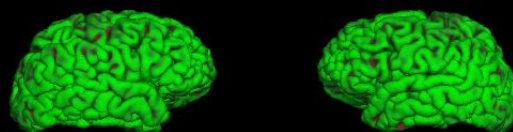
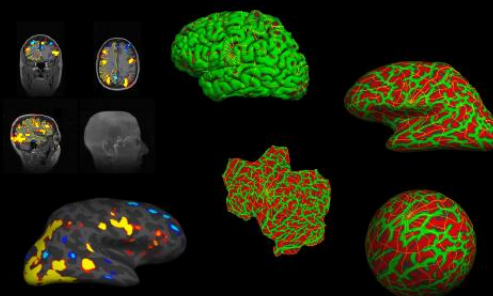


Surface-based Group Analysis in FreeSurfer



FreeSurfer

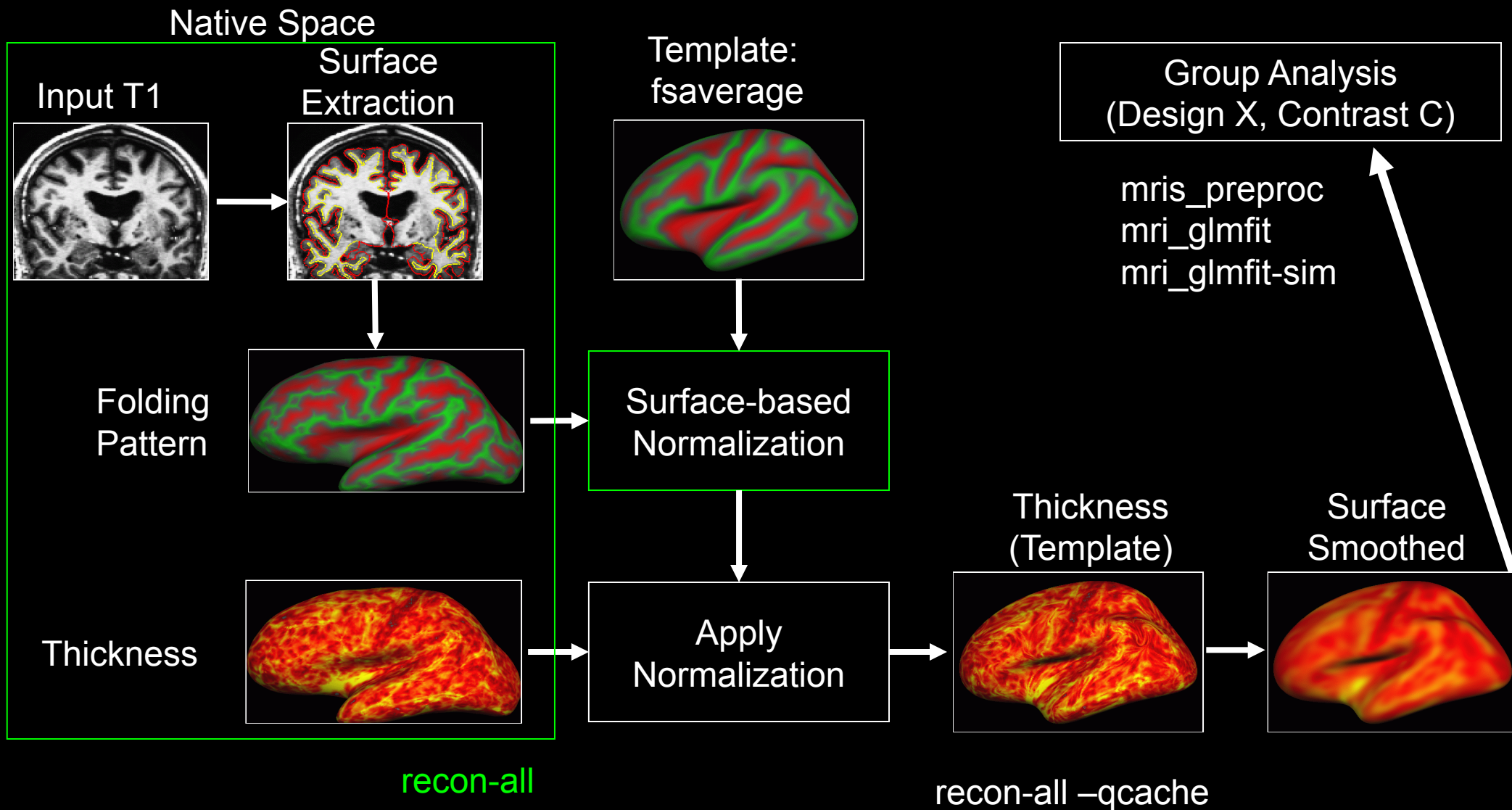


MASSACHUSETTS
GENERAL HOSPITAL

Outline

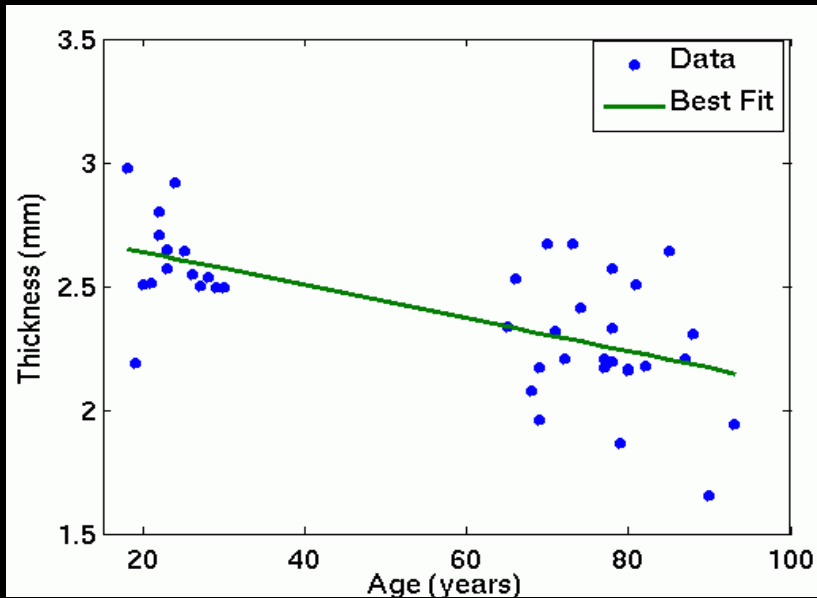
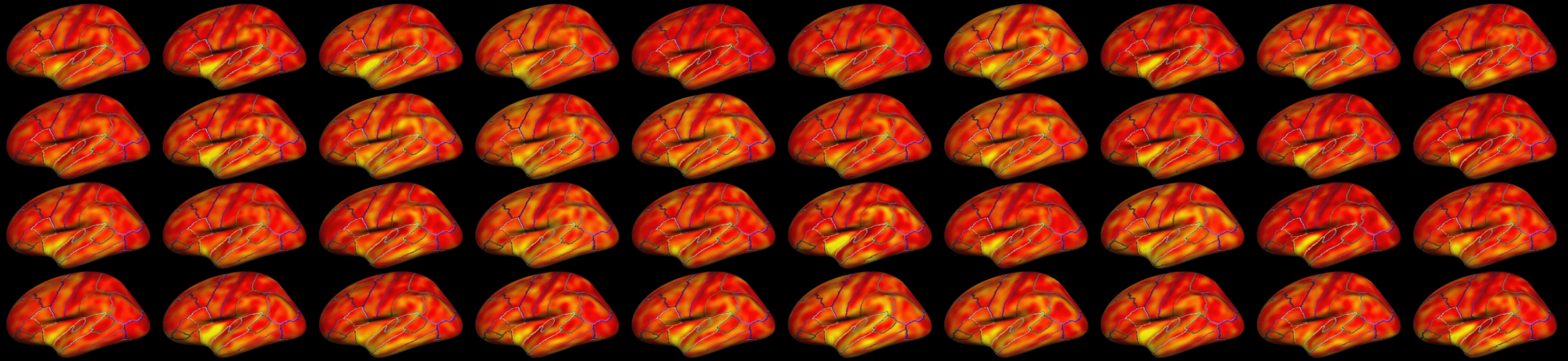
- Group Analysis Overview
- Theory
 - Surface-based Intersubject Registration
 - Surface-based Smoothing
 - General Linear Model (GLM)
- Practice
 - FSGD File
 - Processing Stages
 - Vertex-wise Analysis
 - Correction for multiple comparisons
- Interactive/Automated GUI (QDEC)

Analysis Pipeline

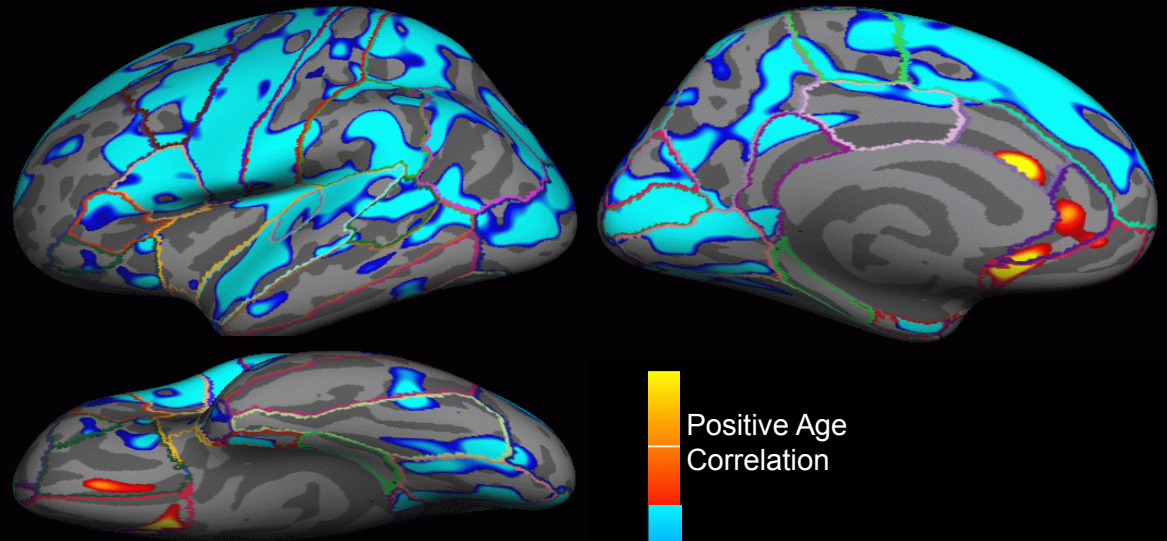


Aging Thickness Study

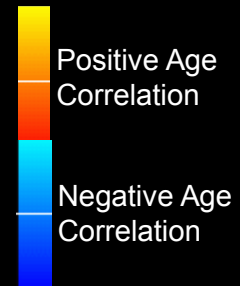
N=40



Design X (slope, intercept)



Contrast C $p < .01$

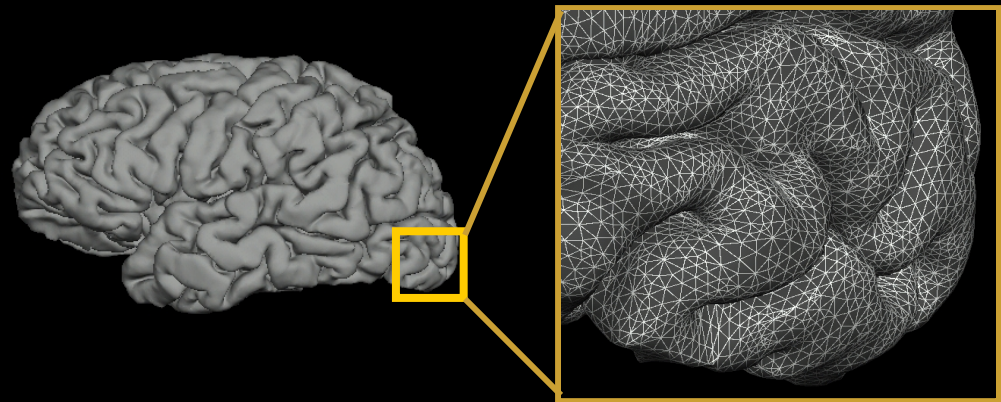


Theory

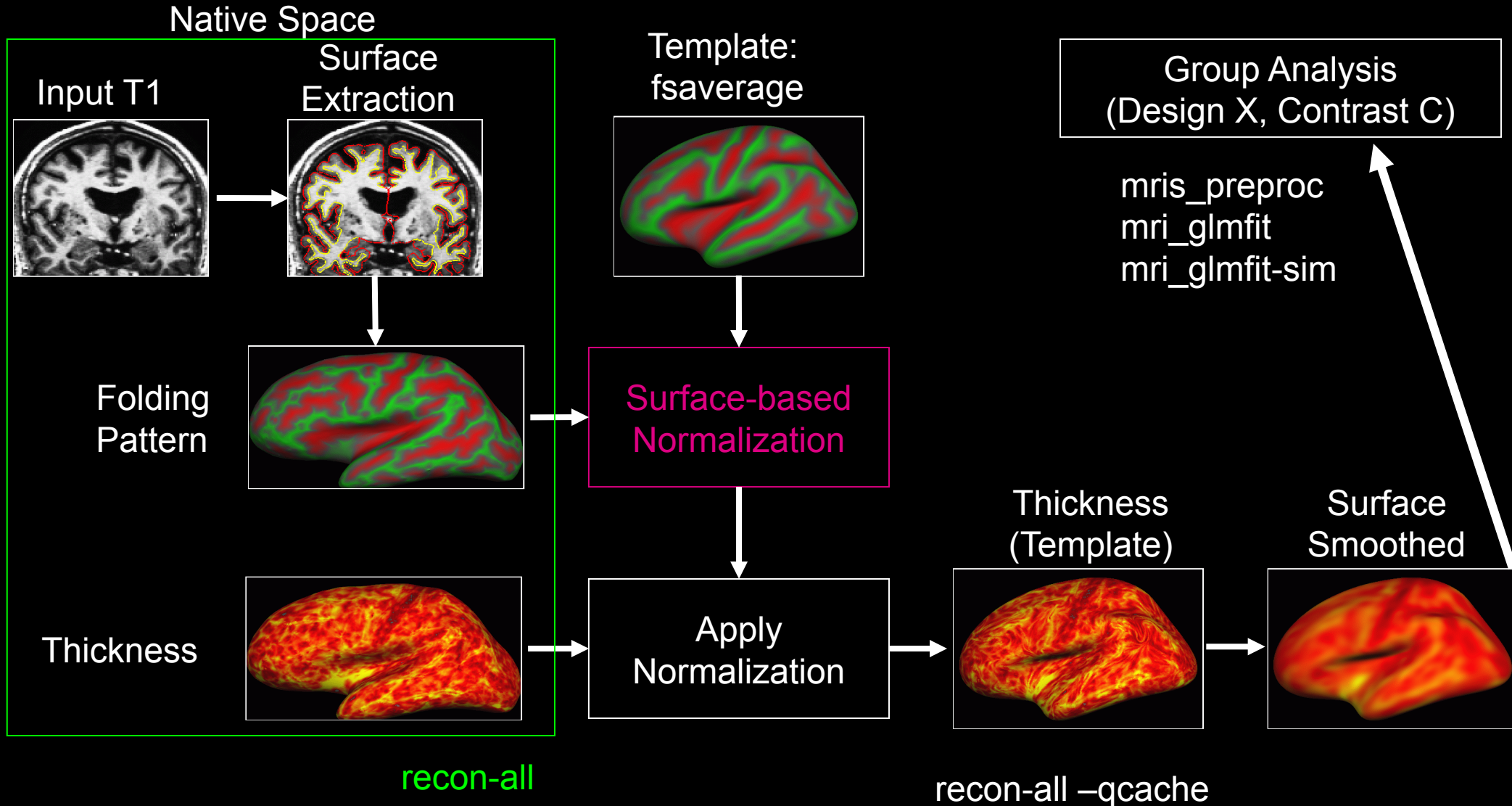
1. Surface-based Intersubject Registration
2. Surface-based Smoothing
3. General Linear Model (GLM)

Surface-based Measures

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

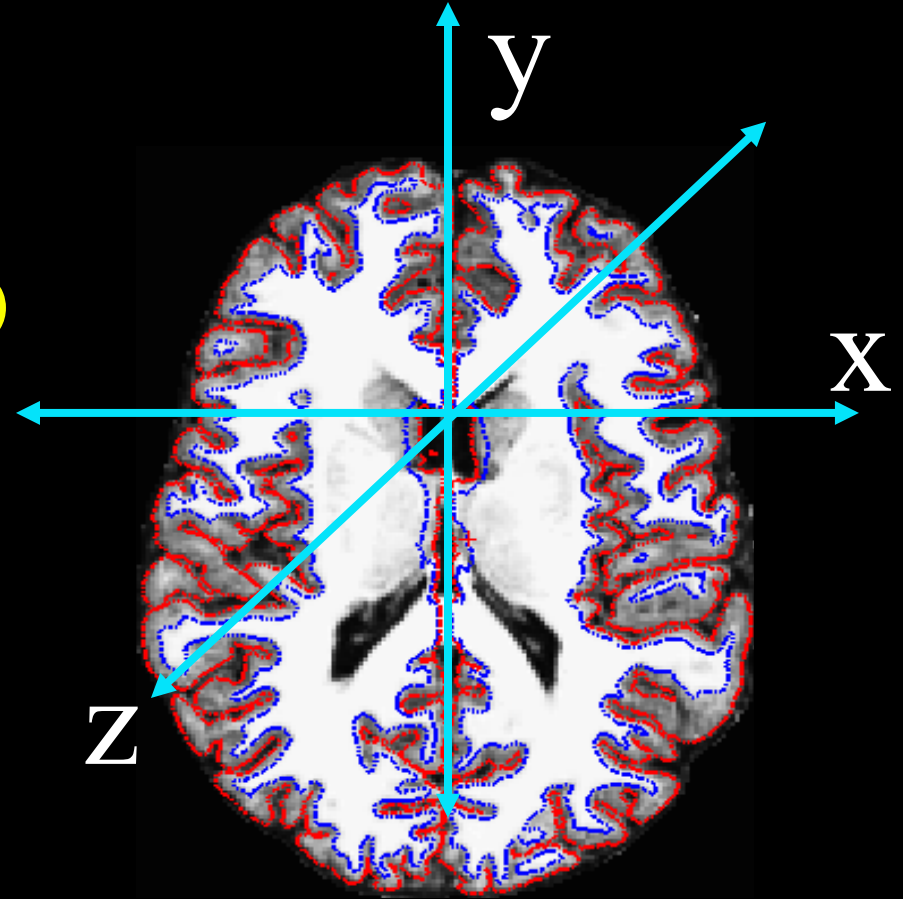


Surface-based Spatial Normalization



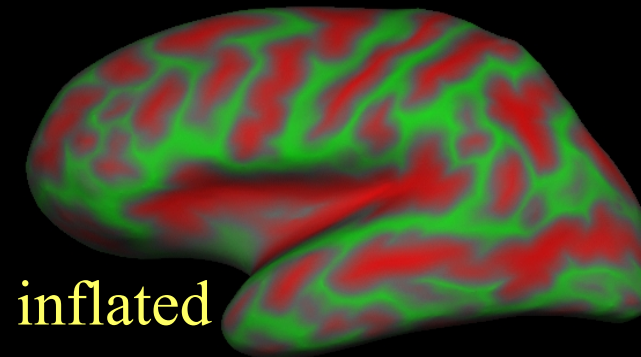
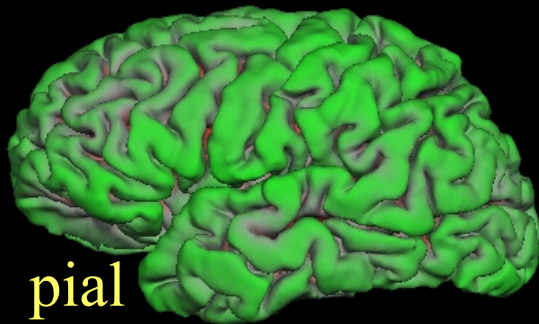
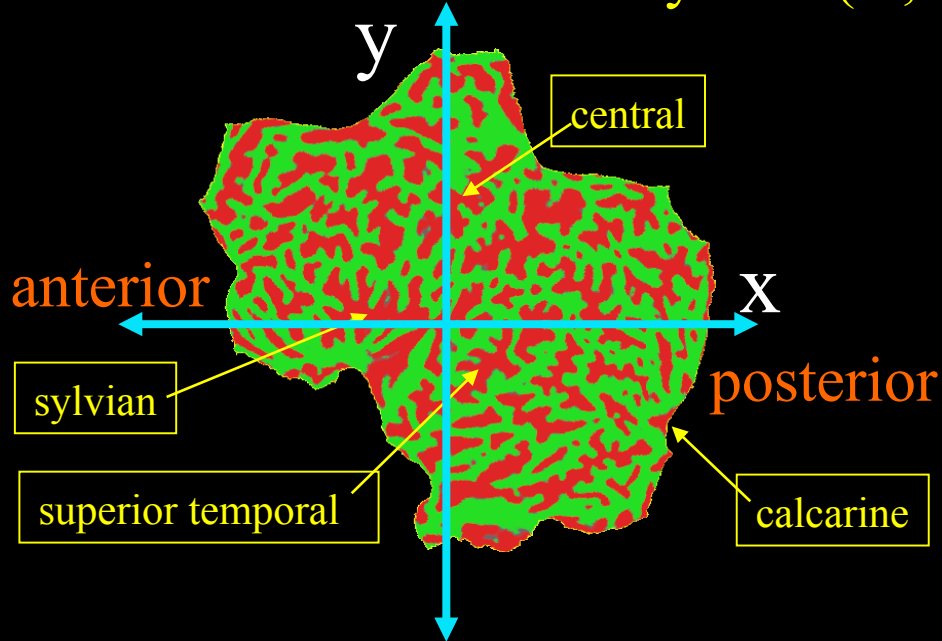
Coordinate Systems: 3D (Volumetric)

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



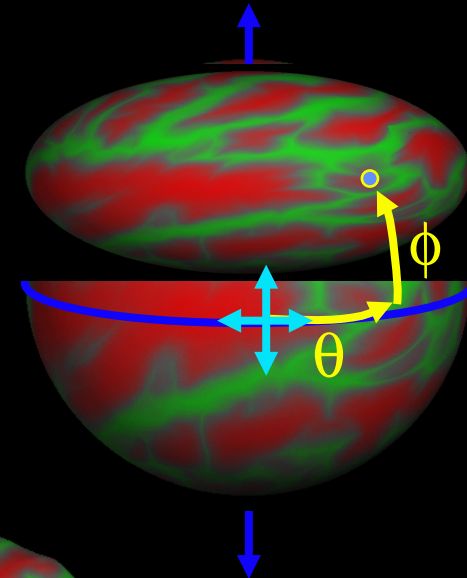
Coordinate Systems: 2D (Surface)

Sheet: 2D Coordinate System (X,Y)



Sphere: 2D Coordinate System

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

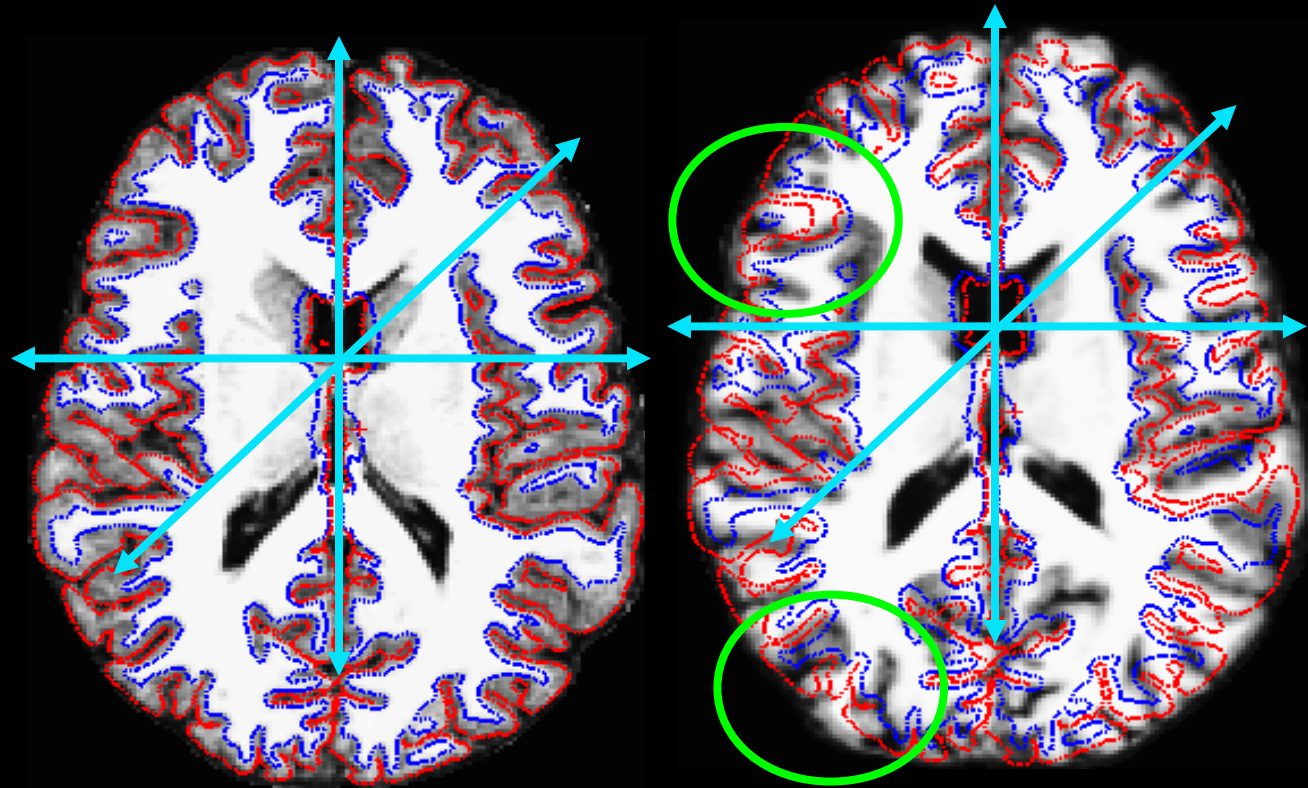


Curvature

- SULCUS (+)
- GYRUS (-)

Volumetric Intersubject Registration

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

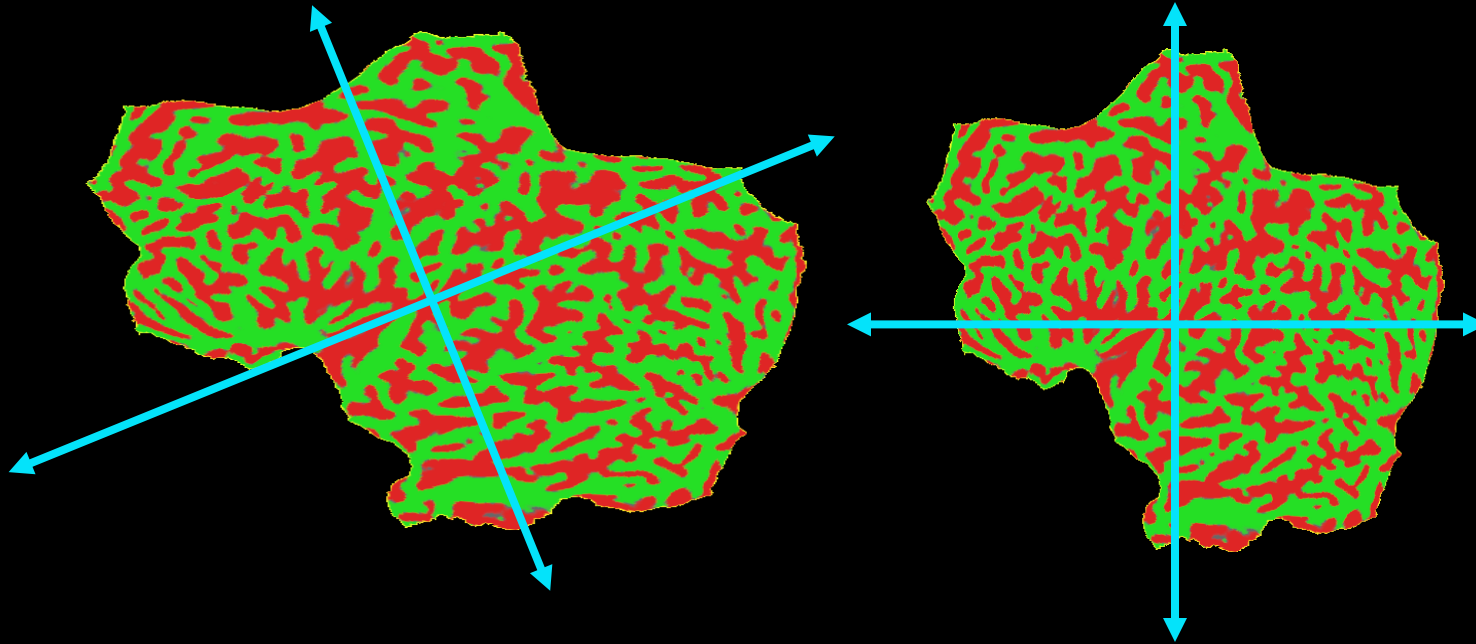


- Match Intensity, Voxel-by-Voxel
- Problems
- Can use nonlinear volumetric (cf CVS)

Surface-based Intersubject Registration

Subject 1

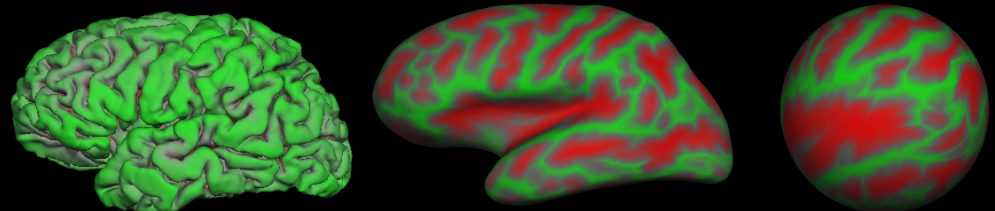
Subject 2



Curvature “Intensity”

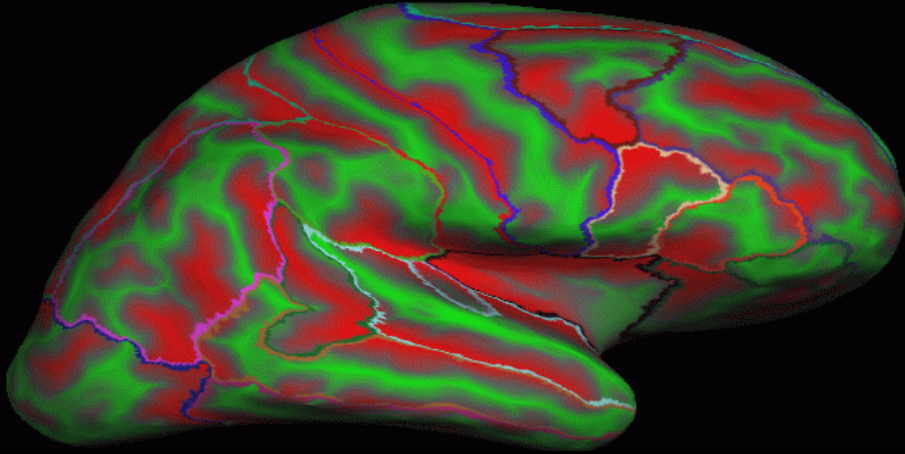
- SULCUS (+)
- GYRUS (-)
- Codes folding pattern

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

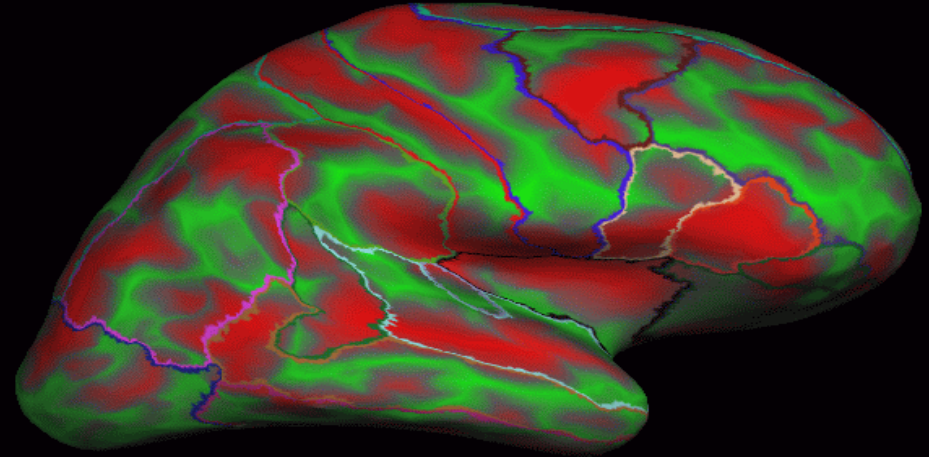


Surface Registration

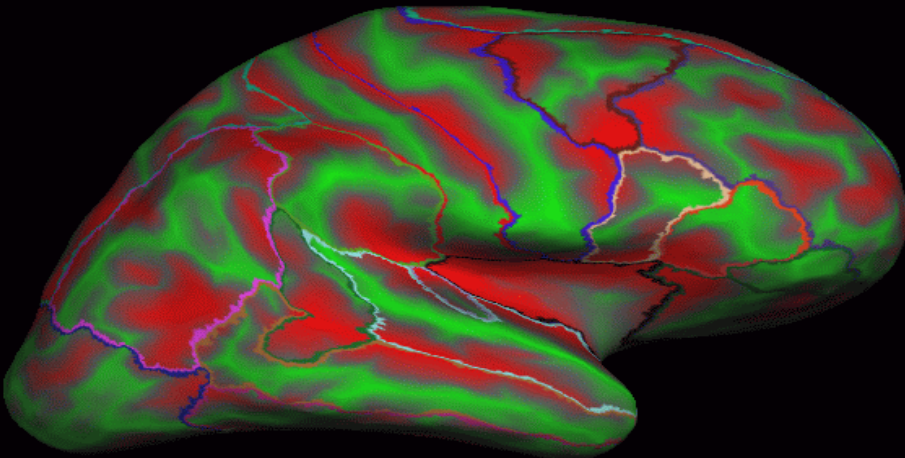
Subject 1



Subject 2 (Before)

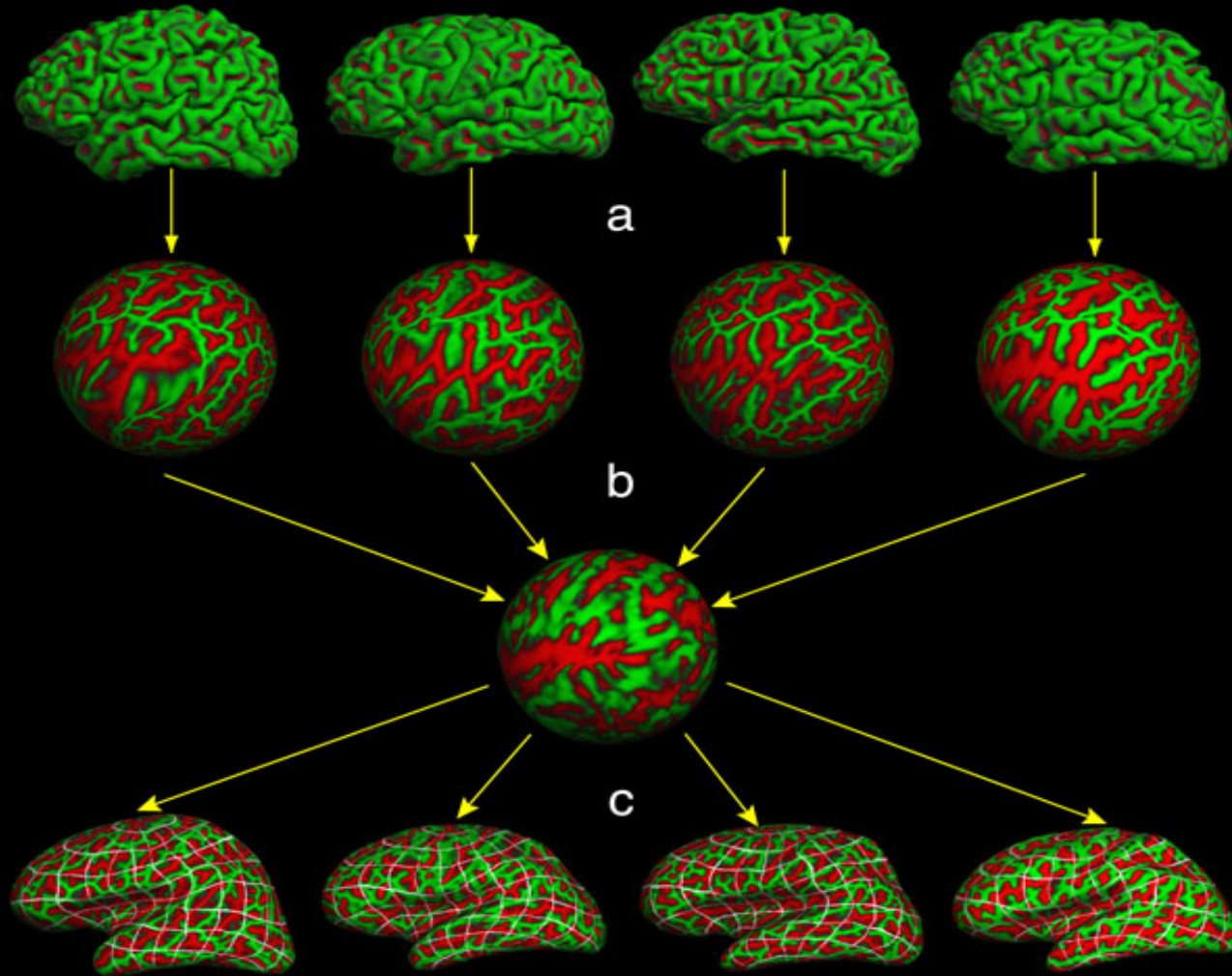


Subject 2 (After)



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

A Surface-Based Coordinate System

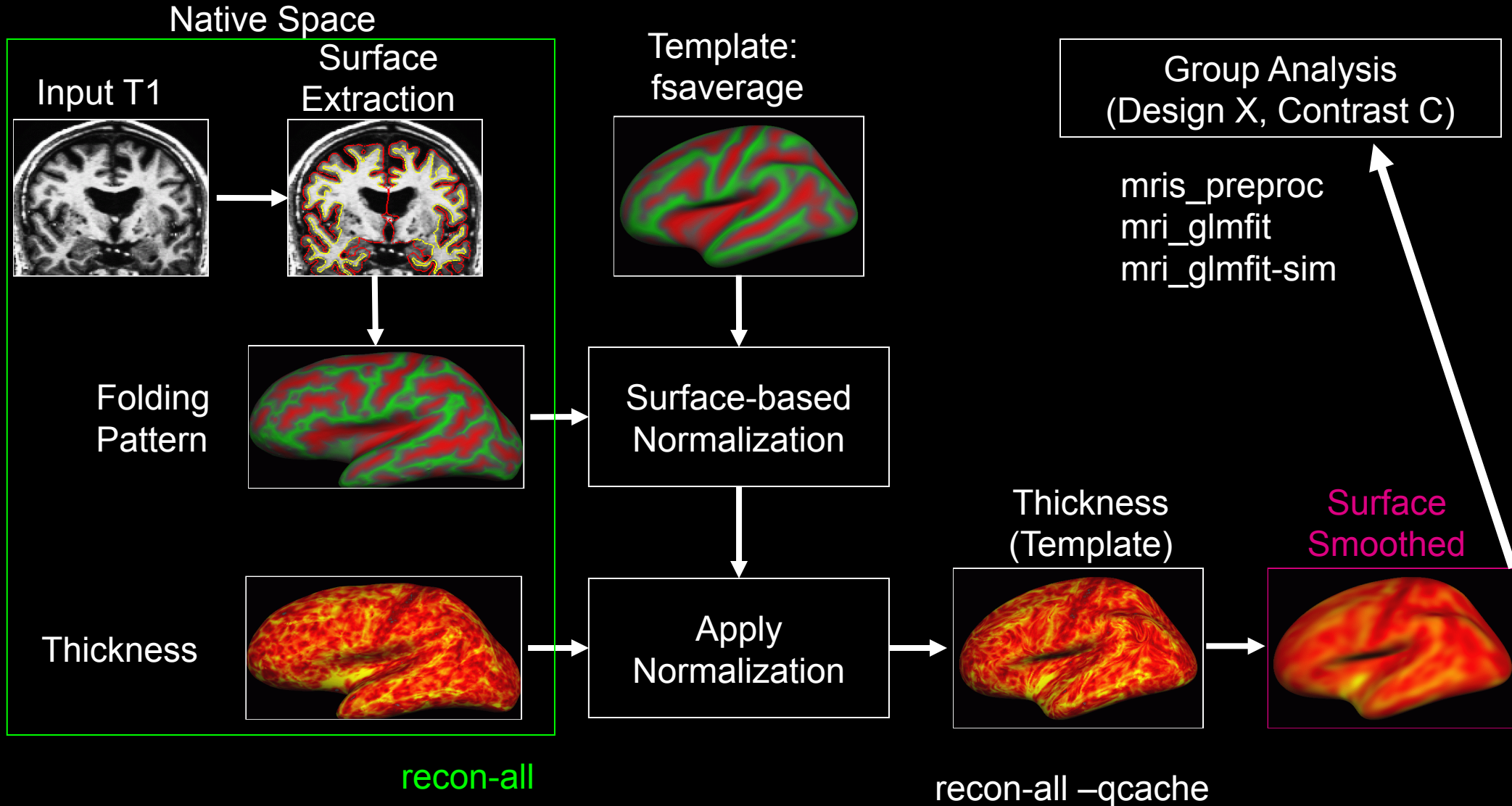


Common space for group analysis (like Talairach) – “fsaverage”

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

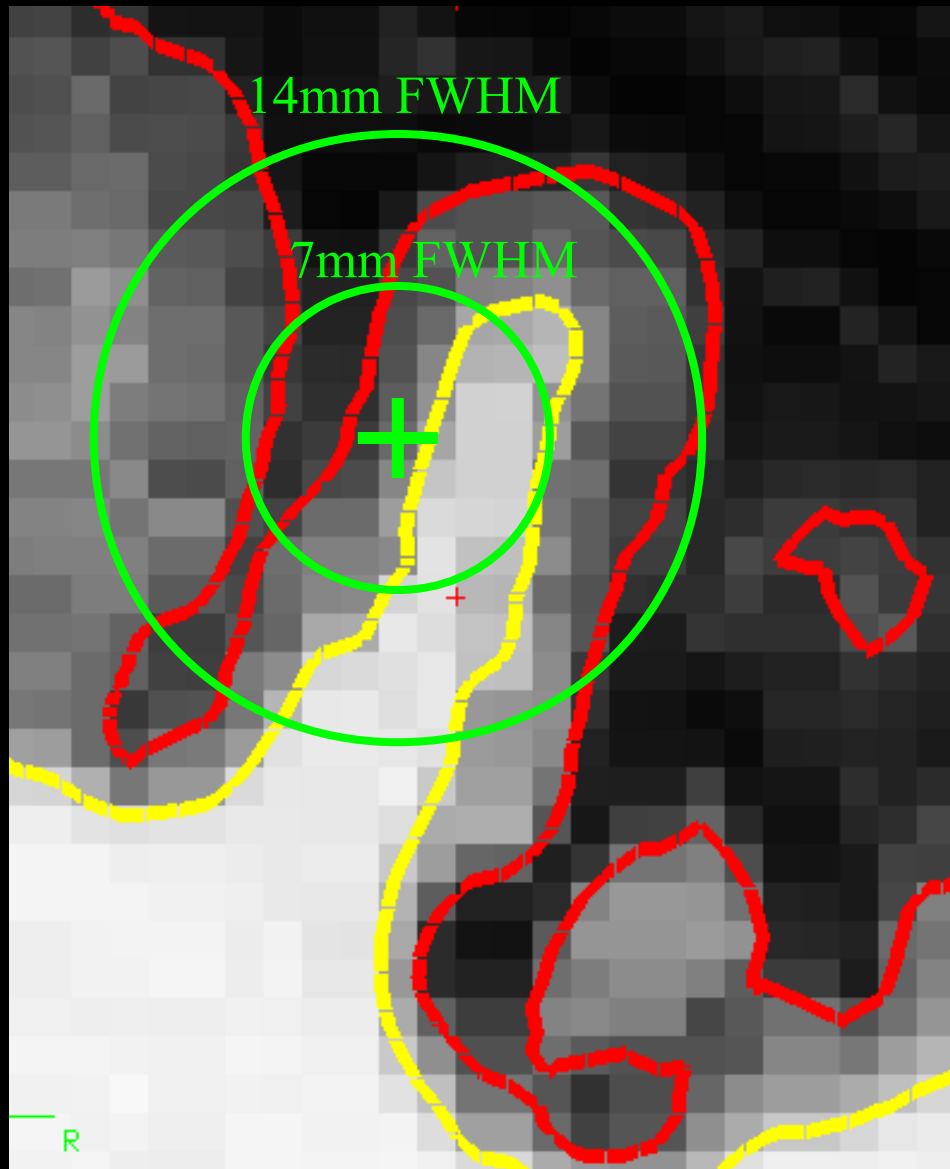
Why should you smooth?

- Might Improve CNR
- Improve intersubject registration (functional)

How much smoothing?

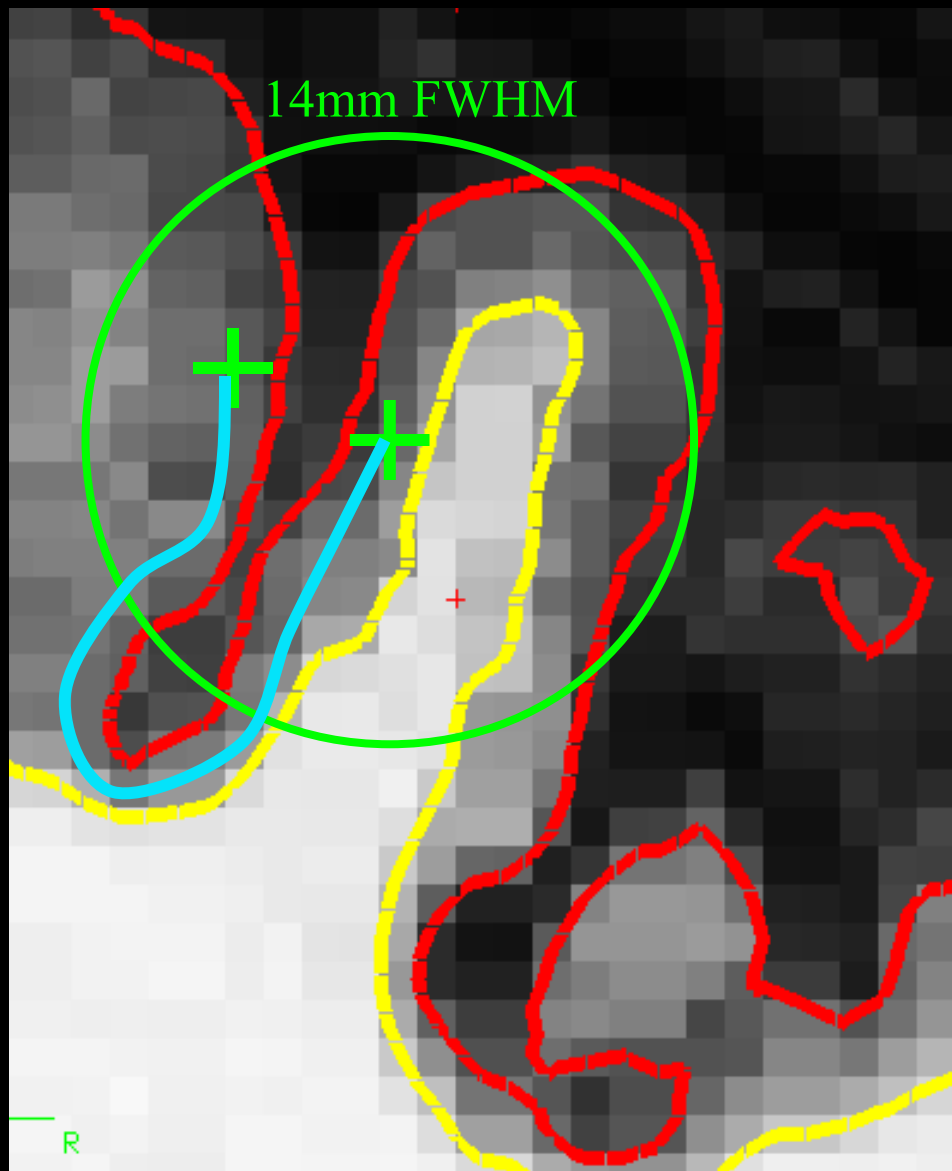
- Blob-size
- Typically 10-20 mm FWHM
- Surface smoothing more forgiving than volume-based

Volume-based Smoothing



- Smoothing is averaging of nearby voxels

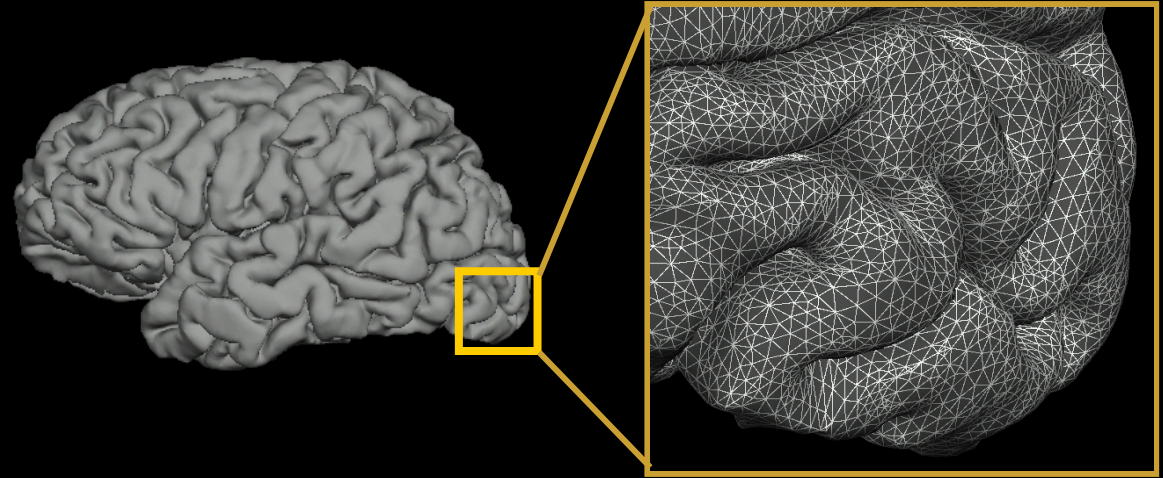
Volume-based Smoothing



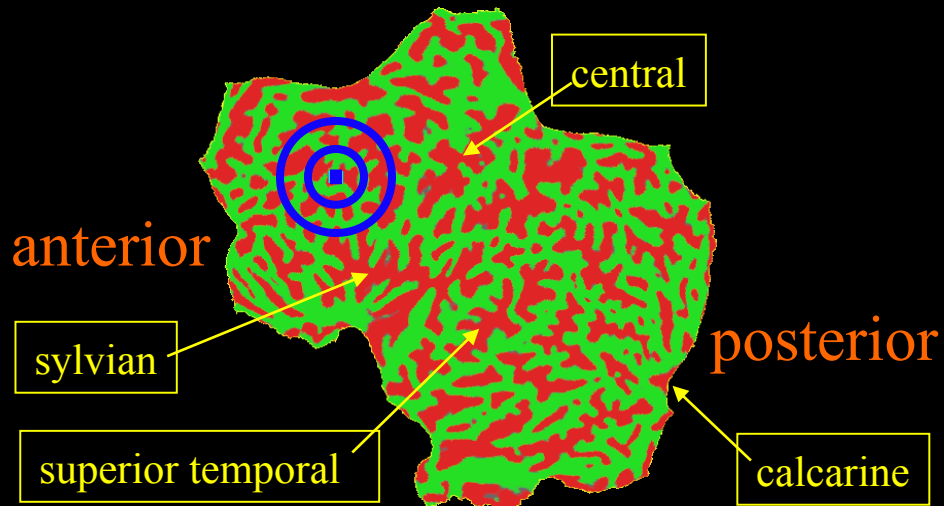
- 5 mm apart in 3D
- 25 mm apart on surface!
- Kernel much larger
- Averaging with other tissue types (WM, CSF)
- Averaging with other functional areas

Surface-based Smoothing

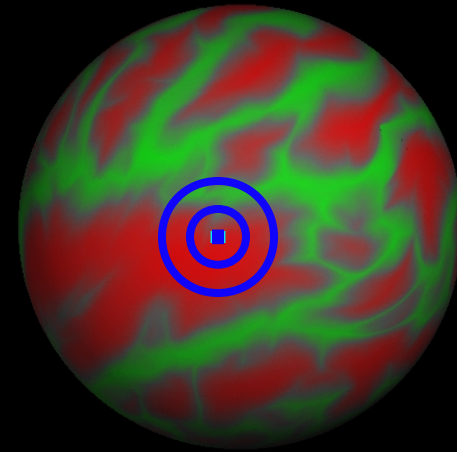
- Smoothing is averaging of nearby vertices



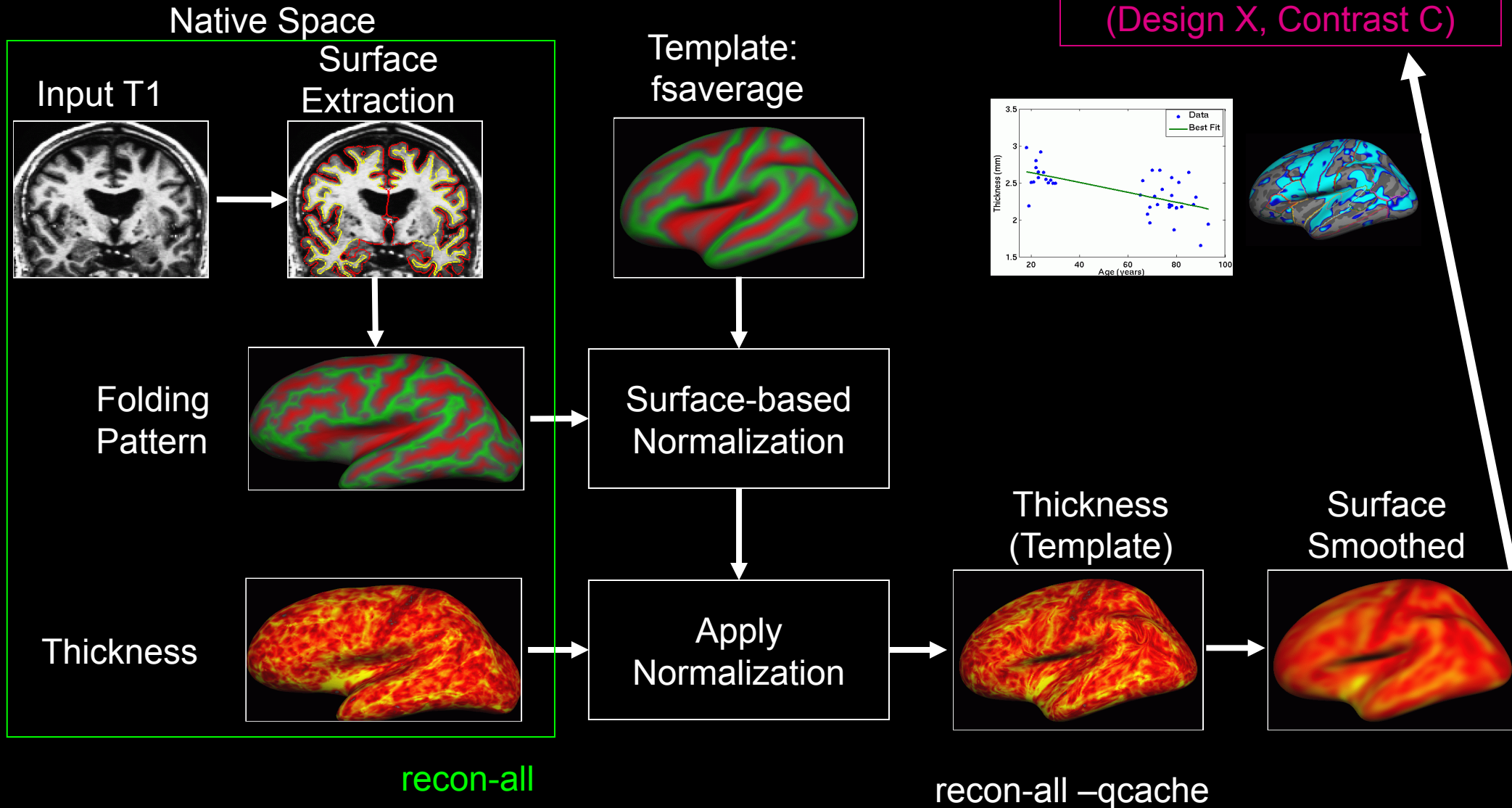
Sheet: 2D Coordinate System (X,Y)



Sphere: 2D Coordinate System (θ , ϕ)



Group Analysis



GLM Theory: Design Matrix

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

Data

Design Matrix

Regression Coefficients

$$y = X * \beta$$

GLM Theory: Contrasts

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

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

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

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

Vertex-wise Analysis and Inference

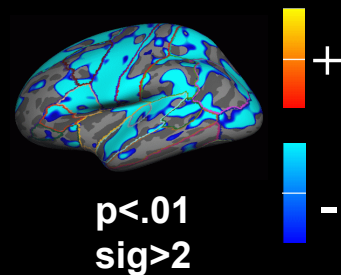
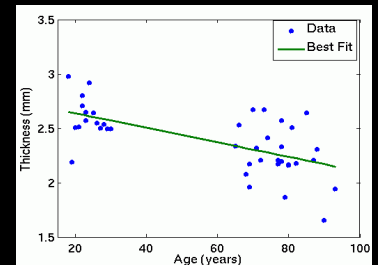
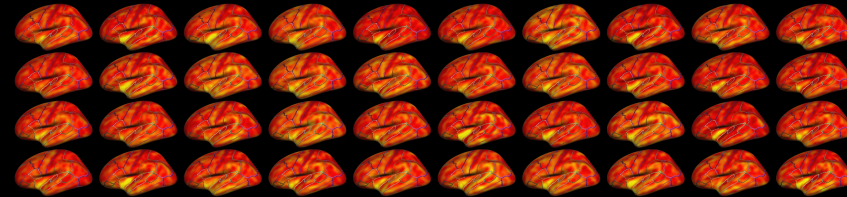
$$\begin{aligned} y &= X * \beta + \varepsilon \\ \beta &= (X^t X)^{-1} X^t y \\ \gamma &= C * \beta \end{aligned}$$

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

Male Group Female*Age

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

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)

$$\text{NRegressors} = \text{NClasses} * (\text{NVariables} + 1)$$

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

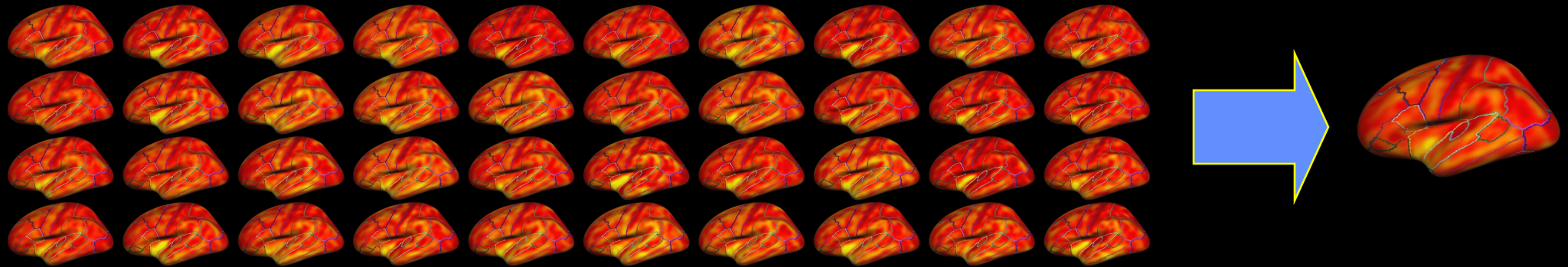
--fsgd FSGDFile : Specify subjects thru FSGD File
--hemi lh : Process left hemisphere
--meas thickness : \$SUBJECTS_DIR/subjectid/surf/hemi.thickness
--target fsaverage : common space is subject fsaverage
--o lh.thickness.mgh : output “volume-encoded surface file”

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 ($C1, C2$, etc)
- Loads data ($y=lh.thickness.sm10.mgh$)
- 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

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

--fsgd gender_age.txt

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

--surf fsaverage lh

--cortex

--glmdir lh.gender_age.glmdir

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

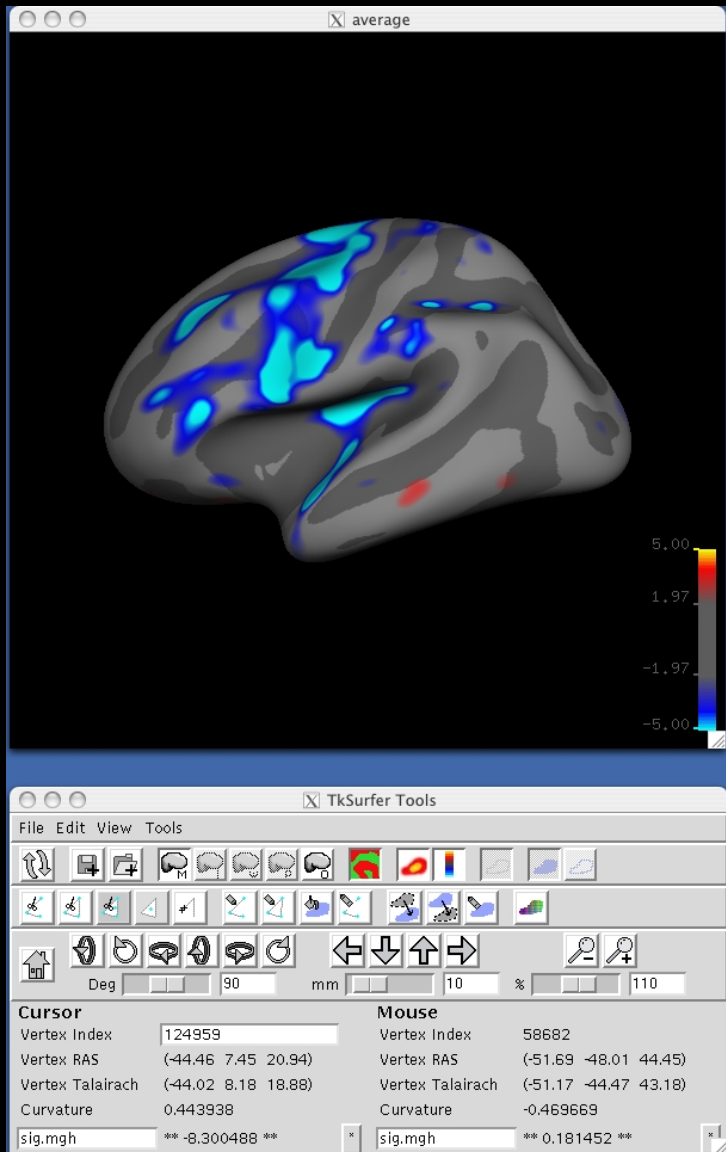
gamma.mgh, F.mgh

gender/

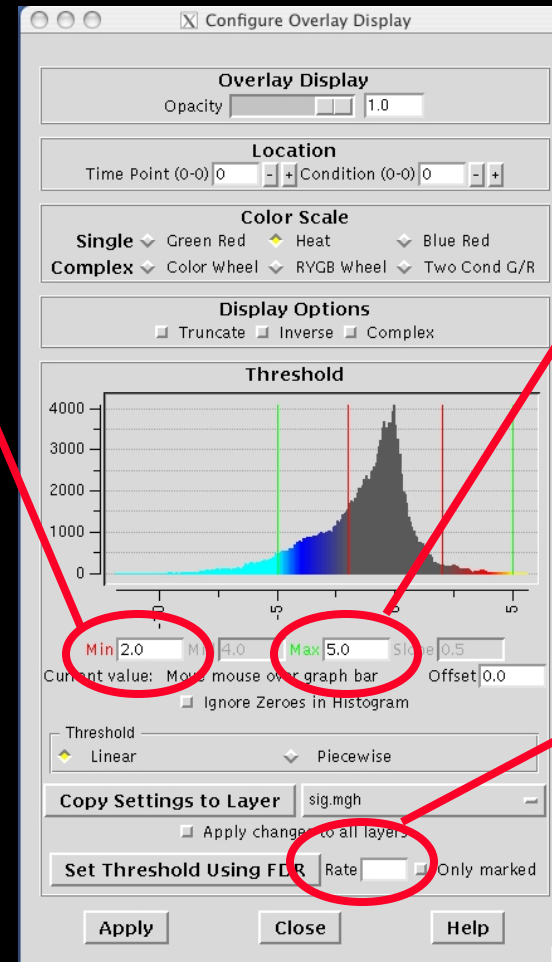
sig.mgh = $-\log_{10}(p)$

gamma.mgh, F.mgh

Visualization with tksurfer



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



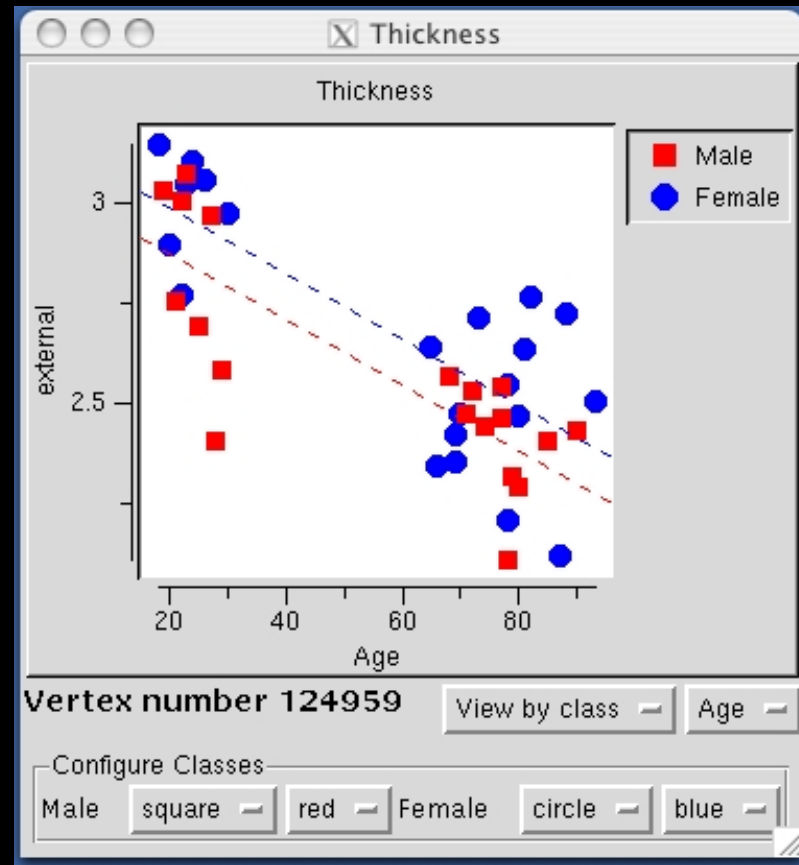
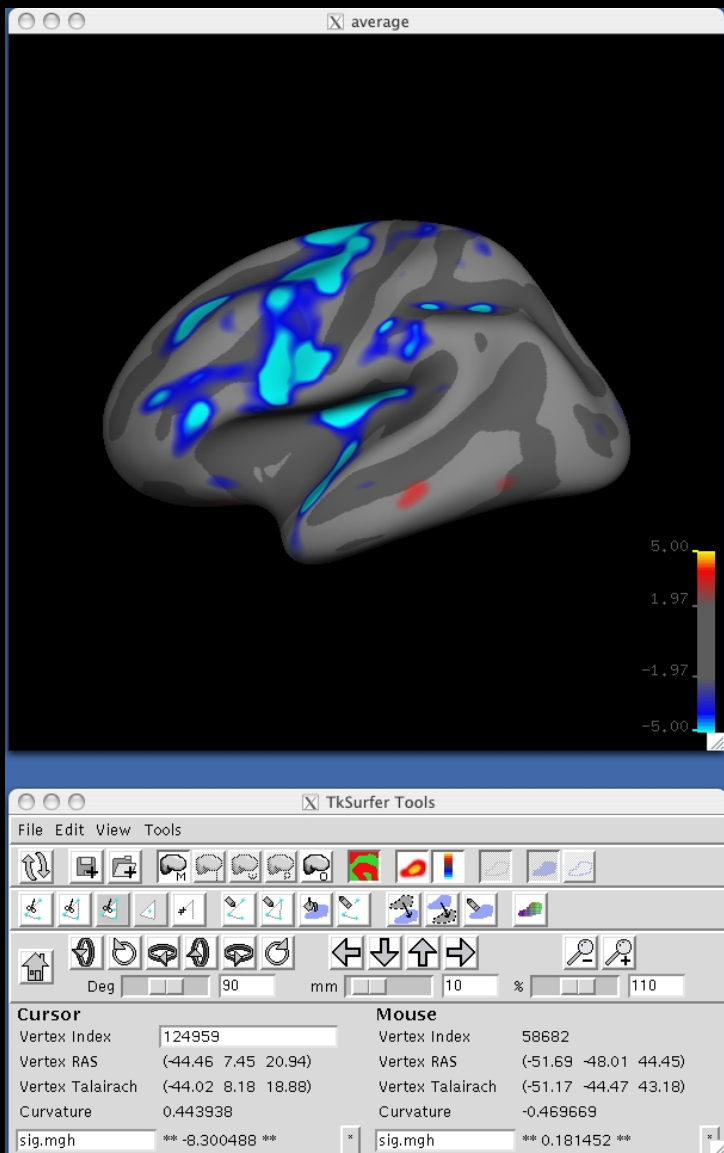
Saturation:
 $-\log_{10}(p)$,
Eg, 5=.00001

False
Discovery
Rate
Eg, .01

View->Configure->Overlay

File->LoadOverlay

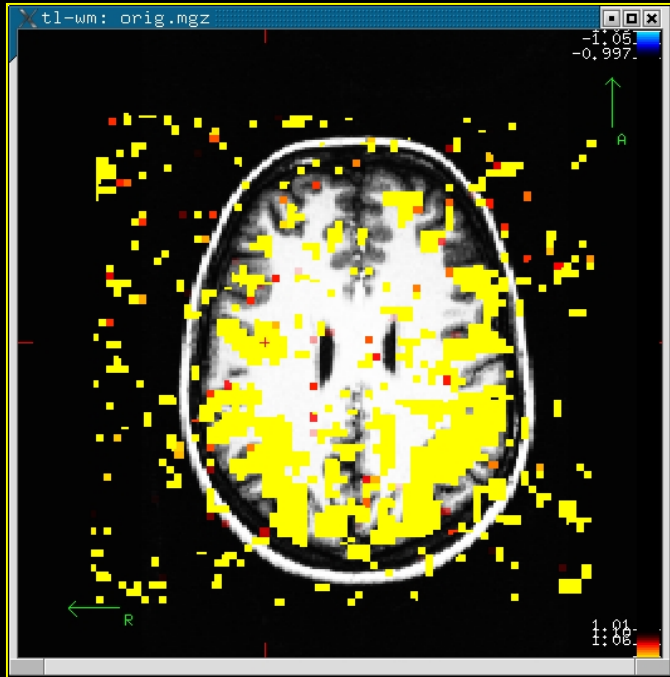
Visualization with tk-surfer



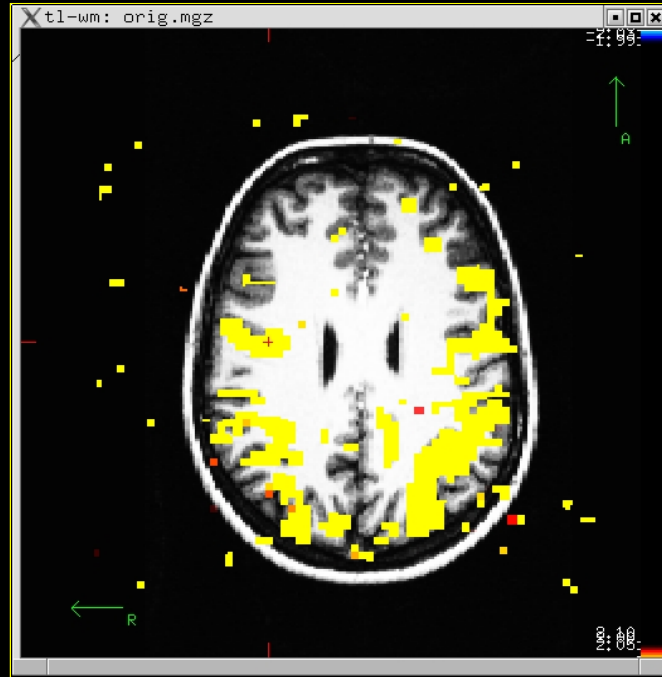
File->

Load Group Descriptor File ...

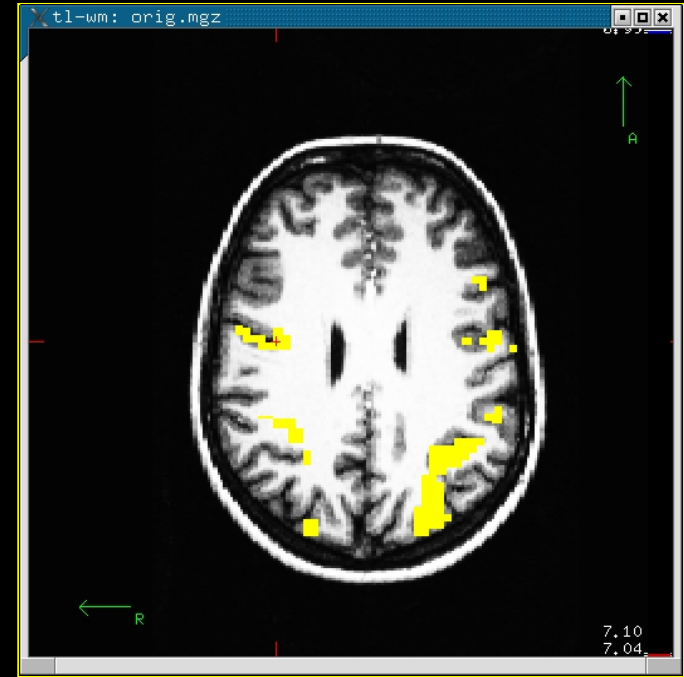
Problem of Multiple Comparisons



$$p < 0.10$$



$$p < 0.01$$



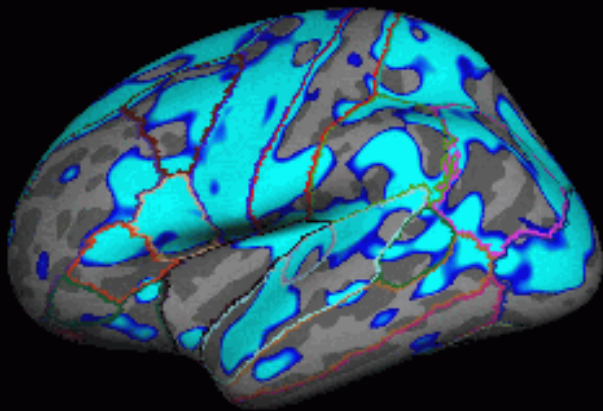
$$p < 10^{-7}$$

Correction for Multiple Comparisons

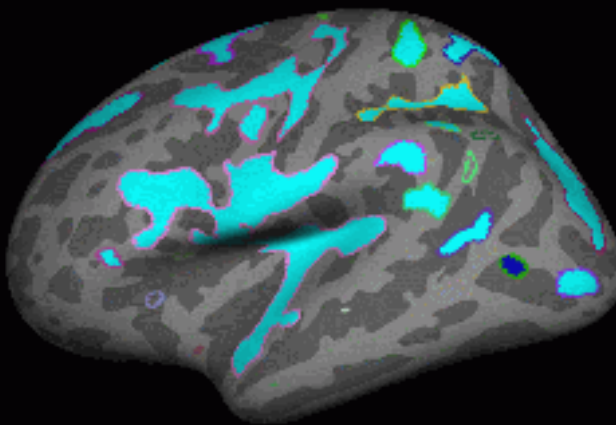
- Cluster-based
 - Monte Carlo simulation
 - Permutation Tests
 - Surface Gaussian Random Fields (GRF)
 - There but not fully tested
- False Discovery Rate (FDR) – built into tksurfer and QDEC. (Genovese, et al, NI 2002)

Clustering

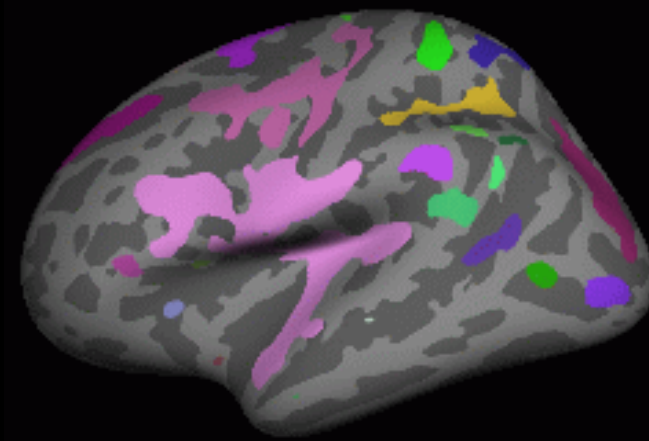
1. Choose a vertex-wise (cluster-forming) threshold
 - Eg, 2 ($p < .01$), or 3 ($p < .001$)
 - Sign (pos, neg, abs)
1. A cluster is a group of connected (neighboring) vertices above threshold
2. Cluster has a size (area in mm^2)



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

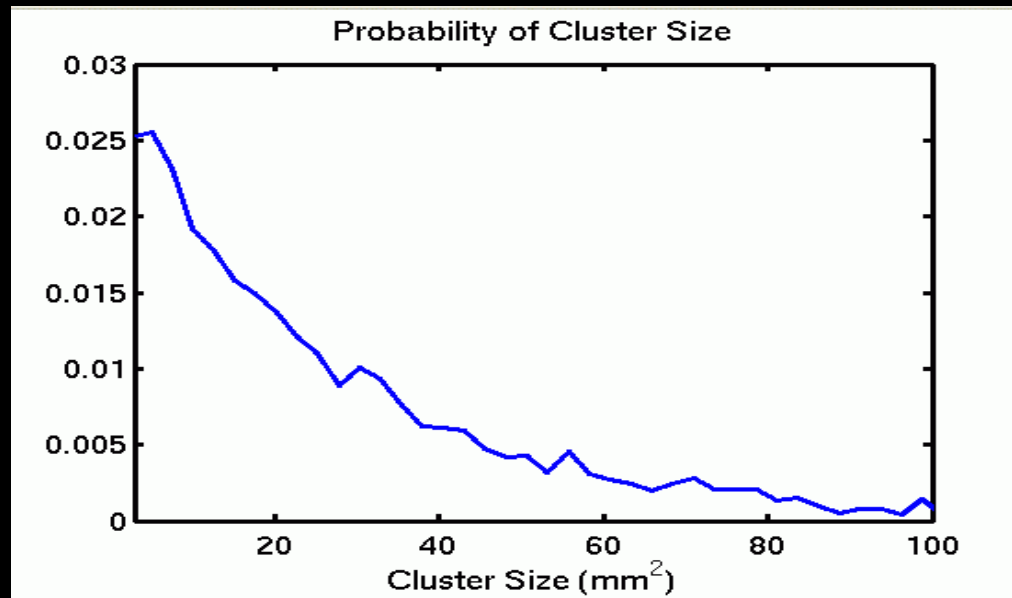
1. Analyze, threshold, cluster, max cluster size

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

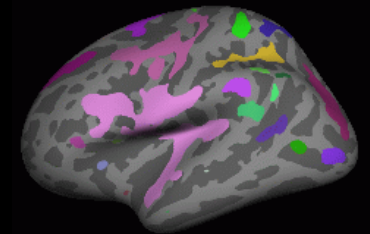
age

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
factormname.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