



## • Group Analysis: Basic concepts

### ¶ Group analysis

- ↳ Make general conclusions about some population
- ↳ Partition/untangle data variability into various sources

### ¶ Why two types of analysis?

- ↳ High computation cost
- ↳ Within-subject variation ignored

### ¶ Mess in terminology

- ↳ Fixed: factor, model (effects)
  - Fixed-effects analysis (sometimes): averaging a few subjects
- ↳ Random: factor, model (effects)
  - Random-effects analysis (sometimes): subject as a random factor
  - But really a mixed-effects analysis
  - Psychologists: Within-subject (repeated measures) / between-subjects factor
- ↳ Mixed: design, model (effects)
  - Mixed design: crossed [e.g., AXBXC] and nested [e.g., BXC(A)]
  - Mixed-effects: model with both types of factors; model with both inter/intra-subject variances

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## • Group Analysis: Basic concepts

### ¶ Fixed factor

- ↳ Treated as a fixed variable in the model
  - Categorization of experiment conditions (modality: visual/audial)
  - Group of subjects (gender, normal/patients)
- ↳ All levels of the factor are of interest and included for all replications
- ↳ Fixed in the sense inferences
  - apply only to the specific levels of the factor
  - don't extend to other potential levels that might have been included

### ¶ Random factor

- ↳ Exclusively subject in FMRI
- ↳ Treated as a random variable in the model
  - average + effects uniquely attributable to each subject:  $N(\mu, \sigma^2)$
- ↳ Each subject is of NO interest
- ↳ Random in the sense
  - subjects serve as a random sample of a population
  - inferences can be generalized to a population

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## • Group Analysis: Types

### • Averaging across subjects

- ↳ Number of subjects  $n < 6$
- ↳ Case study: can't generalize to whole population
- ↳ Simple approach (3dcalc)
  - $T = \sum t_i / \sqrt{n}$
- ↳ Sophisticated approach
  - $B = \sum (b_i / \sqrt{v_i}) / \sum (1 / \sqrt{v_i})$ ,  $T = B \sqrt{\sum (1 / \sqrt{v_i}) / \sqrt{n}}$ ,  $v_i$  = variance for  $i$ -th regressor
  - $B = \sum (b_i / v_i) / \sum (1 / v_i)$ ,  $T = B \sqrt{\sum (1 / v_i)}$
  - Combine individual data and then run regression

### • Mixed-effects analysis

- ↳ Number of subjects  $n > 10$
- ↳ Random effects of subjects
- ↳ Individual and group analyses: separate
- ↳ Within-subject variation ignored
- ↳ Main focus of this talk

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## • Group Analysis: Programs in AFNI

### • Non-parametric analysis

- ↳  $4 < \text{number of subjects} < 10$
- ↳ No assumption of normality; statistics based on ranking
- ↳ Programs
  - 3dWilcoxon (~ paired  $t$ -test)
  - 3dMannWhitney (~ two-sample  $t$ -test)
  - 3dKruskalWallis (~ between-subjects with 3dANOVA)
  - 3dFriedman (~ one-way within-subject with 3dANOVA2)
  - Permutation test: plugin on AFNI under Define Datamode / Plugins /; C program by Tom Holroyd
- ↳ Multiple testing correction with FDR (3dFDR)
- ↳ Can't handle complicated designs
- ↳ Less sensitive to outliers (more robust) and less flexible than parametric tests

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## • Group Analysis: Programs in AFNI

### ¶ Parametric tests

- ↳ Number of subjects > 10
- ↳ Assumption: Gaussian
- ↳ Programs
  - `3dttest` (one-sample, two-sample and paired t)
  - `3dANOVA` (one-way between-subject)
  - `3dANOVA2` (one-way within-subject, 2-way between-subjects)
  - `3dANOVA3` (2-way within-subject and mixed, 3-way between-subjects)
  - `3dRegAna` (regression/correlation, hi-way or unbalanced ANOVA, ANCOVA)
  - `GroupAna` (Matlab package for up to 5-way ANOVA)
  - `3dLME` (R package for all sorts of group analysis)

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## • Group Analysis: Planning

### ¶ How many subjects?

- ↳ Power/efficiency: proportional to  $\sqrt{n}$ ;  $n > 10$
- ↳ Balance: Equal number of subjects across groups if possible

### ¶ Input files

- ↳ Common brain in tlrc space (resolution doesn't have to be  $1 \times 1 \times 1 \text{ mm}^3$ )
- ↳ % signal change (**not** statistics) or normalized variables
  - HRF magnitude: Regression coefficients
  - Contrasts

### ¶ Design

- ↳ Number of factors
- ↳ Number of levels for each factor
- ↳ Factor types
  - Fixed (factors of interest) vs. random (subject)
  - Cross/nesting: Balanced? Within-subject/repeated-measures vs. between-subjects
- ↳ Which program?
  - `3dttest`, `3dANOVA/2/3`, `GroupAna`, `3dLME`

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• **Group Analysis: Planning**

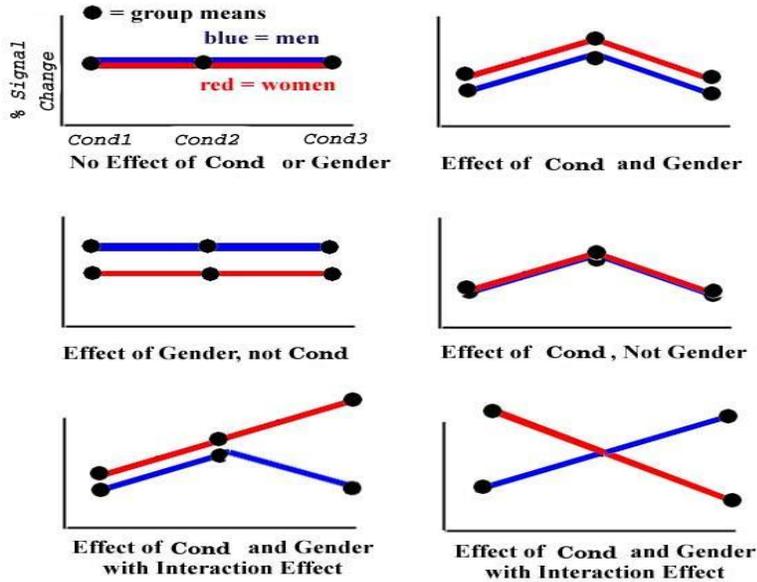
• **Output**

- ↳ Main effect F
  - F: general information about all levels of a factor
  - Any difference response between two sexes
- ↳ Interaction F
  - Mutual/reciprocal influence among 2 or more factors
  - Effect for each factor depends on levels of other factors
- ↳ Example
  - Dependent variable: income
  - Factor A: sex (men vs. women); factor B: race (whites vs. blacks)
  - Main effects: men > women; whites > blacks
  - Is it fair to only focus on main effects? Interaction!
    - Black men < black women;
    - Black women almost the same as white women;
    - Black men << white men

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• **Group Analysis: Main effect and interaction**

Main effects and Interactions Between Gender and Condition



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## • Group Analysis: Planning

### ¶ Output

- ↳ General linear test
  - Contrast
  - General linear test
  - Trend analysis

### ¶ Thresholding

- ↳ Two-tail by default
- ↳ For a desirable one-tail  $p$ , look for  $2p$  on AFNI

### ¶ Scripting – 3dANOVA3

- ↳ **Three-way between-subjects** (type 1)
  - 3 categorizations of groups: sex, disease, age
- ↳ **Two-way within-subject** (type 4): Crossed design AXBXC
  - One group of subjects: 16 subjects
  - Two categorizations of conditions: A – category; B - affect
- ↳ **Two-way mixed** (type 5): BXC(A)
  - Nesting (between-subjects) factor (A): subject classification, e.g., sex
  - One category of condition (within-subject factor B): condition (visual vs. audial)
  - Nesting: balanced

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## • Group Analysis: Example

```

3dANOVA3 -type 4 -alevels 3 -blevels 3 -clevels 16 \
-dset 1 1 1 stats.sb04.beta+tlrc'[0]' \
-dset 1 2 1 stats.sb04.beta+tlrc'[1]' \
-dset 1 3 1 stats.sb04.beta+tlrc'[2]' \
-dset 2 1 1 stats.sb04.beta+tlrc'[4]' \
...
-fa Category \
-fb Affect \
-fab CatXAff \
-amean 1 T \ (coding with indices)
-adiff 1 2 TvsE \ (coding with indices)
-acontr 1 0 -1 TvsF \ (coding with coefficients)
-bcontr 0.5 0.5 -1 non-neu \ (coefficients)
-aBdiff 1 2 : 1 TvsE-pos \ (indices)
-aBcontr 1 -1 0 : 1 TvsE-pos \ (coefficients)
-Abdiff 1 : 1 2 TMvsTP \ (indices)
-Abcontr 2 : 1 -1 0 HMvsHP \ (coefficients)
bucket_anova33

```

Model type, Factor levels

Input for each cell in ANOVA table: totally 3X3X8 = 32

F tests: Main effects & interaction

F tests: 1<sup>st</sup> order Contrasts

F tests: 2<sup>nd</sup> order Contrasts

Output: bundled

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## • Group Analysis: GroupAna

### ¶ Multi-way ANOVA

- ↳ Matlab script package for up to 5-way ANOVA
- ↳ Can handle both volume and surface data
- ↳ Can handle up to 4-way unbalanced designs
  - No missing data allowed
- ↳ Downsides
  - Requires Matlab plus Statistics Toolbox
  - GLM approach: regression through dummy variables
  - Complicated design, and compromised power
- ↳ Heavy duty computation
  - minutes to hours
  - Input with lower resolution recommended
  - Resample with `adwarp -dxyz #` or `3dresample`
- ↳ See <http://afni.nimh.nih.gov/sscc/gangc> for more info

### ¶ Alternative: **3dLME**

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## • Group Analysis: ANCOVA (ANalysis of COVAriances)

### ¶ Why ANCOVA?

- ↳ Subjects might not be an ideally randomized representation of a population
- ↳ If not controlled, cross-subject variability will lead to loss of power and accuracy
- ↳ Different from amplitude modulation: cross-subject vs. within-subject variation
- ↳ Direct control through experiment design: balanced selection of subjects (e.g., age group)
- ↳ Indirect (statistical) control: add covariates in the model
- ↳ Covariate (variable of no interest): uncontrollable/confounding, usually continuous
  - Age, IQ, cortex thickness
  - Behavioral data, e.g., response time, correct rate, symptomatology score, ...
  - Even categorical factors such as gender

### ¶ ANCOVA = Regression + ANOVA

- ↳ Assumption: linear relation between HDR and the covariate
- ↳ GLM approach: accommodate both categorical and quantitative variables

### ¶ Programs

- ↳ **3dRegAna**: for simple ANCOVA
  - If the analysis can be handled with `3dtttest` without covariates
- ↳ **3dLME**: R package (versatile)

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• **Group Analysis:** ANCOVA Example

• **Example: Running ANCOVA**

- ↳ Two groups: 15 normal vs. 13 patients
- ↳ Analysis
  - Compare two group: without covariates, two-sample *t* with `3dttest`
  - Controlling age effect
- ↳ GLM model
  - $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \epsilon_i, i = 1, 2, \dots, n (n = 28)$
  - Code the factor (group) with dummy coding
    - 0, when the subject is a patient – control group;
    - $X_{2i} = \{$ 
      - 1, when the subject is normal.
  - **Centralize** covariate (age)  $X_1$  so that
    - $\beta_0$  = patient effect;  $\beta_1$  = age effect (correlation coef);  $\beta_2$  = normal vs patient
  - $X_{3i} = X_{1i} X_{2i}$  models interaction (optional) between covariate and factor (group)
    - $\beta_3$  = interaction

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• **Group Analysis:** ANCOVA Example

```
3dRegAna -rows 28 -cols 3 \
-xydata 0.1 0 0 patient/Pat1+tlrc.BRIK \
-xydata 7.1 0 0 patient/Pat2+tlrc.BRIK \
...
-xydata 7.1 0 0 patient/Pat13+tlrc.BRIK \
-xydata 2.1 1 2.1 normal/Norm1+tlrc.BRIK \
-xydata 2.1 1 2.1 normal/Norm2+tlrc.BRIK \
...
-xydata 0.1 1 0.1 normal/Norm15+tlrc.BRIK \
-model 1 2 3 : 0 \
-bucket 0 Pat_vs_Norm \
-brick 0 coef 0 'Pat' \
-brick 1 tstat 0 'Pat t' \
-brick 2 coef 1 'Age Effect' \
-brick 3 tstat 1 'Age Effect t' \
-brick 4 coef 2 'Norm-Pat' \
-brick 5 tstat 2 'Norm-Pat t' \
-brick 6 coef 3 'Interaction' \
-brick 7 tstat 3 'Interaction t'
```

Model parameters: 28 subjects, 3 independent variables

Input: Covariates, factor levels, interaction, and input files

Specify model for F and R<sup>2</sup>

Output: #subbricks = 2\*#coef + F + R<sup>2</sup>

Label output subbricks for  $\beta_0, \beta_1, \beta_2, \beta_3$

See <http://afni.nimh.nih.gov/sscc/gangc/ANCOVA.html> for more information

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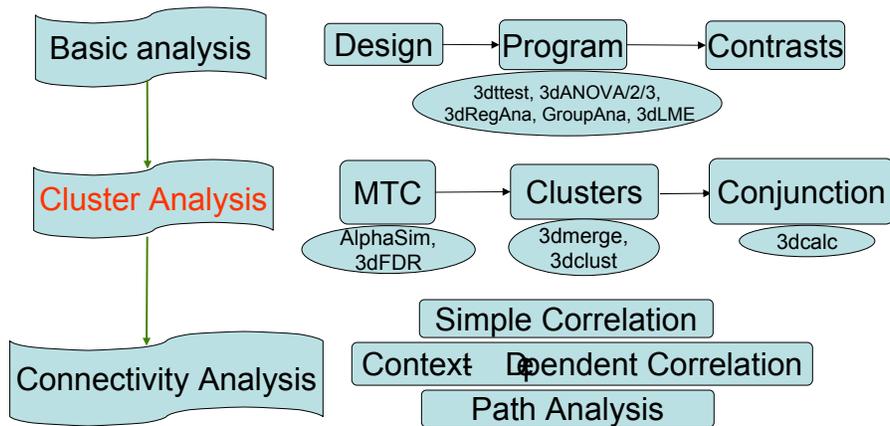
## • Group Analysis: 3dLME

### 📄 An R package

- ↳ Open source platform
- ↳ Linear mixed-effects modeling (LME)
- ↳ Handles various situations in one package
  - Unbalanced designs
  - Missing data
  - ANOVA and ANCOVA
  - Modeling with basis functions
  - Heteroscedasticity, variance-covariance structure
- ↳ Disadvantages
  - High computation cost
  - Sometimes difficult to compare with traditional ANOVA
- ↳ Still under development
- ↳ See <http://afni.nimh.nih.gov/sscc/gangc/lme.html> for more information

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## Group Analysis



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## • Cluster Analysis: Multiple testing correction

### • Two types of errors

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

### • Family-Wise Error (FWE)

- ↳ Birth rate  $H_0$ : sex ratio at birth = 1:1
  - ↳ What is the chance there are 5 boys (or girls) in a family?  $(1/2)^5 \sim 0.03$
  - ↳ In a pool of 10000 families with 5 kids, expected #families with 5 boys =?  
 $10000 \times (1/2)^5 \sim 300$
- ↳ Multiple testing problem: voxel-wise statistical analysis
  - ↳ With  $n$  voxels, what is the chance to mistake  $\geq$  one voxel?  
Family-Wise Error:  $\alpha_{FW} = 1 - (1 - p)^n \rightarrow 1$  as  $n$  increases
  - ↳  $n \sim 20,000$  voxels in the brain

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## • Cluster Analysis: Multiple testing correction

### • Multiple testing problem in FMRI

- ↳ 3 occurrences of multiple tests: individual, group, and conjunction
- ↳ Group analysis is the most concerned

### • Approaches

- ↳ Control FWE
  - ↳ Overall significance:  $\alpha_{FW} = P(\geq \text{one false positive voxel in the whole brain})$
  - ↳ Bonferroni correction:  $\alpha_{FW} = 1 - (1 - p)^n \sim np$ , if  $p \ll 1/n$ 
    - \* Use  $p = \alpha/n$  as individual voxel significance level to achieve  $\alpha_{FW} = \alpha$
    - \* Too stringent and overly conservative:  $p = 10^{-8} \sim 10^{-6}$
  - ↳ Something to rescue?
    - \* Correlation: Voxels in the brain are not independent
    - \* Cluster: Structures in the brain
    - \* Control FWE based on spatial correlation and cluster size
- ↳ Control false discovery rate (FDR)
  - ↳ FDR = expected proportion of false + voxels among all detected voxels

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• **Cluster Analysis: AlphaSim**

‣ FWE in AFNI

- ↳ Monte Carlo simulations with **AlphaSim**
- ↳ Named for Monte Carlo, Monaco, where the primary attractions are casinos
- ↳ Program: **AlphaSim**
  - Randomly generate some number (e.g., 1000) of brains with white noise
  - Count the proportion of voxels are false + in **ALL** (e.g., 1000) brains
  - Parameters:
    - \* ROI - mask
    - \* Spatial correlation - FWHM
    - \* Connectivity – radius: how to identify voxels belong to a cluster?
    - \* Individual voxel significant level - uncorrected  $p$
  - Output
    - \* Simulated (estimated) **overall significance level** (corrected  $p$ -value)
    - \* Corresponding **minimum cluster size**

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• **Cluster Analysis: AlphaSim**

- ↳ Program: **AlphaSim**
  - See detailed steps at <http://afni.nimh.nih.gov/sscc/gangc/mcc.html>

‣ Example

```
AlphaSim \
-mask MyMask+orig \
-fwhmx 8.5 -fwhmy 7.5 -fwhmz 8.2 \
-rmm 6.3 \
-pthr 0.0001 \
-iter 1000
```

The screenshot shows a GUI for the AlphaSim program with several input fields. Red arrows from the command line point to these fields:
 

- Program**: Points to the 'Program' field.
- Restrict correcting region: ROI**: Points to the 'Restrict correcting region: ROI' field.
- Spatial correlation**: Points to the 'Spatial correlation' field.
- Connectivity: how clusters are defined**: Points to the 'Connectivity: how clusters are defined' field.
- Uncorrected p**: Points to the 'Uncorrected p' field.
- Number of simulations**: Points to the 'Number of simulations' field.

- Output: 5 columns
  - \* Focus on the 1<sup>st</sup> and last columns, and ignore others
  - \* 1<sup>st</sup> column: minimum cluster size in voxels
  - \* Last column: alpha ( $\alpha$ ), overall significance level (corrected  $p$  value)

Cl Size	Frequency	Cum Prop	p/Voxel	Max Freq	Alpha
2	1226	0.999152	0.00509459	831	0.859
5	25	0.998382	0.00015946	25	0.137
10	3	1.0	0.00002432	3	0.03

- May have to run several times with different uncorrected  $p$

uncorrected  $p \uparrow \leftrightarrow$  cluster size  $\uparrow$

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## Cluster Analysis: 3dFDR

### Definition

FDR = % false + voxels among all detected voxels in **ONE** brain

$$FDR = \frac{N_{ia}}{D_a} = \frac{N_{ia}}{N_{ia} + N_{aa}}$$

- ↳ FDR only focuses on individual voxel's significance level within the ROI, but doesn't consider any spatial structure
  - spatial correlation
  - cluster size

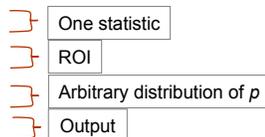
### Algorithm

↳ statistic ( $t$ ) →  $p$  value → FDR ( $q$  value) →  $z$  score

### Example

```
3dFDR -input 'Group+tlrc[6]' \
      -mask_file mask+tlrc \
      -cdep -list \
      -output test
```

	Declared Inactive	Declared Active	
Truly Inactive	$N_{ii}$	$N_{ia}(I)$	$T_i$
Truly Active	$N_{ai}(II)$	$N_{aa}$	$T_a$
	$D_i$	$D_a$	



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## Cluster Analysis: FWE or FDR?

### FWE or FDR? Correct type I error in different sense

- ↳ FWE:  $\alpha_{FW} = P(\geq \text{one false positive voxel in the whole brain})$ 
  - Frequentist's perspective: Probability among **many** hypothetical activation brains
  - Used usually for parametric testing
- ↳ FDR = expected % false + voxels among all detected voxels
  - Focus: controlling false + among detected voxels in **one** brain
  - More frequently used in non-parametric testing
- ↳ Concrete example
  - Individual voxel  $p = 0.001$  for a brain of 25,000 EPI voxels
  - Uncorrected → 25 false + voxels in the brain
  - FWE: corrected  $p = 0.05$  → 5% false + hypothetical brains for a fixed voxel location
  - FDR: corrected  $p = 0.05$  → 5% voxels in those **positively** labeled ones are false +

### Fail to survive correction?

- ↳ Tricks
  - One-tail?
  - ROI – e.g., grey matter or whatever ROI you planned to look into
- ↳ Analysis on surface

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- **Cluster Analysis:** Conjunction analysis

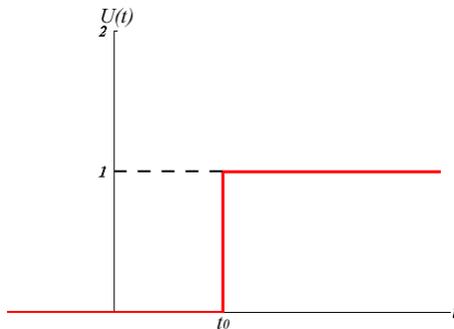
- **Conjunction analysis**

- ↳ Common activation area: intersection
    - ↳ Exclusive activations
    - ↳ With  $n$  entities, we have  $2^n$  possibilities (review your combinatorics!)

- **Tool: 3dcalc**

- ↳ Heaviside unit (step function) defines a *On/Off* event

$$U(t-t_0) = \begin{cases} 1 & t \geq t_0 \\ 0 & t < t_0 \end{cases}$$



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- **Cluster Analysis:** Conjunction analysis

- **Example**

- ↳ 3 contrasts A, B, and C
    - ↳ Assign each based on binary system: A: 001( $2^0=1$ ); B: 010( $2^1=2$ ); C: 100( $2^2=4$ )
    - ↳ Create a mask with 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 - 8 ( $=2^3$ ) scenarios:

```
000(0): none;
001(1): A but no others;
010(2): B but no others;
011(3): A and B but not C;
100(4): C but no others;
101(5): A and C but not B;
110(6): B and C but not A;
111(7): A, B and C
```

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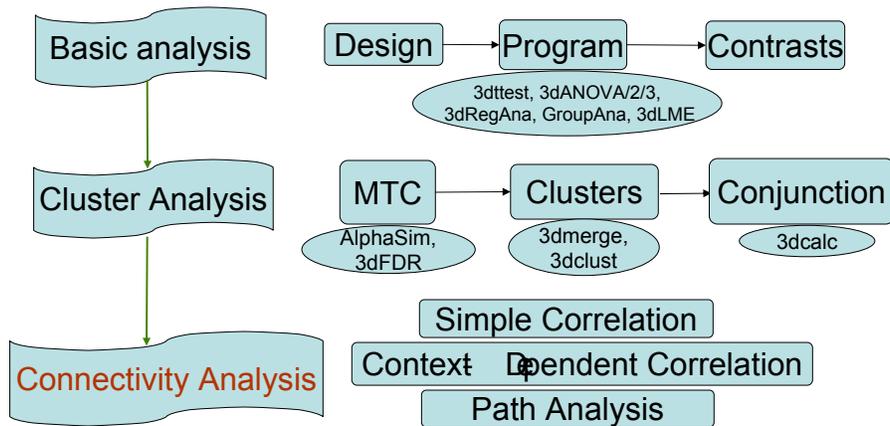
- **Cluster Analysis:** Conjunction analysis

- **Multiple testing correction issue**

- How to calculate the  $p$ -value for the conjunction map?
    - No problem if each entity was corrected before conjunction analysis
    - But that may be too stringent and over-corrected
    - With 2 or 3 entities, analytical calculation possible
      - Each can have different uncorrected  $p$
      - Double or triple integral of Gaussian distribution
    - With more than 3 entities, may have to resort to simulations
      - Monte Carlo simulations
      - A program in the pipeline?

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## Group Analysis



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## • **Connectivity: Correlation Analysis**

### ¶ Correlation analysis (a.k.a. functional connectivity)

- ↳ Similarity between a seed region and the rest of the brain
- ↳ Says nothing about causality/directionality
- ↳ Voxel-wise analysis
- ↳ Both individual subject and group levels
- ↳ Two types: simple and context-dependent correlation (a.k.a. PPI)

### ¶ Steps at individual subject level

- ↳ Create ROI
- ↳ Isolate signal for a condition/task
- ↳ Extract seed time series
- ↳ Correlation analysis through regression analysis
- ↳ More accurately, partial (multiple) correlation

### ¶ Steps at group level

- ↳ Convert correlation coefficients to Z (Fisher transformation): 3dcalc
- ↳ One-sample t test on Z scores: 3dttest

¶ More details: <http://afni.nimh.nih.gov/sscc/gangc>

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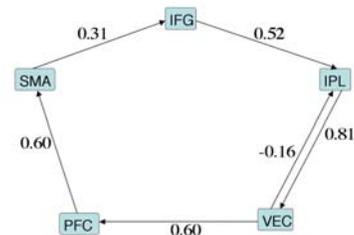
## • **Connectivity: Path Analysis or SEM**

### ¶ Causal modeling (a.k.a. structural connectivity)

- ↳ Start with a network of ROI's
- ↳ Path analysis
  - Assess the network based on correlations (covariances) of ROI's
  - Minimize discrepancies between correlations based on data and estimated from model
  - Input: Model specification, correlation matrix, residual error variances, DF
  - Output: Path coefficients, various fit indices

### ↳ Caveats

- Valid only with the data and model specified
- No proof: modeled through correlation (covariance) analysis
- Even with the same data, an alternative model might be equally good or better
- If one critical ROI is left out, things may go awry



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## • **Connectivity: Path Analysis or SEM**

### ¶ Path analysis with 1dSEM

- ↳ Model validation: 'confirm' a theoretical model
  - Accept, reject, or modify the model?
- ↳ Model search: look for 'best' model
  - Start with a minimum model (can be empty)
  - Some paths can be excluded
  - Model grows by adding one extra path a time
  - 'Best' in terms of various fit criteria
- ↳ More information <http://afni.nimh.nih.gov/sscc/gangc/PathAna.html>

### ¶ Difference between causal and correlation analysis

- ↳ Predefined network (model-based) vs. network search (data-based)
- ↳ Modeling: causation (and directionality) vs. correlation
- ↳ ROI vs. voxel-wise
- ↳ Input: correlation (covariance) vs. original time series
- ↳ Group analysis vs. individual + group