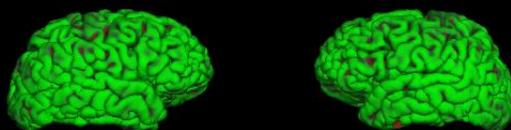
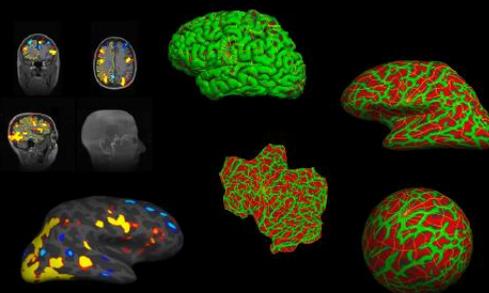


Surface-based Group Analysis in FreeSurfer



FreeSurfer

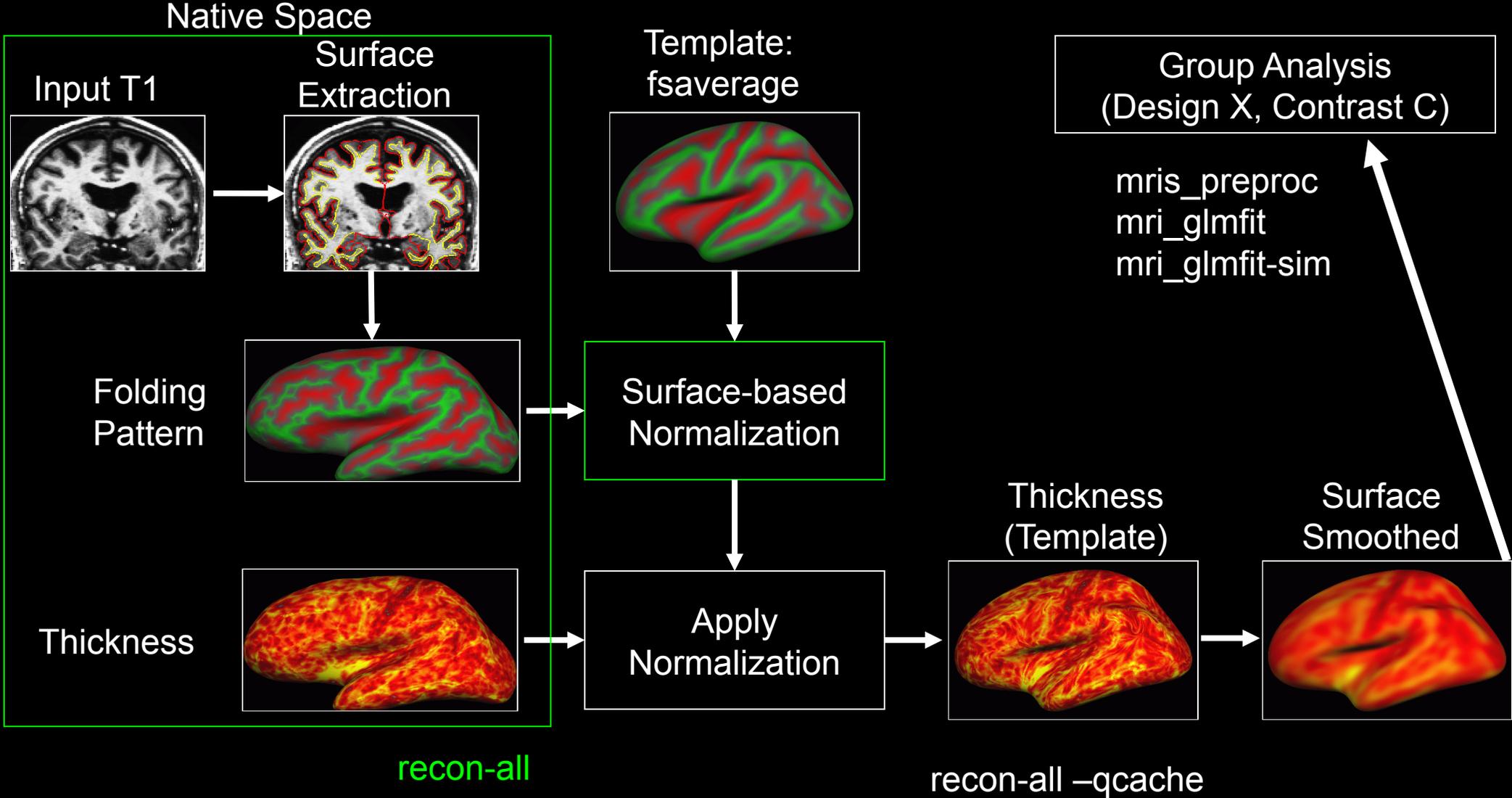


MASSACHUSETTS
GENERAL HOSPITAL

Outline

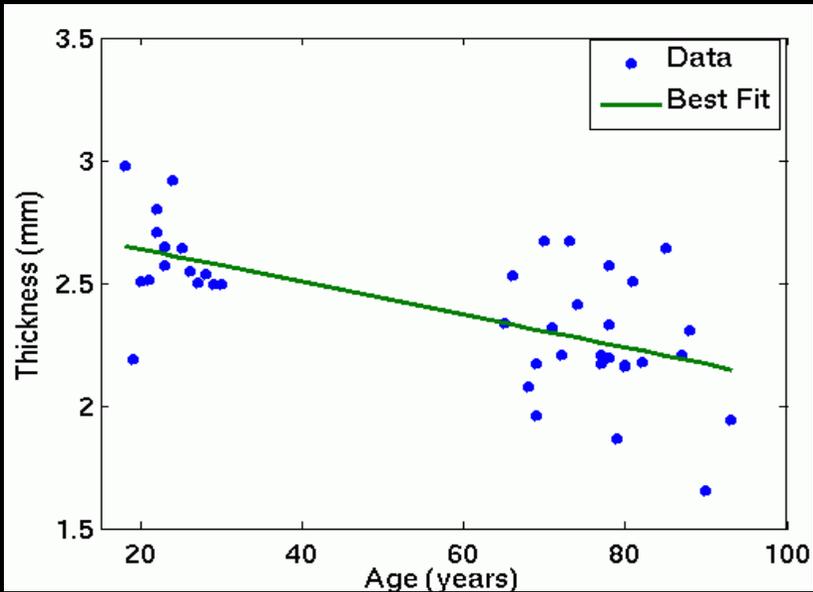
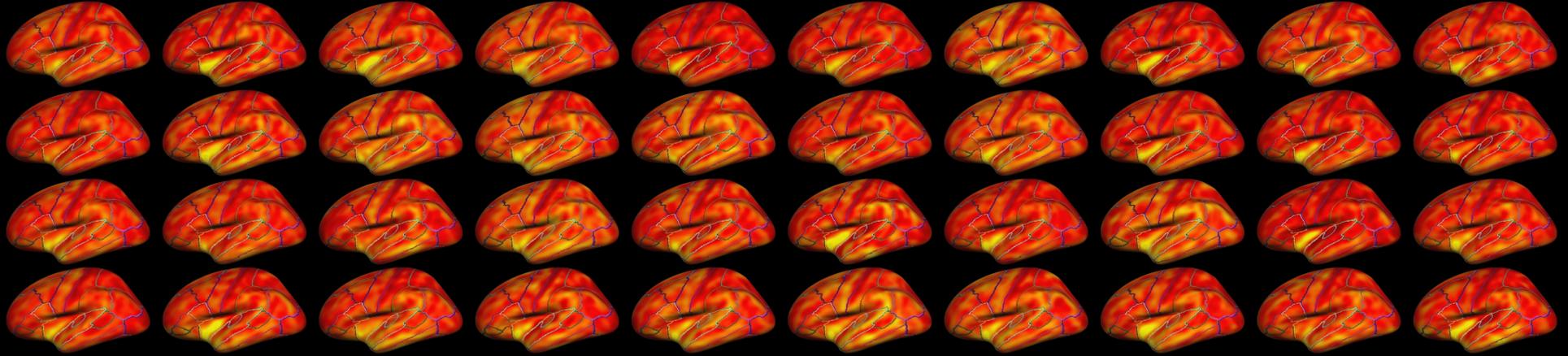
- Group Analysis Overview
- Theory
 - Surface-based Intersubject Registration
 - Surface-based Smoothing
 - General Linear Model (GLM)
- Practice
 - FSGD File
 - Processing Stages
 - Vertex-wise Analysis
 - Correction for multiple comparisons
- Interactive/Automated GUI (QDEC)

Analysis Pipeline

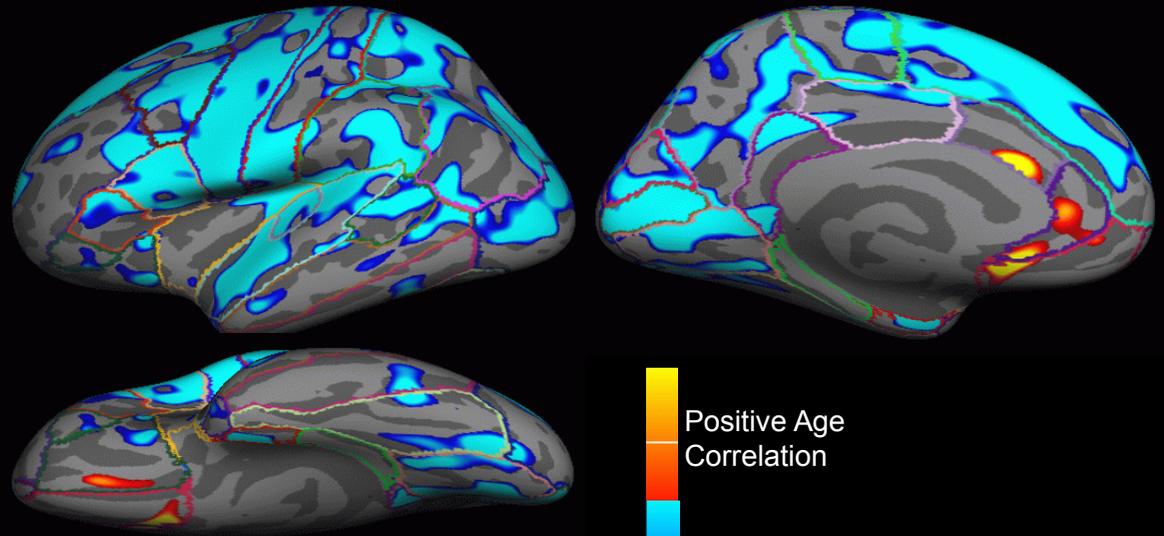


Aging Thickness Study

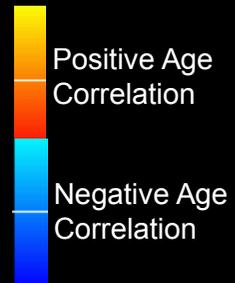
N=40



Design X (slope, intercept)



Contrast C $p < .01$

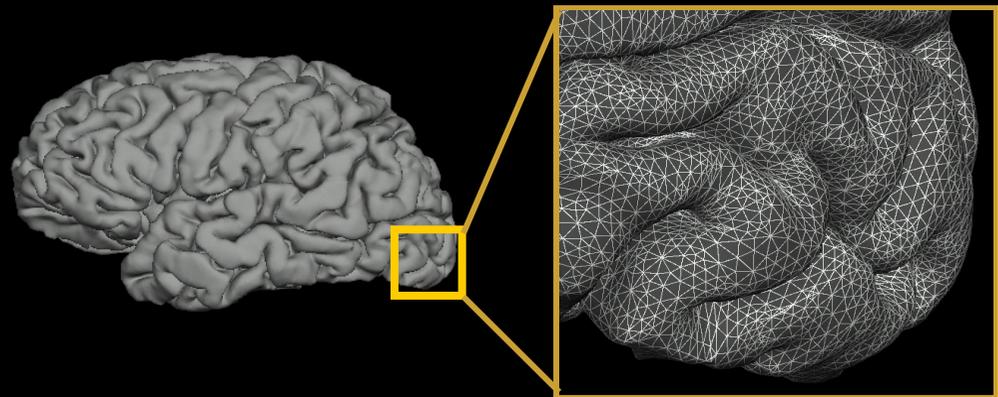


Theory

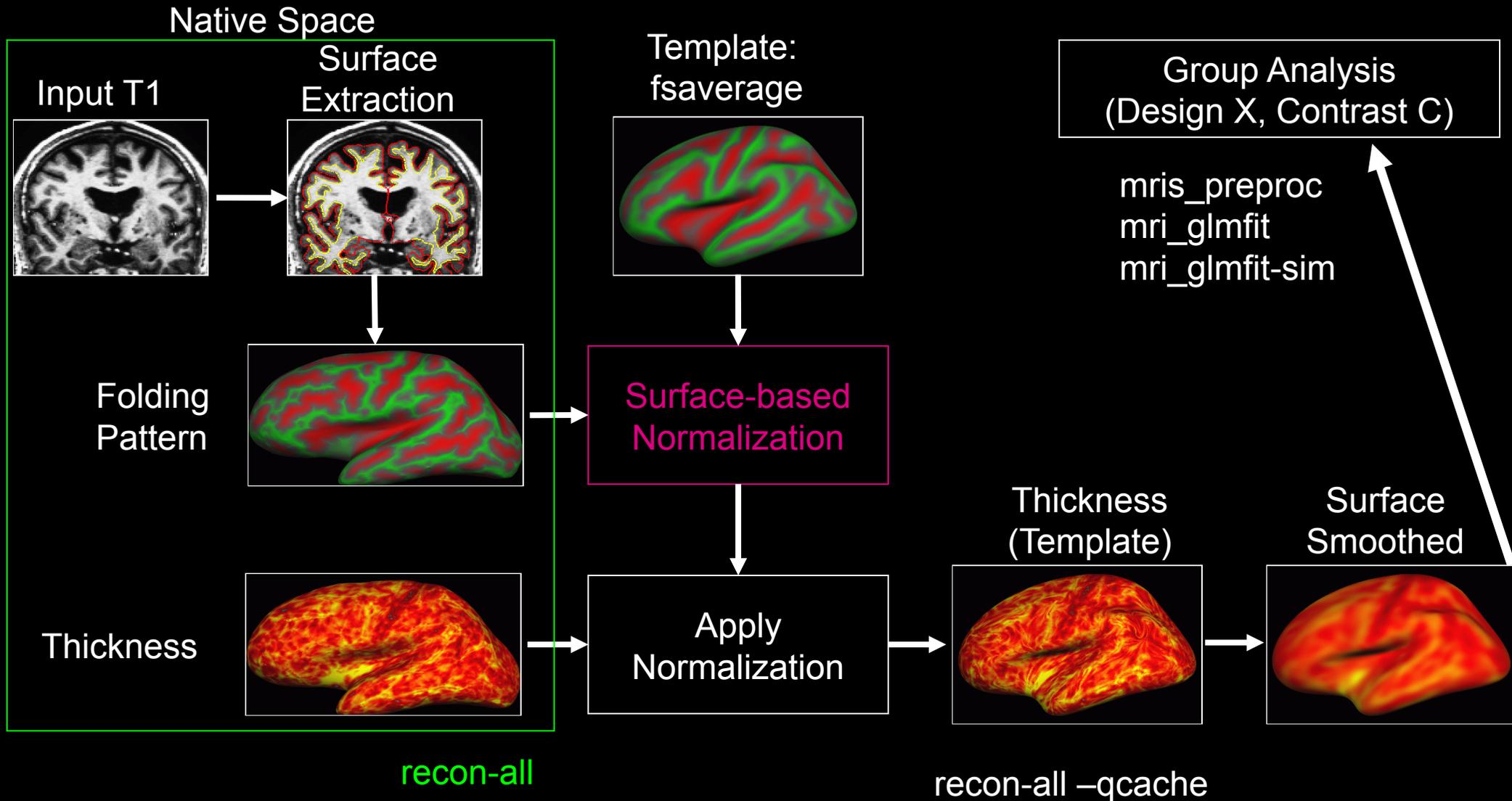
1. Surface-based Intersubject Registration
2. Surface-based Smoothing
3. General Linear Model (GLM)

Surface-based Measures

- Morphometric (eg, thickness)
- Functional
- PET
- MEG/EEG
- Diffusion (?) sampled just under the surface

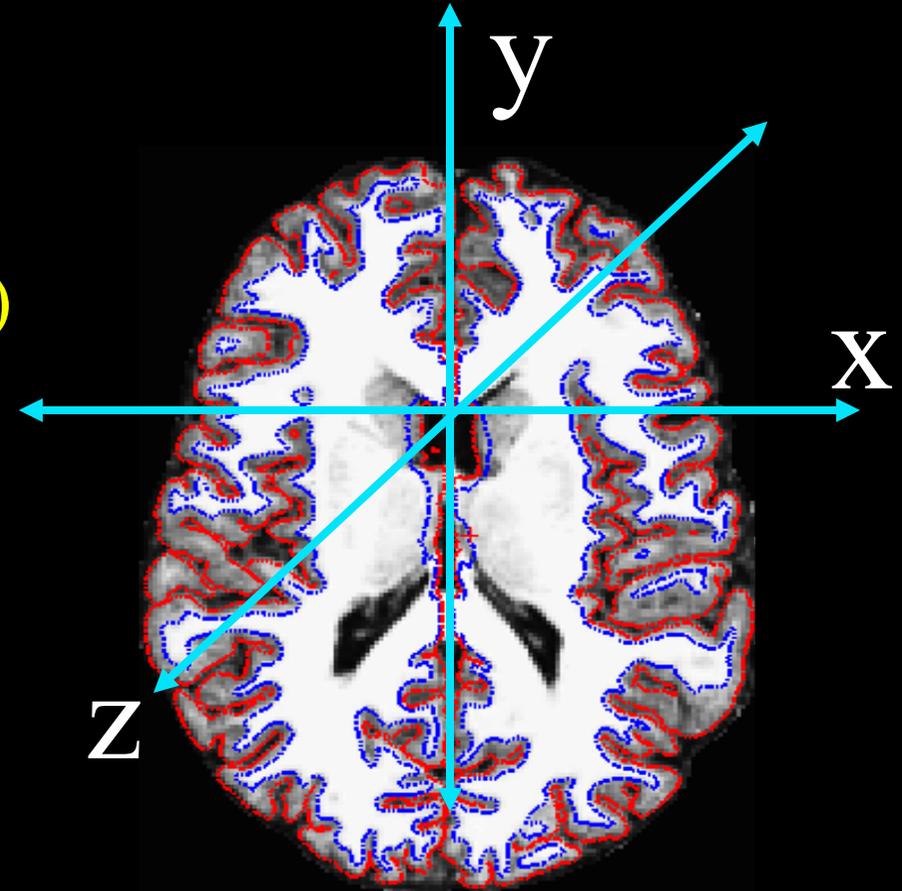


Surface-based Spatial Normalization



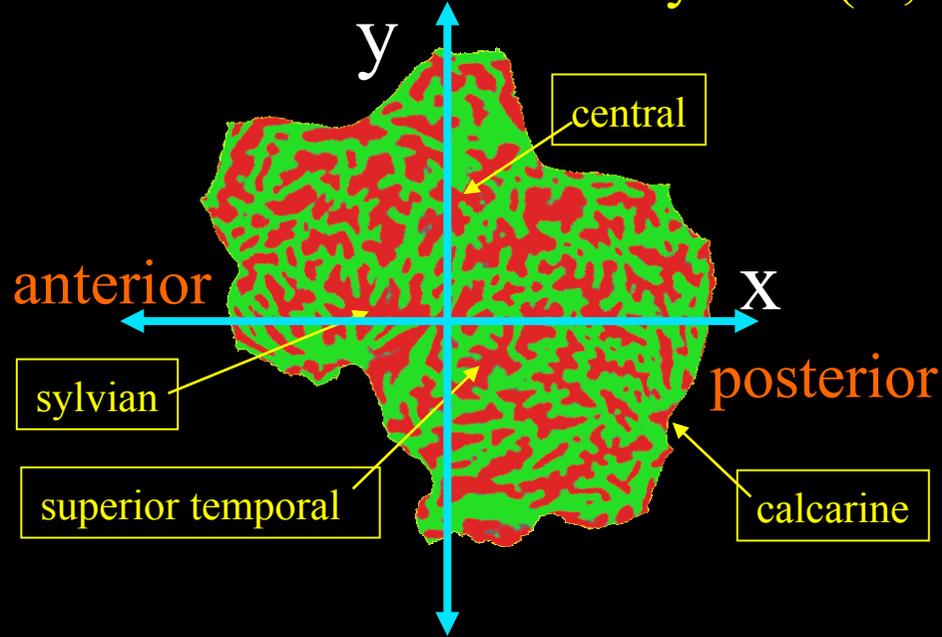
Coordinate Systems: 3D (Volumetric)

- 3D Coordinate System
 - XYZ
 - RAS (Right-Anterior-Superior)
 - CRS (Column-Row-Slice)
 - Origin ($XYZ=0$, eg, AC)
 - MR Intensity at each XYZ



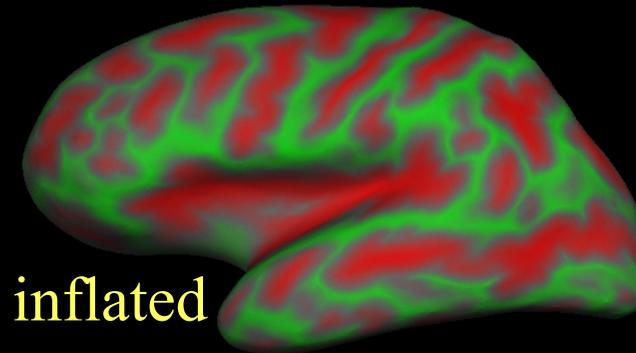
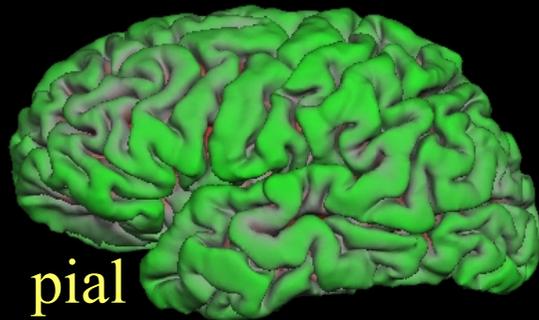
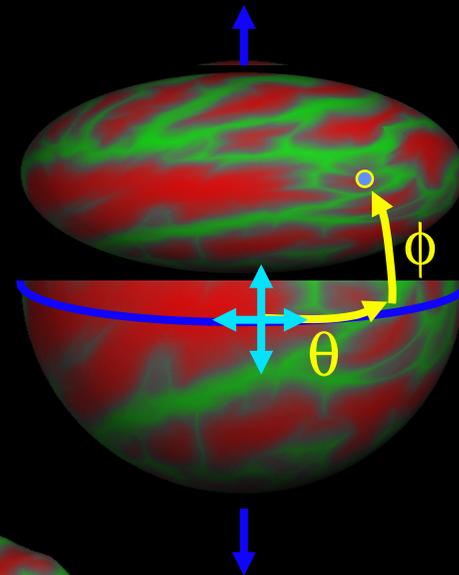
Coordinate Systems: 2D (Surface)

Sheet: 2D Coordinate System (X,Y)



Sphere: 2D Coordinate System

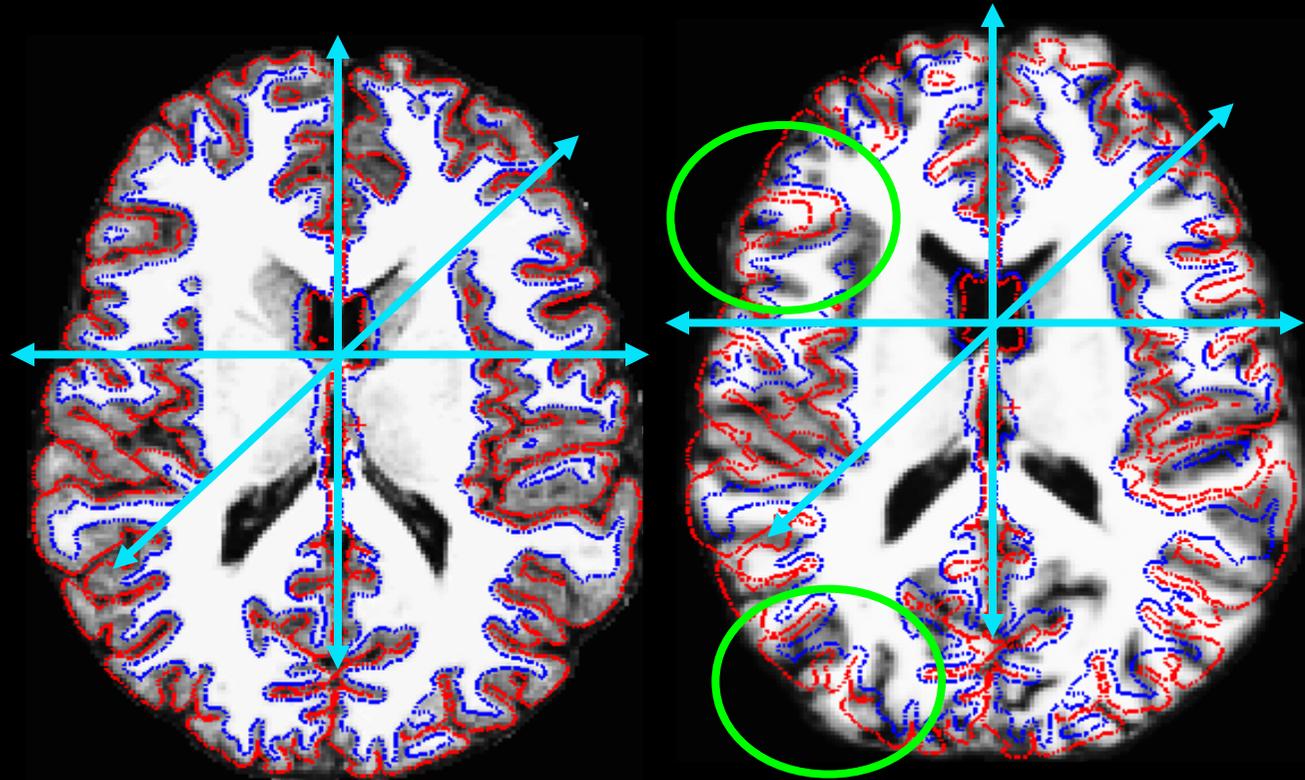
- Latitude and Longitude (θ , ϕ)
- Continuous, no cuts
- Value at each point (eg, thickness)



- Curvature
- SULCUS (+)
 - GYRUS (-)

Volumetric Intersubject Registration

- Affine/Linear
 - Translate
 - Rotate
 - Stretch
 - Shear
 - (12 DOF)

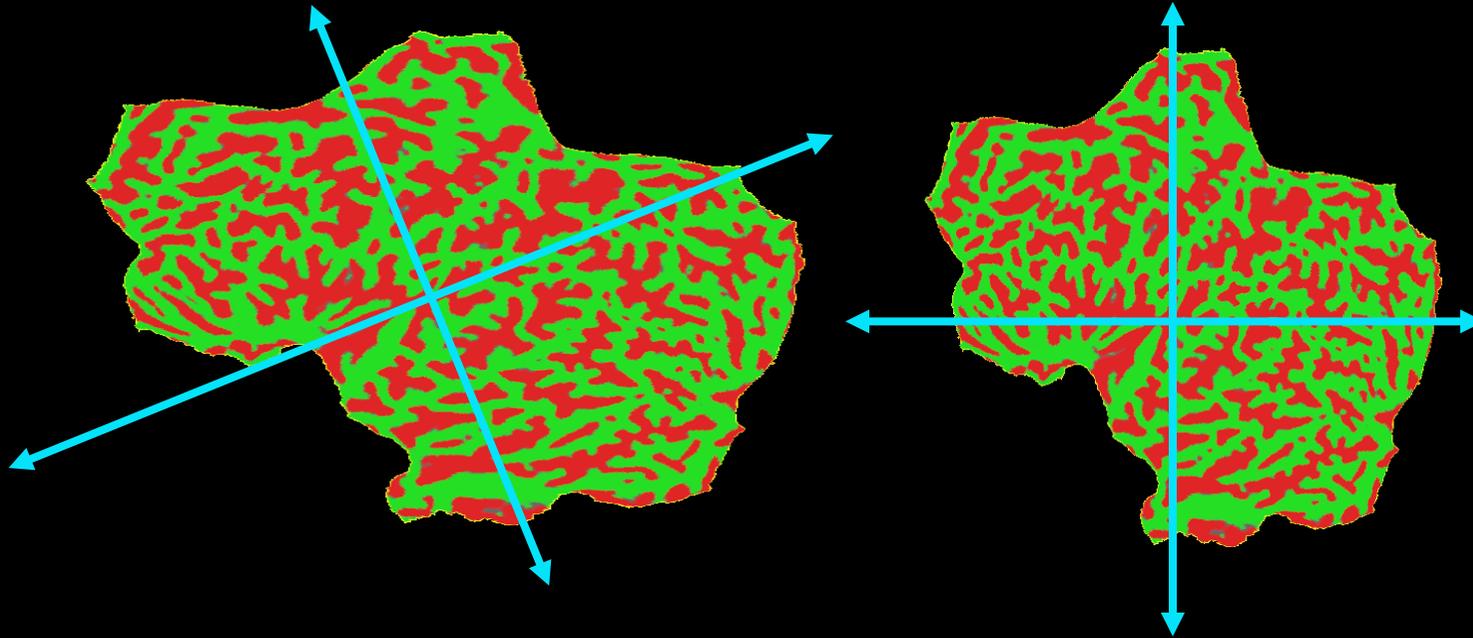


- Match Intensity, Voxel-by-Voxel
- Problems
- Can use nonlinear volumetric (cf CVS)

Surface-based Intersubject Registration

Subject 1

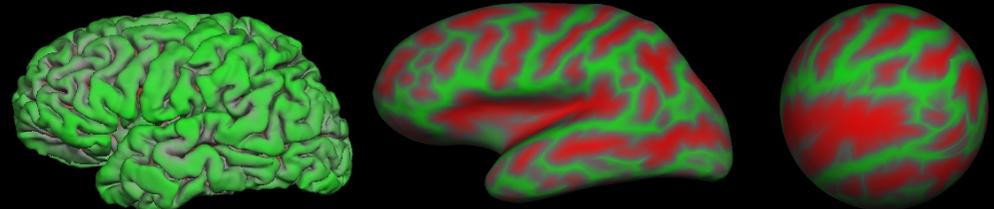
Subject 2



Curvature “Intensity”

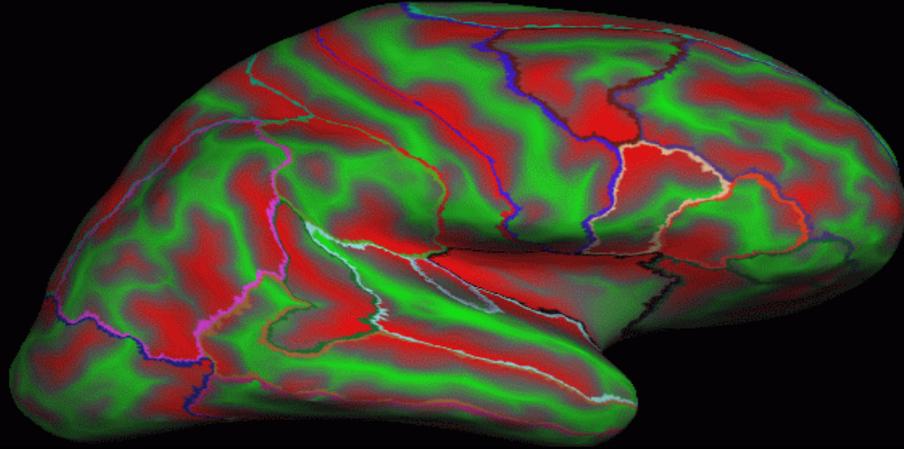
- SULCUS (+)
- GYRUS (-)
- Codes folding pattern

- Translate, Rotate, Stretch, Shear (12 DOF)
- Match Curvature, Vertex-by-Vertex
- Nonlinear Stretching (“Morphing”) allowed (area regularization)
- Actually done on sphere
- “Spherical Morph”

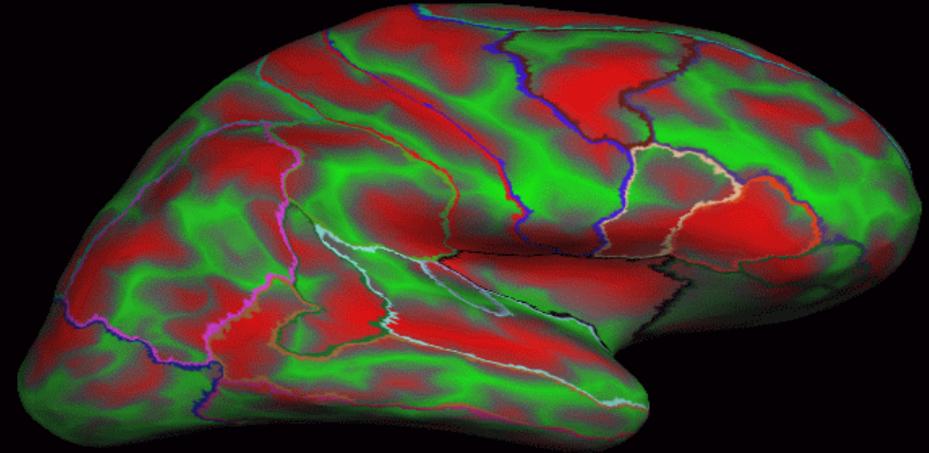


Surface Registration

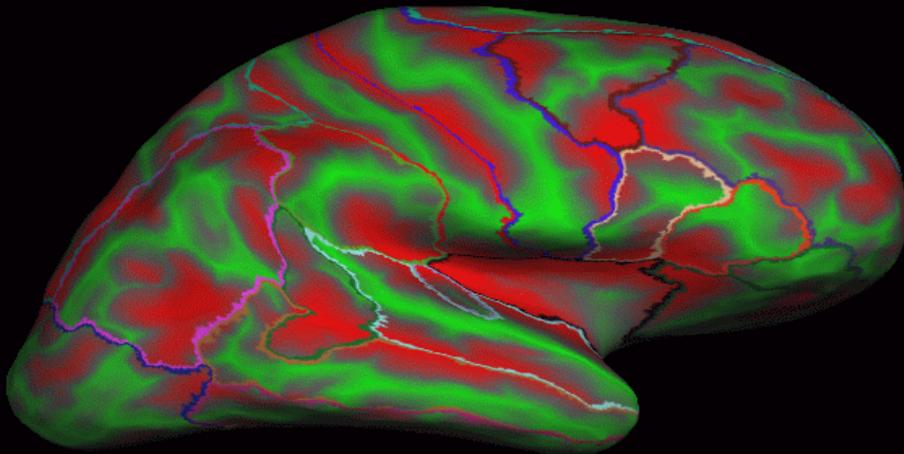
Subject 1



Subject 2 (Before)

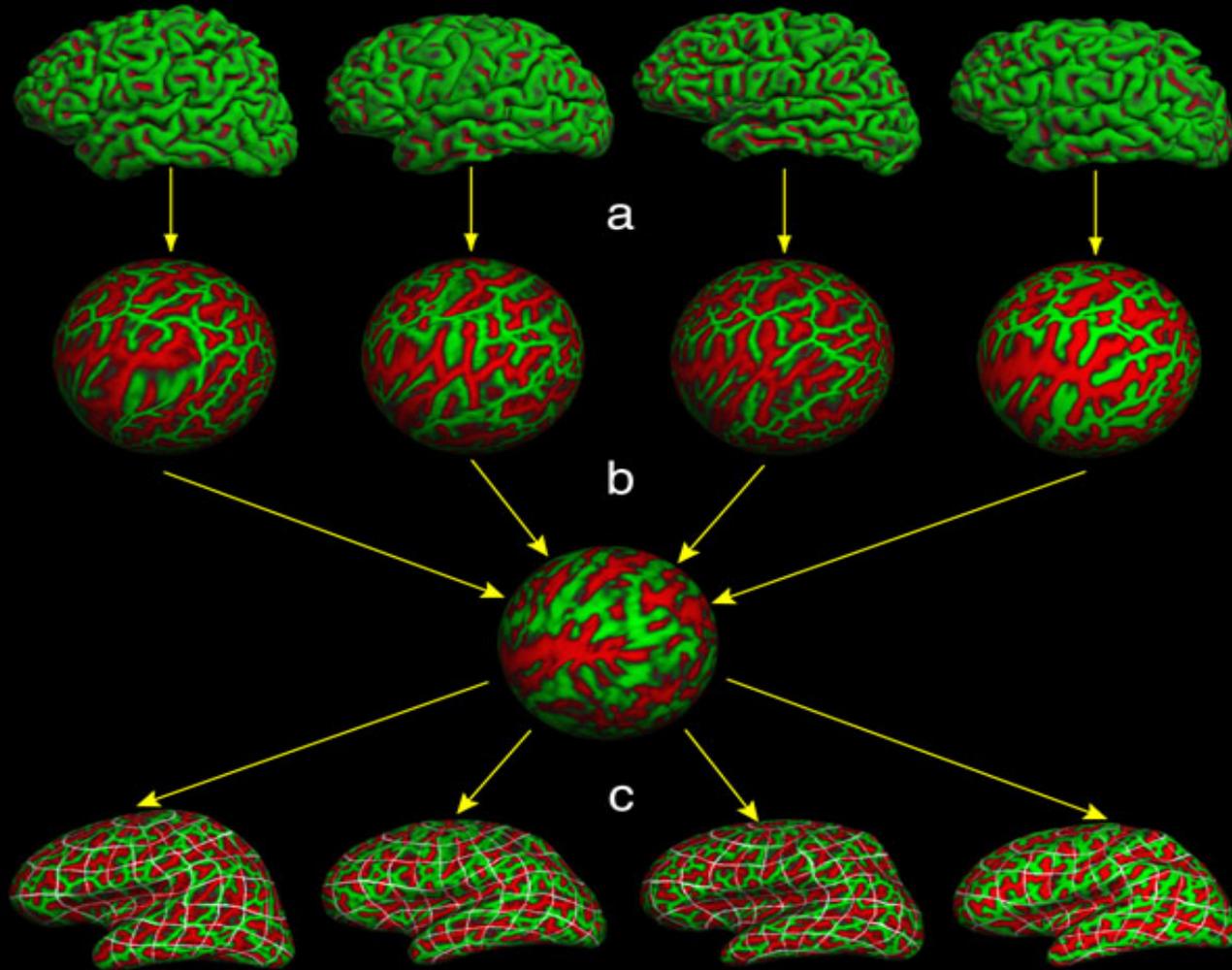


Subject 2 (After)



- Shift, Rotate, Stretch
- High dimensional (~500k)
- Preserve metric properties
- Take variance into account

A Surface-Based Coordinate System

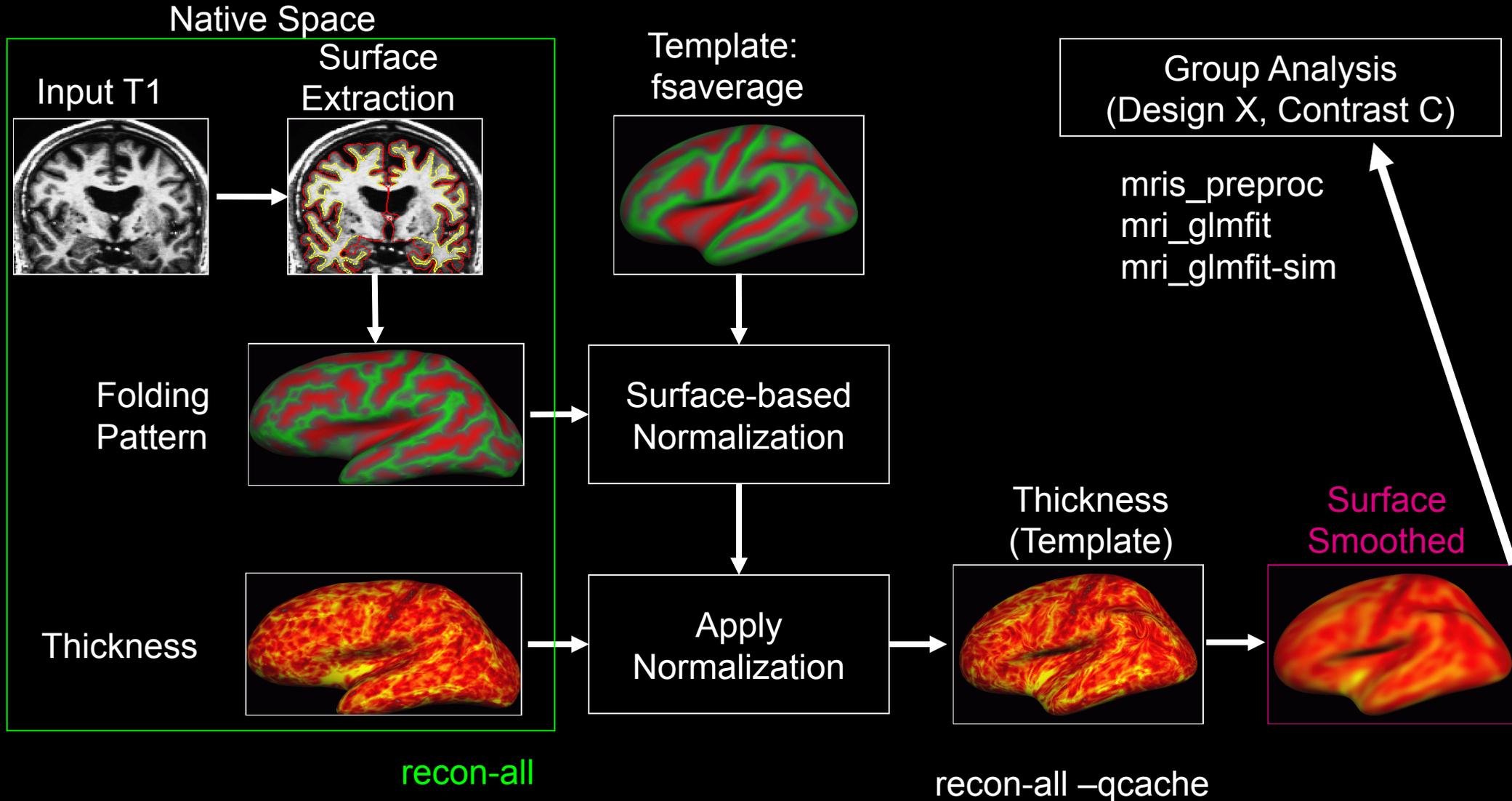


Common space for group analysis (like Talairach) – “fsaverage”

Surface-based Intersubject Registration

- Gray Matter-to-Gray Matter (it's all gray matter!)
- Gyrus-to-Gyrus and Sulcus-to-Sulcus
- Some minor folding patterns won't line up
- Fully automated, no landmarking needed
- Atlas registration is probabilistic, most variable regions get less weight.
- Done automatically in recon-all

Spatial Smoothing



Spatial Smoothing

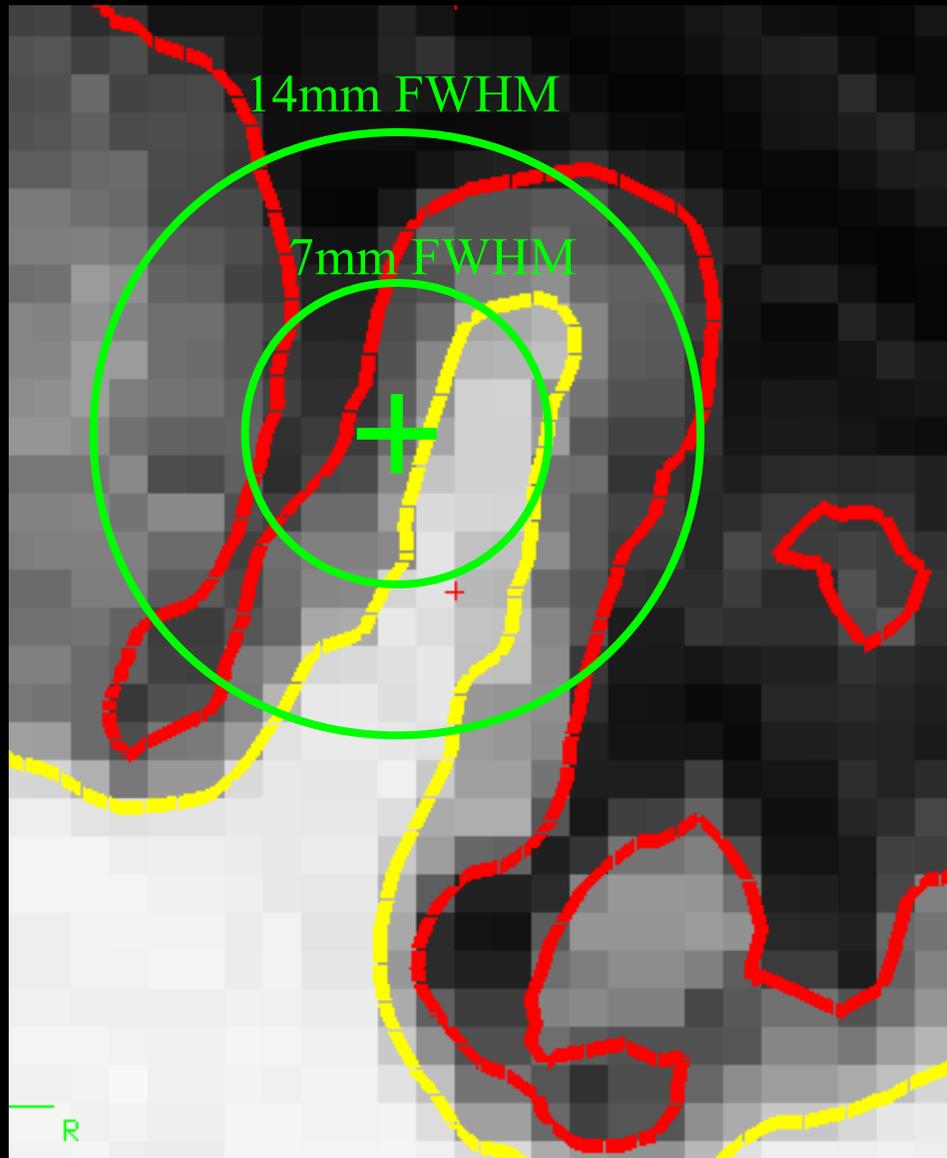
Why should you smooth?

- Might Improve CNR
- Improve intersubject registration (functional)

How much smoothing?

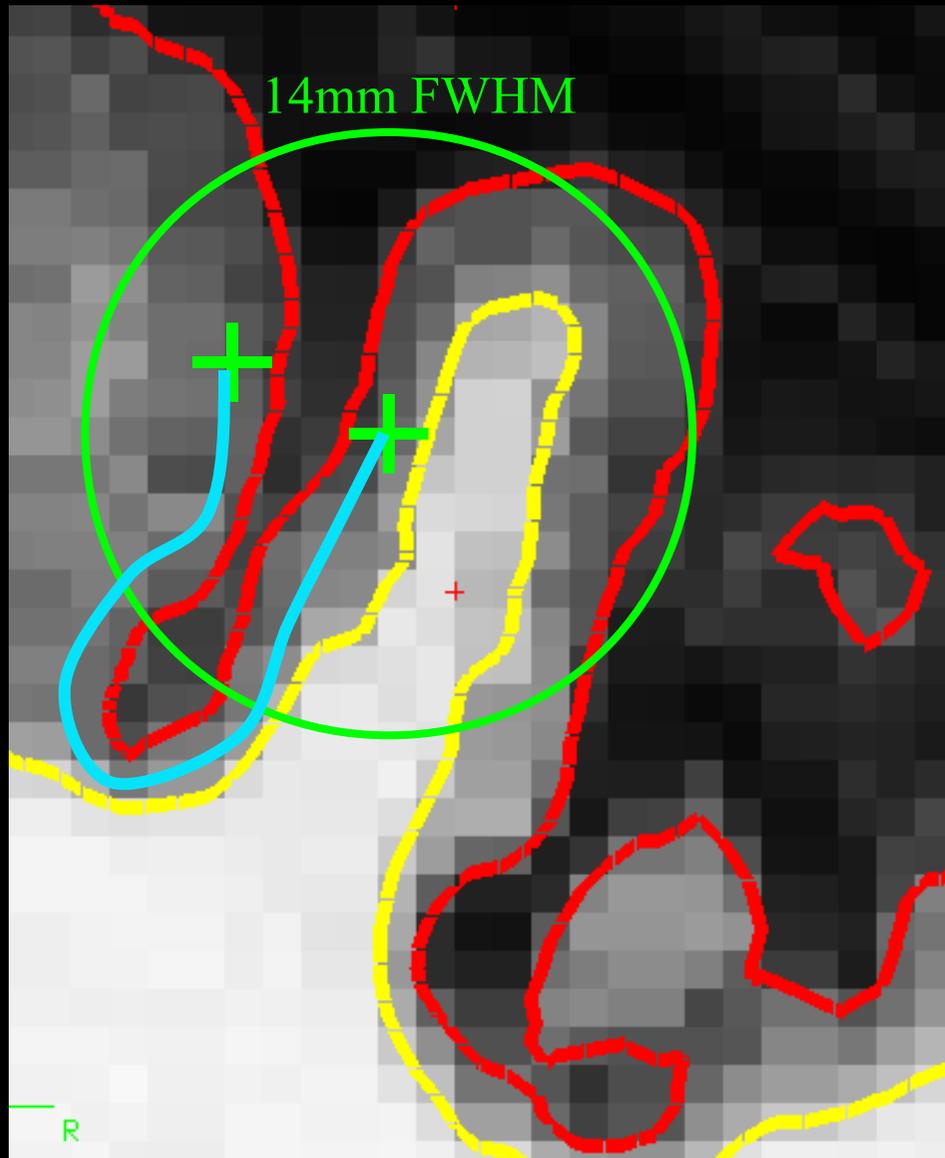
- Blob-size
- Typically 10-20 mm FWHM
- Surface smoothing more forgiving than volume-based

Volume-based Smoothing



- Smoothing is averaging of nearby voxels

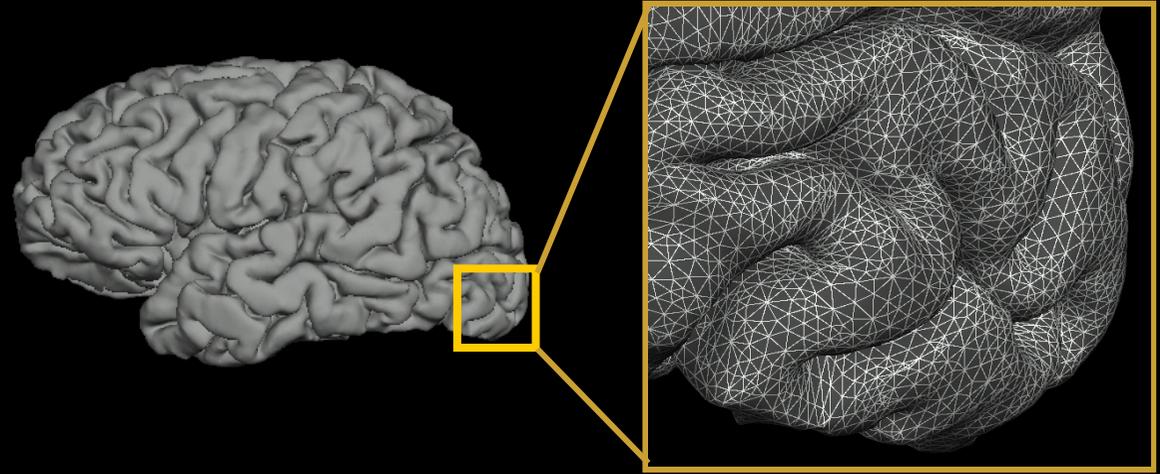
Volume-based Smoothing



- 5 mm apart in 3D
- 25 mm apart on surface!
- Kernel much larger
- Averaging with other tissue types (WM, CSF)
- Averaging with other functional areas

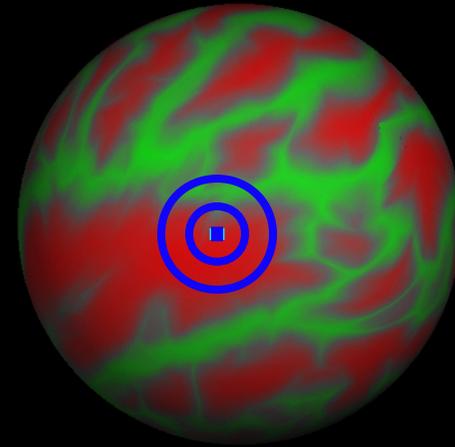
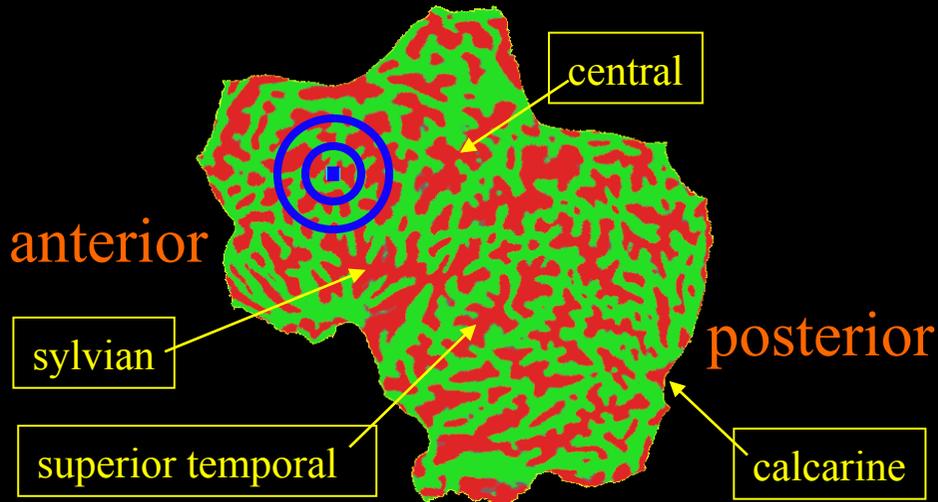
Surface-based Smoothing

- Smoothing is averaging of nearby vertices



Sheet: 2D Coordinate System (X,Y)

Sphere: 2D Coordinate System (θ , ϕ)

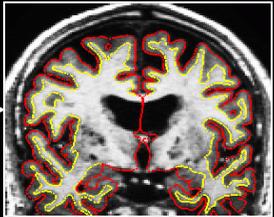


Group Analysis

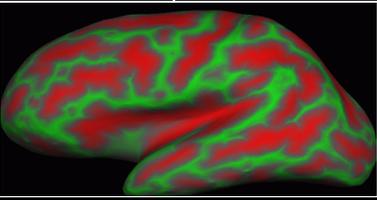
Native Space

Surface
Extraction

Input T1

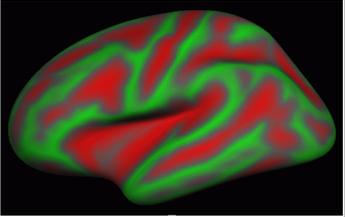


Folding
Pattern



Surface-based
Normalization

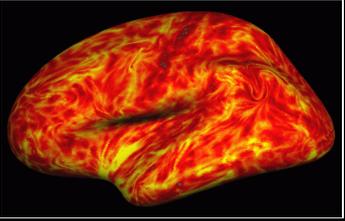
Template:
fsaverage



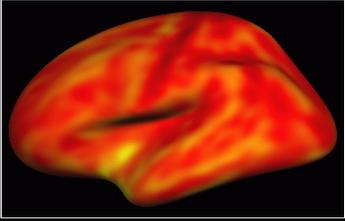
Apply
Normalization



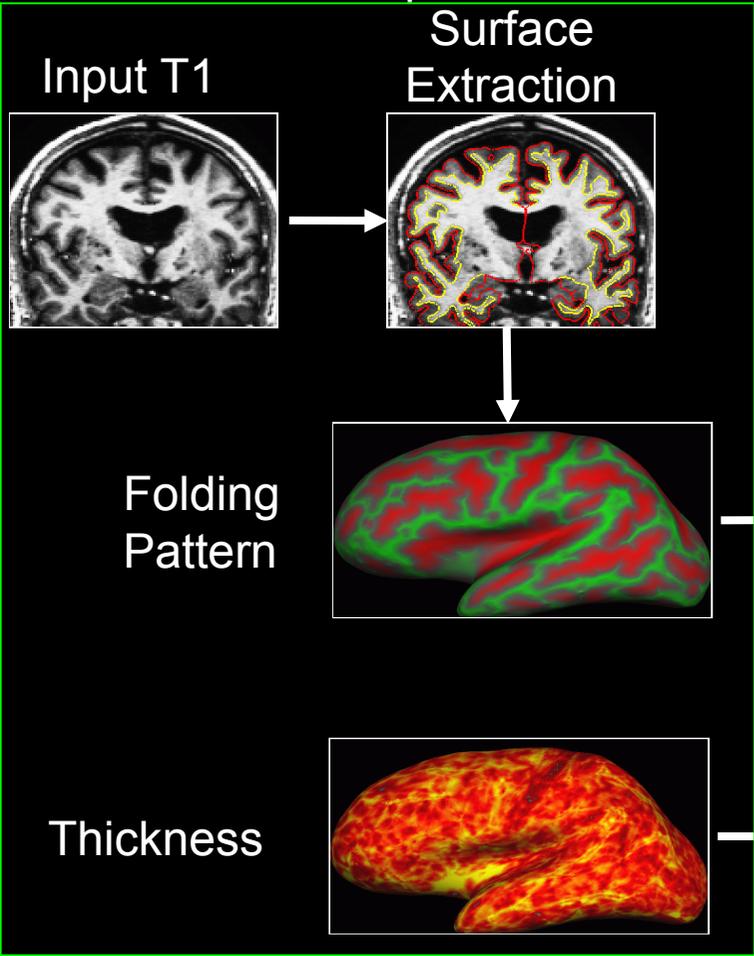
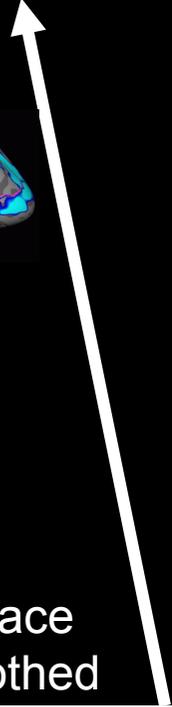
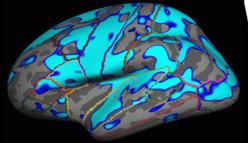
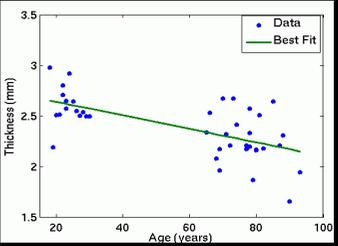
Thickness
(Template)



Surface
Smoothed



Group Analysis
(Design X, Contrast C)



recon-all

recon-all -qcache

GLM Theory: Design Matrix

$$\begin{bmatrix} y_{11} \\ y_{12} \\ y_{13} \\ y_{21} \\ y_{22} \\ y_{23} \end{bmatrix} = \begin{array}{c} \begin{matrix} \text{G1} & \text{G2} & \text{G1*Age} & \text{G2*Age} \end{matrix} \\ \begin{bmatrix} 1 & 0 & 20 & 0 \\ 1 & 0 & 25 & 0 \\ 1 & 0 & 30 & 0 \\ 0 & 1 & 0 & 18 \\ 0 & 1 & 0 & 23 \\ 0 & 1 & 0 & 35 \end{bmatrix} \end{array} \cdot \begin{bmatrix} b_1 \\ b_2 \\ m_1 \\ m_2 \end{bmatrix}$$

Data

Design Matrix

Regression Coefficients

$$y = X * \beta$$

GLM Theory: Contrasts

$$\begin{bmatrix} y_{11} \\ y_{12} \\ y_{13} \\ y_{21} \\ y_{22} \\ y_{23} \end{bmatrix} = \begin{matrix} & \text{G1} & \text{G2} & \text{G1*Age} & \text{G2*Age} \\ \begin{bmatrix} 1 & 0 & 20 & 0 \\ 1 & 0 & 25 & 0 \\ 1 & 0 & 30 & 0 \\ 0 & 1 & 0 & 18 \\ 0 & 1 & 0 & 23 \\ 0 & 1 & 0 & 35 \end{bmatrix} & \bullet & \begin{bmatrix} b_1 \\ b_2 \\ m_1 \\ m_2 \end{bmatrix} \end{matrix}$$

$$C_{\text{Group}} = [1 \ -1 \ 0 \ 0] \quad \text{Group diff}=0?$$

$$C_{\text{Age}} = [0 \ 0 \ 0.5 \ 0.5] \quad \text{Mean slope}=0?$$

$$C_{\text{GroupXAge}} = [0 \ 0 \ +1 \ -1] \quad \text{Group/Age InterX?}$$

Vertex-wise Analysis and Inference

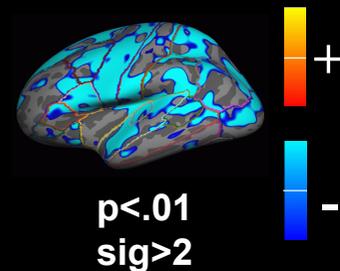
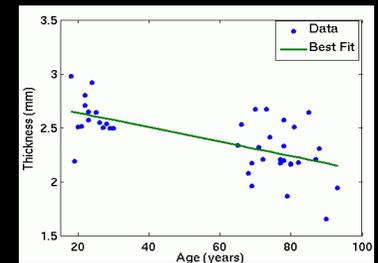
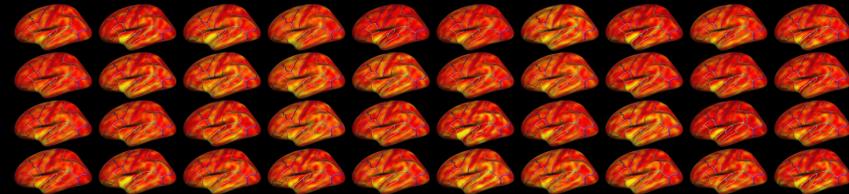
$$y = X * \beta + \epsilon$$
$$\beta = (X^t X)^{-1} X^t y$$
$$\gamma = C * \beta$$

p-value/significance

- value between 0 and 1
- closer to 0 means more significant

FreeSurfer stores p-values as $-\log_{10}(p)$:

- $0.1 = 10^{-1} \rightarrow \text{sig}=1$, $0.01 = 10^{-2} \rightarrow \text{sig}=2$
- sig.mgh files
- Signed by sign of γ
- p-value is for an unsigned test



Practice

1. X (Design Matrix)
2. C (Contrast Matrix or Matrices)
3. y (Data)
4. Analysis
5. Correction for multiple comparisons

Specifying X and C

1. Manually create X – text editor
 - Manually create C
1. FSGD file – text editor
 - FreeSurfer creates X
 - Manually create C
1. QDEC – spread sheet, etc
 - FreeSurfer creates X and C

FreeSurfer Group Descriptor (FSGD) File

- Simple text file (ascii, clear text)
- List of all subjects in the study
- Accompanying demographics

FSGD Format

```
GroupDescriptorFile 1
```

```
Class Male
```

```
Class Female
```

```
Variables
```

		Age	Weight	IQ
Input bert	Male	10	100	1000
Input fred	Male	15	150	1500
Input jenny	Female	20	200	2000
Input margaret	Female	25	250	2500

- One Discrete Factor (Gender) with Two Levels (M&F)
- Three Continuous Variables: Age, Weight, IQ
- \$SUBJECTS_DIR/bert

FSGD \rightarrow X (Automatic)

Female Group		Male*Age		Female*Age		1000	0
Male Group						1500	0

$$X = \begin{bmatrix} 1 & 0 & 10 & 0 & 100 & 0 & 1000 & 0 \\ 1 & 0 & 15 & 0 & 150 & 0 & 1500 & 0 \\ 0 & 1 & 0 & 20 & 0 & 200 & 0 & 2000 \\ 0 & 1 & 0 & 25 & 0 & 250 & 0 & 2500 \end{bmatrix}$$

Age
Weight
IQ

Manual



$$C = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

Tests for the difference in intercept/offset between groups

$$C = \begin{bmatrix} 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \end{bmatrix}$$

Tests for the difference in age slope between groups

FSGD Terminology

Continuous Variables/Factors: Age, IQ, Volume, etc

Discrete Variables/Factors: Gender, Handedness, Diagnosis

Levels of Discrete :

Handedness: Left and Right

Gender: Male and Female

Diagnosis: Normal, MCI, AD

Cannot specify Discrete Factors in FSGD

Group or Class: Specification of All Discrete Factors:

- Left-handed Male MCI
- Right-handed Female Normal

Number of Regressors

Each Group/Class:

- Has its own Intercept
- Has its own Slope (for each continuous variable)

$$N\text{Regressors} = N\text{Classes} * (N\text{Variables} + 1)$$

Data (y): Thickness Study

1. `$$SUBJECTS_DIR/bert/surf/lh.thickness`
2. `$$SUBJECTS_DIR/fred/surf/lh.thickness`
3. `$$SUBJECTS_DIR/jenny/surf/lh.thickness`
4. `$$SUBJECTS_DIR/margaret/surf/lh.thickness`
5. ...

Assemble Data: mris_preproc

mris_preproc --help

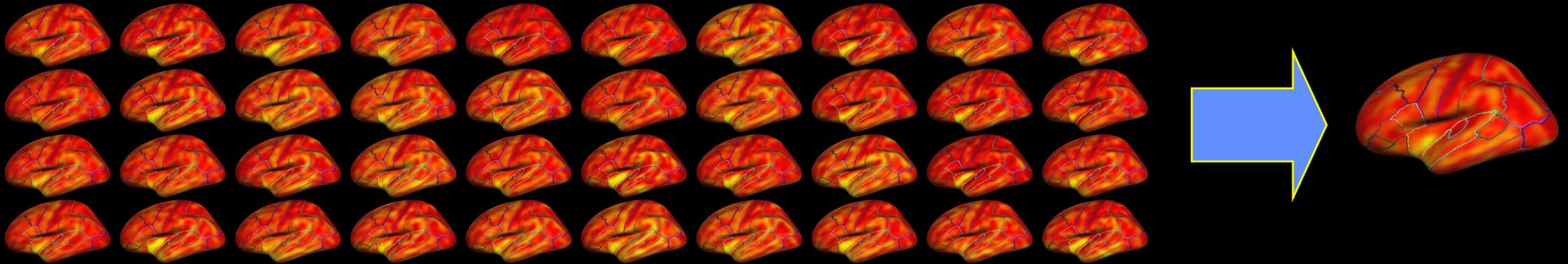
- fsgd FSGDFile** : Specify subjects thru FSGD File
- hemi lh** : Process left hemisphere
- meas thickness** : \$SUBJECTS_DIR/subjectid/surf/hemi.thickness
- target fsaverage** : common space is subject fsaverage
- o lh.thickness.mgh** : output “volume-encoded surface file”

Lots of other options!

lh.thickness.mgh – file with thickness maps for all subjects → Input to Smoother or GLM

See also: recon-all -qcache

A Stack of Images



lh.thickness.mgh – file with thickness maps for all subjects → Input to Smoother or GLM

Surface Smoothing

- `mri_surf2surf --help`
- Loads `lh.thickness.mgh`
- 2D surface-based smoothing
- Specify FWHM (eg, `fwhm = 10 mm`)
- Saves `lh.thickness.sm10.mgh`
- Can be slow (~10-30min)
- `recon-all -qcache`

Estimation and Inference: `mri_glmfit`

- Reads in FSGD File and constructs X
- Reads in your contrasts ($C1, C2, \text{etc}$)
- Loads data ($y = \text{lh.thickness.sm10.mgh}$)
- Fits GLM (ie, computes β)
- Computes contrasts ($\gamma = C * \beta$)
- t or F ratios, significances
- Significance $-\log_{10}(p)$ ($.01 \rightarrow 2, .001 \rightarrow 3$)

mri_glmfit

mri_glmfit

--y lh.thickness.sm10.mgh

--fsgd gender_age.txt

--C age.mtx --C gender.mtx

--surf fsaverage lh

--cortex

--glmdir lh.gender_age.glmdir

mri_glmfit --help

mri_glmfit

mri_glmfit

--y lh.thickness.sm10.mgh

--fsgd gender_age.txt

--C age.mtx --C gender.mtx

--surf fsaverage lh

--cortex

--glmdir lh.gender_age.glmdir

Input file (output from smoothing).
Stack of subjects, one frame per subject

mri_glmfit

```
mri_glmfit
```

```
--y lh.thickness.sm10.mgh
```

```
--fsgd gender_age.txt
```

```
--C age.mtx --C gender.mtx
```

```
--surf fsaverage lh
```

```
--cortex
```

```
--glmdir lh.gender_age.glmdir
```

- FreeSurfer Group Descriptor File (FSGD)
- Group membership
- Covariates

mri_glmfit

```
mri_glmfit  
--y lh.thickness.sm10.mgh  
--fsgd gender_age.txt  
--C age.mtx --C gender.mtx  
--surf fsaverage lh  
--cortex  
--glmdir lh.gender_age.glmdir
```

- Contrast Matrices
- Simple text/ASCII files
- Test hypotheses

mri_glmfit

mri_glmfit

--y lh.thickness.sm10.mgh

--fsgd gender_age.txt

--C age.mtx --C gender.mtx

--surf fsaverage lh

--cortex

--glmdir lh.gender_age.glmdir

- Perform analysis on left hemisphere of fsaverage subject
- Masks by fsaverage cortex.label
- Computes FWHM in 2D

mri_glmfit

```
mri_glmfit
--y lh.thickness.sm10.mgh
--fsgd gender_age.txt
--C age.mtx --C gender.mtx
--surf fsaverage lh
--cortex
--glmdir lh.gender_age.glmdir
```

Output directory:

lh.gender_age.glmdir/

beta.mgh – parameter estimates
rvar.mgh – residual error variance
etc ...

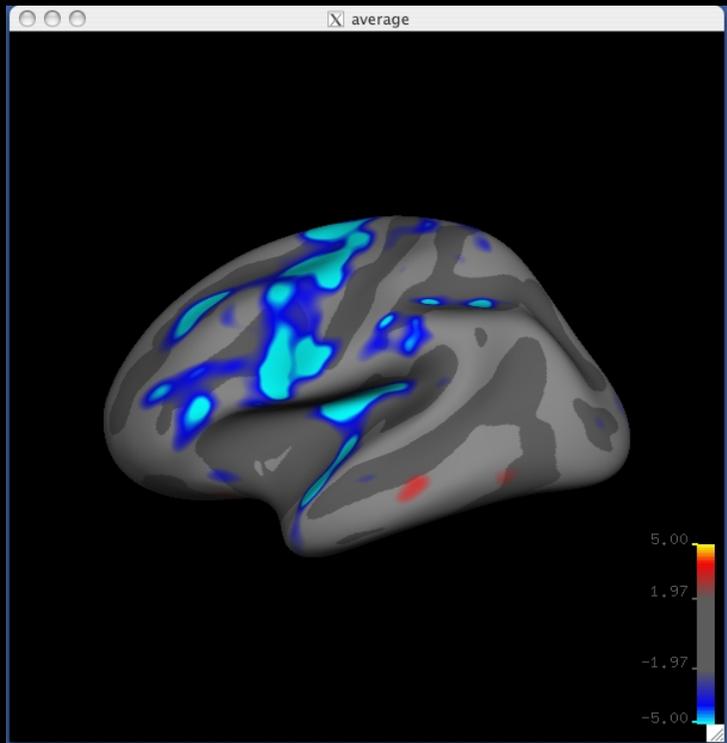
age/

sig.mgh = $-\log_{10}(p)$, uncorrected
gamma.mgh, F.mgh

gender/

sig.mgh = $-\log_{10}(p)$
gamma.mgh, F.mgh

Visualization with tksurfer



Threshold:
 $-\log_{10}(p)$,
Eg, 2=.01

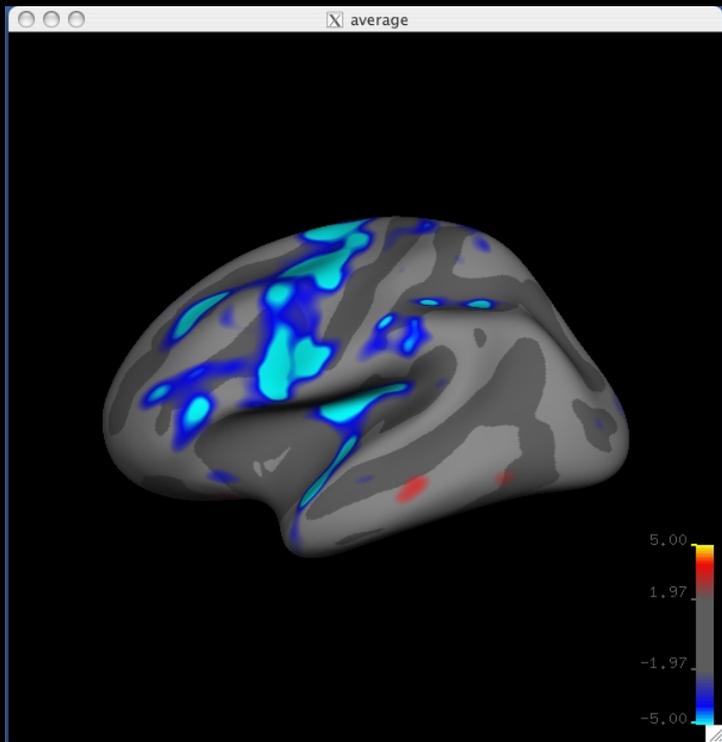
File->LoadOverlay

Saturation:
 $-\log_{10}(p)$,
Eg, 5=.00001

False
Discovery
Rate
Eg, .01

View->Configure->Overlay

Visualization with tksurfer



TkSurfer Tools

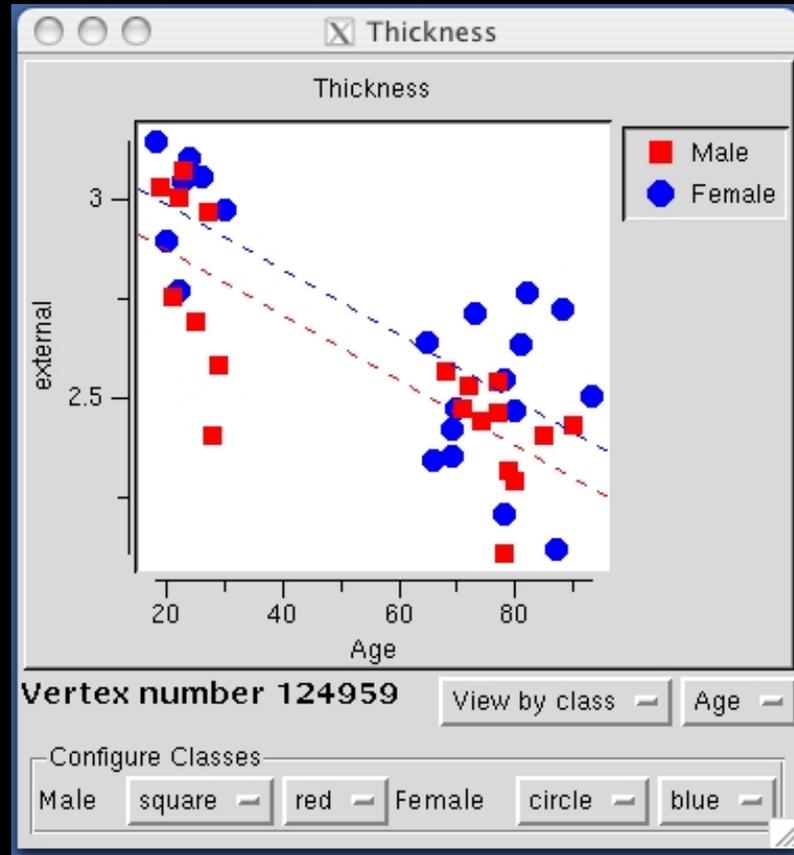
File Edit View Tools

Cursor

Vertex Index	124959
Vertex RAS	(-44.46 7.45 20.94)
Vertex Talairach	(-44.02 8.18 18.88)
Curvature	0.443938
sig.mgh	** -8.300488 **

Mouse

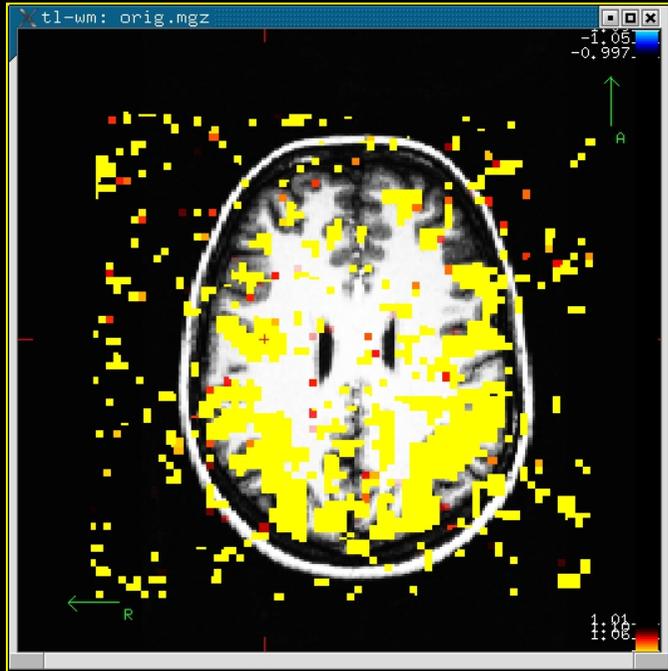
Vertex Index	58682
Vertex RAS	(-51.69 -48.01 44.45)
Vertex Talairach	(-51.17 -44.47 43.18)
Curvature	-0.469669
sig.mgh	** 0.181452 **



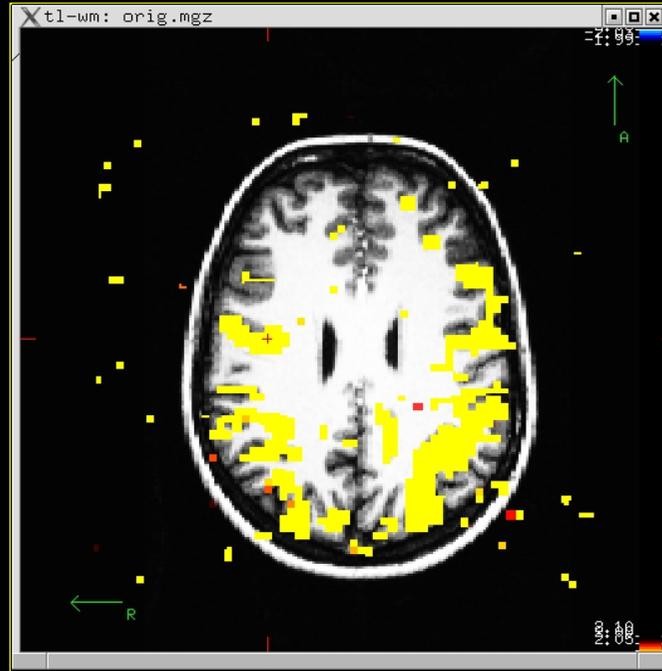
File->

Load Group Descriptor File ...

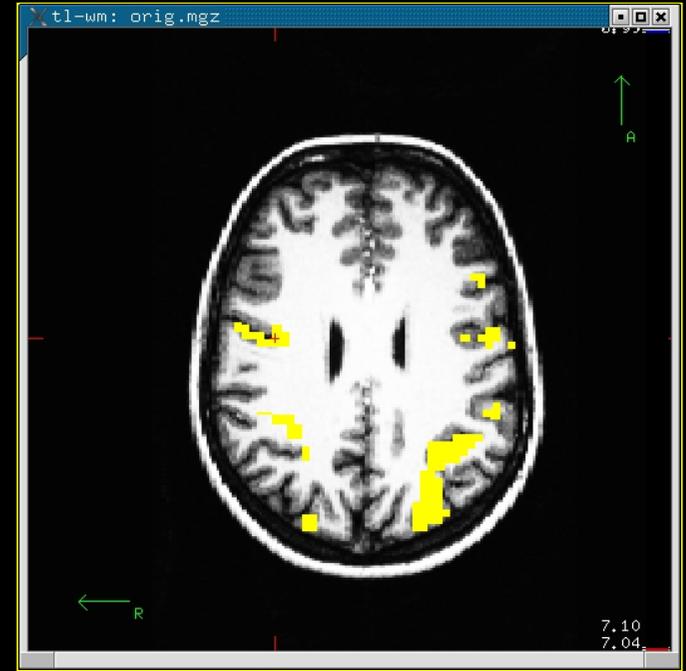
Problem of Multiple Comparisons



$p < 0.10$



$p < 0.01$



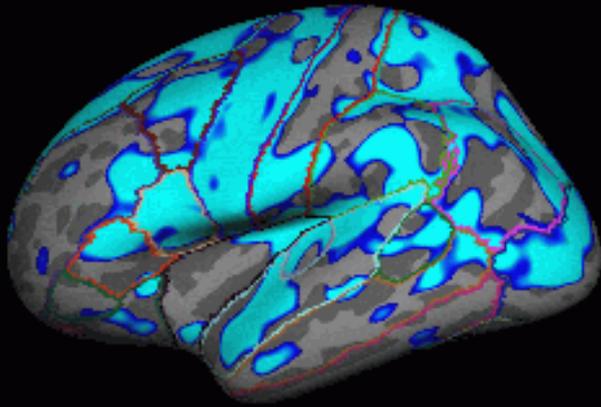
$p < 10^{-7}$

Correction for Multiple Comparisons

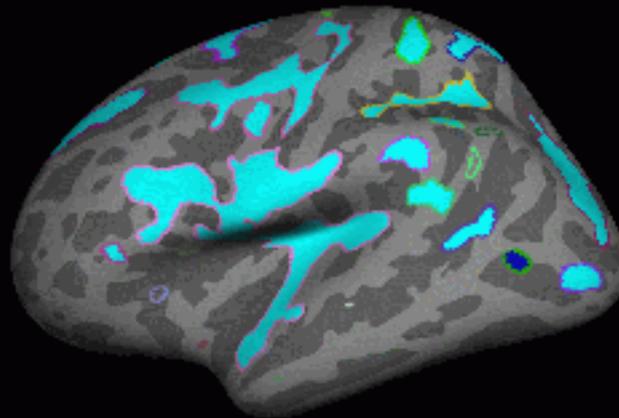
- Cluster-based
 - Monte Carlo simulation
 - Permutation Tests
 - Surface Gaussian Random Fields (GRF)
 - There but not fully tested
- False Discovery Rate (FDR) – built into tksurfer and QDEC. (Genovese, et al, NI 2002)

Clustering

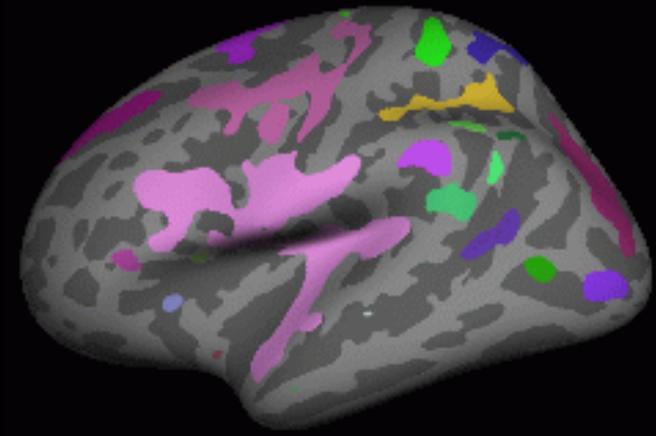
1. Choose a vertex-wise (cluster-forming) threshold
 - Eg, 2 ($p < .01$), or 3 ($p < .001$)
 - Sign (pos, neg, abs)
1. A cluster is a group of connected (neighboring) vertices above threshold
2. Cluster has a size (area in mm^2)



$p < .01$ ($-\log_{10}(p) = 2$)
Negative



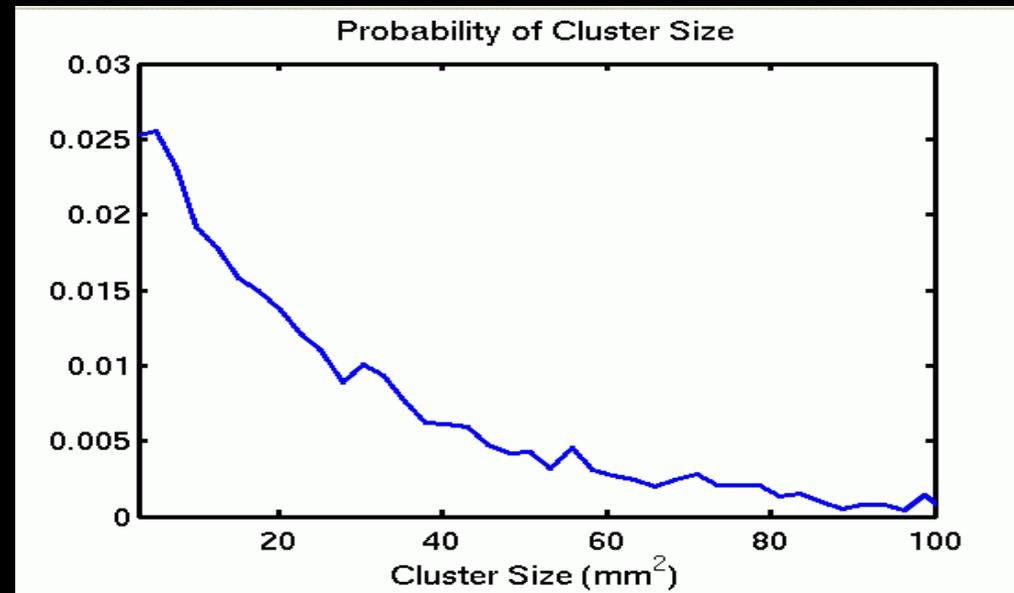
$p < .0001$ ($-\log_{10}(p) = 4$)
Negative



Cluster-based Correction for Multiple Comparisons

1. Simulate data under Null Hypothesis:
 - Synthesize Gaussian noise and then smooth (Monte Carlo)
 - Permute rows of design matrix (Permutation, orthog)
1. Analyze, threshold, cluster, max cluster size
2. Repeat 10,000 times
3. Analyze real data, get cluster sizes
4. $P(\text{cluster}) = \# \text{MaxClusterSize} > \text{ClusterSize} / 10000$

mri_glmfit-sim



Surface-based Correction for Multiple Comparisons

- 2D Cluster-based Correction at $p < .05$

```
mri_glmfit-sim  
  --glmdir lh.gender_age.glmdir  
  --cache pos 2  
  --cwpvalthresh .025
```

Original mri_glmfit command:

```
mri_glmfit  
  --y lh.thickness.sm10.mgh  
  --fsgd gender_age.txt  
  --C age.mtx -C gender.mtx  
  --surf fsaverage lh  
  --cortex  
  --glmdir lh.gender_age.glmdir
```

Surface-based Correction for Multiple Comparisons

- 2D Cluster-based Correction at $p < .05$

mri_glmfit-sim

--glmdir lh.gender_age.glmdir

--cache pos 2

--cwpvalthresh .025

Original mri_glmfit command:

mri_glmfit

--y lh.thickness.sm10.mgh

--fsgd gender_age.txt

--C age.mtx -C gender.mtx

--surf fsaverage lh

--cortex

--glmdir lh.gender_age.glmdir

lh.gender_age.glmdir/

beta.mgh – parameter estimates

rvar.mgh – residual error variance

etc ...

age/

sig.mgh – $-\log_{10}(p)$, uncorrected

gamma.mgh, F.mgh

gender/

sig.mgh – $-\log_{10}(p)$

gamma.mgh, F.mgh

Surface-based Group Analysis

Surface-based Correction for Multiple Comparisons

- 2D Cluster-based Correction at $p < .05$

```
mri_glmfit-sim  
--glmdir lh.gender_age.glmkdir  
--cache pos 2  
--cwpvalthresh .025
```

- Use pre-cached simulation results
- positive contrast
- voxelwise threshold = 2 ($p < .01$)
- Can use another simulation or permutation

Surface-based Correction for Multiple Comparisons

- 2D Cluster-based Correction at $p < .05$

```
mri_glmfit-sim
```

```
--glmdir lh.gender_age.glmmdir
```

```
--cache pos 2
```

```
--cwpvalthresh .025
```

Cluster-wise threshold $p < .025$

Why Cluster-wise threshold $p < .025$ when I wanted $p < .05$?

Correction across 2 spaces: lh and rh: $.025 = .05/2$

Bonferroni Correction

Correction for Multiple Comparisons Output (Surface)

```
mri_glmfit-sim
--glmdir lh.gender_age.glmdir
--cache pos 2
--cwpvalthresh .025
```

lh.gender_age.glmdir

mri_glmfit
output directory

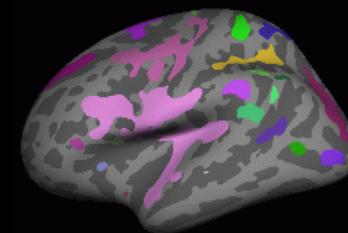
age

gender

contrast
directories

sig.mgh – pre-existing uncorrected p-values
cache.th20.pos.sig.cluster.mgh – map of significance of clusters
cache.th20.pos.sig.ocn.annot – annotation of significant clusters
cache.th20.pos.sig.cluster.summary – text file of cluster table
 (clusters, sizes, MNI305 XYZ, and their significances)

- Only shows clusters $p < .025$
- Cluster significance values not corrected for 2 spaces!



Command-line Processing Stages

- Model (X, FSGD) and Contrasts (C)
- Assemble Data (mris_preproc)
 - Resample into Common Space (fsaverage)
 - Concatenate into one file
- Smooth (mri_surf2surf)
- Fit Model (Estimate) (mri_glmfit)
- Correct for multiple comparisons (mri_glmfit-sim)
- Visualize (tksurfer)

recon-all -qcache

QDEC – An Interactive Statistical Engine GUI

Query – Select subjects based on Match Criteria

Design – Specify discrete and continuous factors

Estimate – Fit Model

Contrast – Automatically Generate Contrast Matrices

Interactive – Makes easy things easy (that used to be hard)

...a work in progress

- No Query yet
- Two Discrete Factors (Two Levels)
- Two Continuous Factors
- Surface only

QDEC – Spreadsheet

qdec.table.dat – spreadsheet with subject information – spreadsheet can be huge!

fsid	gender	age	diagnosis	Left-Cerebral-White-Matter-Vol
011121_vc8048	Female	70	Demented	202291
021121_62313-2	Female	71	Demented	210188
010607_vc7017	Female	73	Nondemented	170653
021121_vc10557	Male	75	Demented	142029
020718_62545	Male	76	Demented	186087
020322_vc8817	Male	77	Nondemented	149810

gender.levels

Female
Male

diagnosis.levels

Demented
Nondemented

Discrete Factors need a
factormame.level file

Tutorial

1. Command-line Stream

- Create an FSGD File for a thickness study
- Age and Gender
- Run
 - mris_preproc
 - mri_surf2surf
 - mri_glmfit
 - mri_glmfit-sim
 - tksurfer

1. QDEC – same data set