumanMovie** and **HumanPoint**) from simpler rigid motions (**ToolMovie** and **ToolPoint**)

Text output from programs goes to screen *and* file

Master Script for Data Analysis

```
afni_proc.py                                                    \
  -dsets ED/ED_r??+orig.HEAD                                    \
  -subj_id ED.8.glt                                             \
  -copy_anat ED/EDspgr                                          \
  -tcat_remove_first_trs 2                                      \
  -volreg_align_to first                                        \
  -regress_stim_times misc_files/stim_times.*.1D                \
  -regress_stim_labels ToolMovie HumanMovie                     \
                       ToolPoint HumanPoint                     \
  -regress_basis 'TENT(0,14,8)'                                 \
  -regress_opts_3dD                                             \
  -gltsym ../misc_files/glt1.txt -glt_label 1 FullF             \
  -gltsym ../misc_files/glt2.txt -glt_label 2 HvsT              \
  -gltsym ../misc_files/glt3.txt -glt_label 3 MvsP              \
  -gltsym ../misc_files/glt4.txt -glt_label 4 HMvsHP            \
  -gltsym ../misc_files/glt5.txt -glt_label 5 TMvsTP            \
  -gltsym ../misc_files/glt6.txt -glt_label 6 HPvsTP            \
  -gltsym ../misc_files/glt7.txt -glt_label 7 HMvsTM
```

- Master script program
- 10 input datasets
- Set output filenames
- Copy anat to output dir
- Discard first 2 TRs
- Where to align all EPIs
- Stimulus timing files (4)
- Stimulus labels
- HRF model
- Specifies that next lines are options to be passed to `3dDeconvolve` directly (in this case, the GLTs we want computed)

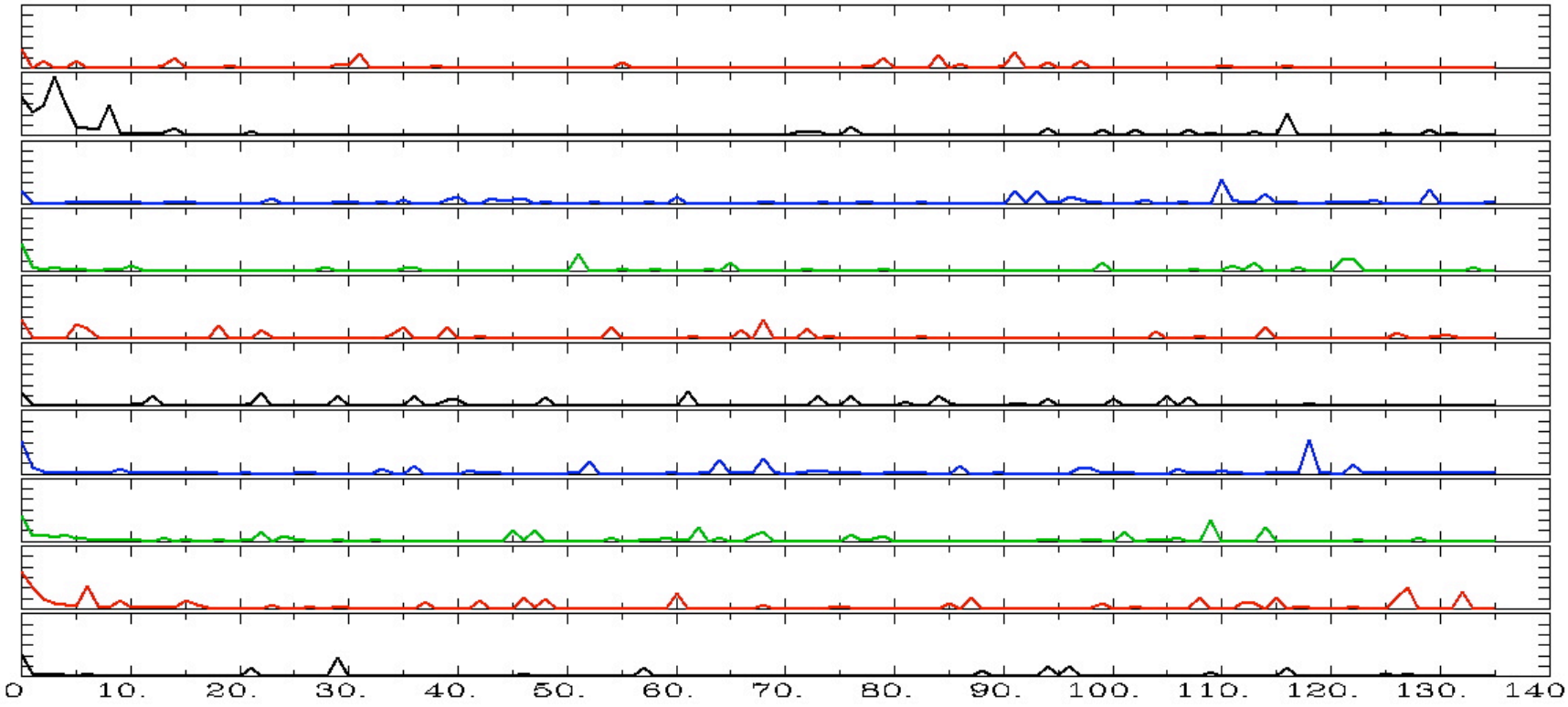
This script generates file `proc.ED.8.glt` (180 lines), which contains all the AFNI commands to produce analysis results into directory `ED.8.glt.results/` (148 files)

Shell Script for Deconvolution - Outline

- Copy datasets into output directory for processing
- Examine each imaging run for outliers: **3dToutcount**
- Time shift each run's slices to a common origin: **3dTshift**
- Registration of each imaging run: **3dvolreg**
- Smooth each volume in space (136 sub-bricks per run): **3dmerge**
- Create a brain mask: **3dAutomask** and **3dcalc**
- Rescale each voxel time series in each imaging run so that its average through time is 100: **3dTstat** and **3dcalc**
  - ⋆ If baseline is 100, then a β_q of 5 (say) indicates a 5% signal change in that voxel at tent function knot #q after stimulus
  - ⋆ Biophysics: believe % signal change is relevant physiological parameter
- Catenate all imaging runs together into one big dataset (1360 time points): **3dTcat**
  - ⋆ This dataset is useful for plotting **-fitts** output from **3dDeconvolve** and visually examining time series fitting
- Compute HRFs and statistics: **3dDeconvolve**

Script - 3dToutcount

```
# set list of runs
set runs = (`count -digits 2 1 10`)
# run 3dToutcount for each run
foreach run ( $runs )
  3dToutcount -automask pb00.$subj.r$run.tcat+orig > outcount_r$run.1D
end
```



Via `1dplot outcount_r??.1D`

`3dToutcount` searches for “outliers” in data time series;
You should examine noticeable runs & time points

Script - 3dTshift

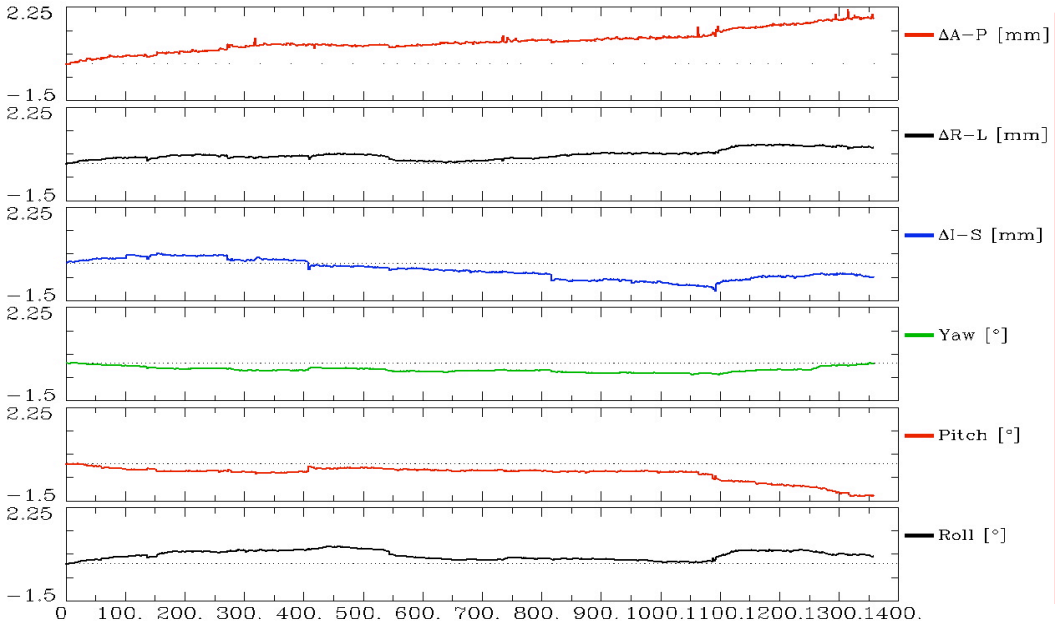
```
# run 3dTshift for each run
foreach run ( $runs )
  3dTshift -tzero 0 -quintic -prefix pb01.$subj.r$run.tshift \
           pb00.$subj.r$run.tcat+orig
end
```

- Produces new datasets where each time series has been shifted to have the same time origin
- `-tzero 0` means that all data time series are interpolated to match the time offset of the first slice
  - Which is what the slice timing files usually refer to
  - Quintic (5^{th} order) polynomial interpolation is used
- `3dDeconvolve` will be run on these time-shifted datasets
  - This is mostly important for Event-Related FMRI studies, where the response to the stimulus is briefer than for Block designs
    - (Because the stimulus is briefer)
  - Being a little off in the stimulus timing in a Block design is not likely to matter much

Script - 3dvolreg

```
# align each dset to the base volume
foreach run ( $runs )
  3dvolreg -verbose -zpad 1 -base pb01.$subj.r01.tshift+orig'[0]'  \
           -1Dfile dfile.r$run.1D -prefix pb02.$subj.r$run.volreg  \
           pb01.$subj.r$run.tshift+orig
end
```

- Produces new datasets where each volume (one time point) has been aligned (registered) to the #0 time point in the #1 dataset
- Movement parameters are saved into files **dfile.r$run.1D**
  - Will be used as extra regressors in **3dDeconvolve** to reduce motion artifacts



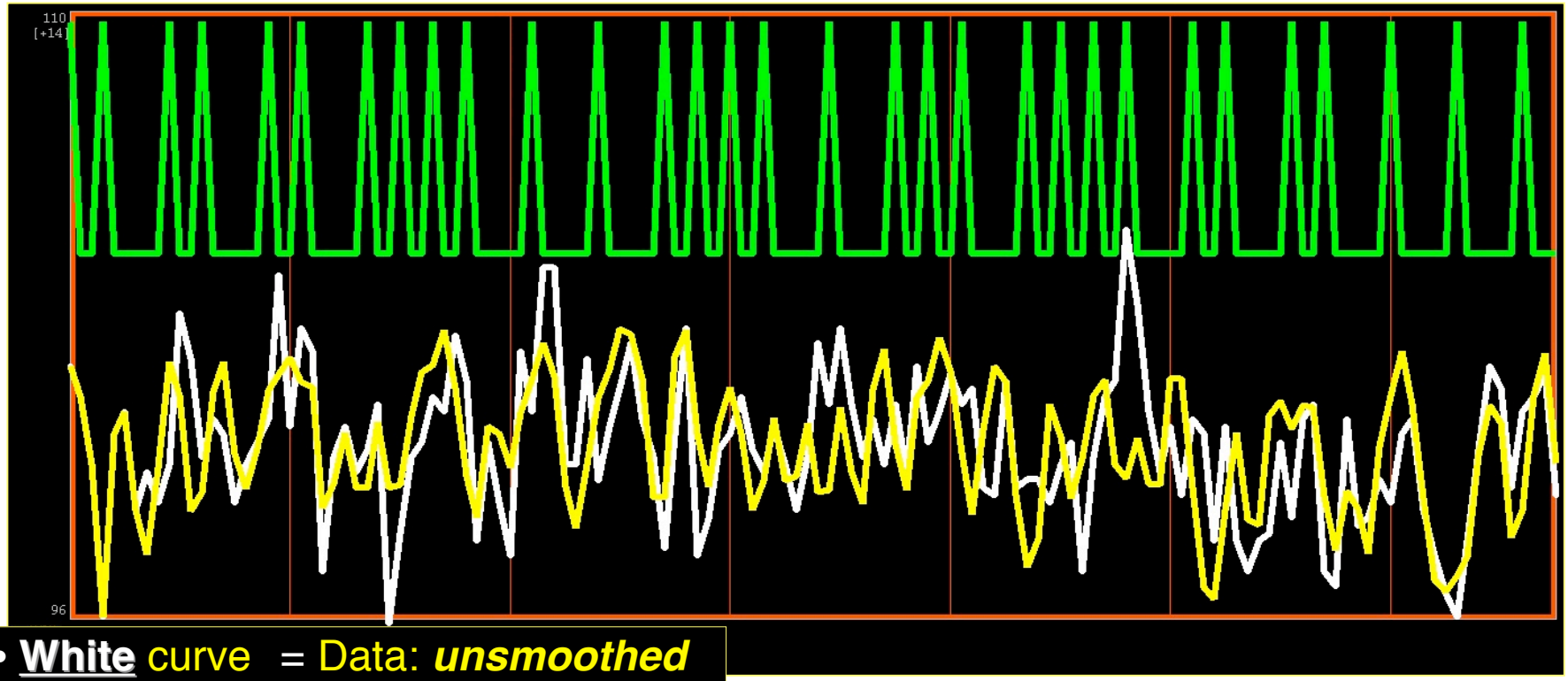
1dplot -volreg dfile.rall.1D

- Shows movement parameters for all runs (1360 time points) in degrees and millimeters
- **Very** important to look at this graph!
- Excessive movement can make an imaging run useless — **3dvolreg** won't be able to compensate
  - Pay attention to scale of movements: more than about 2 voxel sizes in a short time interval is usually bad

Script - 3dmerge

```
# blur each volume
foreach run ( $runs )
    3dmerge -1blur_fwhm 4 -doall -prefix pb03.$subj.r$run.blur   \
            pb02.$subj.r$run.volreg+orig
end
```

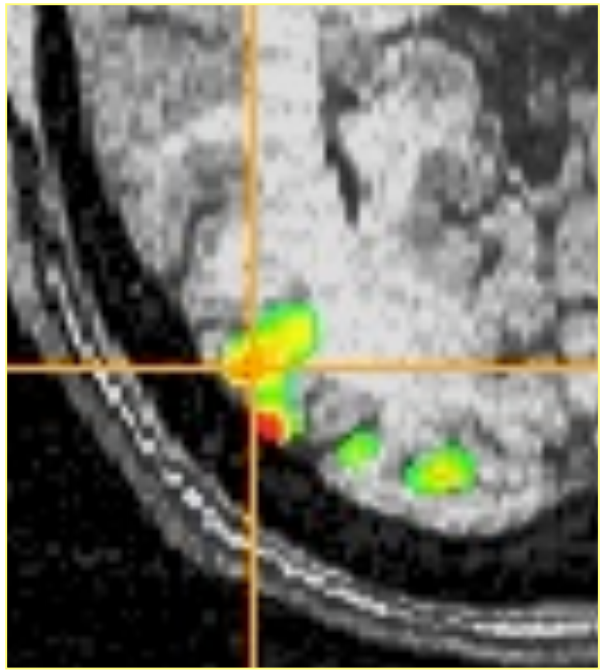
- **Why Blur**? Reduce noise by averaging neighboring voxels time series



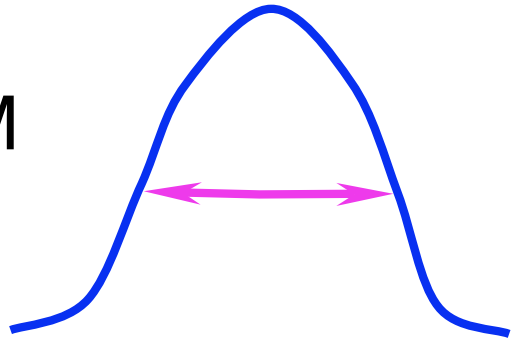
- **White** curve = Data: ***unsmoothed***
- **Yellow** curve = Model fit ($R^2 = 0.50$)
- **Green** curve = Stimulus timing

This is an extremely good fit for ER FMRI data!

Why Blur? - 2



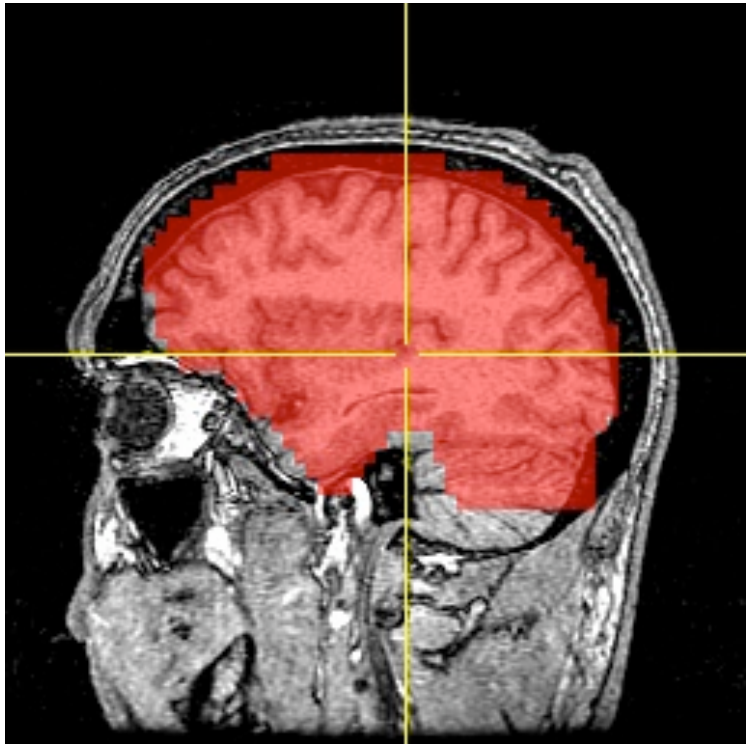
- fMRI activations are (usually) blob-ish (several voxels across)
- Averaging neighbors will also reduce the fiendish multiple comparisons problem
  - ★ Number of independent "resels" will be smaller than number of voxels (*e.g.*, 2000 *vs*. 20000)
- Why not just acquire at lower resolution?
  - ★ To avoid averaging across brain/non-brain interfaces
  - ★ To project onto surface models
- Amount to blur is specified as FWHM (**F**ull **W**idth at **H**alf **M**aximum) of spatial averaging filter (4 mm in script)



Script - 3dAutomask

```
# create 'full_mask' dataset (union mask)
foreach run ( $runs )
  3dAutomask -dilate 1 -prefix rm.mask_r$run pb03.$subj.r$run.blur+orig
end
# get mean and compare it to 0 for taking 'union'
3dMean -datum short -prefix rm.mean rm.mask*.HEAD
3dcalc -a rm.mean+orig -expr 'ispositive(a-0)' -prefix full_mask.$subj
```

- **3dAutomask** creates a mask of contiguous high-intensity voxels (with some hole-filling) from each imaging run separately
- **3dMean** and **3dcalc** are used to create a mask that is the union of all the individual run masks
- **3dDeconvolve** analysis will be limited to voxels in this mask
  - Will run faster, since less data to process

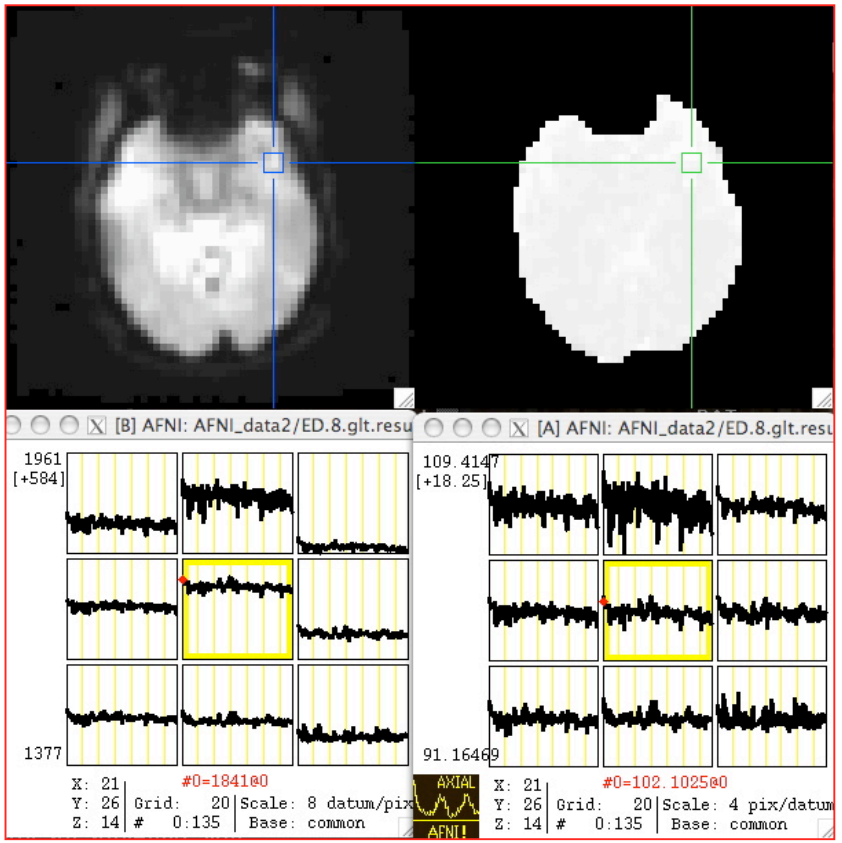


Automask from EPI shown in red

Script - Scaling

```
# scale each voxel time series to have a mean of 100
# (subject to maximum value of 200)
foreach run ( $runs )
  3dTstat -prefix rm.mean_r$run pb03.$subj.r$run.blur+orig
  3dcalc -a pb03.$subj.r$run.blur+orig -b rm.mean_r$run+orig  \
         -c full_mask.$subj+orig                              \
         -expr 'c * min(200, a/b*100)' -prefix pb04.$subj.r$run.scale
end
```

- **3dTstat** calculates the mean (through time) of each voxel's time series data
- For voxels in the mask, each data point is scaled (multiplied) using **3dcalc** so that it's time series will have mean=100
- If an HRF regressor has max amplitude =1, then its β coefficient will represent the percent signal change (from the mean) due to that part of the signal model
- Scaled images are very boring to view
  - No spatial contrast by design!
  - Graphs have common baseline now →



Script - 3dDeconvolve

```
3dDeconvolve -input pb04.$subj.r??.scale+orig.HEAD -polort 2          \
  -mask full_mask.$subj+orig -basis_normall 1 -num_stimts 10          \
  -stim_times 1 stimuli/stim_times.01.1D 'TENT(0,14,8)'               \
  -stim_label 1 ToolMovie                                             \
  -stim_times 2 stimuli/stim_times.02.1D 'TENT(0,14,8)'               \
  -stim_label 2 HumanMovie                                            \
  -stim_times 3 stimuli/stim_times.03.1D 'TENT(0,14,8)'               \
  -stim_label 3 ToolPoint                                             \
  -stim_times 4 stimuli/stim_times.04.1D 'TENT(0,14,8)'               \
  -stim_label 4 HumanPoint                                            \
  -stim_file 5 dfile.rall.1D'[0]' -stim_base 5 -stim_label 5 roll     \
  -stim_file 6 dfile.rall.1D'[1]' -stim_base 6 -stim_label 6 pitch    \
  -stim_file 7 dfile.rall.1D'[2]' -stim_base 7 -stim_label 7 yaw      \
  -stim_file 8 dfile.rall.1D'[3]' -stim_base 8 -stim_label 8 dS       \
  -stim_file 9 dfile.rall.1D'[4]' -stim_base 9 -stim_label 9 dL       \
  -stim_file 10 dfile.rall.1D'[5]' -stim_base 10 -stim_label 10 dP    \
  -iresp 1 iresp_ToolMovie.$subj -iresp 2 iresp_HumanMovie.$subj      \
  -iresp 3 iresp_ToolPoint.$subj -iresp 4 iresp_HumanPoint.$subj      \
  -gltsym ../misc_files/glt1.txt -glt_label 1 FullF                   \
  -gltsym ../misc_files/glt2.txt -glt_label 2 HvsT                    \
  -gltsym ../misc_files/glt3.txt -glt_label 3 MvsP                    \
  -gltsym ../misc_files/glt4.txt -glt_label 4 HMvsHP                  \
  -gltsym ../misc_files/glt5.txt -glt_label 5 TMvsTP                  \
  -gltsym ../misc_files/glt6.txt -glt_label 6 HPvsTP                  \
  -gltsym ../misc_files/glt7.txt -glt_label 7 HMvsTM                  \
  -fout -tout -full_first -x1D Xmat.x1D -fitts fitts.$subj -bucket stats.$subj
```

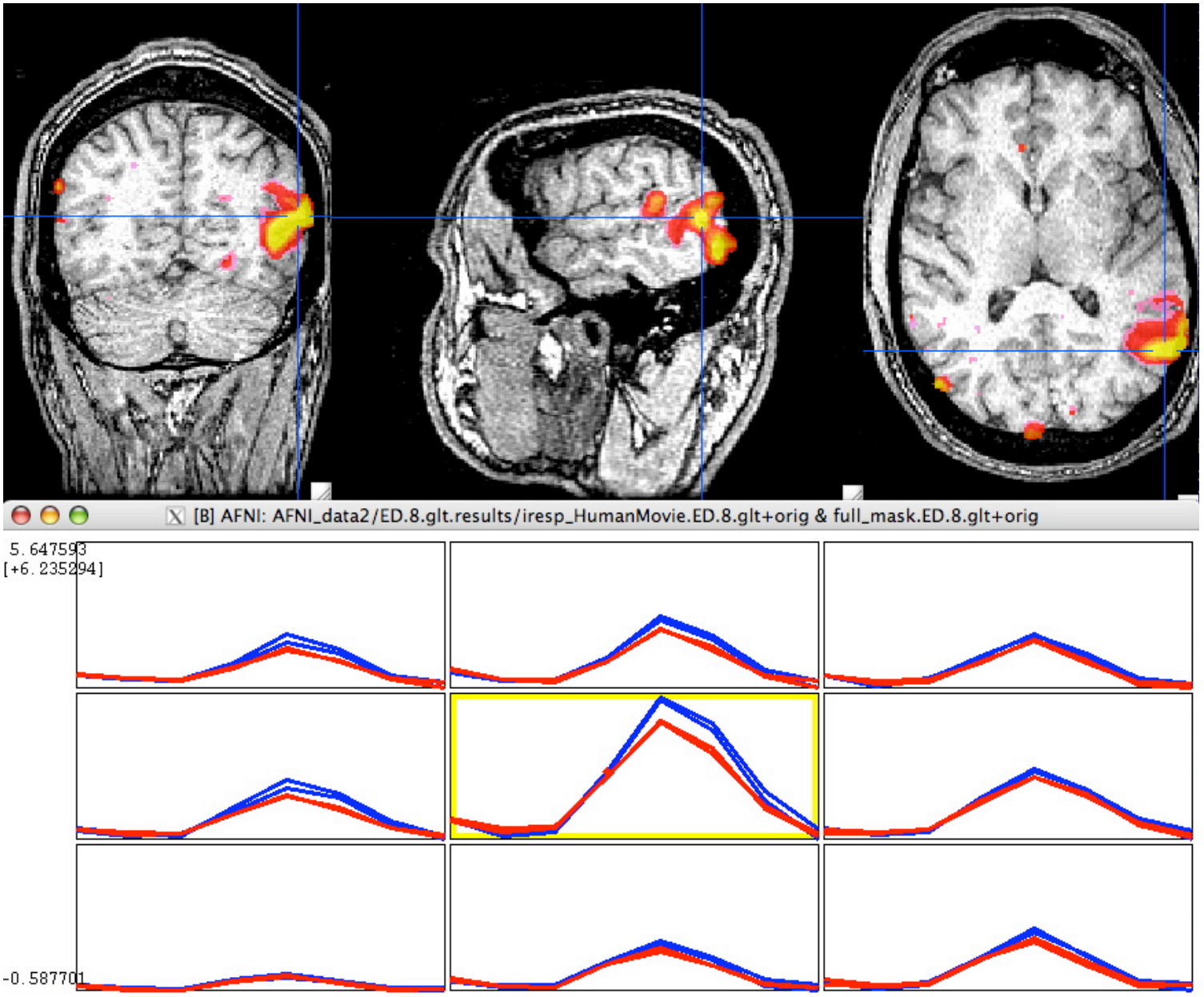
4 stim types

motion params

HRF outputs

GLTs

Results: Humans vs. Tools



- Color overlay: **HvsT** GLT contrast
- **Blue** (upper) graphs: Human HRFs
- **Red** (lower) graphs: Tool HRFs

Script - X Matrix

Via `1grayplot -sep Xmat.x1D`

Script - Random Comments

- **`-polort 2`**
  - ★Sets baseline (detrending) to use quadratic polynomials—in each run
- **`-mask full_mask.$subj+orig`**
  - ★Process only the voxels that are nonzero in this mask dataset
- **`-basis_normall 1`**
  - ★Make sure that the basis functions used in the HRF expansion all have maximum magnitude=1
- **`-stim_times 1 stimuli/stim_times.01.1D`**
  `'TENT(0,14,8)'`
  `-stim_label 1 ToolMovie`
  - ★The HRF model for the **ToolMovie** stimuli starts at 0 s after each stimulus, lasts for 14 s, and has 8 basis tent functions
    - o Which have knots (breakpoints) spaced 14/(8-1) = 2 s apart
- **`-iresp 1 iresp_ToolMovie.$subj`**
  - ★The HRF model for the **ToolMovie** stimuli is output into dataset **`iresp_ToolMovie.ED.8.glt+orig`**

Script - GLTs

- **`-gltsym ../misc_files/glt2.txt -glt_label 2 HvsT`**
  - ⋆ File **`../misc_files/glt2.txt`** contains 1 line of text:
    - **`-ToolMovie +HumanMovie -ToolPoint +HumanPoint`**
    - This is the "Humans vs. Tools" **HvsT** contrast shown on Results slide
- This GLT means to take all 8 β coefficients for each stimulus class and combine them with additions and subtractions as ordered:

$$\mathrm{LC} = -\beta_0^{\mathrm{TM}} - \cdots - \beta_7^{\mathrm{TM}} + \beta_0^{\mathrm{HM}} + \cdots + \beta_7^{\mathrm{HM}} - \beta_0^{\mathrm{TP}} - \cdots - \beta_7^{\mathrm{TP}} + \beta_0^{\mathrm{HP}} + \cdots + \beta_7^{\mathrm{HP}}$$

  - This test is looking at the integrated (summed) response to the "Human" stimuli and subtracting it from the integrated response to the "Tool" stimuli
- Combining subsets of the β weights is also possible with **`-gltsym`**:
  - **`+HumanMovie[2..6] -HumanPoint[2..6]`**
  - This GLT would add up just the #2,3,4,5, & 6 β weights for one type of stimulus and subtract the sum of the #2,3,4,5, & 6 β weights for another type of stimulus
    - And also produce *F*- and *t*-statistics for this linear combination

Script - Multi-Row GLTs

- GLTs presented up to now have had one row
  - ★ Testing if some linear combination of β weights is nonzero; test statistic is t or F ($F=t^2$ when testing a single number)
  - ★ Testing if the $\mathbf{X}$ matrix columns, when added together to form one column as specified by the GLT (**+** and **–**), explain a significant fraction of the data time series (equivalent to above)
- Can also do a single test to see if several different combinations of β weights are ***all*** zero

```
-gltsym ../misc_files/glt1.txt
-glt_label 1 FullF
```

4 rows

```
+ToolMovie
+HumanMovie
+ToolPoint
+HumanPoint
```

  - ★ Tests if ***any*** of the stimulus classes have nonzero integrated HRF (each name means "add up those β weights") : DOF = (4,1292)
  - ★ Different than the default "`Full F-stat`" produced by `3dDeconvolve`, which tests if any of the ***individual*** β weights are nonzero: DOF = (32,1292)

Two Possible Formats for `-stim_times`

- If you don't use **`-local_times`** or **`-global_times`**, 3dDeconvolve will *guess* which way to interpret numbers:

```
 4.7
 9.6
11.8
19.4
```

- A single column of numbers (GLOBAL times)
  - ★ One stimulus time per row
  - ★ Times are relative to first image in dataset being at ***t*=0**
  - ★ May not be simplest to use if multiple runs are catenated
- One row for each run within a catenated dataset (LOCAL times)
  - ★ Each time in j^{th} row is relative to start of run #*j* being ***t*=0**
  - ★ If some run has NO stimuli in the given class, just put a single "*****" in that row as a filler
    - o Different numbers of stimuli per run are OK
    - o At least one row must have more than 1 time (so that the LOCAL type of timing file can be told from the GLOBAL)

```
4.7 9.6 11.8 19.4
*
8.3 10.6
```

- Two methods are available because of users' diverse needs
  - ★ **N.B.**: if you chop first few images off the start of each run, the inputs to **`-stim_times`** must be adjusted accordingly***!***
    - o Better to use **`-CENSORTR`** to tell **`3dDeconvolve`** just to ignore those points

More information about -stim_times and its variants is in the afni07_advanced talk

Spatial Models of Activation

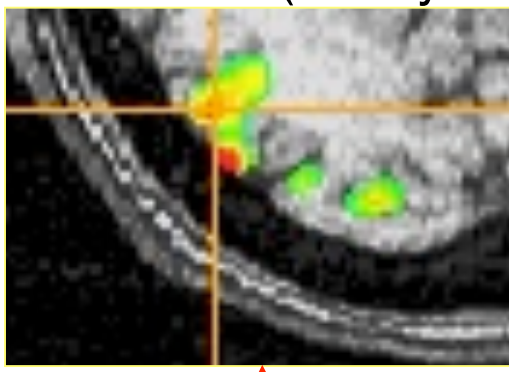
- Smooth data in space before analysis
- Average data across anatomically-selected regions of interest ROI (before or after analysis)
  - Labor intensive (*i.e.*, hire more students)
- Reject isolated small clusters of above-threshold voxels after analysis

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
    - o 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

Multi-Voxel Statistics

Spatial Clustering
&
False Discovery Rate:

"Correcting" the Significance

Basic Problem

- Usually have 30–200K FMRI voxels in the brain
- Have to make at least one decision about each one:
  - ★ Is it “active”?
    - o That is, does its time series match the temporal pattern of activity we expect?
  - ★ Is it differentially active?
    - o 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 = 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
    - o 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
    - o For $N \cdot p$ small (compared to 1), $\alpha_{FW} \approx N \cdot p$
    - o $N \approx$ 20,000+ voxels in the brain
    - o To keep probability of even one false positive $\alpha_{FW} < 0.05$ (the "corrected" p-value), need to have $p < 0.05 / 2 \times 10^4 = 2.5 \times 10^{-6}$
    - o 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
    - o Overall significance: α_{FW} = Prob(≥ one false positive voxel in the whole brain)
    - o **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: $p = 10^{-8} \ldots 10^{-6}$
    - o 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)
    - o 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`

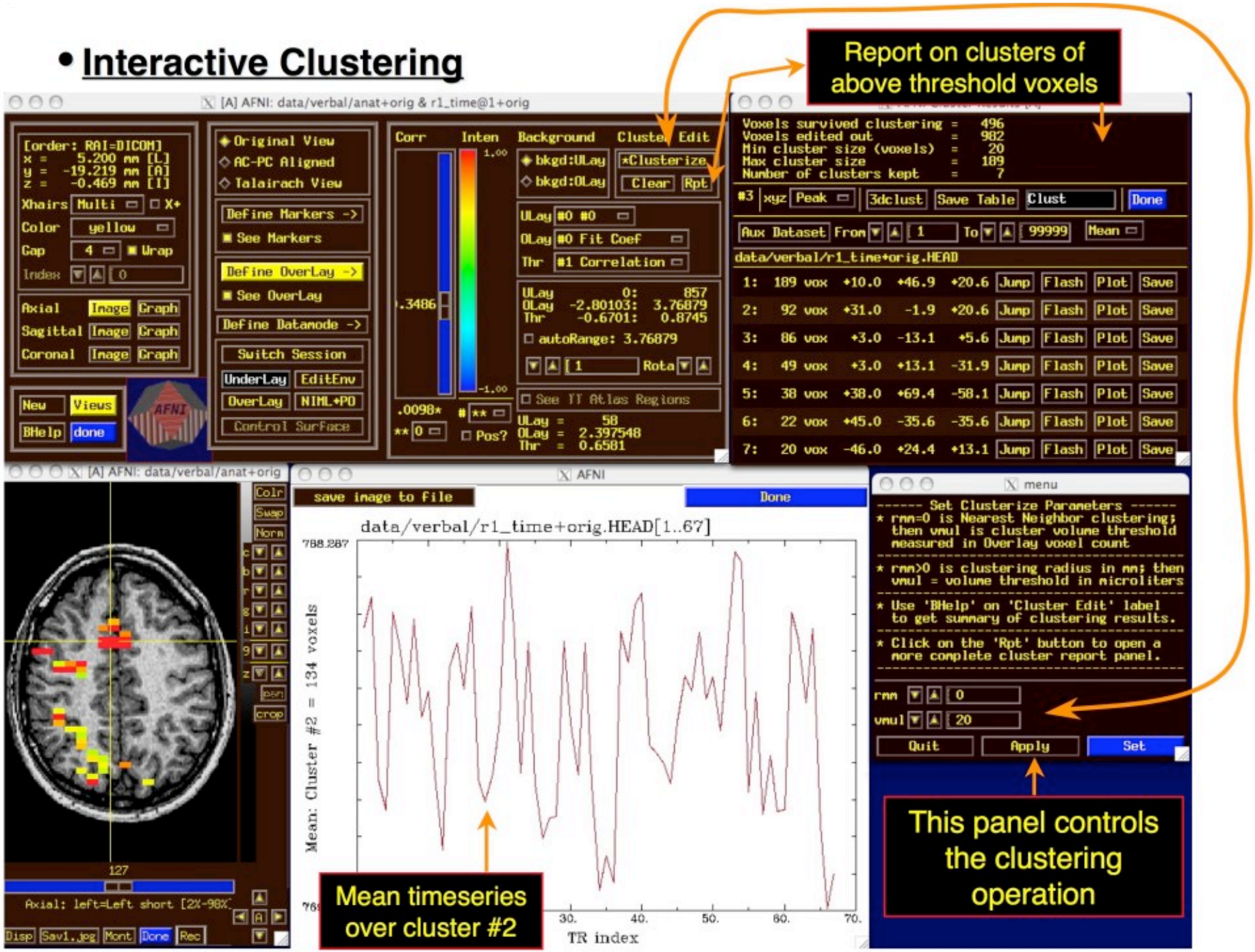
- **FWE control in AFNI**
  - ★ Monte Carlo simulations with program **`AlphaSim`**
    - o Named for a place where primary attractions are randomization experiments
    - o Randomly generate some number (*e.g.*, 1000) of brain volumes with white noise (spatially uncorrelated)
      - That is, each "brain" volume is purely in H_0 = no activation
      - Noise images can be blurred to mimic the smoothness of real data
    - o 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, …)
    - o 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
    - o 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 1st and 6th columns (ignore others)
  - ★ 1st column: cluster size in voxels
  - ★ 6th column: alpha (α) = 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	0.029000
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`) $p\uparrow$ ⇔ required minimum cluster size ↑
  - See detailed steps at **http://afni.nimh.nih.gov/sscc/gangc/mcc.html**

• Interactive Clustering



False Discovery Rate in 

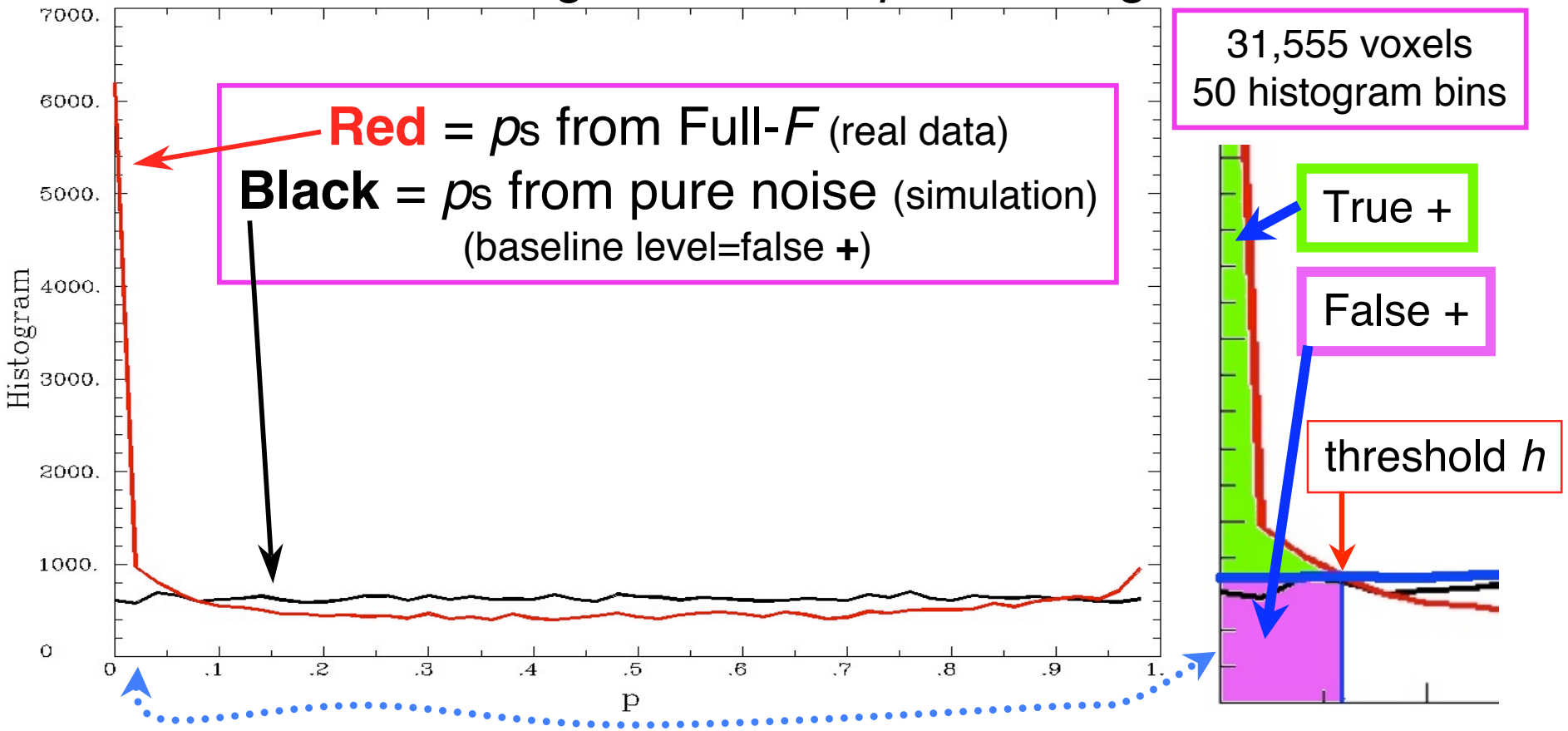
- Situation: making *many* statistical tests at once
  - e.g, Image voxels in FMRI; associating genes with disease
- Want to set threshold on statistic (e.g., *F*- or *t*-value) to control ***false positive*** error rate
- Traditionally: set threshold to control probability of making a ***single*** false 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*

- 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)

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

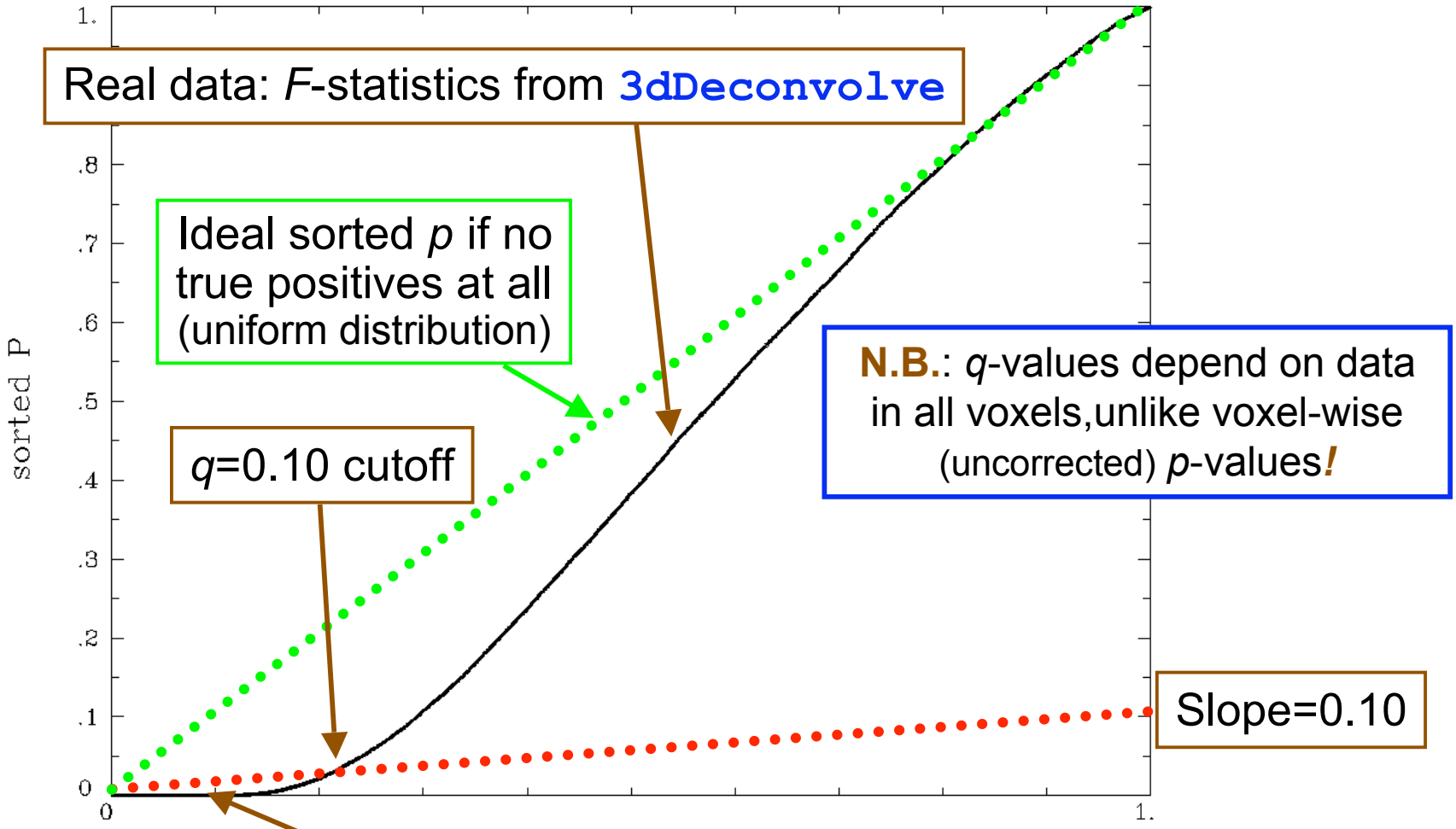


How *q* is Calculated from Data

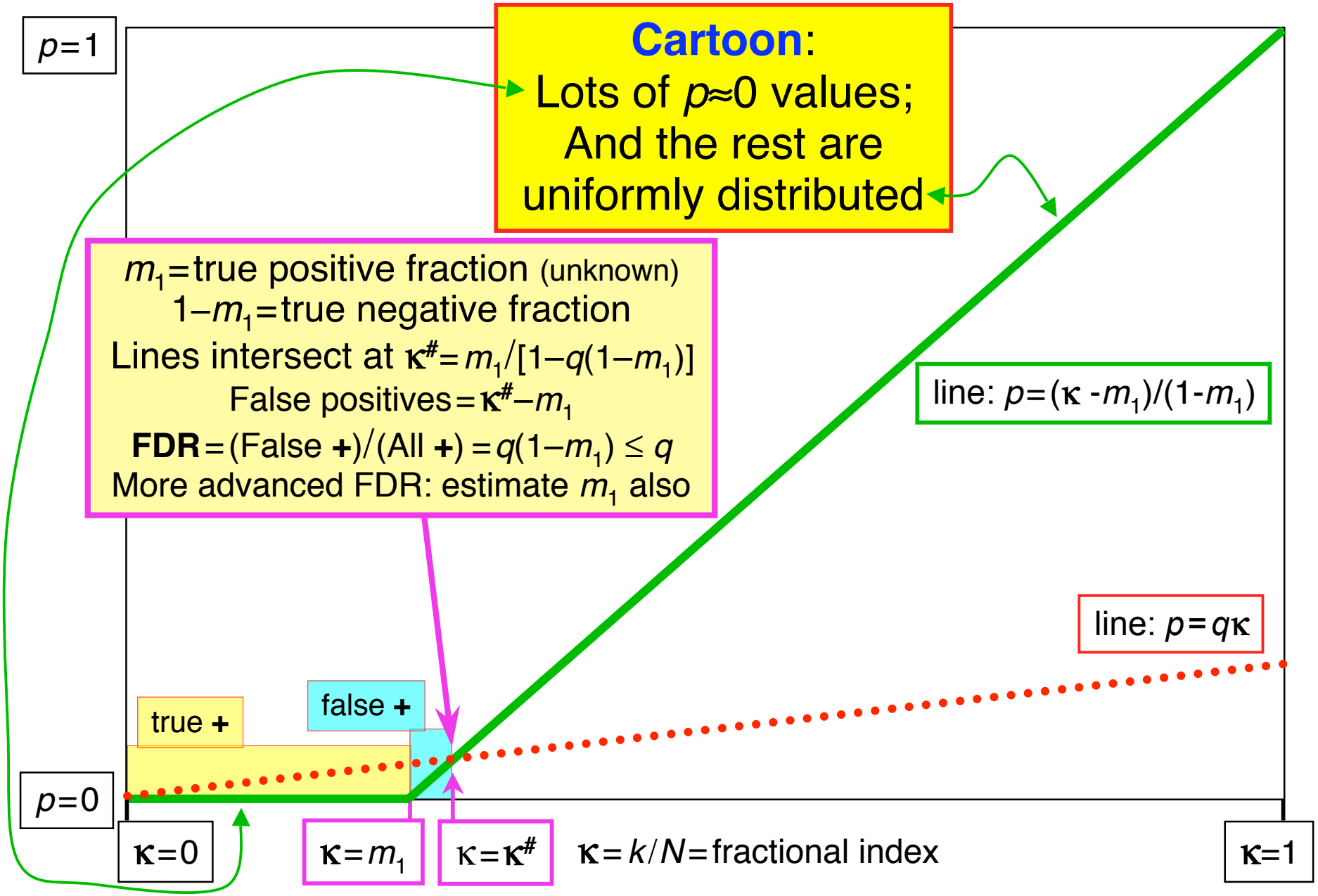
- Compute *p*-values of each statistic: $P_1, P_2, P_3, \cdots, P_N$
- Sort these: $P_{(1)} \le P_{(2)} \le P_{(3)} \le \cdots \le P_{(N)}$ {subscript$_{()}$ ≡ sorted}
- For $k = 1..N$, $q_{(k)} = \min_{m \ge k} [\, N \cdot P_{(m)} / m]$
  - Easily computed from sorted *p*-values by looping downwards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

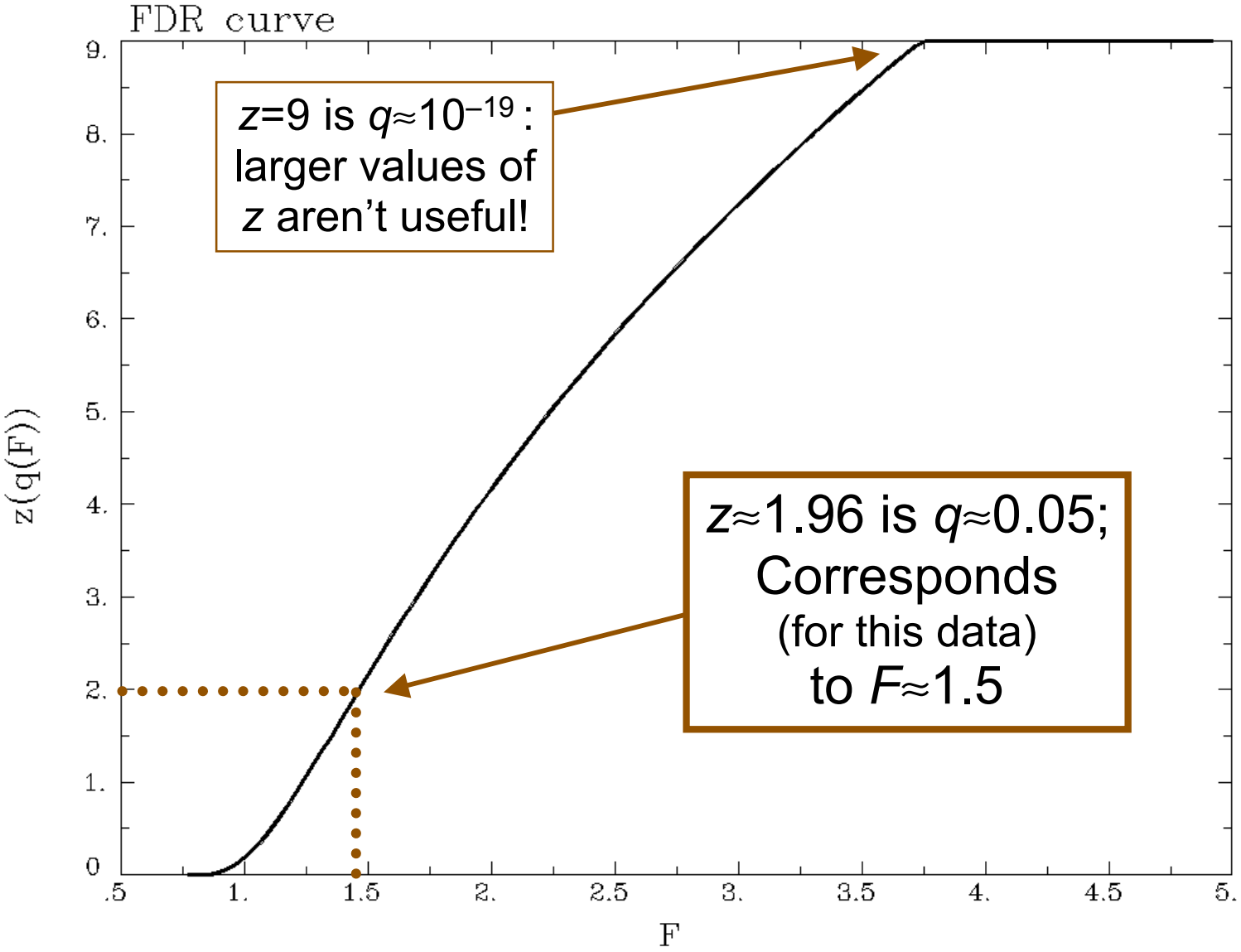
- Graph sorted *p*-values of voxel #*k* vs. $\kappa = k/N$ (the cumulative histogram of *p*, flipped sideways) and draw some lines from origin



Why This Line-Drawing Works



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

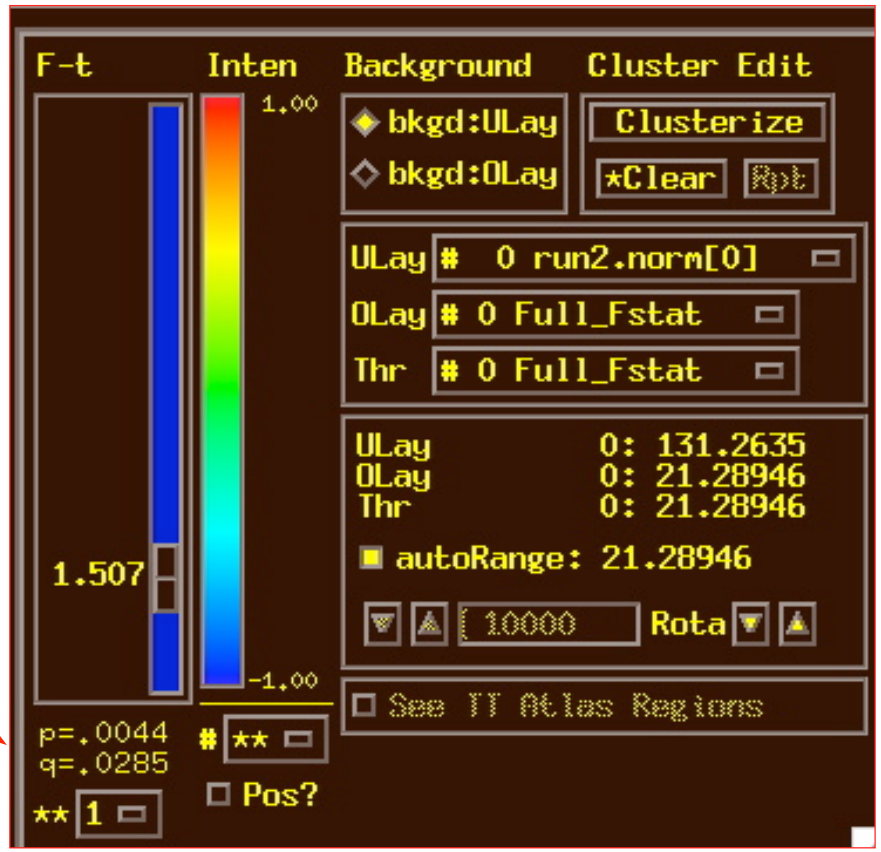


Recent Changes to `3dFDR`

- Don't include voxels with p=1 (e.g., F=0), even if they are in the `-mask` supplied on the command line
  - This changes 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^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. *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

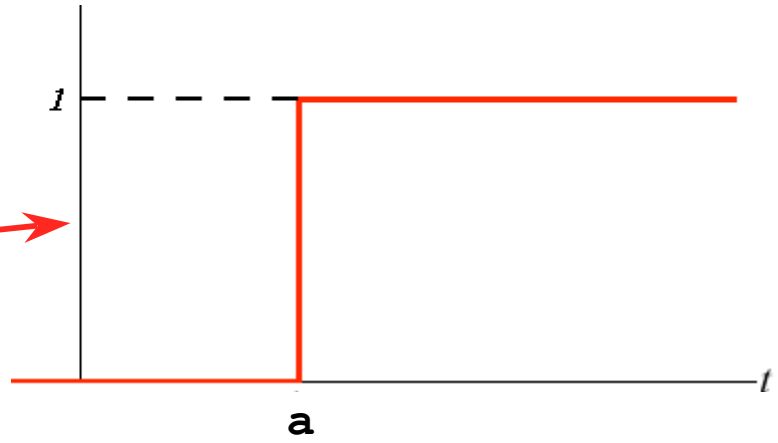
- FDR is conservative (*q*-values are too large) when voxels are positively correlated (e.g., from spatially smoothing)
  - Correcting for this is not so easy, since *q* depends on data (including true positives), so a simulation like **`AlphaSim`** is hard to conceptualize
  - At present, FDR is an 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: 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 25,000 EPI voxels
    - Uncorrected → ≈25 false positive voxels in the brain
    - FWE: corrected $p = 0.05$ → ≈5% of the time would expect one or more 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 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

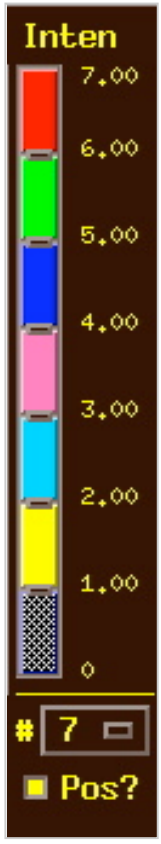
- **Conjunction**
  - ★ Dictionary: “a compound proposition that is true if and only if all of its component propositions are true”
  - ★ FMRI: areas that are active under 2 or more conditions (**AND** logic)
    - o e.g, in a visual language task and in an auditory language task
  - ★ Can also be used 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
    - o Heaviside step function defines a *On/Off* logic
    - o **step(t-a)** = 0 if **t** < **a**
      = 1 if **t** > **a**
    - o Can be used to apply more than one 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:
    - o 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 **`AlphaSim`**
  - ★ 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!