

FreeSurfer processing pipeline for laminar fMRI

Anna I Błażejewska

Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital & Department of Radiology, Harvard Medical School, MA, USA

FreeSurfer for laminar fMRI

single subject analysis, submillimeter resolution data @7T

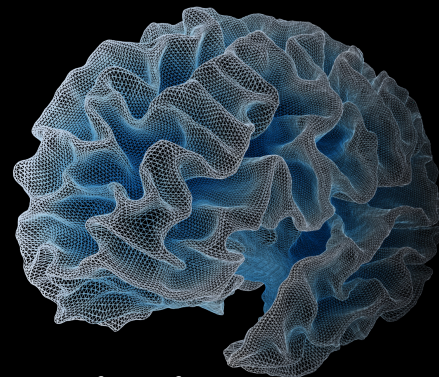
anatomical data – surface reconstruction:

- acquisition
- bias field correction & tissue segmentation
- surface reconstruction
- segmentation edits & highres stream
- family of surfaces across cortical depth

functional data – *laminar* activations:

- pre-processing
- registration to anatomical data
- projection of volume data onto surfaces
- anatomically-informed smoothing

scripts – overview



freesurfer.net

FreeSurfer for laminar fMRI

single subject analysis, submillimeter resolution data @7T

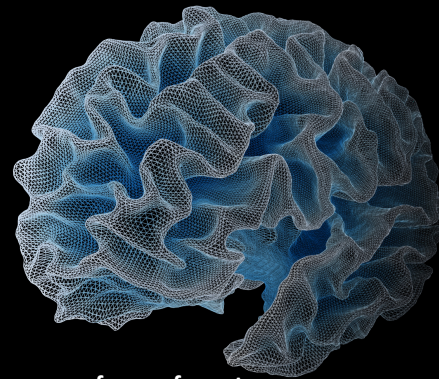
anatomical data – surface reconstruction:

- acquisition
- bias field correction & tissue segmentation
- surface reconstruction
- segmentation edits & highres stream
- family of surfaces across cortical depth

functional data – *laminar* activations:

- pre-processing
- registration to anatomical data
- projection of volume data onto surfaces
- anatomically-informed smoothing

scripts – overview



freesurfer.net

anatomical data: acquisition

MPRAGE = magnetization-prepared T1-weighted:
strong tissue contrast, high resolution in reasonable imaging time

MEMPRAGE multi-echo MPRAGE¹:

- partial suppression of dura → improved precision of pial surface placement
- root mean squared (RMS) echoes combination to increase SNR

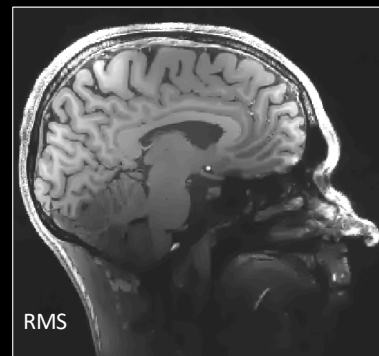
TR = 2530 s, FA = 6.3, 0.75 mm iso, PAT = 2

RF field inhomogeneity @ 7T

problematic for magnetization preparation

frequency offset corrected inversion (FOCI) pulse²

optimized to minimize effects
of B_0 & transmit field inhomogeneity



1. van der Kouwe et al. NI, 2008; 2. Hurley et al. MRM, 2010; 3. Fujimoto et al. NI, 2014;

anatomical data: bias field correction with SPM

Segment

RF field inhomogeneity @ 7T

- due to dielectric properties of tissue, transmit coil design & geometry
- spatially varying transmit efficiency & flip angles affect excitation pulses
- **bias field**: smoothly varying intensity bias & degraded tissue contrast in inhomogeneity regions
- ! challenge for segmentation & surface reconstruction
- conventional correction: fitting low order spatial trends, not complex enough (ANTs N4 in FreeSurfer)
- SPM: joint estimation of bias & tissue segmentation using gaussian mixture model¹

after

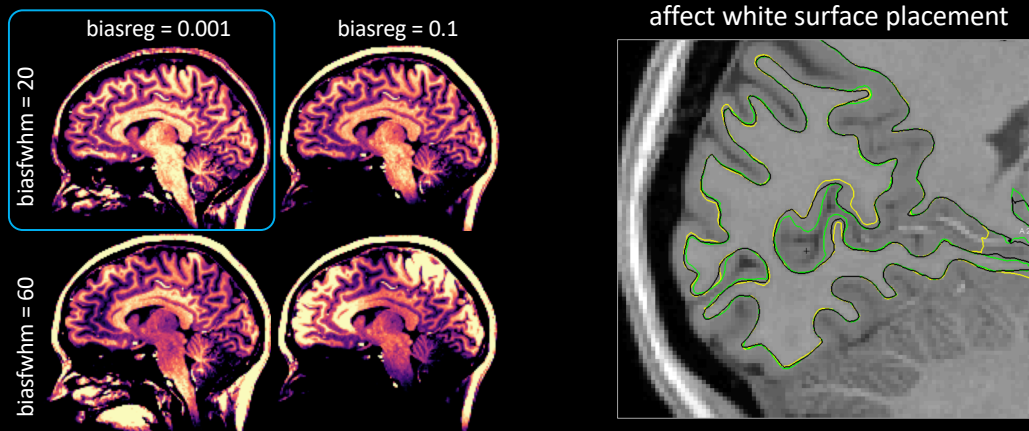


1. Ashburner & Friston, NI, 2005; 26:839–851

anatomical data: bias field correction with SPM

Segment

- trade-off between mitigating bias & preserving tissue contrast
- parameters to consider for 7T data:
 - bias regularization**: Should we penalize large variations in the bias field?
 - bias fwhm**: How smooth is the bias field?



"Knowing what works best should be a matter of empirical exploration." SPM Manual

courtesy of Daniel Gomez

anatomical data: tissue segmentation with SPM

Segment

bone



- 5 tissue classes: probability maps for each tissue
- gaussian mixture model: ≥ 1 gaussians represent intensity distribution of tissue class
- can be helpful for correction of FreeSurfer segmentations

1. Ashburner & Friston, NI, 2005; 26:839–851

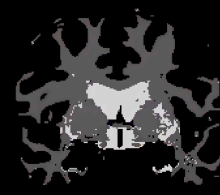
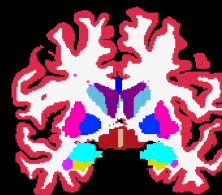
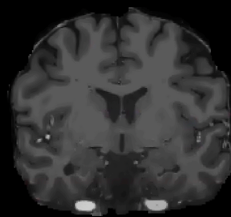
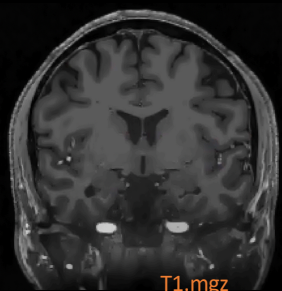
anatomical data: surface reconstruction with FreeSurfer

recon-all

volume-based stream:

- intensity correction & normalization
- skull-stripping (brain segmentation)
- automatic subcortical labeling based on atlas
- white matter segmentation ('inside cortex' mask)

\$SDIR/mri:

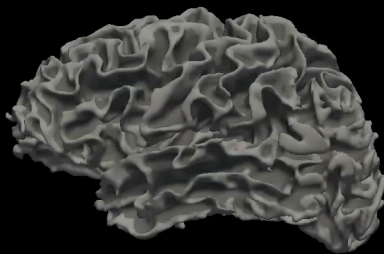


anatomical data: surface reconstruction with FreeSurfer

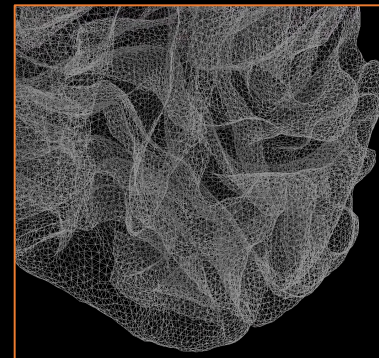
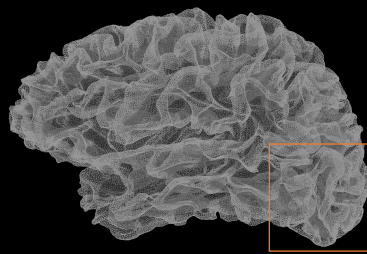
recon-all

volume-based → surface-based stream

- left & right hemisphere processed separately
- tessellation = covering with triangles of **wm.mgz**
- **lh.orig** & **rh.orig** surfaces: rough, jagged



surface: **lh.orig**

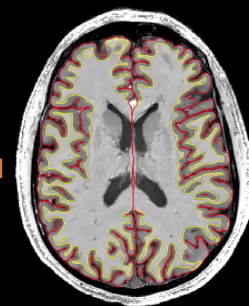


anatomical data: surface reconstruction with FreeSurfer

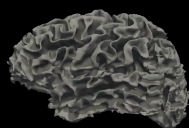
recon-all

surface-based stream:

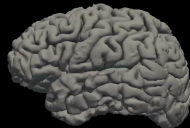
- adjusting surface placement with smoothness constraint, following T_1 intensity gradient → **{lh,rh}.white**
- expanding following T_1 intensity gradients → **{lh,rh}.pial**
- inflating with distortion constraints, no intensity constraints → **{lh,rh}.inflated**
- more inflation to sphere → **{lh,rh}.sphere** for fixing topological errors



\$SDIR/surf:



lh.white



lh.pial



lh.inflated

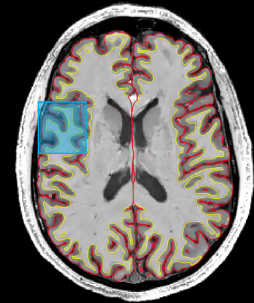


lh.sphere

anatomical data: surface reconstruction with FreeSurfer

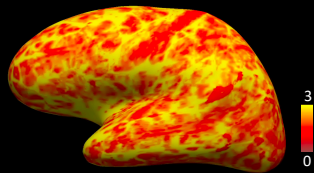
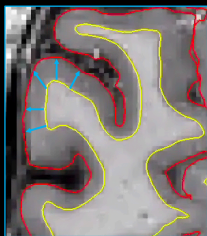
recon-all

- same number of vertices & vertex correspondence across all surfaces
- **surface overlay**: a value assigned to each vertex
 - cortical thickness**: distance between **white** & **pial** surface [mm]
 - cortical curvature**: circle tangent to surface [1/radius]

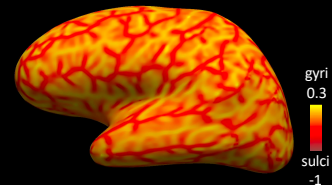
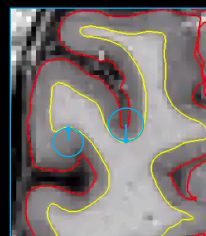


\$SDIR/surf:

lh.thickness



lh.curv



anatomical data: surface reconstruction with FreeSurfer

freeview

opening volumes:

```
freeview -v volume.mgz
```

opening surfaces:

```
freeview -f {lh,rh}.surface:edgecolor=red
```

opening surface overlays:

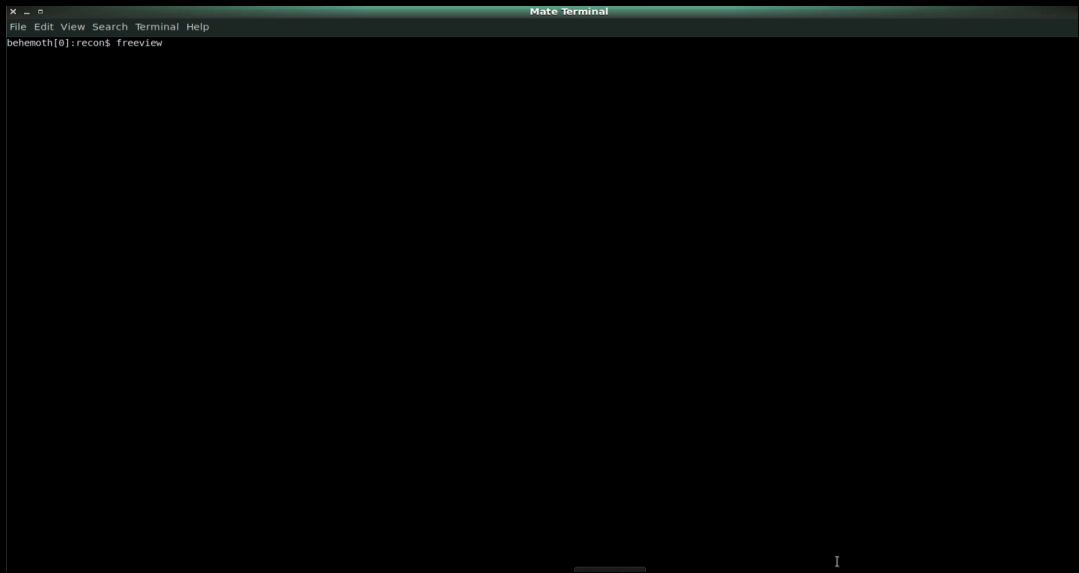
```
freeview -f {lh,rh}.surface:overlay={lh,rh}.overlayfile.mgz
```

the most helpful command:

```
freeview --help
```

anatomical data: surface reconstruction with FreeSurfer

freeview



anatomical data: surface reconstruction with FreeSurfer

recon-all

other useful outcomes:

`$$DIR/labels`: atlas-based labels, subject specific

`$$DIR/scripts`: log files, recon-all.log, recon-all.done, recon-all.cmd

to learn
about
mailing
over 1

about
about

about
about
→ mis

recon-all step	Individual Flag	Input	Command Line	Output	
recon-all - autorecon1 <subj <subjid>	-i <invol1>	invol1.dcm or .nii or .mgz	mri_convert invol1.dcm orig/001.mgz	orig/001.mgz	
	-i <invol2> optional	invol2.dcm or .nii or .mgz	mri_convert invol2.dcm orig/002.mgz	orig/002.mgz	
	-T2 <invol> or -FLAIR <invol> optional	invol.dcm or .nii or .mgz	mri_convert --no_scale 1 invol.dcm orig/T2raw.mgz	orig/T2raw.mgz	
	-motioncor	orig/001.mgz	mri_robust_template --mov 001.mgz 002.mgz --average 1 --template rawavg.mgz --sdlit -- inittp 1 --fixtp --noit --scale --scaleout --subsample 200 --lta	rawavg.mgz	
		orig/002.mgz			
		rawavg.mgz	mri_convert rawavg.mgz orig.mgz --conform	orig.mgz	
		orig.mgz	mri_add_xfm_to_header -c transforms/talairach.xfm orig.mgz orig.mgz	orig.mgz	
	-talairach	orig.mgz	mri_nu_correct.mni --n 1 --proto-its 1000 --distance 50 --no-rescale --i orig.mgz --o orig_nu.mgz	orig_nu.mgz	
		orig_nu.mgz	talairach_avi --i orig_nu.mgz --xfm transforms/talairach.auto.xfm	transforms/talairach.auto.xfm	
		transforms/talairach.auto.xfm	cp transforms/talairach.auto.xfm transforms/talairach.xfm	transforms/talairach.xfm	
		transforms/talairach.xfm	talairach_ald -T 0.005 -xfm transforms/talairach.xfm		
	-nuintensitycor	orig.mgz	awk -f \$FREESURFER_HOME/bin/extract_talairach_avi_QA.awk transforms/talairach_avi.log	transforms/talairach_avi.log	
		talairach.xfm			
	-normalization	nu.mgz	mri_nu_correct.mni --i orig.mgz --o nu.mgz --uchar transforms/talairach.xfm --n 2	nu.mgz	
	-skullstrip	nu.mgz	mri_normalize -g 1 -mprage nu.mgz T1.mgz	T1.mgz	
		nu.mgz	mri_em_register -skull nu.mgz \$FREESURFER_HOME/average/RB_all_withskull_2016-05-10.vc700.gca transforms/talairach_with_skull.lta	transforms/talairach_with_skull.lta	
		T1.mgz	mri_watershed -T1 -brain_atlas \$FREESURFER_HOME/average/RB_all_withskull_2016-05-10.vc700.gca transforms/talairach_with_skull.lta T1.mgz brainmask.auto.mgz	brainmask.auto.mgz	
brainmask.auto.mgz		cp brainmask.auto.mgz brainmask.mgz	brainmask.mgz		
recon-all step	Individual Flag	Input	Command Line	Output	

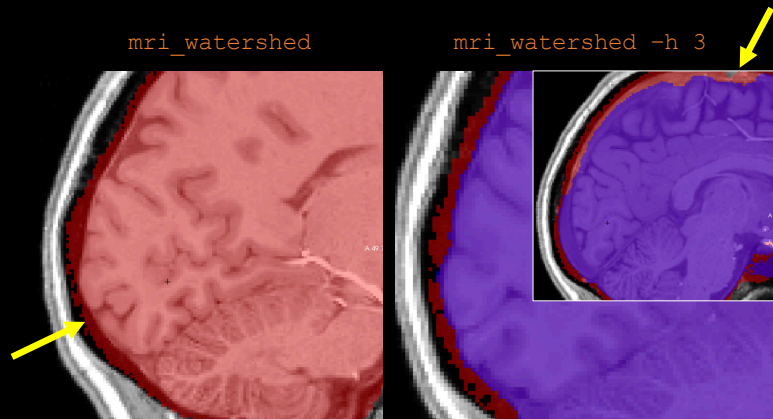
anatomical data: brain segmentation

`mri_watershed`

problem: brain mask includes sagittal sinus/dura → misplaced pial surface

changing exposed parameters of `mri_watershed`

may improve brain mask locally but damage in other locations



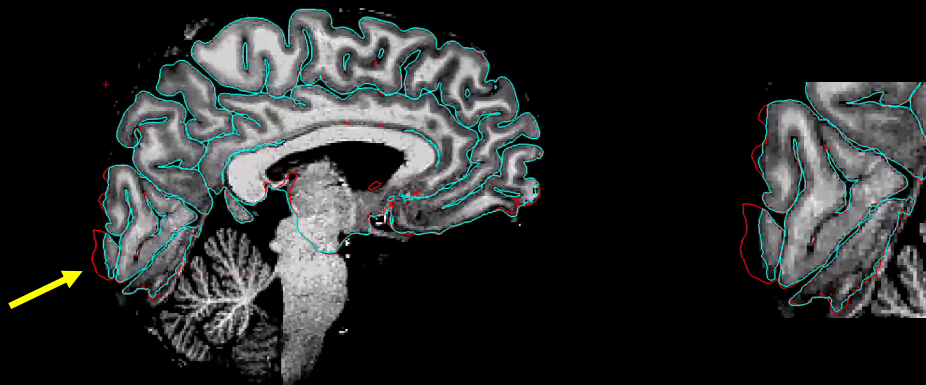
courtesy of Daniel Gomez

anatomical data: brain segmentation

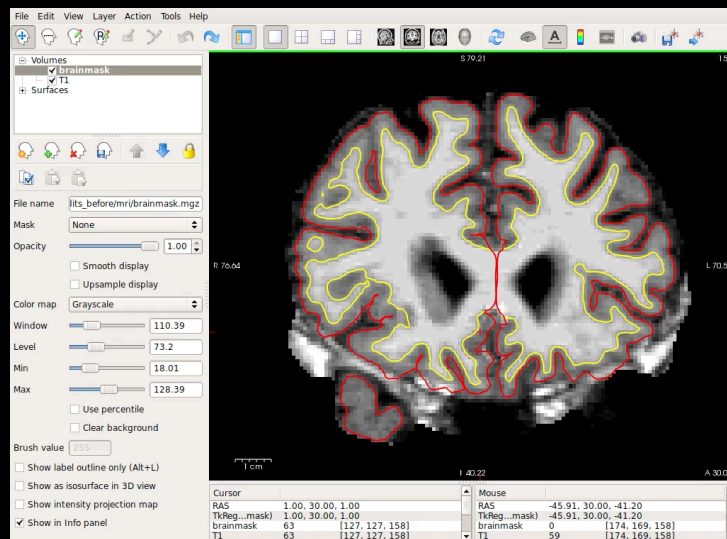
problem: brain mask includes sagittal sinus/dura → misplaced pial surface

1. SPM bone & soft tissue segmentation → *nobrain* mask subtracted from `brainmask.mgz`
2. additional manual brain mask edits if needed – in 3 planes

`recon-all ... -autorecon2-pial`



anatomical data: brain segmentation



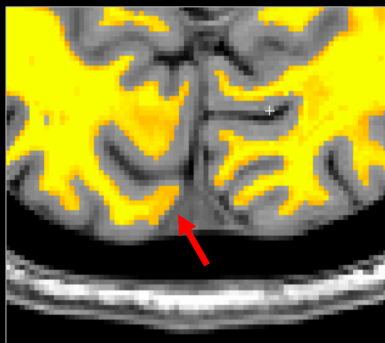
surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/PialEdits_freeview

anatomical data: white matter segmentation

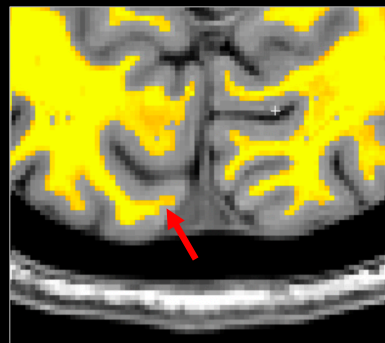
mri_segment

problem: white matter mask includes some gray matter → misplaced white surface
fine tuning `mri_segment` may improve segmentation

`mri_segment`



`mri_segment -gray_lo 35
-gray_hi 100 -wm_low 105`



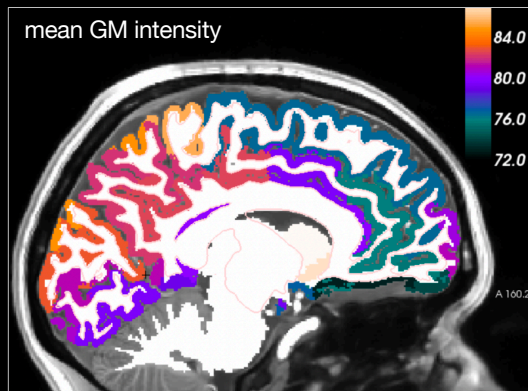
courtesy of Daniel Gomez

anatomical data: white matter segmentation

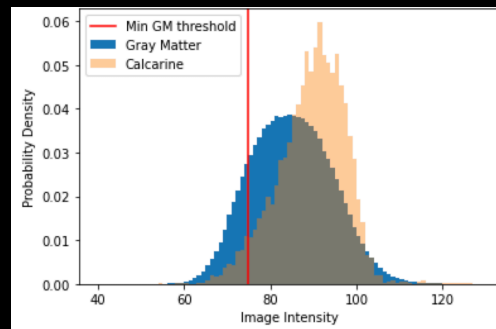
mri_segment

problem: white matter mask includes some gray matter → misplaced white surface

- mean intensity of GM across different brain regions
- Freesurfer's global thresholds work OK for whole brain, but not necessarily for a specific region



intensity histograms
for GM in whole brain vs. in calcarine



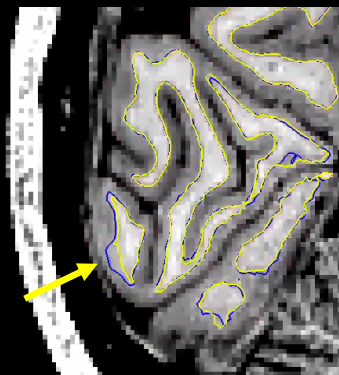
courtesy of Daniel Gomez

anatomical data: white matter segmentation

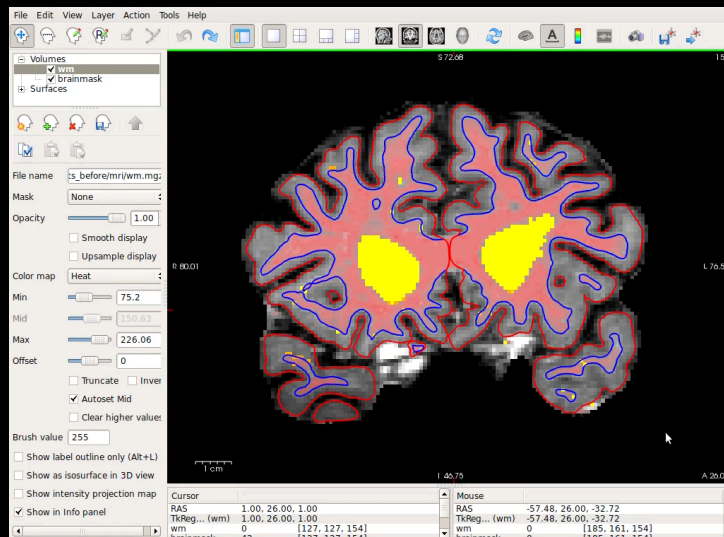
problem: white matter mask includes some gray matter → misplaced white surface

additional manual white matter mask edits – in 3 planes

```
recon-all ... -autorecon2-wm -autorecon3
```



anatomical data: white matter segmentation



anatomical data: FreeSurfer highres stream

recon-all

surface reconstruction flags specific for 7T submillimeter resolution data:

-highres to avoid down-sampling of input anatomical images to 1 mm

-expert \$EXPPFILE → mris_inflate -n 100

high resolution data produce high vertex density meshes

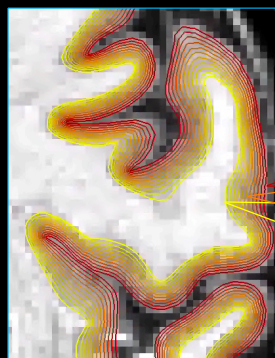
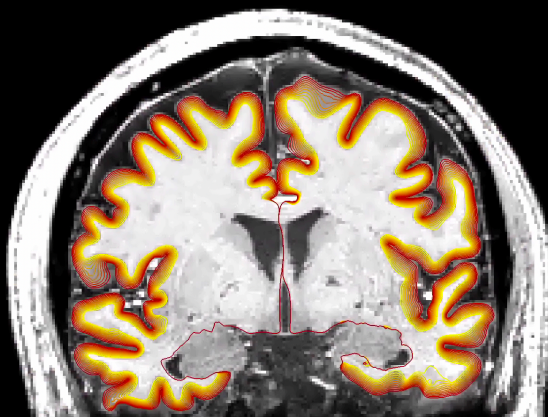
→ increased number of iterations needed

to inflate the surface sufficiently for projection to sphere & fixing topological defects

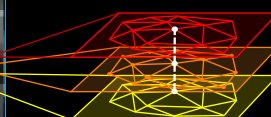
anatomical data: family of surface meshes

mrisc_expand

evenly-spaced surface meshes → local coordinate frame
e.g. 11 surfaces total: white - 9 intermediate surfaces - pial



topological correspondence
of meshes across surfaces



FreeSurfer for laminar fMRI

single subject analysis, submillimeter resolution data @7T

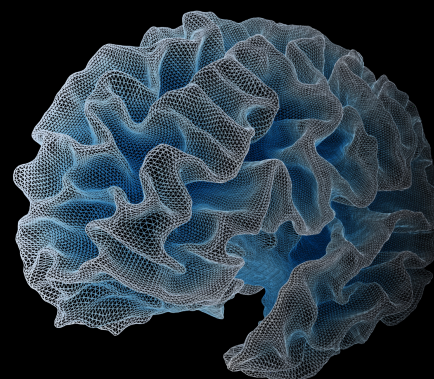
anatomical data – surface reconstruction:

- acquisition
- bias field correction & tissue segmentation
- surface reconstruction
- segmentation edits & highres stream
- family of surfaces across cortical depth

functional data – laminar activations:

- pre-processing
- registration to anatomical data
- projection of volume data onto surfaces
- anatomically-informed smoothing

[scripts – overview](#)



freesurfer.net

functional data: pre-processing

input/output: 4D BOLD fMRI dataset

purpose: ensure that data meets the assumptions made by the analysis

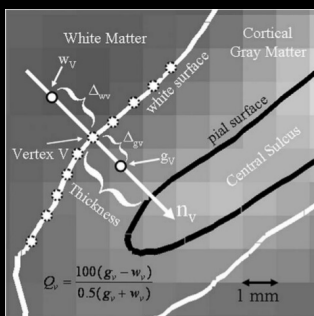
- **motion correction** – time course comes from the same anatomical location across the run (AFNI)
- **slice timing correction** – voxels in a given time frame were sampled in the same instance
- **B₀ distortion correction** – correct warping due to the air/tissue magnetic susceptibility interfaces
- **spatial smoothing?** (see later slides)
- correct order?
- interpolation affects spatial & temporal structure of noise

functional data: registration to anatomical data

bbregister

boundary-based registration

- uses boundary-based cost function applied to WM/GM border
- based on local tissue contrast – robust to slowly varying bias fields

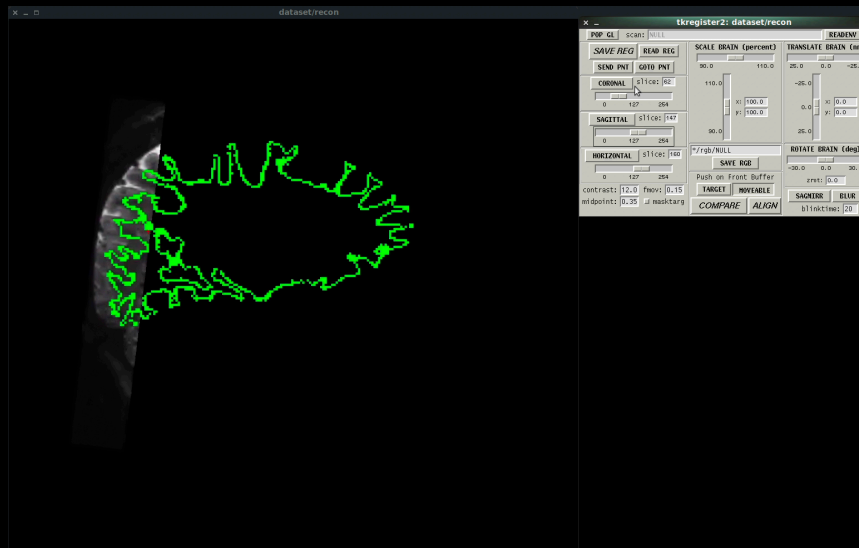


- inter-subject, cross-modal, 6 DOF (rigid body)
- especially robust for aligning partial volumes to whole brain
- great for laminar fMRI studies where accuracy is important

functional data: registration to anatomical data

bbregister

--init-reg init.dat manual initialization: 1 min/brain, in 3 planes, save registration *.dat file

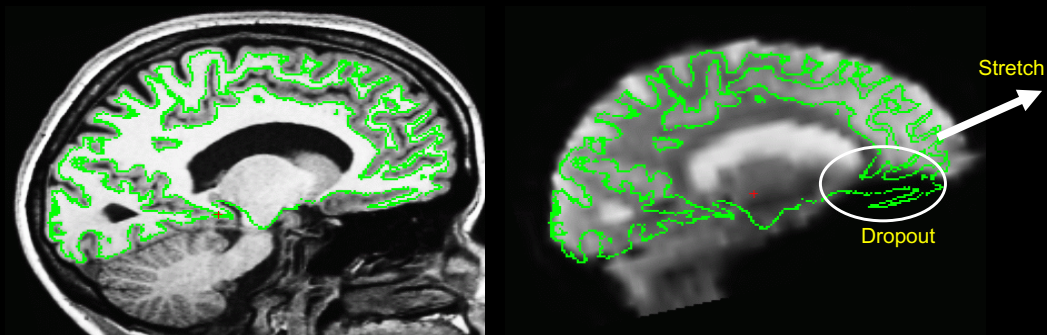


functional data: registration to anatomical data

bbregister

B_0 distortion is incompatible with the rigid body model – will ruin registration

--epi-mask ignores brain edge & B_0 distorted regions based on FreeSurfer labels

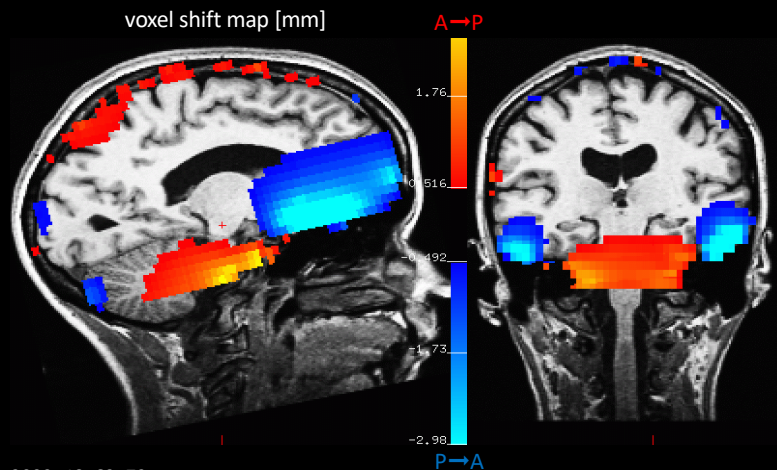


functional data: registration to anatomical data

bbregister

B_0 distortion is incompatible with the rigid body model – will ruin registration

--vsm `VoxelShiftMap` to include B_0 distortion correction while performing registration
will shift (i.e., distort) the surface to match fMRI volume (reduce resampling/interpolation)



Greve & Fischl NI, 2009; 48, 63–72.

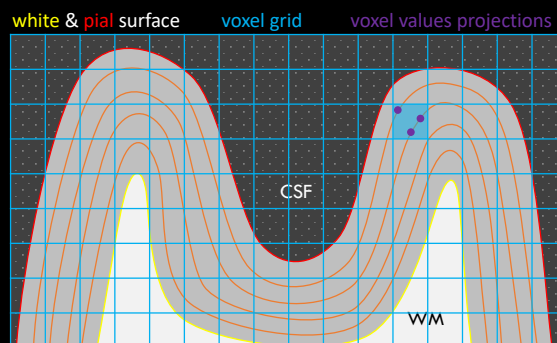
courtesy of Doug Greve

functional data: projection onto surfaces

mri_vol2surf

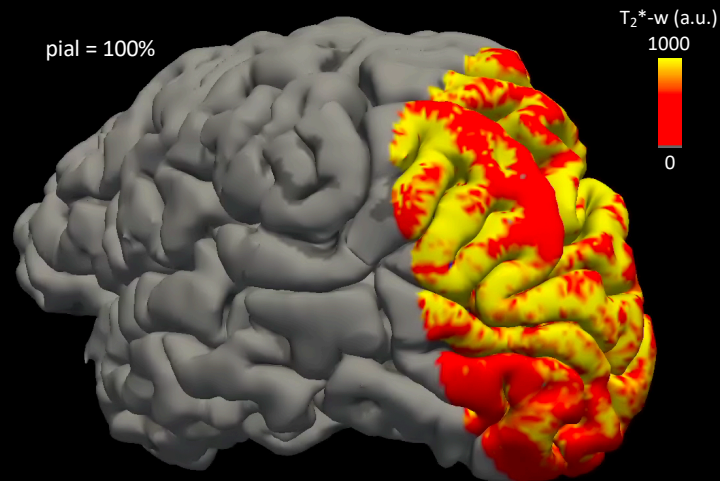
surface-based approach projects voxels onto each surface mesh in the family:
assigns voxel values to surface vertices → surface overlays

- ✓ allows for visualization of tangential activation pattern as a function of depth
- ✓ topological correspondence of meshes → easy to generate depth profiles at each vertex
- ✗ voxel counted multiple times (omitted for sparse meshes & small voxels)



functional data: projection onto surfaces

mri_vol2surf



output transformation can be used to transform:
labels, statistical maps etc. calculated in volume space

functional data: conventional volume-based smoothing

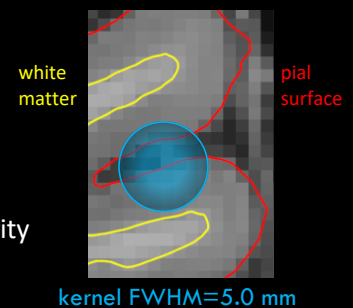
why do we smooth?

- increase SNR & detection sensitivity
- remove across-subject variability & help spatial normalization
- remove false positives – activation is assumed to be smooth

convolutional 3D (Gaussian) kernel which averages over:

- distant voxels from the opposite banks of a sulcus
- different tissue types
- different functional areas

→ activation mislocalization: biased away from the site of neuronal activity



! applied to reduce errors but may introduce errors

surface-based approaches restricting smoothing to cortical ribbon^{1,2}

functional data: surface-mesh navigated 3D intracortical smoothing

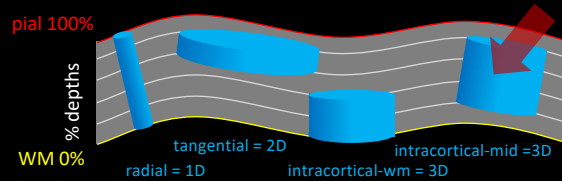
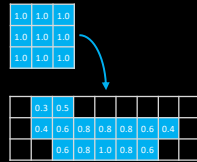
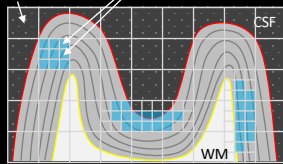
`mris_smooth_intracortical`

now: sub-millimeter voxels → cortex viewed as a **3D structure**

→ small redistributed voxels to avoid signals originating in CSF & WM

new: family of surface-meshes & steerable kernels tracking folds, tangential & radial extent

3.0 mm³ vs 1.0 mm³ voxels



anatomically-informed smoothing avoiding CSF (noise!) & WM (no fMRI activation)

→ increase **sensitivity (tSNR)** & **tissue specificity (fCNR)** of fMRI measurements

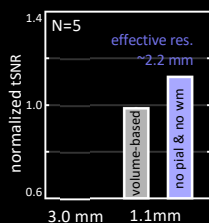
Blazejewska et al., NI, 2019

functional data: surface-mesh navigated 3D intracortical smoothing

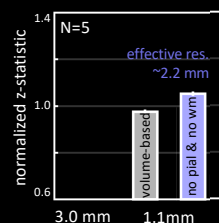
`mris_smooth_intracortical`

- resting-state & breath-hold task fMRI data, projected on *FreeSurfer* surface meshes
- volume-based & surface-based intracortical equivalent smoothing applied to 1.1 mm isotropic data

sensitivity (tSNR)
resting-state fMRI



tissue specificity (fCNR)
breath-hold task fMRI



- best balance between high tSNR & fCNR
- “no pial & no wm” intracortical kernel
- appropriately smoothed high-res data
→ higher tSNR, fCNR than 3.0 mm data !

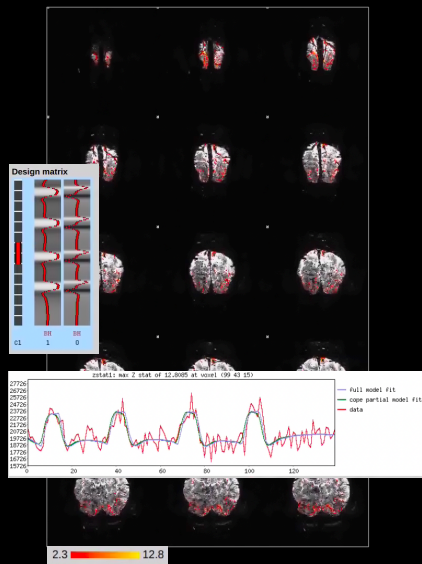
- prior information about spatial structure of activation pattern: laminar/columnar
- to create ROI from a separate functional localizer scan
- to aid visualization of results

Blazejewska et al., NI, 2019

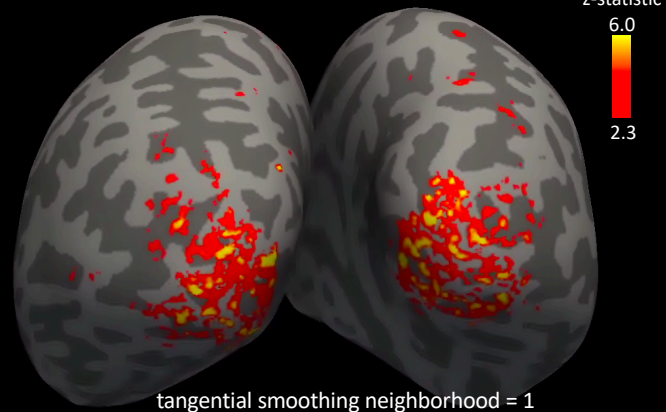
functional data: projection of statistics onto surfaces

feat

statistical maps calculated in volume space (feat, FSL) – projected on surfaces



inflated mid = 50%
average of 10 runs



FreeSurfer for laminar fMRI

single subject analysis, submillimeter resolution data @7T

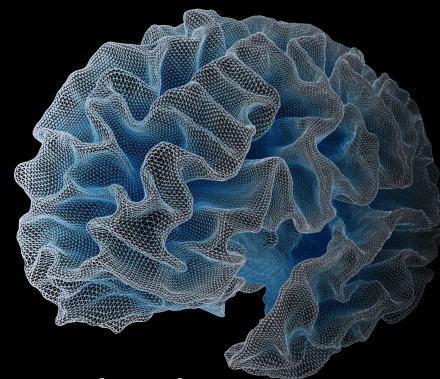
anatomical data – surface reconstruction:

- acquisition
- bias field correction & tissue segmentation
- surface reconstruction
- segmentation edits & highres stream
- family of surfaces across cortical depth

functional data – *laminar* activations:

- pre-processing
- registration to anatomical data
- projection of volume data onto surfaces
- anatomically-informed smoothing

scripts – overview



freesurfer.net

scripts – overview

`PIPELINE.sh` – main script

TODO: paths/file names that need to be modified by the user

LONG: this command may run for several hours.. proceed with caution..

CHECK: a command (usually `freeview`) to open the results of most recent commands

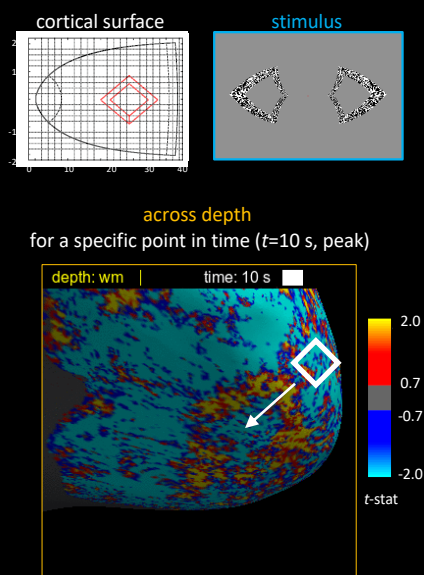
anatomical:

- bias correction (**SPM**) – already performed
- surface reconstruction (**recon-all**) – already performed (**recon** subdirectory), LONG
- manual edits of brain/WM segmentation + partial recon-all – potentially LONG
- generating a family of surfaces (**mris_expand**)

functional: to be performed for each run (you can use a loop ;)

- motion correction (**AFNI**) & linear detrending (**FSL**)
- registration (manual initialization + **bbregister**)
- GLM (**feat**, **FSL**), averaging statistics over multiple runs
- projection of data/statistics onto surfaces (**mri_vol2surf**)
- surface-based smoothing (**mris_smooth_intracortical**)

thank you!



Jonathan R Polimeni



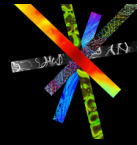
Bruce Fischl



Doug Greve



Daniel Gomez



Athinoula A.
Martinos
Center
For Biomedical Imaging



postdoctoral research fellow positions available

ultra-high field human *ex vivo* & *in vivo* MRI

with **Anna I Blazejewska**
ablazejewska@mgh.harvard.edu
www.ismrm.org/jobs/j08343.pdf

