

FMRI Analysis

Experiment Design



Scanning



Pre-Processing



Individual Subject Analysis

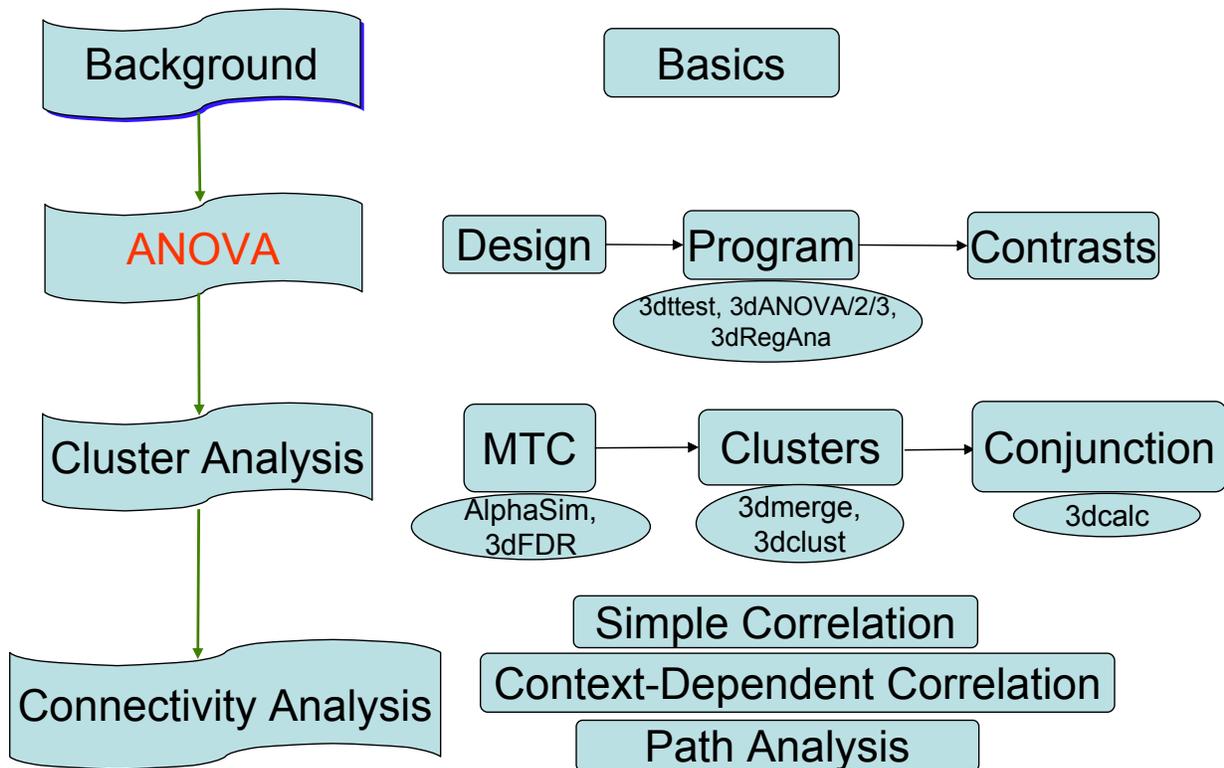


Group Analysis



Post-Processing

Group Analysis



• Group Analysis: types

☞ Fixed effect

- ↳ Only a few subjects
- ↳ Case study: can't generalize to whole population
- ↳ Simple approach: $T = \sum t / \sqrt{n}$
- ↳ Sophisticated approach
 - $B = \sum [b_i / \sqrt{v_i}] / \sum [1 / \sqrt{v_i}]$, $T = B \sum [1 / \sqrt{v_i}] / \sqrt{n}$
 - $B = \sum (b_i / v_i) / \sum (1 / v_i)$, $T = B \sqrt{\sum (1 / v_i)}$
 - Concatenate individual subject data

☞ Random effect

- ↳ Individual subject and group analysis: separate
- ↳ Assumption: within-subject variation is negligible compared to between-subjects
- ↳ Focus of this talk

☞ Mixed effect

- ↳ Bring within-subject variances to group analysis
- ↳ Currently not easy to do in FMRI analysis

• Group Analysis: Overview

¶ Parametric Tests

- ⌞ `3dttest` (one-sample, unpaired and paired t)
- ⌞ `3dANOVA` (one-way between-subject)
- ⌞ `3dANOVA2` (one-way within-subject, 2-way between-subjects)
- ⌞ `3dANOVA3` (2-way between-subjects, within-subject and mixed, 3-way between-subjects)
- ⌞ `3dRegAna` (regression/correlation, unbalanced ANOVA, ANCOVA)
- ⌞ `GroupAna` (Matlab script for up to 5-way ANOVA)

¶ Non-Parametric Analysis

- ⌞ No assumption of normality; Statistics based on ranking
- ⌞ Appropriate when number of subjects too few
- ⌞ Programs
 - `3dWilcoxon` (~ paired t -test)
 - `3dMannWhitney` (~ two-sample t -test)
 - `3dKruskalWallis` (~`3dANOVA`)
 - `3dFriedman` (~`3dANOVA2`)
 - Permutation test
 - plugin on AFNI under Define Datamode / Plugins /
 - C program by Tom Holroyd
- ⌞ Can't handle complicated designs
- ⌞ Less sensitive to outliers (more robust) and less flexible than parametric tests

• Group Analysis: Overview

☞ How many subjects?

- ⌞ Power: proportional to \sqrt{n} ; $n > 10$
- ⌞ Efficiency increases by the square root of # subjects
- ⌞ Balance: Equal number of subjects across groups if possible

☞ Input

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

☞ Design

- ⌞ Number of factors
- ⌞ Number of levels for each factor
- ⌞ Within-subject / repeated-measures vs. between-subjects
 - Fixed (factors of interest) vs. random (subject)
 - Nesting: Balanced?
- ⌞ Which program?

☞ Contrasts and trend analysis: One-tail or two-tail?

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• Group Analysis : 3dttest

• Basic usage

↳ One-sample t

- One group: simple effect
- Example: 15 subjects under condition A with $H_0: \mu_A = 0$

↳ Two-sample t

- Two groups: Compare one group with another
- ~ 1-way between-subject (3dANOVA)
- Unequal sample sizes allowed
- Assumption of equal variance
- Example: 15 subjects under A and 13 other subjects under B - $H_0: \mu_A = \mu_B$

↳ Paired t

- Two conditions of one group: Compare one condition with another
- ~ one-way within-subject (3dANOVA2 -type 3)
- ~ one-sample t on individual contrasts
- Example: Difference between conditions A and B for 15 subjects with $H_0: \mu_A = \mu_B$

• Output: 2 values (% and t) at each voxel

• Versatile program: Most tests can be done with 3dttest -piecemeal vs. bundled

• Group Analysis: 3dANOVA

- ⌘ Generalization of two-sample t -test
 - ↳ One-way between-subject
 - ↳ H_0 : no difference across all levels (groups)
 - ↳ Examples of groups: gender, age, genotype, disease, *etc.*
 - ↳ Unequal sample sizes allowed
- ⌘ Assumptions
 - ↳ Normally distributed with equal variances across groups
- ⌘ Results: 2 values ($\%$ and t)
- ⌘ **3dANOVA vs. 3dttest**
 - ↳ Equivalent with 2 levels (groups)
 - ↳ More than 2 levels (groups): Can run multiple two-sample t -test

• Group Analysis: 3dANOVA2

☞ Designs

↳ One-way within-subject (type 3)

- Major usage
- Compare conditions in one group
- Extension and equivalence of paired t

↳ Two-way between-subjects (type 1)

- 1 condition, 2 classifications of subjects
- Extension and equivalence two-sample t
- Unbalanced designs disallowed: Equal number of subjects across groups

☞ Output

↳ Main effect (-fa): F

↳ Interaction for two-way between-subjects (-fab): F

↳ Contrast testing

- Simple effect (-amean)
- 1st level (-acontr, -adiff): one-sample or paired t among factor levels
- 2nd level (interaction) for two-way between-subjects
- 2 values per contrast: % and t

• Group Analysis : 3dANOVA3

☞ Designs

- ↳ Three-way between-subjects (**type 1**)
 - 3 categorizations of groups
- ↳ **Two-way within-subject (type 4)**: Crossed design AXBXC
 - Generalization of paired *t*-test
 - One group of subjects
 - Two categorizations of conditions: A and B
- ↳ **Two-way mixed (type 5)**: Nested design BXC(A)
 - Nesting factor: ≥ 2 groups of subjects (Factor A): subject classification, e.g., gender
 - One category of condition (Factor B)
 - Nesting: balanced

☞ Output

- ↳ Main effect (**-fa** and **-fb**) and interaction (**-fab**): *F*
- ↳ Contrast testing
 - 1st level: **-amean, -adiff, -acontr, -bmean, -bdiff, -bcontr**
 - 2nd level: **-abmean, -aBdiff, -aBcontr, -Abdiff, -Abcontr**
 - 2 values per contrast : % and *t*

• Group Analysis: GroupAna

- ¶ Multi-way ANOVA
 - ↳ Matlab script package for up to 5-way ANOVA
 - ↳ Requires Matlab plus Statistics Toolbox
 - ↳ GLM approach (slow)
 - ↳ Powerful: Test for interactions
 - ↳ Downside
 - Difficult to test and interpret simple effects/contrasts
 - Complicated design, and compromised power
 - ↳ Heavy duty computation: minutes to hours
 - Input with lower resolution recommended
 - Resample with `adwarp -dxyz #` or `3dresample`
 - ↳ Can handle both volume and surface data
 - ↳ Can handle following unbalanced designs (two-sample *t* type):
 - 3-way ANOVA type 3: BXC(A)
 - 4-way ANOVA type 3: BXCXD(A)
 - 4-way ANOVA type 4: CXD(AXB)
- ¶ See <http://afni.nimh.nih.gov/sscc/gangc> for more info
- ¶ Alternative: `3dRegAna`

• Group Analysis: Example

¶ Design

- ↳ 4 conditions (TM, TP, HM, HP) and 8 subjects
- ↳ 2-way within-subject: 2x2x8
 - A (Object), 2 levels: Tool vs Human
 - B (Animation), 2 levels: Motion vs Point
 - C (subject), 8 levels
 - AxBxC: Program? **3dANOVA3 -type 4**

¶ Main effects (A and B): 2 *F* values

¶ Interaction AXB: 1 *F*

¶ Contrasts

- ↳ 1st order: TvsH, MvsP
- ↳ 2nd order: TMvsTP, HMvsHP, TMvsHM, TPvsHP
- ↳ 6 contrasts x 2 values/contrast = 12 values

¶ Logistic

- ↳ Input: 2x2x8 = 32 files (4 from each subject)
- ↳ Output: 18 subbricks

• Group Analysis: Example

Script

```

3dANOVA3 -type 4 -alevels 2 -blevels 2 -clevels 8 \
-dset 1 1 1 ED_TM_irf_mean+tlrc \
-dset 1 2 1 ED_TP_irf_mean+tlrc \
-dset 2 1 1 ED_HM_irf_mean+tlrc \
-dset 2 2 1 ED_HP_irf_mean+tlrc \
...
-adiff 1 2 Tvsh1 \ (indices for difference)
-acontr 1 -1 Tvsh2 \ (coefficients for contrast)
-bdiff 1 2 MvsP1 \
-aBdiff 1 2 : 1 TMvsHM \ (indices for difference)
-aBcontr 1 -1 : 1 TMvsHM \ (coefficients for contrast)
-aBcontr -1 1 : 2 HPvsTP \
-Abdiff 1 : 1 2 TMvsTP \
-Abcontr 2 : 1 -1 HMvsHP \

-fa ObjEffect \
-fb AnimEffect \
-fab ObjXAnim \

-bucket Group

```

Model type, number of levels for each factor

Input for each cell in ANOVA table: totally 2X2X8 = 32

1st order Contrasts, paired t test

2nd order Contrasts, paired t test

Main effects & interaction F test; Equivalent to contrasts

Output: bundled

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• Group Analysis: Example

‣ Alternative approaches

‣ GroupAna

‣ 3dRegAna

‣ Paired *t*: 6 tests

‣ Program: 3dttest **-paired**

‣ For TM vs HM: 16 (2x8) input files (β coefficients: %) from each subject

```
3dttest -paired -prefix TMvsHM \
-set1 ED_TM_irf_mean+tlrc ... ZS_TM_irf_mean+tlrc \
-set2 ED_HM_irf_mean+tlrc ... ZS_HM_irf_mean+tlrc
```

‣ One-sample *t*: 6 tests

‣ Program: 3dttest

‣ For TM vs HM: 8 input files (contrasts: %) from each subject

```
3dttest -prefix TMvsHM \
-base1 0 \
-set2 ED_TMvsHM_irf_mean+tlrc ... ZS_TMvsHM_irf_mean+tlrc
```

- **Group Analysis**: ANCOVA (ANalysis of COVAriances)

- ☞ Why ANCOVA?

- ⌞ Subjects might not be an ideally randomized representation of a population
 - ⌞ If no controlled, cross-subject variability will lead to loss of power and accuracy
 - ⌞ Direct control: balanced selection of subjects
 - ⌞ Indirect (statistical) control: untangling covariate effect
 - ⌞ Factor of no interest - covariate: uncontrollable/confounding variable, usually continuous
 - Age
 - Behavioral data, e.g., response time
 - Cortex thickness
 - Gender

- ☞ ANCOVA = Regression + ANOVA

- ⌞ Assumption: linear relation between % signal change and the covariate
 - ⌞ GLM approach
 - ⌞ **Centralize** covariate so that it would not confound with other effects

- ☞ 3dRegAna

- ⌞ Flexible program that can run all sorts of group analysis
 - ⌞ Miserable to write script, but hopeful: python scripting in future

• Group Analysis: ANCOVA Example

☞ Example: Running ANCOVA

- ⌞ Two groups: 15 normal vs. 13 patients
- ⌞ Analysis: comparing the two groups
- ⌞ Running what test?
 - Two-sample t with 3dttest
 - Controlling age effect?
- ⌞ GLM model
 - $Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \varepsilon_i, i = 1, 2, \dots, n (n = 28)$
 - Demean covariate (age) X_1
 - Code the factor (group) with a dummy variable
 - 0, when the subject is a patient;
 - $X_{2i} = \{$
 - 1, when the subject is normal.
 - With covariate X_1 centralized:
 - β_0 = effect of patient; β_1 = age effect (correlation coef); β_2 = effect of normal
 - $X_{3i} = X_{1i} X_{2i}$ models interaction (optional) between covariate and factor (group)
 - β_3 = interaction

• Group Analysis: ANCOVA Example

3dRegAna -rows 28 -cols 3 \

Model parameters: 28 subjects,
3 independent variables

-workmem 1000 \

Memory

-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 -8.9 1 -8.9 normal/Norm14+tlrc.BRIK \

-xydata 0.1 1 0.1 normal/Norm15+tlrc.BRIK \

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

-model 1 2 3 : 0 \

Specify model for F and R²

-bucket 0 Pat_vs_Norm \

Output: #subbricks = 2*#coef + F + R²

-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' \

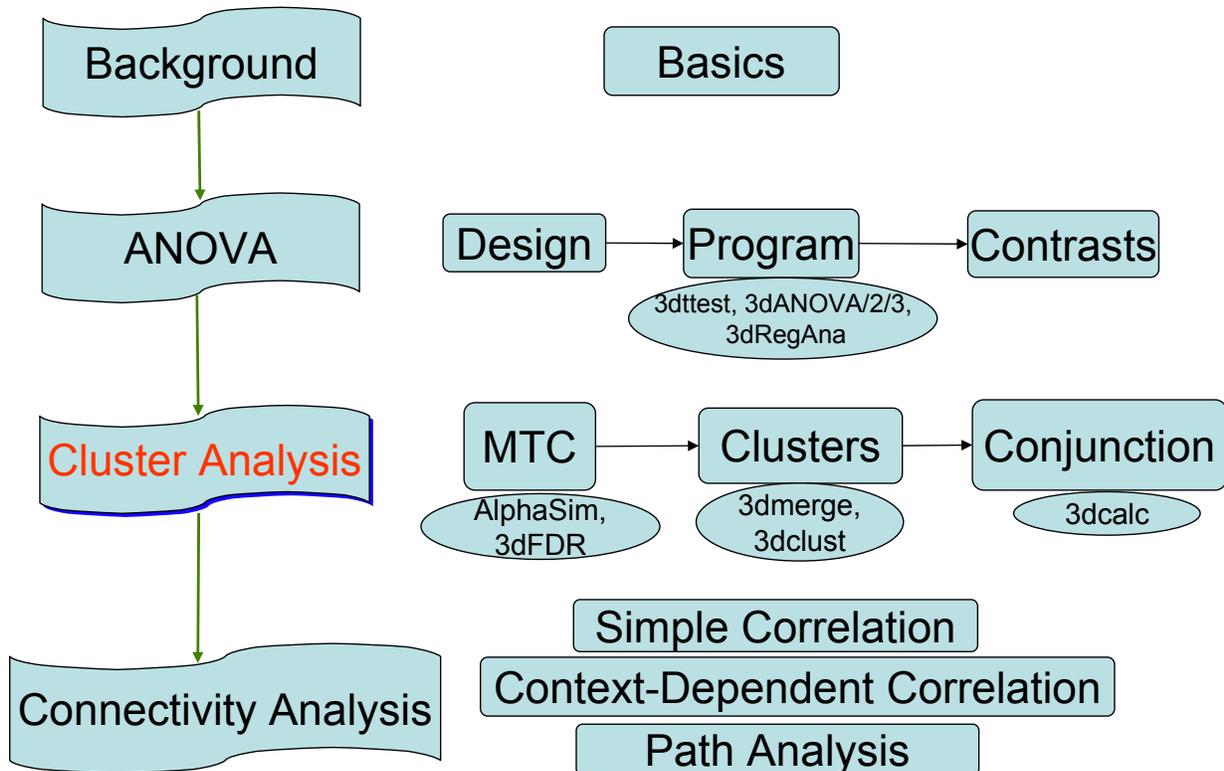
-brick 7 tstat 3 'Interaction t'

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



• Cluster Analysis: Multiple testing correction

• 2 types of errors in statistical tests

↳ What is H_0 in fMRI studies?

↳ 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 = β

↳ Usual strategy: controlling type I error

(power = $1 - \beta$ = probability of detecting true activation)

↳ Significance level = α : $p < \alpha$

• 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?

$$(2)^5 \sim 0.03$$

➢ In a community with 100 families with 5 kids, expected #families with 5 boys =?

$$100 \times (2)^5 \sim 3$$

↳ In fMRI H_0 : no activation at a voxel

➢ What is the chance a voxel is mistakenly labeled as activated (false +)?

➢ Multiple testing problem: With n voxels, what is the chance to mistakenly label at least one voxel? Family-Wise Error: $\alpha_{FW} = 1 - (1 - p)^n \rightarrow 1$ as n increases

➢ 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 $\alpha_{FW} = \alpha$

• Cluster Analysis: Multiple testing correction

- ⌘ Multiple testing problem in fMRI: voxel-wise statistical analysis
 - ⌞ Increase of chance at least one detection is wrong in cluster analysis
 - ⌞ 3 occurrences of multiple testings: individual, group, and conjunction
 - ⌞ Group analysis is the most concerned
- ⌘ Two approaches
 - ⌞ Control **FWE**: $\alpha_{FW} = P(\geq \text{one false positive voxel in the whole brain})$
 - Making α_{FW} small but without losing too much power
 - Bonferroni correction doesn't work: $p=10^{-8}\sim 10^{-6}$
 - *Too stringent and overly conservative: Lose statistical power
 - Something to rescue? Correlation and structure!
 - *Voxels in the brain are not independent
 - *Structures in the brain
 - ⌞ Control false discovery rate (**FDR**)
 - **FDR** = expected proportion of false + voxels among all **detected** voxels
 - ⌞ 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 +

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• Cluster Analysis: AlphaSim

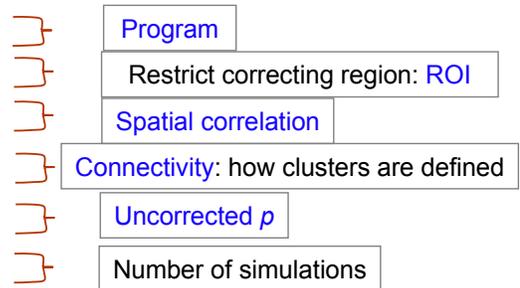
- ⌘ FWE: Monte Carlo simulations
 - ↳ Named for Monte Carlo, Monaco, where the primary attractions are casinos
 - ↳ Program: **AlphaSim**
 - Randomly generate some number (e.g., 1000) of brains with white noise
 - Count the proportion of voxels are false + in all brains
 - Parameters:
 - * ROI - mask
 - * Spatial correlation - FWHM
 - * Connectivity - radius
 - * Individual voxel significant level - uncorrected p
 - Output
 - * Simulated (estimated) **overall significance level** (corrected p -value)
 - * Corresponding **minimum cluster size**
 - Decision: Counterbalance among
 - * Uncorrected p
 - * Minimum cluster size
 - * Corrected p

• Cluster Analysis: AlphaSim

¶ See detailed steps at <http://afni.nimh.nih.gov/sscc/gangc/mcc.html>

¶ Example

```
AlphaSim \
-mask MyMask+orig \
-fwhmx 4.5 -fwhmy 4.5 -fwhmz 6.5 \
-rmm 6.3 \
-pthr 0.0001 \
-iter 1000
```



¶ Output: 5 columns

- ↳ Focus on the 1st and last columns, and ignore others
- ↳ 1st column: minimum cluster size in voxels
- ↳ Last column: alpha (α), 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**

• Cluster Analysis: 3dFDR

¶ Definition:

FDR = proportion of false + voxels among all detected voxels

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

¶ Doesn't consider

- ↳ spatial correlation
- ↳ cluster size
- ↳ connectivity

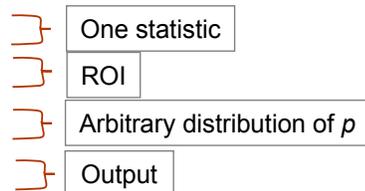
¶ Again, only controls the **expected** % false positives **among** declared active voxels

¶ 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	



- **Cluster Analysis**: 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

- ✦ Fail to survive correction?

- ✦ At the mercy of reviewers

- ✦ Analysis on surface

- ✦ Tricks

- One-tail?
- ROI – cheating?

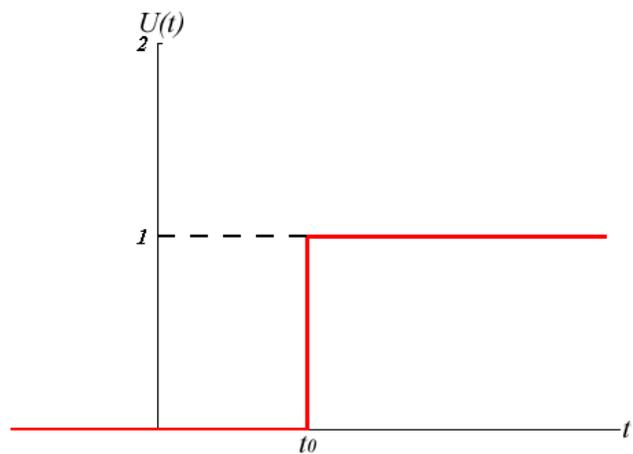
- ✦ Many factors along the pipeline

- Experiment design: power?
- Sensitivity (power) vs specificity (small regions)
- Poor spatial alignment among subjects

- **Cluster Analysis:** Conjunction analysis

- Conjunction analysis: HM vs TM
 - Common activation area
 - Exclusive activations
- Double/dual thresholding with AFNI GUI
 - Tricky
 - Only works for two contrasts
 - Common but not exclusive areas
- Conjunction analysis with `3dcalc`
 - Flexible and versatile
 - **Heaviside unit (step function)** defines a *On/Off* event

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



- **Cluster Analysis**: Conjunction analysis

- Example with 3 contrasts: A vs D, B vs D, and C vs D

- Map 3 contrasts based on binary system: A > D: 000(1); B > D: 010(2); C > D: 100(4)

- Create a mask with 3 subbricks of t (threshold = 4.2)

```
3dcalc -a func+tlrc'[5]' -b func+tlrc'[10]' -c func+tlrc'[15]' \  
-expr 'step(a-4.2)+2*step(b-4.2)+4*step(c-4.2)' \  
-prefix ConjAna
```

- Interpret output - 8 (=2³) scenarios:

- 000(0): none;

- 001(1): A > D but no others;

- 010(2): B > D but no others;

- 011(3): A > D and B > D but not C > D;

- 100(4): C > D but no others;

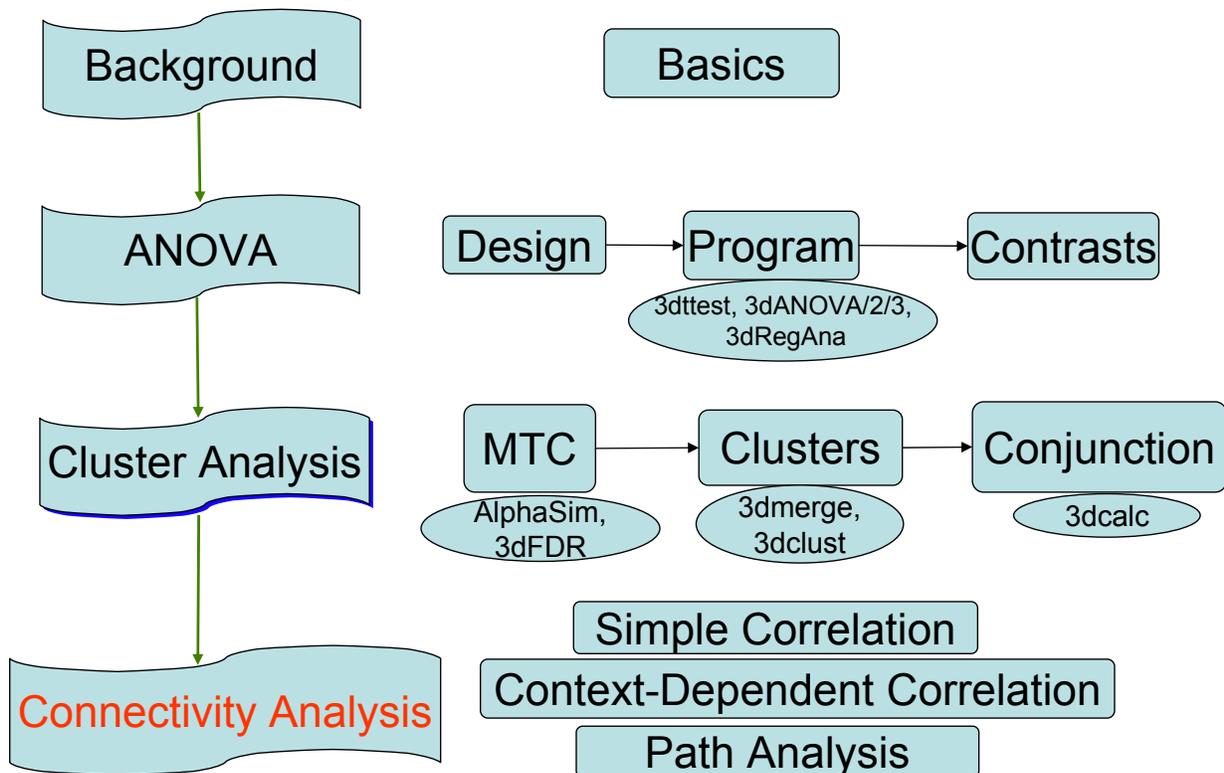
- 101(5): A > D and C > D but not B > D;

- 110(6): B > D and C > D but not A > D;

- 111(7): A > D, B > D and C > D

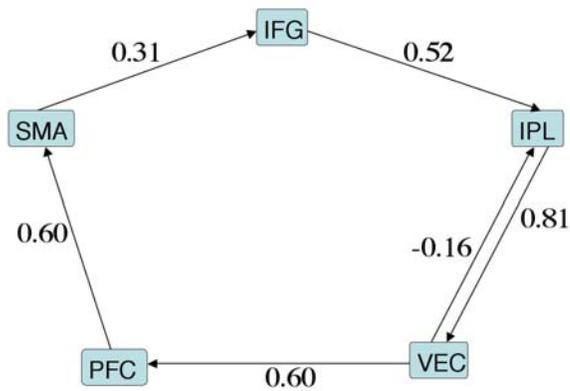
- Downside: no p associated conjunctions with and no MTC

Group Analysis



• Connectivity: Path Analysis

- ⌘ Causal model approach on a network of ROI's
- ⌘ Minimizing discrepancies
 - ↳ btw correlation based on data and one estimated from model



- ⌘ Input: Model specification, correlation matrix, residual error variances, DF
- ⌘ Output: Path coefficients, various fit indices

• Connectivity: Path Analysis – 1dSEM

- ⌘ AFNI program 1dSEM
 - ↳ Written in C
 - ↳ Not dependent on FMRI analysis platform
- ⌘ Two modes
 - ↳ Validate a theoretical model
 - Accept, reject, or modify the model?
 - ↳ Search for 'best' model
 - Start with a minimum model (can be empty)
 - Some paths can be excluded
 - 'Best' in terms of various fit criteria
- ⌘ Script: *1dSEM -theta testthetasfull.1D -C testcorr.1D -psi testpsi.1D -DF 30*
- ⌘ Pitfalls
 - ↳ Causal relationship modeled through correlation (covariance) analysis
 - ↳ Valid only with the data and model specified
 - ↳ If one critical ROI is left out, things may go awry
- ⌘ More details
 - ↳ <http://afni.nimh.nih.gov/sscc/gangc/PathAna.html>
 - ↳ 1dSEM -help

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- **Need Help?**

- ☼ Command with “-help”

- `3dANOVA3 -help`

- ☼ Manuals

- <http://afni.nimh.nih.gov/afni/doc/manual/>

- ☼ Web

- <http://afni.nimh.nih.gov/sscc/gangc>

- ☼ Examples: HowTo#5

- <http://afni.nimh.nih.gov/afni/doc/howto/>

- ☼ Message board

- <http://afni.nimh.nih.gov/afni/community/board/>

- ☼ Appointment

- **Contact us @1-800-NIH-AFNI**