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”?
    - 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)**
  - ★ Simple probability example: sex ratio at birth = 1:1
    - o What is the chance there are 5 boys in a family with 5 kids? $(1/2)^5 \approx 0.03$
    - o In a pool of 10,000 families with 5 kids, expected #families with 5 boys =? $10{,}000 \times (2)^{-5} \approx 312$
  - ★ 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)

- **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 $p << 1/N$
      - 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
      - Cluster: 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`

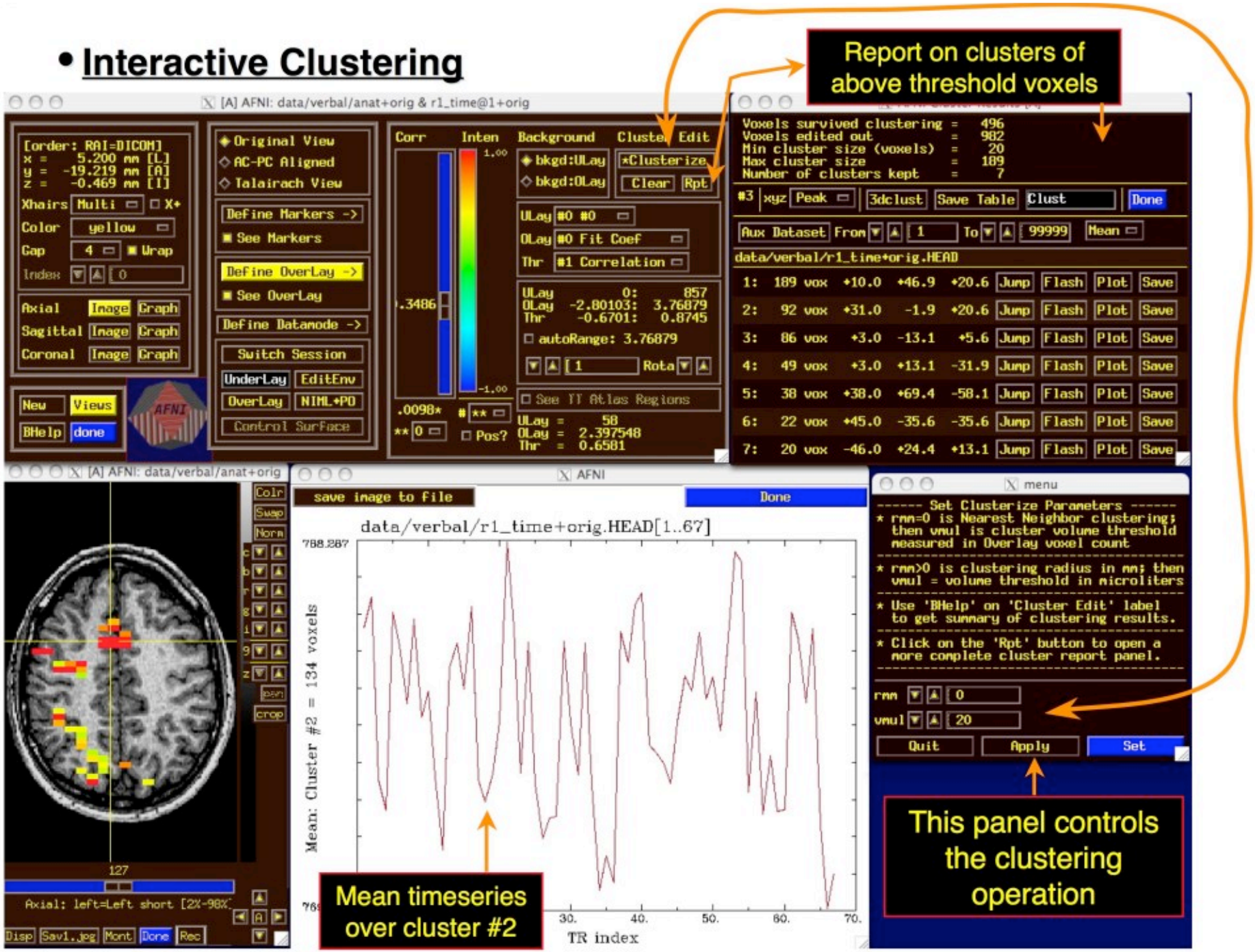
- **FWE control in AFNI**
  - ★ Monte Carlo simulations with program **`AlphaSim`**
    - o Named for a place where the primary attractions are casinos
    - o Randomly generate some number (*e.g.*, 1000) of brain volumes with white noise (spatially uncorrelated)
      - That is, each "brain" volume 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 p↑ ⇔ 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 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 $z(q)$]

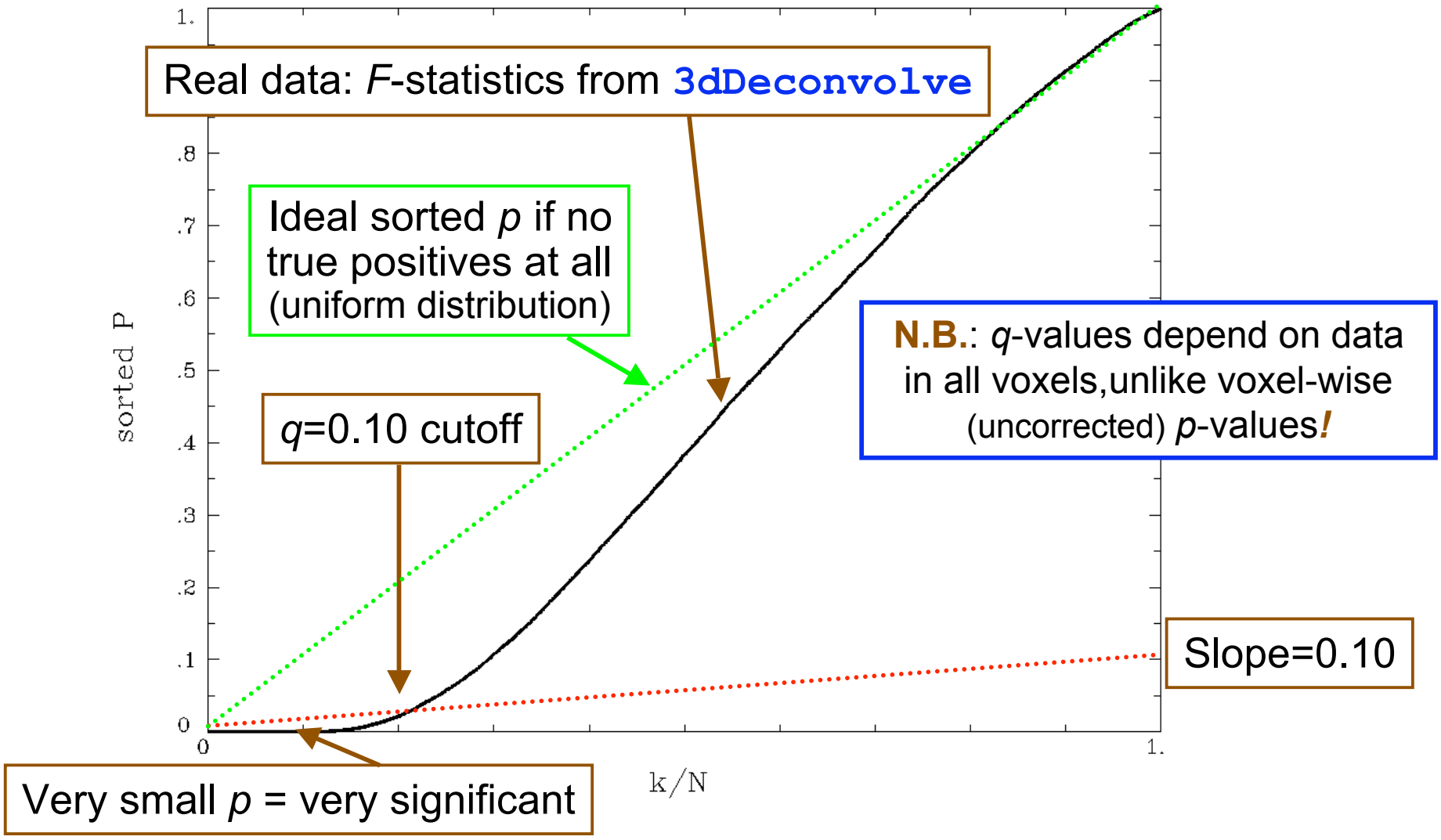
- Given some collection of statistics (say, *F*-values from **`3dDeconvolve`**), set a threshold *h*
- The **uncorrected *p*-value** of *h* is the probability $F > h$ when the null hypothesis is true (no activation)
  - "Uncorrected" means "per-voxel"
  - The "corrected" *p*-value is the probability 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*-score: e.g, $z(0.05) \approx 1.95996$
    - So that larger is "better" (in the same sense): e.g, $z(0.01) \approx 2.57583$

How *q* is Calculated from Data

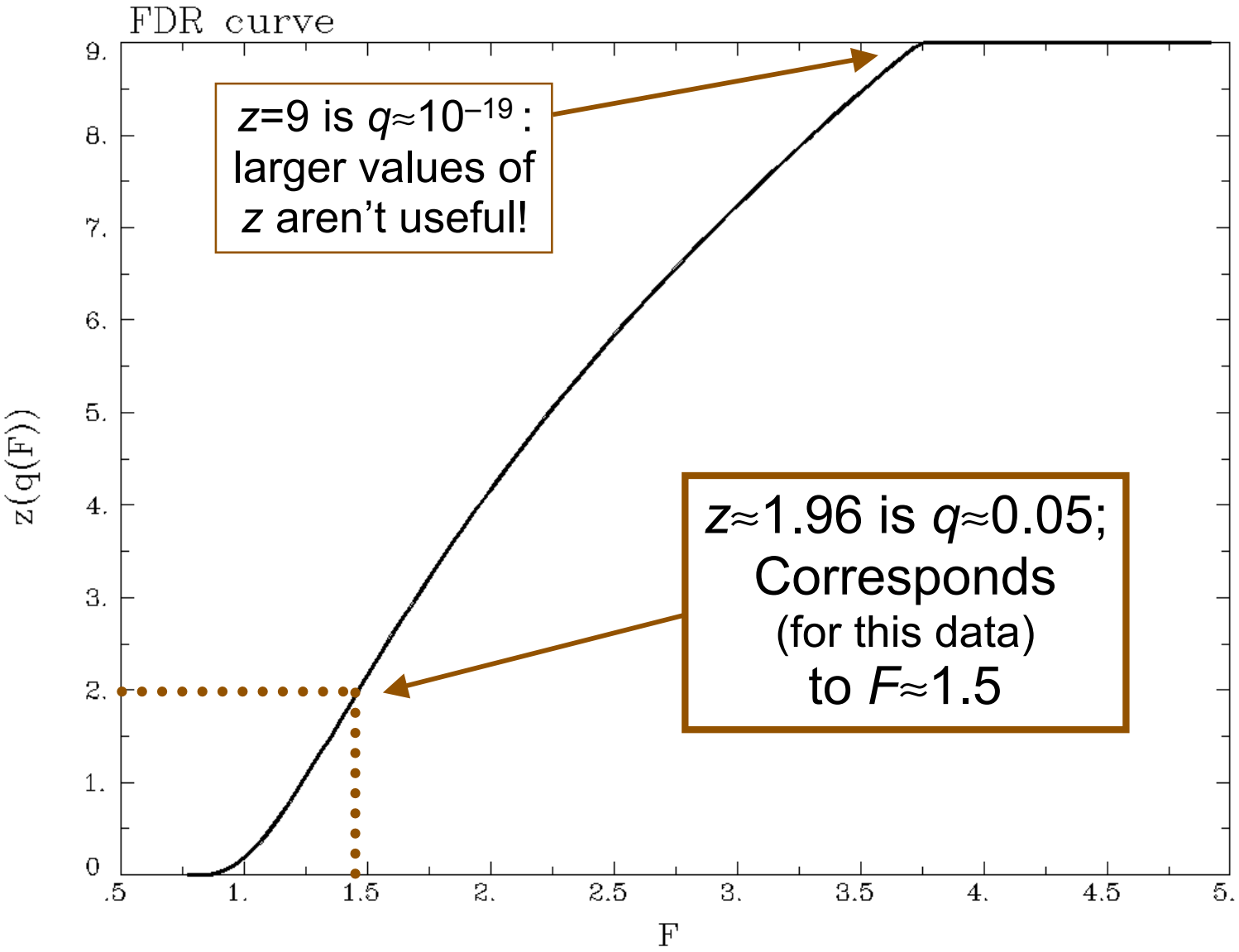
- Compute *p*-values of each statistic: $P_1, P_2, P_3, \cdots, P_N$
- Sort these: $P_{(1)} \le P_{(2)} \le P_{(3)} \le \cdots \le P_{(N)}$ {subscript$_{()}$ ≡ sorted}
- For $k = 1..N$, $q_{(k)} = \min_{m \ge k} [\, N \cdot P_{(m)} / m]$
  - Easily computed from sorted *p*-values by looping downwards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

Graphical Calculation of *q*

- Graph sorted *p*-values of voxel #*k* vs. *k*/*N* and draw lines from origin



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

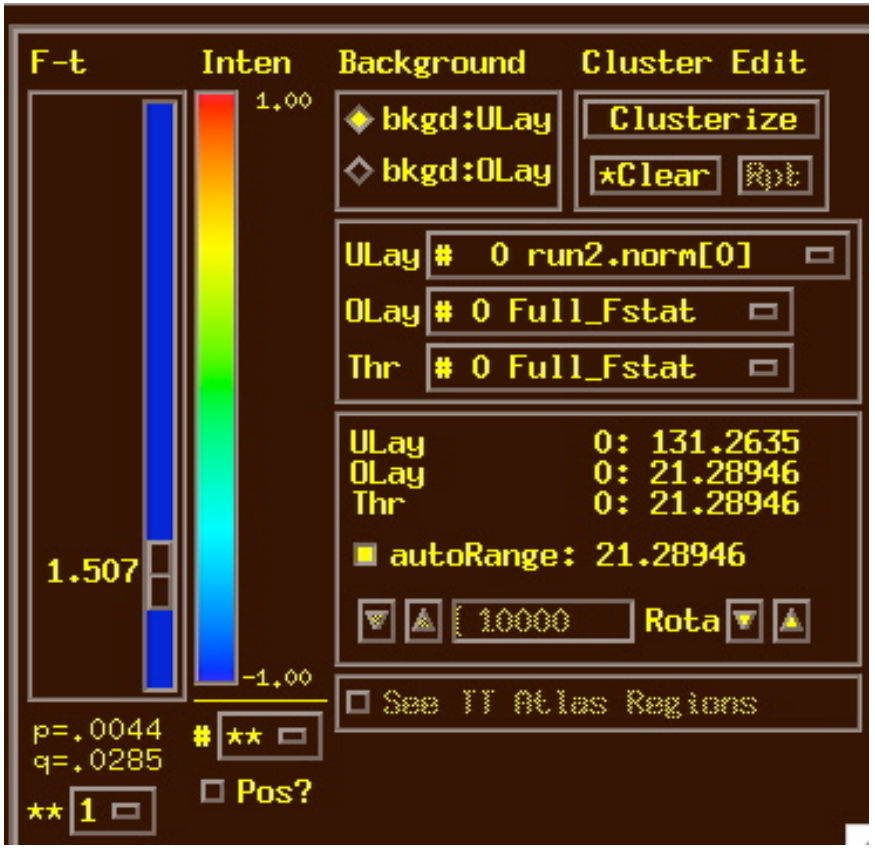


Recent Changes to `3dFDR`

- 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. *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 older 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"



FDR Statistical Issues

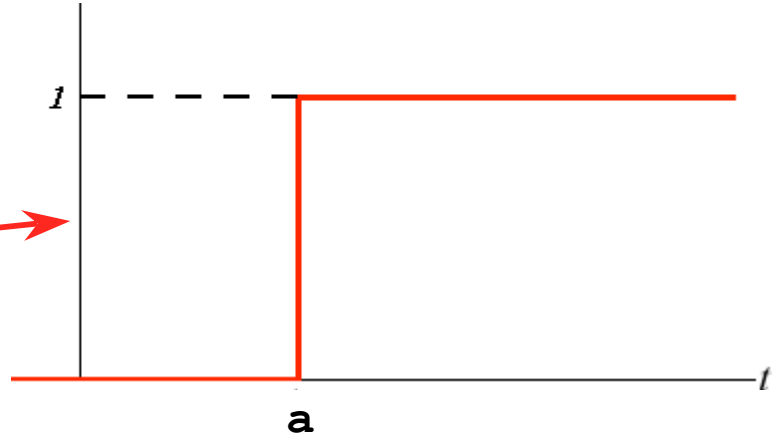
- FDR is conservative (*q*-values are too large) when voxels are positively correlated (e.g., from spatially smoothing)
  - Correcting for this is not so easy, since *q* depends on data (including true positives), so a simulation like **`AlphaSim`** is hard to conceptualize
  - At present, FDR is an 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
    - Trial calculations show that this may not be a significant effect, compared to spatial smoothing (which tends to make *q* too large)

FWE or FDR?

- These 2 methods control Type I error in different sense
  - ★ FWE: α_{FW} = Prob (≥ one false positive voxel 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 + 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

Conjunction Analysis

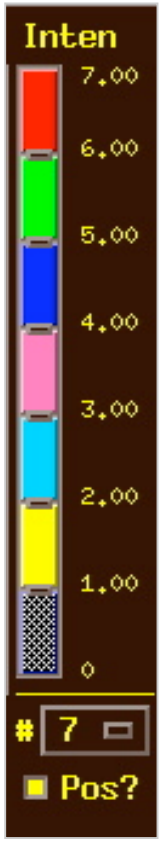
- **Conjunction**
  - ★ Dictionary: “a compound proposition that is true if and only if all of its component propositions are true”
  - ★ FMRI: areas that are active under 2 or more conditions (AND logic)
    - o e.g, in a visual language task and in an auditory language task
  - ★ Can also be used to 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 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:
    - 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 before conjunction analysis using **`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 Gaussian distributions
  - ★ With more than 3 entities, may have to resort to simulations
    - Monte Carlo simulations?
    - Will Gang write such a program? Only time will tell!