FreeSurfer Tutorial & Workshop

September 26-29, 2016
http://freesurfer.net/fswiki/FsTutorial

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Charlestown, MA 02129
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<td>talk/demo</td>
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<td>** Intro to FreeSurfer Jargon</td>
<td>talk</td>
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<td>Introduction to Freesurfer</td>
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<td>talk</td>
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<td>Freeview demonstration</td>
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<td>3:55 - 4:25</td>
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<td>talk</td>
<td>Dylan Tisdall</td>
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<td>* MRI Acquisition Methods</td>
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<tr>
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<td>Group Analysis</td>
<td>talk</td>
<td>Emily Lindemer</td>
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<td>10:50 - 11:10</td>
<td>Multiple Comparisons</td>
<td>talk</td>
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<td>1:30 - 2:15</td>
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<td>Lilla Zöllei</td>
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<td>Longitudinal Tutorial</td>
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<td>FreeSurfer Troubleshooting</td>
<td>talk</td>
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<td>Troubleshooting Tutorial</td>
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<td>6:30 - ?</td>
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<td>Multi-Modal Integration, Part 1</td>
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<td>10:35 - 11:15</td>
<td>Introduction to Diffusion MRI</td>
<td>talk</td>
<td>Anastasia Yendiki</td>
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<td>11:15 - 12:00</td>
<td>Diffusion Processing Tutorial</td>
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<td>12:00 - 1:00</td>
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<td>TRAActs Constrained by UnderLying Anatomy (TRACULA)</td>
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<td>Anastasia Yendiki</td>
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<td>Anastasia Yendiki</td>
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<td>break</td>
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<td>Basics of fMRI Analysis</td>
<td>talk</td>
<td>Jon Polimeni</td>
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<td>FSFAST, Part 1: Preprocessing</td>
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<td>9:00 - 9:40</td>
<td>FSFAST, Part 2: GLM Analysis</td>
<td>talk</td>
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<td>talk</td>
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<td>Multi-Modal Integration Tutorial: fMRI Integration &amp; Surface-based Group fMRI Analysis</td>
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<td>* An Overview of Registration Methods</td>
<td>talk</td>
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<td>Future Directions</td>
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<td>Question &amp; Answer Session</td>
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FreeSurfer Course, September 26-29 Talk Descriptions

* Indicates an optional technical talk. These talks have great content. They are related to, but not directly about, the FreeSurfer processing stream.

** Indicates an optional talk that covers basic/beg inner material.

Day 1: Introduction / Single Subject Analysis / ROI Analysis / MR Acquisition

- **Intro to Linux for FreeSurfer Users
  - This linux tutorial is very basic. **Scripting will not be covered**. This tutorial will cover how to navigate (cd) through directories, how to copy files, how to make directories, how to use emacs, and how to set variables. For those who are not familiar with Linux, this tutorial is meant to get you comfortable enough to at least get through the FreeSurfer tutorials that are part of the course. If you have never used Linux before, this session is highly recommended.

- **Introduction to FreeSurfer Jargon
  - Intended for those new to imaging software. Explains basic vocabulary such as voxel, surface, vertex, volume, segmentation, parcellation, registration, and recon.

- Introduction to FreeSurfer
  - Overview of FreeSurfer's abilities.

- Analyzing the Individual Subject in FreeSurfer
  - Description of each step of the FreeSurfer processing stream.

- Freeview demonstration
  - The basics of using Freeview.

- Interaction with Individual Subject Data - Tutorial
  - Using FreeSurfer's visualization tools to look at FreeSurfer output.

- Surface-based Smoothing/Surface-based Registration
  - Volume vs. Surface-based analysis

- Region of Interest (ROI) Analysis - Talk and Tutorial
  - A description of the FreeSurfer atlases used in subcortical segmentation and cortical parcellation as well as the measures FreeSurfer provides. Information on creating and analyzing ROIs (volume vs. intensity studies).

- **A Non-physicist's Intro to MR
  - A short introduction to the basic processes underlying MR imaging, with an emphasis on intuitive explanations for non-physicist MR users.

- *MRI Acquisition Methods for Morphometry
  - A description of how using different scan protocols can affect data quality and FreeSurfer reconstruction.

- *Motion-compensated neuroanatomical imaging
  - While real-time fMRI motion correction has been available with some MRI scanners for years (e.g. Siemens' PACE), effective real-time motion correction in neuroanatomical imaging is just now becoming available. This talk focuses on the motion-correction
system we have developed for the Siemens platform, but also discusses the current developments on the GE platform.

Day 2: Group Analysis / Longitudinal Analysis / Troubleshooting

- **Group Analysis - Talk and Tutorial**
  - Review of linear algebra & other group analysis basics. Covers the basics of group analysis in the context of imaging data.
- **Multiple Comparisons - Talk and Tutorial**
  - How to correct group analysis findings for false discoveries.
- **QDEC (group analysis software) - Demonstration and Tutorial**
  - Introduction to a user-friendly tool for conducting group analyses.
- **Longitudinal FreeSurfer - Talk and Tutorial**
  - How to process longitudinal data with FreeSurfer and what's going on behind the scenes.
- **FreeSurfer Troubleshooting - Talk and Tutorial**
  - Discussion of the possible errors one may find in their FreeSurfer output and how to fix them.
- **Quality Checking a Recon - Demonstration**
  - Live demo of how to scroll through FreeSurfer output and look for errors.

Day 3: Multimodal Analysis / Diffusion Analysis / fMRI Analysis

- **Multi-Modal Integration, Part 1: Registration – Talk and Tutorial**
  - Introduction on how FreeSurfer output can be used with other modalities (i.e. fMRI data, diffusion data). How to register surface data with other modalities using FreeSurfer.
- **A Non-physicist's Intro to Diffusion MR**
  - A short introduction to the basic processes underlying Diffusion MR imaging, with an emphasis on intuitive explanations for non-physicist MR users.
- **Introduction to Diffusion MRI, Diffusion Data Processing – Talk and Tutorial**
  - How to process diffusion MRI data to extract basic diffusion measures.
- **TRActs Constrained by UnderLying Anatomy (TRACULA), Longitudinal TRACULA – Talks and Tutorial**
  - How to reconstruct white-matter pathways using FreeSurfer's new tractography tool.
- **Basics of fMRI Analysis**
  - Covers the basics of functional MRI data analysis, spanning preprocessing to single-subject and group-level analyses. Topics include motion correction, B0 distortion correction, spatial normalization, spatial smoothing, hemodynamic modeling, GLM analysis, contrasts and hypothesis testing, and Random-, Fixed-, and Mixed-Effects group analyses.
- **FSFAST, Part 1: Preprocessing**
Preprocessing of fMRI (motion correction, slice-timing correction, smoothing, registration to the anatomical, sampling to the common group space in the surface and volume), setting up and running block and event-related analysis, and adding nuisance variables to the analysis.

Day 4: fMRI Analysis / Multimodal Analysis / Future Directions

- FSFAST, Part 2: GLM Analysis – Talk and Tutorial
  - Performing the individual fMRI time series fMRI analysis and performing group fMRI analysis in the volume and on the surface.

- Multi-Modal Integration, Part 2 - Talk and Tutorial
  - Introduction on how FreeSurfer output can be used with other modalities (i.e. fMRI data, diffusion data). How to integrate fMRI with other modalities, and how to run surface-based group fMRI analysis.

- *An Overview of Registration Methods
  - Overview of different registration methods discussed so far (flirt, robust_register, bbregister) and introduction of a new registration tool, CVS (combined volume and surface registration).

- Future Directions of FreeSurfer
  - A look at some of the current research and features the FreeSurfer Development team has been working on.

- Question and Answer Session
  - Open forum to resolve any unanswered questions
FreeSurfer System Requirements

Summary of Requirements:
Operating System: Linux, Mac OS X, Windows (via VirtualBox)
Processor Speed: 2GHz at least
RAM: 8GB recommended
Graphics card: 3D graphics card with its own graphics memory & accelerated OpenGL drivers
Size of installation package: 14GB
Typical size of a processed subject: 370MB
Tutorial dataset size: 5Gigs
Other requirements: Matlab (only needed to run FS-FAST, the fMRI analysis stream)

Freesurfer is available for the Linux and Mac OS X operating systems. It can also be used on Windows with VirtualBox (but runs a bit slower). The download page lists the supported platforms. Matlab is required to run the FS-FAST component of Freesurfer (but Matlab is not required to run the reconstruction utilities).

A 2GHz or faster processor, at least 4GB of RAM, and a 3D graphics card (with its own graphics memory) with accelerated OpenGL drivers, is recommended. Freesurfer is highly CPU and memory intensive (and moderately disk intensive), so concentrate on boosting those performance aspects (more memory is better...**8GB is highly recommended!**).

With the inclusion of new OpenMP-enabled code in freesurfer, multiple-cores can now be accessed by recon-all. This means you should also consider purchasing a multi-core machine if you intend to process multiple subjects at the same time. If you do this, be sure to purchase 4GB per subject, that is, if you would like to process four subjects at once, your machine must have four cores and 16GB of memory. The flag you would include with recon-all to do this is: `-openmp 4` which tells recon-all to use four cores (cpus) when the special parallelized code is run (ie. the 'gcareg' stage).

Most modern video graphics card will perform fine, but be aware that graphics cards that use CPU memory as video memory will have a noticeably slow redraw rate. NVidia graphics cards have been found to work best with freesurfer. While freesurfer includes some GPU support, it is not actively supported anymore, so purchasing a GPU card explicitly for freesurfer is not recommended, in lieu of purchasing multiple cores and using the OpenMP functionality (`-openmp <num_threads> flag`). A 19" (or greater!) monitor is also recommended.

If you're running Freesurfer on a server, then allocate 4GB of memory to your job.

Freesurfer requires about 8.5GB of disk space for the full installation, which includes the Freesurfer binaries, support libraries, the MINC toolkit, and sample MRI data.

The volume and surface data files produced by Freesurfer for a typical subject (assuming two structural MRI scans of source data) consume about 370MB of disk space. The full tutorial
dataset is 18GB.

Mailing list notes regarding **suggested specs for a Linux box:**

- CPU: Intel vs AMD: no preference. also, CPU speed is not critical. It is better to have at least 4GB, and budget 4GB per subject you want to process simultaneously. Try to get an 'Ivy Bridge' motherboard architecture or whatever is newer. This allows better addressing of scattered memory which is common in freesurfer, and it alone accounts for a 5% or so speedup. AMD might have a similar memory controller.
- Graphics card:
  - ATI (AMD) vs nVidia: We still spec nVidia because we don't have problems with its OpenGL-X driver under linux. Perhaps ATI has finally supplied one that works, but this hasn't been attempted in a couple years. ATI cards on the Mac work fine with freesurfer though.
  - GPU: We no longer support [further] CUDA or GPU development because of lack of resources and difficulty, in preference to using OpenMP, which uses CPU cores. We will continue to support the existing GPU CUDA code that runs on recon-all with the -use-gpu switch. By support, keeping it running with each new nvidia cuda driver release as best as we can.
- Disk: Freesurfer is not disk intensive, so SSD is not a benefit. Budget 400MB per subject for storage.
- Linux: Both freesurfer and fsl groups use CentOS 6 in our Centers. It seems to work well.
Processing in FreeSurfer: An example pipeline from start to finish

INDIVIDUAL SUBJECT:
   >> recon-all -all -s <>
      > Edit output
   >> recon-all -make all -s <>

LONGITUDINAL:
   >> recon-all -base <> -tp <> -all
   >> recon-all -long <> -tp <> -all

1ST LEVEL ANALYSIS:
   >> recon-all -s <> -qcache
      > Create FSGD and contrast files
   >> mris_preproc --fsgd <> --cache-in <> --target fsaverage --hemi <> --out <> --fwhm <>
   >> mri_glmfit --y <> --fsgd <> --C <> --surf fsaverage <> --cortex --glmdir <>
   >> mri_glmfit-sim --glmdir <> --cache <> <> --cwp <> --2spaces

MULTIMODAL INTEGRATION:
   >> bbregister --mov <> --s <> --lta <>
   >> mri_vol2vol or mri_vol2surf
   >> mris_preproc --iv <> <> --iv <> <>
   >> mri_glmfit --y <> --fsgd <> --C <> --surf fsaverage <> --cortex --glmdir <>
   >> mri_glmfit-sim --glmdir <> --cache <> <> --cwp <> --2spaces
Processing Your Own Data

With FreeSurfer, certain variables must be set in order to use it correctly:

**FREESURFER_HOME**

*tell Operating System where FreeSurfer is*

**SUBJECTS_DIR**

*tell FreeSurfer where data is*

Required Variables

To use FreeSurfer you’ll have to do:

- Set `FREESURFER_HOME` to the path where FreeSurfer is located:
  ```
  setenv FREESURFER_HOME /home/apps/freesurfer
  ```
- Source the `SetUpFreeSurfer.csh` script to get your computer ready to use FreeSurfer:
  ```
  source $FREESURFER_HOME/SetUpFreeSurfer.csh
  ```
- Set `SUBJECTS_DIR` to the path where your data is located:
  ```
  setenv SUBJECTS_DIR /path/to/data
  ```

Getting Started with FreeSurfer

Download freesurfer:

https://surfer.nmr.mgh.harvard.edu/fswiki/QuickInstall

Note: Anything in red below means you should substitute it with the correct info.

For Macs only:
- Use X11 or XQuartz
  - Go to X11 or XQuartz > Preferences > Check “Emulate 3 button mouse”
- For other machines:
  - Open a terminal window

To get started using FreeSurfer:

```bash
setenv FREESURFER_HOME /Applications/freesurfer
source $FREESURFER_HOME/SetUpFreeSurfer.csh
setenv SUBJECTS_DIR /path/to/your/subject/data
```

From Scanner to FreeSurfer

For dcm format:
- If you do not know which of a subject’s dicoms is the MPRAGE/T1 scan:
  ```
  cd to directory with subject’s dicoms
  unpacksdcmdir -scanonly ./scan.log -src /location/of/dicoms -targ /location/to/save/log
  ```
  The scan.log file will show the first and last slice/series numbers
- You will need the full path to the MPRAGE(s) and the name of the 1st series in the MPRAGE to run recon-all.

Create a directory for your subject data. This will be your SUBJECTS_DIR.

```bash
mkdir study1
```

To get started:

```bash
setenv SUBJECTS_DIR /path/to/study1
cd $SUBJECTS_DIR
recon-all -i /path/to/subject's/mprage.dcm -i /if/have/second/mprage.dcm -all -s subj001
```
anatomically derived defect

- A topological defect in the cortical surface that arises from a feature of normal neuroanatomy to be distinguished from defects arising entirely from segmentation errors. See topological defect

artifact

- A feature that appears in an image but is not actually present in the imaged object.

average convexity

- The signed distance that a vertex moves during the inflation process.

brain volume

- The T1 volume after the skull and other non-brain structures have been removed. This volume can be viewed using tkmedit.

canonical surface

- Surface-based atlas constructed from the cortical surfaces of 40 normal individuals (used for inter-subject averaging).

conversion/averaging

- Process of converting and averaging multiple structural acquisitions from the native magnet format into the native FreeSurfer format (see COR files).

COR files

- The native file format used by FreeSurfer to store 3D structural image data.

Euler number

- After Leonhard Euler (1707-83). A topological invariant of a surface that can be computed from the number of edges, vertices and faces in a polygonal tessellation (command mris_euler_number ). The Euler number of a sphere will equal 2; the Euler number of a surface with n handles is 2 - 2n.

filled volume

- The wm volume after separation of the left and right hemispheres and filling of each hemisphere. This volume can be viewed using tkmedit.
flattening

- Producing a planar (flat) representation of a patch of the cortical surface that has minimal metric distortion.

gyrus

- A fold or convolution of brain tissue (an outward folded region).

inflated surface

- The smoothwm surface after inflation. This surface can be viewed using surfer.

inflation

- The process of smoothing the cortex while minimizing metric distortion, so that all sulci are fully visible and surface distances are apparent to visual inspection.

intensity

- Measured amount of magnetic field at a given spatial location, represented by a voxel (higher SNR signal to noise ratio means voxels will have a higher intensity relative to the background noise, and appear brighter).

label

- A particular region of interest. e.g. in tksurfer the label would be a region of interest in the surface. In tkmedit a label is a region of interest in the volume.

morphing

- Computer graphics technique whereby a mapping is computed that smoothly transforms one image or surface into another.

morphometrics

- The study of geometric properties of the human brain.

motion correction

- Processing multiple structural volumes so that the effects of subject movement are minimized. This is typically done by aligning multiple images/volume to an initial image/volume (see conversion/averaging).
MRI volume

- The three dimensional volumetric data set collected from a MRI scanner.

orig volume

- The original MRI volume. This volume can be viewed using tkmedit.

orig surface

- The first surface constructed by covering the labeled voxels in the filled volume. This surface can be viewed using surfer.

pial

- Pertaining to the delicate pia mater which envelops the brain (gray matter). Also, the model of the pial surface (?h.pial).

pial surface

- The refined estimate of the gray/CSF boundary (pial surface). This surface can be viewed using surfer.

region growing process

- An algorithm that groups voxels or sub-regions into larger regions.

RF-field inhomogeneities

- Spatial variations in the Radio Frequency (RF) excitation pulse. These variations result in changes in the measured intensity for a given tissue class that are related to the spatial location of the voxel.

segmentation

- Labeling of tissue classes from MRI data (e.g. white matter).

smoothing

- Process of producing a relatively even and regular cortical surface.

smoothwm surface

- The orig surface after smoothing. This surface can be viewed using surfer.
sulcus

- A groove or furrow in brain tissue (an inward folded region).

supertessellated icosahedron

- Polygonal approximation to a sphere.

T1

- Longitudinal relaxation constant.

T1 volume

- The MRI volume after intensity normalization. This volume can be viewed using tkmedit.

T1 Weighted Image

- A magnetic resonance image where the contrast is predominantly dependent on T1.

T2 Weighted Image

- A magnetic resonance image where the contrast is predominantly dependent on T2.

T2

- Transverse relaxation constant.

Talairach coordinate

- The corresponding location in the Talairach atlas for a given point in a brain that has been coregistered with the atlas (Talairach et al, 1967).

tessellation

- Covering of a surface by repeated use of a single shape.

topology

- The properties of a surface related to its connectivity that are unaffected by geometric (i.e. rubber sheet) transformations.

topological defect

- A portion of a surface that results in the surface topology differing from that of a sphere.
**volume**

- A 3-D data set that typically contains either intensity information derived from the original MRI, or the results of segmenting this data into tissue classes.

**voxel**

- The basic element of an MRI volume (analogous to a pixel in a 2-D image). The volume of a structural voxel is approximately 1 mm$^3$.

**white surface**

- The refined estimate of the gray/white boundary. This surface can be viewed using surfer.

**wm volume**

- The brain volume after white matter segmentation. This is also the volume that is manually edited. This volume can be viewed using tkmedit.
What is Linux?

- Most common household computer
- 90% of all internet traffic comes from Windows based machines*
- Especially popular in the gaming community

Open source operating system
- 1% of all internet traffic comes from Linux based machines
- Widely used in academic, supercomputers, and web servers


9% of all internet traffic comes from OSX based machines
Especially popular in the photo, video, and music editing communities

Linux Desktop

Terminal does not mean “hacking”

Terminal gives you access to your computer via typing commands rather than using the mouse and clicking

Demonstration of commands

**Task:** Navigate to the freesurfer directory, list its content, then create a new directory called Practice and create a simple text file called Notes.txt.

Demovo
Using Freesurfer

- Up to this point, we have not done anything freesurfer related
- Once Freesurfer is installed, many more commands become available to you
- With Freesurfer, certain variables must be set in order to use it correctly

Exercise: Use Freesurfer to display header information of an mri image file, then convert it to nifti format, then display the resulting image in the freeview application.

Exercise

Use Freesurfer to display header information of an mri image file, and convert it to nifti:

```
$> export FREESURFER_HOME=/home/nmrclass/freesurfer
$> source $FREESURFER_HOME/SetUpFreeSurfer.sh
$> export SUBJECTS_DIR=$FREESURFER_HOME/subjects
$> cd $SUBJECTS_DIR
$> mri_info sample-001.mgz
... 
$> mri_convert sample-001.mgz sample-001.nii
$> freeview sample-001.nii
```

Demo

More Help

```
$> mri_info --help
USAGE: mri_info fname1 <fname2> <options>
$> man pwd
NAME
   pwd - print name of current/working directory

UNIX Tutorial For Beginners:
http://www.ee.surrey.ac.uk/Teaching/Linux/

Linux in a Nutshell:
http://docstore.mik.ua/orelly/linux/ch01_01.htm

UNIX Cheat Sheet:
http://tux.cs.unlv.edu/refs/linux_commands.html

Command Line Tutorial:
http://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/CommandLineNavigation
```
<table>
<thead>
<tr>
<th>File Commands</th>
<th>System Info</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ls</em> - directory listing</td>
<td><em>date</em> - show the current date and time</td>
</tr>
<tr>
<td><em>ls</em> -al - formatted listing with hidden files</td>
<td><em>cal</em> - show this month’s calendar</td>
</tr>
<tr>
<td><em>cd</em> dir - change directory to dir</td>
<td><em>uptime</em> - show current uptime</td>
</tr>
<tr>
<td><em>cd</em> - change to home</td>
<td><em>w</em> - display who is online</td>
</tr>
<tr>
<td><em>pwd</em> - show current directory</td>
<td><em>whoami</em> - who you are logged in as</td>
</tr>
<tr>
<td><em>mkdir</em> dir - create a directory dir</td>
<td><em>finger user</em> - display information about user</td>
</tr>
<tr>
<td><em>rm</em> file - delete file</td>
<td><em>uname</em> -a - show kernel information</td>
</tr>
<tr>
<td><em>rm</em> -r dir - delete directory dir</td>
<td><em>cat</em> /proc/cpuinfo - cpu information</td>
</tr>
<tr>
<td><em>rm</em> -f file - force remove file</td>
<td><em>cat</em> /proc/meminfo - memory information</td>
</tr>
<tr>
<td><em>rm</em> -rf dir - force remove directory dir *</td>
<td><em>man</em> command - show the manual for command</td>
</tr>
<tr>
<td><em>cp</em> file1 file2 - copy file1 to file2</td>
<td><em>df</em> - show disk usage</td>
</tr>
<tr>
<td><em>cp</em> -r dir1 dir2 - copy dir1 to dir2; create dir2 if it doesn't exist</td>
<td><em>du</em> - show directory space usage</td>
</tr>
<tr>
<td><em>mv</em> file1 file2 - rename or move file1 to file2 if file2 is an existing directory, moves file1 into directory file2</td>
<td><em>free</em> - show memory and swap usage</td>
</tr>
<tr>
<td><em>ln</em> -s file link - create symbolic link to file</td>
<td><em>whereis</em> app - show possible locations of app</td>
</tr>
<tr>
<td><em>touch</em> file - create or update file</td>
<td><em>which</em> app - show which app will be run by default</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compression</th>
<th>Network</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>tar</em> cf file.tar files - create a tar named file.tar containing files</td>
<td><em>ping</em> host - ping host and output results</td>
</tr>
<tr>
<td><em>tar</em> xf file.tar - extract the files from file.tar</td>
<td><em>whois</em> domain - get whois information for domain</td>
</tr>
<tr>
<td><em>tar</em> czf file.tar.gz files - create a tar with Gzip compression</td>
<td><em>dig</em> domain - get DNS information for domain</td>
</tr>
<tr>
<td><em>tar</em> xzf file.tar.gz - extract a tar using Gzip</td>
<td><em>dig</em> -x host - reverse lookup host</td>
</tr>
<tr>
<td><em>tar</em> xjf file.tar.bz2 - create a tar with Bzip2 compression</td>
<td><em>wget</em> file - download file</td>
</tr>
<tr>
<td><em>gzip</em> file - compresses file and renames it to file.gz</td>
<td><em>wget</em> -c file - continue a stopped download</td>
</tr>
<tr>
<td><em>gzip</em> -d file.gz - decompresses file.gz back to file</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>File Permissions</th>
<th>Installation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>chmod</em> octal file - change the permissions of file to octal, which can be found separately for user, group, and world by adding:</td>
<td>Install from source:</td>
</tr>
<tr>
<td>4 - read (r)</td>
<td>./configure</td>
</tr>
<tr>
<td>2 - write (w)</td>
<td>make</td>
</tr>
<tr>
<td>1 - execute (x)</td>
<td>make install</td>
</tr>
<tr>
<td>Examples:</td>
<td>dpkg -i pkg.deb - install a package (Debian)</td>
</tr>
<tr>
<td>chmod 777 - read, write, execute for all</td>
<td>rpm -Uvh pkg.rpm - install a package (RPM)</td>
</tr>
<tr>
<td>chmod 755 - rwx for owner, rx for group and world</td>
<td></td>
</tr>
<tr>
<td>For more options, see <em>man</em> chmod.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SSH</th>
<th>Shortcuts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ssh</em> user@host - connect to host as user</td>
<td><em>Ctrl+C</em> - halts the current command</td>
</tr>
<tr>
<td><em>ssh</em> -p port user@host - connect to host on port as user</td>
<td><em>Ctrl+Z</em> - stops the current command, resume with fg in the foreground or bg in the background</td>
</tr>
<tr>
<td><em>ssh-copy-id</em> user@host - add your key to host for user to enable a keyed or passwordless login</td>
<td><em>Ctrl+D</em> - log out of current session, similar to exit</td>
</tr>
<tr>
<td><em>grep</em> pattern files - search for pattern in files</td>
<td><em>Ctrl+W</em> - erases one word in the current line</td>
</tr>
<tr>
<td><em>grep</em> -r pattern dir - search recursively for pattern in dir</td>
<td><em>Ctrl+R</em> - type to bring up a recent command</td>
</tr>
<tr>
<td><em>command</em></td>
<td><em>grep</em> pattern - search for pattern in the output of command</td>
</tr>
<tr>
<td><em>locate</em> file - find all instances of file</td>
<td><em>exit</em> - log out of current session</td>
</tr>
</tbody>
</table>

* use with extreme caution.
Intro to FreeSurfer Jargon

- voxel
- surface
- volume
- vertex
- surface-based
- recon
- cortical, subcortical
- parcellation/segmentation
- registration, morph, deform, transforms
  (computing vs. resampling)
What FreeSurfer Does...

FreeSurfer creates computerized models of the brain from MRI data.

Input:
T1-weighted (MPRAGE) 1mm$^3$ resolution (.dcm)

Output:
Segmented & parcellated conformed volume (.mgz)

Recon

“recon your data”
...short for reconstruction
...cortical surface reconstruction
...shows up in command recon-all

Volumes

- orig.mgz
- T1.mgz
- brainmask.mgz
- wm.mgz
- filled.mgz (Subcortical Mass)

Cortical vs. Subcortical GM

cortical gm

- coronal
- sagittal

subcortical gm

- coronal
- sagittal
Parcellation vs. Segmentation

(cortical) parcellation  (subcortical) segmentation

Intro to FreeSurfer Jargon

voxel
surface
volume
vertex
surface-based
recon
cortical, subcortical
parcellation/segmentation
registration, morph, deform, transforms
(computing vs. resampling)

Registration

Goal:
to find a common coordinate system for the input data sets

Examples:
• comparing different MRI images of the same individual (longitudinal scans, diffusion vs functional scans)
• comparing MRI images of different individuals

Inter-subject, uni-modal example

Linear registration: 6, 9, 12 DOF

Linear registration: 6, 9, 12 DOF
Linear registration: 6, 9, 12 DOF

Intra-subject, multi-modal example

Inter-subject non-linear example

Some registration vocabulary

- Input datasets:
  - Fixed / template / target
  - Moving / subject
- Transformation models
  - rigid
  - affine
  - nonlinear
- Objective / similarity functions
- Applying the results
  - deform, morph, resample, transform
- Interpolation types
  - (tri)linear
  - nearest neighbor
FreeSurfer Questions

Search for terms and answers to all your questions in the Glossary, FAQ, or FreeSurfer Mailing List Archives.
FreeSurfer Introduction

Course Overview

Day 1
- Individual Subject Analysis
- ROI Analysis
- MR Basics & Acquisition Tips

Day 2
- Group Analysis
- Longitudinal Analysis
- Troubleshooting

Day 3
- Multimodal Analysis
- Diffusion Analysis
- fMRI Analysis

Day 4
- fMRI Analysis
- Multimodal Analysis
- Future Directions

Course Schedule

https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/Sept2016CourseSchedule

Lectures and Practicals

- General format: talk followed by tutorial (both are on the wiki course page, but please don’t download tutorial data or FreeSurfer – it can kill the network)
- Search on YouTube for the FreeSurfer channel!

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Type</th>
<th>Lecturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30</td>
<td>** Intro to Linux for FreeSurfer Users</td>
<td>task</td>
<td>Zeev Kaufman</td>
</tr>
<tr>
<td>8:45</td>
<td>** Intro to FreeSurfer Jargon</td>
<td>talk</td>
<td>Allison Stevens &amp; Lisa Zille</td>
</tr>
<tr>
<td>9:15</td>
<td>break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9:45</td>
<td>Freeview demonstration</td>
<td>demo</td>
<td>Allison Moreau</td>
</tr>
<tr>
<td>10:00</td>
<td>Interaction with Individual Subject Data Tutorial</td>
<td>tutorial</td>
<td>Stuff</td>
</tr>
<tr>
<td>10:15</td>
<td>Lunch # (Suggestions for where to eat)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td>Interaction with Individual Subject Data Tutorial</td>
<td>tutorial</td>
<td>Stuff</td>
</tr>
<tr>
<td>1:45</td>
<td>Surface-based Analysis: Interpolated Registration &amp; Smoothing</td>
<td>talk</td>
<td>Doug Glover</td>
</tr>
<tr>
<td>2:15</td>
<td>break</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:30</td>
<td>ROI Analysis</td>
<td>talk</td>
<td>Doug Glover</td>
</tr>
<tr>
<td>3:05</td>
<td>ROI Analysis</td>
<td>tutorial</td>
<td>Staff</td>
</tr>
</tbody>
</table>

Food and such

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:20</td>
<td>Registration</td>
<td></td>
</tr>
<tr>
<td>12:20</td>
<td>Lunch # (Suggestions for where to eat)</td>
<td></td>
</tr>
</tbody>
</table>
| 12:30 | Optional Lunch Talk: A Non-physicist's Intro to MRI | talk | Dylan Tsai

Cafeteria in main building (Building 149)

Morning coffee and "breakfast" – muffins, bagels, fruit
Afternoon coffee and some snacks
Tuesday evening socializing at Boston Beer Works

To Caffeinate or not to Caffeinate?

Please don’t spill coffee (or anything else!) on the laptops. If you do, please be prepared to fund a replacement!
What is FreeSurfer?

- Neuroimaging analysis software package
  - Open Source
- Detailed characterization of anatomy
  - Cortex – thickness, folding patterns, ROIs
  - Subcortical – structure boundaries
  - Hippocampal subfields
  - Longitudinal analysis – detect changes
- Statistical tools (GLM, LME, …), group comparison
- Multi-modal integration
  - fMRI (task, rest, retinotopy)
  - DWI Tractography
  - PET

What is FreeSurfer?

- Popular...
- Total # licenses distributed as of July: 25,214

Outline

- Anatomical Analysis
  - Surface-based (Cortex)
  - Volume-based
- Multi-modal integration
  - DWI/Tractography
  - fMRI
Outline

- Anatomical Analysis
- Surface-based (Cortex)
- Volume-based
- Multi-modal integration
  - DWI/Tractography
  - fMRI

Cortex

- Outer layer of gray matter
- 1-5mm thick
- Highly folded
- 2 Dimensional, embedded in 3D
- Function follows the surface
  - Visualization
  - Spatial Smoothing
  - Inter-subject Registration

2D Surface in 3D Space

Inflation

Surface Model

- Triangle Mesh ('Finite Element')
- Vertex = point of triangles
- Neighborhood
- XYZ at each vertex
- Triangles/Faces ~ 300,000
- Area, Distance
- Curvature, Thickness
- Movable

Cortical Thickness

- Shortest distance between white and pial surfaces
- 1-5mm in healthy subjects

Function Follows the Surface

- Visual areas mapped using fMRI retinotopy
- Pattern is clear on the surface, but lost in the volume

From (Sereno et al, 1995, Science).
What Can One Do With A Surface Model?

goal: use model to imposed desired activity pattern on V1

desired shape of activity pattern \rightarrow \text{required shape of stimulus}

w=k \log(z+a)

left primary visual cortex

right visual hemifield

Collaboration with Jon Polimeni and Larry Wald.


Tangential Resolution Measured with Surface-based Analysis


NeuroMarketing!

Aim 1 of our NCRR Center Grant, spelling:
"MGH Center for Functional Neuroimaging Technologies;
and NCRR Center for Research Resources."
(just kidding)

Thanks to Larry Wald for this slide.

Problems with Affine (12 DOF) Registration

Subject 1               Subject 2 aligned with Subject 1
                        (Subject 1’s Surface)


A Surface-Based Registration

Common space for group analysis (like Talairach) “fsaverage”
Anatomical Study: Aging

Surface-based Registration Performance

Predicting Brodmann Areas: Talairach Coordinates

Predicting Brodmann Areas from Folding Patterns

Automatic Gyral Segmentation

Outline

- Anatomical Analysis
- Surface-based (Cortex)
- Volume-based
- Multi-modal integration
  - DWI/Tractography
  - fMRI
Volumetric Segmentation (aseg)

Caudate
Pallidum
Putamen
Amygdala
Hippocampus

Not Shown:
Nucleus Accumbens
Cerebellum

ROI Volume Study

Lateral Ventricular Volume
(Percent of Brain)

Healthy
MCI: Did NOT convert
MCI: Did convert
Probable AD


Ex vivo MRI of hippocampal subfields

Resolution as high as 0.1 mm isotropic
• Allows precise manual tracing of hippocampal subfields.
• The delineation only relies on geometry for subdividing the CA.

Joint work with J. Eugenio Iglesias, Koen van Leemput and Jean Augustinack

Automated Segmentation

We use the atlas as a prior, and connect it to the image through a Gaussian likelihood term for each label.
• This makes the segmentation sequence-independent.
0.6 mm isotropic T1 (Winterburn et al.)
1 mm T1 + 0.4x0.4x2 mm T2 (ADNI)

Joint work with J. Eugenio Iglesias, Koen van Leemput and Jean Augustinack

Robust Registration

Target
Target

Reuter et al, 2010 Neuroimage
Robust Registration

1. Create unbiased subject template (iterative registration to median)
2. Process template
3. Initialize time points
4. Let it evolve there
- Avoid Bias: All time points are treated the same
- Increases sensitivity and reliability!

Longitudinal Processing

Outline

- Anatomical Analysis
- Surface-based (Cortex)
- Volume-based
- Multi-modal integration
- DWI/Tractography
- fMRI

Outline

- Anatomical Analysis
- Surface-based (Cortex)
- Volume-based
- Multi-modal integration
- DWI/Tractography
- fMRI – task

Tractography with TRACULA
(TRActs Constrained by the Underlying Anatomy)

- Completely automated modeling of 18 major fascicles
- Uses prior probabilistic information on the anatomical structures that each fascicle goes through or next to

Sampling on the Surface

15 sec ‘ON’, 15 sec ‘OFF’
- Flickering Checkerboard
- Auditory Tone
- Finger Tapping
Spatial 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

Group fMRI Analysis: Volume vs Surface

Affine registration to MNI305
5mm volume smoothing vs. 10mm surface smoothing

What is FreeSurfer?

- Cortical extraction and labeling
- Subcortical Segmentation
- Surface-based Inter-subject Registration
- Fully automated
- Multi-modal integration

Use FreeSurfer Be Happy
Anatomical Analysis with FreeSurfer

[Image of FreeSurfer]

Input: T1 Weighted Image

- T1 Contrast: white matter brighter than gray matter
- ~1mm (no more than 1.5mm)
- Higher resolution may be worse!
- Full Brain
- Usually one acquisition is ok
- MPRAGE or SPGR
- 1.5T or 3T
- 7T might have problems
- Subject age > 5 years old
- Brain has no major problems (ie, tumors, parts missing)
- Non-human primates possible

More MRI Pulse Sequence Parameter Details:
http://www.nmr.mgh.harvard.edu/~andre

Processing Stream Overview

[Diagram showing the processing stream]

Fully Automated Reconstruction*

recon-all –i file.dcm –subject bert –all

* “Reconstruction” here refers to cortical reconstruction, not k-space reconstruction.

[Diagram showing the reconstruction process]

Fully Automated Reconstruction

recon-all –i file.dcm –subject bert –all

If you have more than one T1, then use:
–i file1.dcm –i file2.dcm

You can use NIFTI as well with
–i file.nii

To get a list of acquisitions:
dcmunpack –src /path/to/dicoms

$SUBJECTS_DIR

setenv SUBJECTS_DIR /path/to/space
**Fully Automated Reconstruction**

recon-all
--i file.dcm
--subject bert
--all

-all means to do everything!
Can take 10-20 hours

Later, we will show you how to run subsets of the processing stream to make it faster when correcting errors.

**Individual Steps**

**Volumetric Processing Stages**
1. Motion Cor, Avg, Conform (orig.mgz)
2. Non-uniform inorm (nu.mgz)
3. Talairach transform computation (talarach/talarach.xfm)
4. Intensity Normalization 1 (T1.mgz)
5. Skull Strip (brainmask.mgz)
6. EM Register (linear volumetric registration)
7. CA Intensity Normalization (norm.mgz)
8. CA Non-linear Volumetric Registration
9. CA Label (Volumetric Labeling) (aseg.mgz)
10. Intensity Normalization 2 (T1.mgz)
11. White matter segmentation (wm.mgz)
12. Edit WM With ASeg
13. Fill and cut (filled.mgz)

**Surface Processing Stages**
14. Tessellate (?h.orig.nofix)
15. Smooth1
16. Inflate1
17. Sphere (?h.qsphere)
18. Final Surf (?h.white ?h.pial ?h.thickness)
19. Smooth2 (?h.smooththms)
20. IsInfat2 (?h.inflated)
21. Asseg Statistics (stats/aseg.stats)
22. Cortical Parcellation (Labeling)
23. Cortical Parcellation Statistics
24. Cortical Parcellation mapped to Asseg
25. White Matter Parcellation (wmparc.mgz)

**recon-all -help**

Note: ?h.orig means lh.orig or rh.orig

**Upon Completion…**

$SUBJECTS_DIR/bert

scripts mri surf label stats

recon-all –i file.dcm –subject bert –all ~400MB

Native Anatomical Space
e.g., 1x1x1.2mm, 256x256x128

"Conformed" Anatomical Space
1x1x1mm, 256x256x256

mgz = "compressed mgh" format (like nifti) unique to FreeSurfer

Send us recon-all.log when you have problems!

freesurfer@nmr.mgh.harvard.edu
Native Anatomical Space
1x1x1.1mm, 256x256x128, Sag

Conform Step

Conformed Anatomical Space
1x1x1mm, 256x256x256, Cor

rawavg.mgz
orig.mgz

“Anatomical Space”
Surfaces
Parcellations
Segmentations

Upon Completion…

bert
scripts mri surf label stats

lh.orig lh.white lh.pial lh.inflated
lh.thickness and rh.thickness, lh.curv, lh.sulc

Desikan/Killiany Atlas
Destrieux Atlas

Upon Completion…

scripts mri surf label stats

lh.aparc.annot rh.aparc.annot
lh.aparc.a2009s.annot rh.aparc.a2009s.annot

stats files are text files with summary information, eg:
volume of left amygdala
average thickness in superior temporal gyrus

Some of the Processing Steps…

Motion Correction and Averaging

001.mgz 002.mgz

Does not change native resolution.
Usually only need one.
**Talairach Transform**

- Computes 12 DOF transform matrix
- Does NOT resample
- MNI305 template
- Mostly used to report coordinates

```
transform
  talairach.xfm → text file with matrix
```

**Intensity Bias**

- Left side of the image much brighter than right side
- Worse with many coils
- Makes gray/white segmentation difficult

```
albert
  mri
  T1.mgz
```

**Skull Strip**

- Removes all non-brain
  - Skull, Eyes, Neck, Dura
- `brainmask.mgz` (cf, `brain.mgz`)

```
bert
  mri
  brainmask.mgz
T1.mgz
brainmask.mgz
```

**Automatic Volume Labeling**

- Used to fill in subcortical structures for creating subcortical mass
- Useful in its own right
- `aseg.mgz`
- More in ROI Talk

```
ASeg Volume
  bert
  mri
  aseg.mgz
```

**“White Matter” Segmentation**

- Separates white matter from everything else
- Uses `aseg` to “fill in” subcortical structures
- Cerebellum removed, brain stem still there
- `wm.mgz` -- “wm” not a very good name!

```
WM Volume (wm.mgz)
Filled Volume (filled.mgz)
(Subcortical Mass)
```

**Fill and Cut (Subcortical Mass)**

- Fills in any holes.
- Removes any islands
- Removes brain stem
- Separates hemispheres (each hemi has different value)
- `filled.mgz` = “Subcortical Mass”
Surface Extraction

- Hemispheres separated
- Fit to wm.mgz
- 1mm resolution
- Rough, jagged

Surface Model

- Mesh ("Finite Element")
- Vertex = point of triangles
- Neighborhood
- XYZ at each vertex
- Triangles/Faces ~ 300,000
- Vertices ~ 140,000
- Area, Distance
- Curvature, Thickness

Volume vs Surface Model

Volume
- uniform grid
- voxel is an intersection of grid lines
- columns, rows, slices
- voxel size/distance
- voxel assigned a value
- XYZ

Surface
- NON-uniform grid
- vertex is an intersection of triangles
- each vertex has an index
- distance between vertices ~1mm
- vertex assigned a value
- XYZ

Vector of vertex values (~140,000)

White Matter Surface

- Nudge orig surface
- Follow T1 intensity gradients
- Smoothness constraint
- Vertex identity preserved

Pial Surface

- Nudge white surface
- Follow T1 intensity gradients
- Vertex identity preserved

Pial surf grows from white surf

Errors in pial surface placement are typically caused by underlying errors in the white matter placement, and can be corrected by interventions such as white matter control points.
Non-Cortical Areas of Surface

- Amygdala
- Putamen
- Hippocampus
- Caudate
- Ventricles
- CC

Inflation: 2D Surface in 3D Space
- White Surface
- Pial Surface
- Nudge vertices
- No intensity constraint
- See inside sulci
- Used for sphere

Cortical Thickness
- Distance between white and pial surfaces
- One value per vertex
- Surface-based more accurate than volume-based

Curvature (Radial)
- Circle tangent to surface at each vertex
- Curvature measure is $1/r$ of radius of circle
- One value per vertex
- Signed (sulcus/gyrus)

Spherical Registration
- Sulcal Map
- Spherical Inflation
- High-Dimensional Non-linear Registration to Spherical Template
- Atlas template is called “fsaverage”

Automatic Cortical Parcellation
- Spherical Atlas based on Manual Labeling
- Map to Individual Thru Spherical Reg
- Fine-tune based on individual anatomy
- Note: Similar methodology to volume labeling

More in surface-based analysis talk.
More in the Anatomical ROI talk.
Surface Overlays

- lh.sulc on inflated
- lh.curv on inflated
- lh.thickness on inflated
- lh.sulc on pial
- lh.curv on inflated
- fMRI on inflated
- lh.aparc.annot on inflated
- lh.aparc.annot on inflated

• Value for each vertex
• Color indicates value
• Color: gray, red/green, heat, color table
• Rendered on any surface
• fMRI/Stat Maps too

ROI Summaries:

$SUBJECTS_DIR/bert/stats
aseg.stats – volume summaries
lh.aparc.stats – desikan/killiany surface summaries
 lh.aparc.a2009s.stats – destrieux surface summaries
wm.parc.stats – white matter parcellation

Routines to generate spread sheets of group data
• asegstats2table --help
• aparcstats2table --help

More info in Anatomical ROI talk.

Upon Completion of recon-all

$SUBJECTS_DIR/bert

scripts mri surf label stats

recon-all.log
aseg.stats

recon-all –i file.dcm –subject bert –all

Download & Install

Getting FreeSurfer

• surfer.nmr.mgh.harvard.edu
• Register
• Download
• Mailing List
• Wiki: surfer.nmr.mgh.harvard.edu/fswiki
• Platforms:
  • Linux
  • Mac
  • Windows (VirtualBox)
• Installed in $FREESURFER_HOME

What to do next

Getting FreeSurfer

• surfer.nmr.mgh.harvard.edu
• Register
• Download
• Mailing List
• Wiki: surfer.nmr.mgh.harvard.edu/fswiki
• Platforms:
  • Linux
  • Mac
  • Windows (VirtualBox)
• Installed in $FREESURFER_HOME

What to do next
Getting Answers

Wiki

Mail Archive

recon-all -help
mri_convert -help

$FREESURFER_HOME/docs

Send questions to:
freesurfer@nmr.mgh.harvard.edu

Overview

recon-all –i file.dcm –subject bert –all

Tutorial Tips

• Best not to run multiple instances of Freeview at the same time unless you have > 8GB RAM.
• If you are running a command in the foreground, you should not type additional commands in that terminal (command prompt will be missing)

Volume Viewer (Freeview) Radiological Orientation

Right

Left

End of Presentation
Surface-based Analysis: Intersubject Registration and Smoothing

Exploratory Spatial Analysis

• Don’t know where effect is going to be
• vs ROI analysis
• Analyze each voxel separately
• Create a map
• Find clusters

Aging Exploratory Analysis

Cortical Thickness vs Aging
Salat, et al, 2004, Cerebral Cortex

Aging Thickness Study

Individual Exploratory Analysis

• fMRI Words-vs-Fixation
• Single subject (eg, presurgical planning or functional ROI)
• Outlines are FreeSurfer cortical ROIs
• Yellow and blue blobs are functional activation
• Activation does not lie cleanly within a predefined ROI
Exploratory Spatial Analysis

- Generally requires spatial smoothing of data to increase SNR
- For group analysis, requires that subjects' brains be aligned to each other on a voxelwise basis.
- Neither needed for an ROI analysis
- Smoothing and intersubject registration can be performed in the volume or surface.

Why Is a Model of the Cortical Surface Useful?

Local functional organization of cortex is largely 2-dimensional! Eg, functional mapping of primary visual areas:

From (Sereno et al, 1995, Science).

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

Sphere: 2D Coordinate System
- Continuous, no cuts
- Value at each point (eg, thickness)

Curvature
- SULCUS (+)
- GYRUS (-)

Intersubject Registration

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

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

A Surface-Based Coordinate System

Common space for group analysis (like Talairach)

fsaverage

- Has “subject” folder like individual FS subjects
- “Buckner 40” subjects
- Default registration space
- MNI305 coordinates

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
- fsaverage

Spatial Smoothing

Why should you smooth?
- Might Improve CNR/SNR
- Improve intersubject registration

How much smoothing?
- Blob-size
- Typically 5-20 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

Spatial Smoothing

- Spatially convolve image with Gaussian kernel.
- Kernel sums to 1
- Full-Width/Half-max: \( \text{FWHM} = \sigma/\sqrt{\log(256)} \)
- \( \sigma \) = standard deviation of the Gaussian

Effect of Smoothing on Activation

- Working memory paradigm
- FWHM: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20

Surface-based Smoothing

- Smoothing is averaging of nearby vertices
- Sheet: 2D Coordinate System (X,Y)
- Sphere: 2D Coordinate System (0,\( \theta \))

Group fMRI Analysis: Volume vs Surface

- Surface-based Registration and smoothing
- Averaging registration to MNI305 with volume smoothing

5HT\textsubscript{4} BP Asymmetry Study (N=16)

- Left > Right
  \( p<10^{-3} \)
  \( p<10^{-2} \)
- Right > Left
  \( p<10^{-2} \)
  \( p<10^{-3} \)
Surface-based Clustering

- A cluster is a group of connected (neighboring) vertices above threshold
- Neighborhood is 2D, not 3D
- Cluster has a size (area in mm²)
- Reduced search space (corrections for multiple comparisons)

Summary

- Why Surface-based Analysis?
  - Function has surface-based organization
  - Inter-subject registration: anatomy, not intensity
  - Smoothing
  - Clustering
  - Like 3D, but 2D

Use FreeSurfer  
Be Happy
Working with FreeSurfer Regions-of-Interest (ROIs)

Outline

- Subcortical Segmentation
- Cortical Parcellation
- WM Segmentation
- Preparation/Analysis of Stats

FreeSurfer ROI Terminology

ROI = Region Of Interest
Volume/Image (Subcortical):
- Segmentation
Surface (Cortical):
- Parcellation/Annotation/Surface Segmentation
- Clusters, Masks (from sig.mgh, fMRI)
- Label you created

SUBCORTICAL AUTOMATIC SEGMENTATION (aseg)

ROI Volume Study

Lateral Ventricular Volume (left) (Percent of Intracranial Volume)


Segmentation

- Volume style format (mgz, nii, nii.gz)
- Each voxel has one index (number ID)
- All voxels in the brain are labeled
- Index List found in color lookup table (LUT)
  - $FREESURFER_HOME/FreeSurferColorLUT.txt
- $FREESURFER_HOME/FreesurferColorLUTFile
  - 17 Left Hippocampus 220 216 20 0
  - Name = Left Hippocampus
  - Red=220, Green=216, Blue=20 (out of 255)
  - alpha = 0 (not really used)
- aseg.mgz, aparc+aseg.mgz, wmparc.mgz
Subcortical Segmentation (aseg)

- Caudate
- Pallidum
- Putamen
- Amygdala
- Hippocampus
- Lateral Ventricle
- Thalamus
- White Matter

Not Shown:
- Nucleus Accumbens
- Cerebellum


Volumetric Segmentation Atlas Description

- 39 Subjects
- 14 Male, 25 Female
- Ages 18-87
  - Young (18-22): 10
  - Mid (40-60): 10
  - Old Healthy (69+): 8
  - Old Alzheimer’s (68+): 11
- Siemens 1.5T Vision (Wash U)

FreeSurfer Stats Outputs

- SUBJECTS_DIR
- subject1
- subject2
- subject3 ...
- mri
- label
- stats
- aseg.stats - subcortical volumetric stats
  created by mri_segstats

aseg.stats Global Measures:
Cortical, Gray, White, Intracranial Volumes

Also in aseg.stats header:
- lhCortex, lhCortexVol, Left hemisphere cortical gray matter volume, 192176.447567, mm^3
- rhCortex, rhCortexVol, Right hemisphere cortical gray matter volume, 194153.9526, mm^3
- Cortex, CortexVol, Total cortical gray matter volume, 386330.400185, mm^3
- lhCorticalWhiteMatter, lhCorticalWhiteMatterVol, Left hemisphere cortical white matter volume, 217372.890625, mm^3
- rhCorticalWhiteMatter, rhCorticalWhiteMatterVol, Right hemisphere cortical white matter volume, 219048.187500, mm^3
- CorticalWhiteMatter, CorticalWhiteMatterVol, Total cortical white matter volume, 436421.078125, mm^3
- SubCortGray, SubCortGrayVol, Subcortical gray matter volume, 182006.000000, mm^3
- TotalGray, TotalGrayVol, Total gray matter volume, 568336.400185, mm^3
- SupraTentorial, SupraTentorialVol, Supratentorial volume, 939646.861571, mm^3
- IntraCranialVol, ICV, Intracranial Volume, 1495162.656130, mm^3

Details:
https://surfer.nmr.mgh.harvard.edu/fswiki/MorphometryStats
http://surfer.nmr.mgh.harvard.edu/fswiki/eTIV
Thickness and Area ROI Studies

**Middle Temporal Gyrus**

**Entorhinal Cortex**

Gray matter volume also possible

Surface Mesh (zoom-in)

Parcellation/Annotation

- Surface ONLY
- Annotation format (something.annot)
- Each vertex has only one label/index
- Index List also found in color lookup table (LUT)
- $FREESURFER_HOME/FreeSurferColorLUT.txt

Desikan/Killiany Atlas

- 40 Subjects
  - 14 Male, 26 Female
  - Ages 18-87
  - 30 Non-demented
  - 10 Demented
  - Siemens 1.5T Vision (Wash U)

Desikan/Killiany Atlas: Desikan/Killiany Atlas (35 ROI’s)

Automatic Surface Parcellation:

Destrieux Atlas

- 58 Parcellation Units
- 12 Subjects
FreeSurfer Stats Outputs

SUBJECTS_DIR

subject1 subject2 subject3 ...

mri label stats

lh.aparc.stats — left hemi Destrieux/Killiany surface stats
rh.aparc.stats — right hemi Destrieux/Killiany surface stats
lh.aparc.a2009.stats — left hemi Destrieux
rh.aparc.a2009.stats — right hemi Destrieux

created by mris_anatomical_stats

Parcellation Stats File

<table>
<thead>
<tr>
<th>StructName</th>
<th>NumVert</th>
<th>SurfArea</th>
<th>GrayVol</th>
<th>ThickAvg</th>
<th>ThickStd</th>
<th>MeanCurv</th>
<th>GausCurv</th>
<th>FoldInd</th>
<th>CurvInd</th>
</tr>
</thead>
<tbody>
<tr>
<td>bankssts</td>
<td>1157</td>
<td>811</td>
<td>1992</td>
<td>2.303</td>
<td>0.567</td>
<td>0.117</td>
<td>0.031</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>caudalanteriorcingulate</td>
<td>779</td>
<td>543</td>
<td>1908</td>
<td>3.472</td>
<td>0.676</td>
<td>0.185</td>
<td>0.064</td>
<td>26</td>
<td>1.8</td>
</tr>
<tr>
<td>caudalmiddlefrontal</td>
<td>3145</td>
<td>2137</td>
<td>5443</td>
<td>2.311</td>
<td>0.593</td>
<td>0.132</td>
<td>0.041</td>
<td>35</td>
<td>5.3</td>
</tr>
<tr>
<td>cuneus</td>
<td>1809</td>
<td>1195</td>
<td>2286</td>
<td>1.672</td>
<td>0.411</td>
<td>0.162</td>
<td>0.067</td>
<td>34</td>
<td>4.6</td>
</tr>
<tr>
<td>entorhinal</td>
<td>436</td>
<td>265</td>
<td>1269</td>
<td>2.871</td>
<td>0.881</td>
<td>0.119</td>
<td>0.037</td>
<td>5</td>
<td>0.6</td>
</tr>
<tr>
<td>fusiform</td>
<td>3307</td>
<td>2126</td>
<td>5161</td>
<td>2.109</td>
<td>0.689</td>
<td>0.144</td>
<td>0.064</td>
<td>71</td>
<td>8.7</td>
</tr>
<tr>
<td>inferiorparietal</td>
<td>5184</td>
<td>3514</td>
<td>8343</td>
<td>2.136</td>
<td>0.552</td>
<td>0.146</td>
<td>0.055</td>
<td>82</td>
<td>11.5</td>
</tr>
<tr>
<td>inferiortemporal</td>
<td>3746</td>
<td>2610</td>
<td>8752</td>
<td>2.683</td>
<td>0.748</td>
<td>0.178</td>
<td>0.132</td>
<td>140</td>
<td>18.0</td>
</tr>
</tbody>
</table>

Other ROIs (ex vivo)

Brodmann Areas

6, 4a,4p,3a,3b,1,2

V1, V2

Entorhinal

Brodmann Areas

45,44

MT

Example Label Files

SUBJECTS_DIR

subject1 subject2 subject3 ...

mri label stats

lh.cortex.label
lh.BA1.label
lh.BA2.label
lh.BA3.label

Label File

In Volume

- Easy to draw
- Use “Select Volumes” Tool in tkmedit
- Or use FreeView
- Simple text format

On Surface

Creating Label Files

- Drawing tools:
  - tkmedit, freeview
  - tksurfer
  - QDEC
- Deriving from other data
  - mris_annotation2label: cortical parcellation broken into units
  - mri_volumecluster: a volume made into a cluster
  - mri_surfccluster: a surface made into a cluster
  - mri_vol2label: a volume/segmentation made into a label
  - mri_label2label: label from one space mapped to another
**WHITE MATTER SEGMENTATION**  
(wmparc)

**Gyral White Matter Segmentation**


---

**Merged Cortical + Subcortical**

```
No new information  
For visualization only
```

**ANALYSIS of STATS**

**FreeSurfer Stats Outputs**

```
aseg.stats -- subcortical volumetric stats  
wmparc.stats -- white matter segmentation volumetric stats  
lh.aparc.stats -- left hemi Desikan/Killiany surface stats  
rh.aparc.stats -- right hemi Desikan/Killiany surface stats  
lh.aparc.a2009.stats -- left hemi Destrieux  
rh.aparc.a2009.stats -- right Destrieux
```

**Extract table of subcortical volumes of all structures for all subjects**

```
asegstats2table  
--subjects 001 002 003 004 005  
--meas volume  
--stats aseg.stats  
--tablefile aseg.table.txt
```

Applies to wmparc.stats too:

```
--stats wmparc.stats
```

Output is a simple ASCII text file.
Extract table of average thickness of all cortical structures for all subjects

```
aparcstats2table
- - subjects 001 002 003
- - hemi lh
- - meas thickness
- - parc aparc
- - tablefile aparc_lh_thickness_table.txt
Desikan/Killiany Atlas: --parc aparc
Destrieux Atlas: --parc aparc.a2009s
```

Extract table of surface area of all cortical structures for all subjects

```
aparcstats2table
- - subjects 001 002 003
- - hemi lh
- - meas area
- - parc aparc
- - tablefile aparc_lh_area_table.txt
```

Extract table of GM volume of cortical structures for all subjects

```
aparcstats2table
- - subjects 001 002 003
- - hemi lh
- - meas volume
- - parc aparc
- - tablefile aparc_lh_volume_table.txt
```

Note that the volume of cortical ROIs is extracted with aparcstats2table whereas the volume of subcortical structures is extracted with asegstats2table.

Importing Table Files

- SPSS, oocalc, matlab, R
- Choose: Delimited by spaces

GLM Analysis on Stats Files

- mri_glmfit (used for image-based group analysis)
- Use "-table table.txt" instead of "-y" to specify the input
- Eg. "mri_glmfit -table aparc_lh_vol_stats.txt ..."
- The rest of the command line is the same as you would use for a group study (e.g., FSGD file and contrasts).
- Output is text file sig.table.dat that lists the significances (-log10(p)) for each ROI and contrast.

Summary

- ROIs are individualized
- Subcortical and WM ROIs (Volume)
- Surface ROIs (Volume, Area, Thickness)
- http://freesurfer.net/fswiki/MorphometryStats
- Segmentation vs. Annotation vs. Label File
- Extract to table (asegstats2table, aparcstats2table)
- Now we can do Multimodal Applications
Tutorial

- Load and Inspect:
  - aparc+aseg.mgz
  - aparc.annot
  - FreeSurferColorLUT.txt
- View Individual Stats Files
- Group Table
  - Create
  - Load into spreadsheet
A human head

"pulse"

"flip angle"

main magnetic field
The rate of precession changes linearly with the strength of the magnetic field.
T2 is dephasing

dephasing looks like “less signal”

T1 and T2 relaxation

The fish are what make it interesting....

Using inversion recovery we can weight our measurements for tissues with specific T1.
How do we get spatial information?

Main magnetic field

Two voxels (left and right)

Add up the red lines

Take one measurement (sum)

Gradient

Rate of precession is different in each voxel

Apply a different magnetic field to each half
rate of precession is different in each voxel.

The voxels are out of phase.

Real sequences sum together fractional amounts from all the voxels.

The fractions are changed using the x-, y-, or z-gradients.

The voxels are “unmixed” from all the measurements using an Inverse Fourier Transform.
A Pulse Sequence

“Prepare” (invert, flip)
Localize (Gradients)
Measure
Relax
Go back to 1.

this side dephases faster = less signal

this side’s T2 returns to normal
MRI Acquisition Methods for Brain Morphometry

André J. W. van der Kouwe
Athinoula A. Martinos Center, Massachusetts General Hospital

Avoiding Imaging Artifacts
Small mistakes in the choice of imaging protocol at the beginning of a study can result in a large amount of manual intervention work later on.

Examples of Artifacts
Chemical shift artifact

Examples of Artifacts
Motion artifact

Examples of Artifacts
Intrinsic susceptibility artifact (EPI images)

Examples of Artifacts
Susceptibility artifact from metal
Examples of Artifacts

Wrap in phase encoding direction (3D has two PE directions)

Examples of Artifacts

Data adjacent to cortex

Examples of Artifacts

Poor contrast

MRI Acquisition Methods for Brain Morphometry

1. Contrasts: Bandwidth matched morphometry (PD, T1, T2 and T2*)
2. Artifacts: Distortions (B0 and gradient distortions)
3. Positioning: AutoAlign and motion correction

Contrasts: PD, T1, T2 and T2* weighting

Which is best for brain morphometry/FreeSurfer?

<table>
<thead>
<tr>
<th>Contrast Type</th>
<th>Weighting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton/spin density</td>
<td>PD-weighting</td>
</tr>
<tr>
<td>Gray/white contrast</td>
<td>T1-weighting</td>
</tr>
<tr>
<td>Bright CSF/tumor</td>
<td>T2-weighting</td>
</tr>
</tbody>
</table>

FLASH 5°  
FLASH 30°  
T2-SPACE
Contrasts: PD, T1, T2 and T2* weighting

Which is best for brain morphometry/FreeSurfer?

MPRAGE (FLASH with inversion) has the best contrast for FreeSurfer because:
- MPRAGE parameters chosen for “optimal” gray/white/CSF contrast
- FreeSurfer statistics (priors) based on MPRAGE

Recommended protocol: bandwidth matched

On the Martinos Center scanners under MGH → Morphometry:
- Localizer: 0.13
- AAScout: 0.46
- For cortical thickness (MEMPRAGE):
  - tf_mgh_ma_4echoes_iPAT2 1 x 1 x 1 mm³ 6.03
- For segmentation and PD/T1 estimation (MEFLASH):
  - gre_mgh_ma_5deg_iPAT2 1 x 1 x 1 mm³ 8.28
  - gre_mgh_ma_30deg_iPAT2 1 x 1 x 1 mm³ 8.28
- For T2 contrast (lesion detection):
  - T2_SPACE_iPAT2 1 x 1 x 1 mm³ 4.43

Bandwidths are matched and iPAT/multichannel coils reduce time

Detailed recommended protocols at http://www.nmr.mgh.harvard.edu/~andre

Why multi-echo bandwidth matched?

Geometric distortion with gradient echo sequences is proportional to ∆B0, inversely proportional to bandwidth and follows readout direction

Why multi-echo bandwidth matched?

High bandwidth results in:
- smaller B0 (susceptibility) related geometric distortions
- but lower SNR

With multiecho sequences:
- individual echoes have high bandwidth/low SNR
- but echoes are combined to recover SNR with low distortion

MEMPRAGE, MEFLASH and T2-SPACE can be bandwidth-matched:
- edges of structures align across contrasts
What areas of the cortical surface do B0 distortions affect the most?

Distances between surfaces measured with opposite readout directions (since both surfaces move, cortical thickness changes are much smaller)

Why collect FLASH at different flip angles?

FLASH (spoiled gradient echo) sequence is versatile, simple and easily modeled:

\[
\text{Signal (FLASH)} = \frac{g^2 T1}{1 + T2^{-2}}
\]

Effective T1 and PD (in arbitrary units) can be estimated at each voxel from two FLASH scans with differing flip angles using the FLASH steady-state equation (assume TE << T2*)

T2* can be estimated at each voxel from the signal decay across eight echoes of the multiecho FLASH scan (MFE)

The T1 and PD volumes can be used to synthesize a volume at any flip angle
FreeSurfer command to fit T1, PD and T2* from FLASH volumes:
mri_ms_fitparms [options] [vol_1] [vol_2] ... [output_dir]
For example:
mri_ms_fitparms -tr 20 -te 6 -fa 5 flash5.mgh -tr 20 -te 6 -fa 30 flash30.mgh parameter_maps
(parameters_maps/ is destination directory)
FreeSurfer command to synthesize volume from T1 and PD:
mri_synthesize [options] <TR> <alpha> <TE> <T1 vol> <PD vol> <output>
For example:
mri_synthesize 20 23 0 T1.mgz T2.mgz synth_23.mgz

BW matched protocol: PD

BW matched protocol: T1

BW matched protocol: T2*

Why collect FLASH at different flip angles?
Volumes with arbitrary flip angles can be synthesized from PD and T1

Why collect FLASH at different flip angles?
Flip angle for best contrast varies with pairs of structures and within structures (e.g. gray/white contrast varies across cortex and with age)
T2* in MEMPRAGE can be used to locate dura

In MPRAGE there is little contrast between dura and gray matter and dura is sometimes included within the pial surface.

FreeSurfer can adjust the pial surface so that it excludes dura if MEMPRAGE volumes are available.

T2* in MEMPRAGE can be used to locate dura

FreeSurfer command to correct dura:

```
mris_make_surfaces -dura filename_%d.mgz -l${lastecho} -aseg aseg.auto.mgz -mgz -sdir ${SUBJECTS_DIR} -output dura 1and${lastecho} $({target} ${hemi})
```

where filename_%d.mgz refers to the four separated echoes of the MEMPRAGE and lastecho = 4.

Generates:

lh.pial_dura_1and4, rh.pial_dura_1and4, lh.white_dura_1and4, rh.white_dura_1and4

Dura correction with FreeSurfer

Gradient distortion: uncorrected
Gradient distortion: corrected

GE Whole-Body
CRM NV/CI

Siemens Whole-Body
Symphony/Sonata
Balasubramanian, BU/Tufts; Dale, UCSD

Positioning: AutoAlign and Motion Correction

Background: AutoAlign
Place subject in scanner and acquire AutoAlign localizer (44 s)
Scanner registers acquired brain to average statistical atlas (10 s)
Scan prescriptions for subsequent scans in session are prospectively positioned in standard orientation - therefore also aligned to scans from previous sessions

AutoAlign position

Actual position

Motion correction: Cloverleaf navigators
Cloverleaf navigators are designed to enable a rigid body position estimate in a single readout of less than 5 ms

Cloverleaf k-space trajectory
Navigator signal magnitude

Motion correction: Cloverleaf navigators
Cloverleaf navigators assess and correct for the position of an object in the scanner every TR of a modified FLASH scan (e.g. every 20 ms)

Human results
Cloverleaf navigator correction substantially improved image quality in volunteers performing deliberate head motions

Average
No motion correction
Real-time motion corr.

Average (equal weight)
Real-time motion corr.

Average (MSE weighted)
Real-time motion corr.

3D FLASH (TR=20 ms, TE=10 ms, 1.3 x 1.3 x 1.3 mm, Tsp=745, BW=160 Hz/pixel)
3D radial imaging (UTE) may be used to image bone for attenuation correction in MR-PET. Motion during acquisition and subsequent position changes may invalidate the attenuation correction map. This method may be extended to fetal imaging.

**Motion correction: Radial imaging (UTE)**

CT

MRI

Motion, no correction

Motion, correction

Motion during a 1 min 18 s radial 3D acquisition

Protocol: TR 2.39 ms, effective echo time TE 50 μs, BW 1002 Hz/px, flip angle 2°, FoV 256 mm, resolution 43 mm3.

System: 3 T Siemens (Erlangen, Germany) Tim Trio, 32 channel head coil.

**MPRAGE with EPI navigators**

EPI navigators inserted every TR of MEMPRAGE capture a "snapshot" of subject’s head and allow real-time tracking/correction, also for T2-SPACE. This work will be presented on Friday.

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M. Dylan Tisdall
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Larry White
Paul Wighton
Motion-Compensated Neuroanatomical Imaging

Dylan Tisdall
April 2013

Motion-compensated MRI sequences allow you to image subjects even if they move, without discarding scans and rescanning.

There are two basic types of motion-compensation:

**Retrospective**
Post-process to estimate data that would have been measured if the subject hadn’t moved.
Examples: PROPELLER, SNAILS

**Prospective**
Track the subject and alter the acquisition “on-the-fly” to account for subject motion.
Examples: PACE, vNavs, PROMO

MPRAGE of subject prompted to change position every 45 seconds

No Motion vs. Motion
Red/Yellow: Thinning, Blue: Thickening with motion
Yellow: 30% Thinning

No Motion vs. Motion Correction: No Acquisition
Red/Yellow: Thinning, Blue: Thickening with motion
Yellow: 30% Thinning
Who should use these sequences? Everyone!

- Our vNav sequences are available now on Siemens scanners (WIP 711).
- Other groups are developing similar techniques on GE scanners (e.g., PROMO).

Overview

- Following the subject: EPI-navigated prospective motion correction
- More motion-resistance: automatic retrospective reacquisition
- Using FreeSurfer for validation: longitudinal, cross-contrast analysis

A single TR

- Inv. pulse
- TI gap
- Readout block
- TR gap
- MPRAGE & T2SPACE FLAIR
- previous TR gap
- Readout block
- T2SPACE

A single TR + EPI Navigator

- Inv. pulse
- EPI Nav 275 ms
- Readout block
- TR gap
- MPRAGE & T2SPACE FLAIR
- EPI Nav 275 ms
- Readout block
- T2SPACE

A single TR + EPI Navigator + Registration and Feedback

- Inv. pulse
- EPI Nav 275 ms
- Reg. 80 ms
- Readout block
- TR gap
- MPRAGE & T2SPACE FLAIR
- EPI Nav 275 ms
- Reg. 80 ms
- Readout block
- = updated imaging coordinates
- T2SPACE

A single TR + EPI Navigator + Registration and Feedback

- Inv. pulse
- EPI Nav 275 ms
- Reg. 80 ms
- Readout block
- TR gap
- MPRAGE & T2SPACE FLAIR
- EPI Nav 275 ms
- Reg. 80 ms
- Readout block
- T2SPACE
The Navigator

- $32^3$ EPI
- 8 mm iso
- 256 mm FOV
- 25 shots
- TE 5.2 ms, TR 11 ms
- $\sim 275$ ms

Register each EPI nav volume back to first TR using Siemens’ PACE registration algorithm.

At 3T, observed variance of 50 microns with stationary subject (a pineapple).
Accuracy estimated to be **better than 300 microns** in real-world examples.

Overview

- Following the subject: EPI-navigated prospective motion correction
- More motion-resistance: automatic retrospective reacquisition
- Using FreeSurfer for validation: longitudinal, cross-contrast analysis
Users configure the number of TRs to reacquire as part of their protocol.

Overview

- Following the subject:
  EPI-navigated prospective motion correction
- More motion-resistance:
  automatic retrospective reacquisition
- Using FreeSurfer for validation:
  longitudinal, cross-contrast analysis
Three non-standard FreeSurfer uses

1. "Longitudinal" analysis of same-subject, same-day, motion-free T1 scans without navigators, with navigators but without motion-correction, and with navigators and motion-correction.

2. Registration of same-subject, same-day, with-motion T1 scans to a fully segmented same-subject, same-day, without-motion T1 scan.

3. Cross-contrast registration of same-subject, same-day, with- and without-motion T2 scans to a fully segmented same-subject, same day without motion T1 scan.

"Longitudinal" analysis of same-subject, same-day, motion-free T1 scans without navigators, with navigators but without motion-correction, and with navigators and motion-correction.

Registration of same-subject, same-day, with-motion T1 scans to a fully segmented same-subject, same-day, without-motion T1 scan.

“Longitudinal" analysis of same-subject, same-day, motion-free T1 scans without navigators, with navigators but without motion-correction, and with navigators and motion-correction.

Registration of same-subject, same-day, with-motion T1 scans to a fully segmented same-subject, same-day, without-motion T1 scan.

now we have voxel-wise equivalence

now we have voxel-wise equivalence
We can use `mri_robust_register` to **extrapolate a segmentation** to a subsequent acquisition.

Cross-contrast registration of same-subject, same-day, with- and without-motion T2 scans to a fully segmented same-subject, same day without motion T1 scan.

Now we have voxel-wise equivalence.

We can use `bbregister` to **extrapolate a segmentation** to a subsequent acquisition with a different contrast.

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Surface-based Group Analysis in FreeSurfer

Outline
• Objectives & Example
• GLM Theory & Linear Algebra Review
• Command-line Stream
  • Assemble Data
  • Design/Contrast
  • Analyze
  • Visualize

Group Analysis Objective
• To create a model that can describe patterns of interactions and associations
• The parameters of the model provide measures of the strength of associations
• A General Linear Model (GLM) focuses on estimating the parameters of the model such that they can be applied to new data sets to create reasonable inferences.

Types of Questions
• Does a specific variable have a significant association with an outcome?
• If we control for the effects of a second variable, is the association still significant?
• Is there a group difference in outcome?
• Does a specific variable affect individual outcome differently between groups of individuals?

Aging Exploratory Analysis
In which areas does thickness change with age?

Aging Thickness Study
N=40 (all in fsaverage space)
Surface-based Measures

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

The General Linear Model (GLM)

GLM Theory

Is Thickness correlated with Age?

Independent Variable
- Thickness
- IQ, Height, Weight, etc.

Dependent Variable, Measurement
- Thickness
- Age

Subject 1
Subject 2

Thickness
Age

Of course, you would need more than two subjects...

Linear Algebra Review (stay calm...)

We can put this in matrix format:

\[ y = mx + b \]

Design Matrix
Regression Coefficients (parameters)

\[ \begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{bmatrix} = \begin{bmatrix} 1 & x_1 \\ 1 & x_2 \\ 1 & x_3 \\ 1 & x_4 \end{bmatrix} \begin{bmatrix} b \\ m \end{bmatrix} \]

System of Linear Equations

\[ y_1 = 1b + x_1m \]
\[ y_2 = 1b + x_2m \]
\[ y_3 = 1b + x_3m \]
\[ y_4 = 1b + x_4m \]

Matrix Multiplication
Linear Model

System of Linear Equations
\[ y_1 = 1*b + x_1*m \]
\[ y_2 = 1*b + x_2*m \]

Matrix Formulation
\[ \begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} 1 \\ 1 \\ 1 \\ 1 \end{bmatrix} \begin{bmatrix} b \\ m \end{bmatrix} \]

- One row per subject
- \( x \) values are independent variable (age)
- Column of 1's is the 'offset' term (to multiply by \( b \))

Intercept: \( b \) (= Offset)
Slope: \( m \)

Thickness
\( X = \) Design Matrix
\( b = \) Regression Coefficients
\( \hat{b} = \) Parameter estimates
\( \beta = \) beta.mgh (mri_glmfit output)

More than Two Data Points

\[ y_1 = 1*b + x_1*m + n_1 \]
\[ y_2 = 1*b + x_2*m + n_2 \]
\[ y_3 = 1*b + x_3*m + n_3 \]
\[ y_4 = 1*b + x_4*m + n_4 \]

\[ Y = X*b + n \]

Forming a Hypothesis
- Now, we can fit our parameters, but we need a hypothesis
- Our example: Is there a significant association between age and thickness?
- Formal Hypothesis: The slope of age v. thickness (\( m \)) is significantly different from zero

Null hypothesis: \( m = 0 \)

Testing Our Hypothesis
- Once we fit our model for the optimal regression coefficients (\( m \) and \( b \)), we need to test them for significance as well as test the direction of the effect
- We do this by forming something called a contrast matrix, that isolates our parameter of interest
- We can multiply our contrast matrix by our regression coefficient matrix to compute a variable \( g \), which tells us the direction of our effect
- In this example, since our hypothesis is about the slope \( m \) we will design our contrast matrix to be \( [0 \ 1] \)

Testing our Hypothesis
- We still need to test for significance
- We'll use our contrast matrix \( [0 \ 1] \) again here in a t-test:

This t-value corresponds to a p-value that depends on your sample size. This p-value is between 0 and 1, values closer to 0 indicate a more significant result.
p-values

**p-value/significance**
- value between 0 and 1
- depends on your sample size
- 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 \(g\)
- p-value is for an unsigned test

Putting it all together

1. We used our empirical data to form a design matrix: \(X\)
2. We fit regression coefficients (\(b\) and \(m\)) to our \(x,y\) data
3. We created a contrast matrix: \(C\) to test our hypothesis for:
   1. Direction of effect: \(g = C^*\beta\)
   2. Significance of effect: t-test

Two Groups

- Do groups differ in Intercept?
- Do groups differ in Slope?
- Is average slope different from 0?
- ...

Two Groups

\[
\begin{aligned}
\text{Thickness} & & \text{Intercept: } b_1 \\
\text{Slope: } m_1 & & \text{Slope: } m_2 \\
\text{Age} & & \text{Intercept: } b_2 \\
\end{aligned}
\]

Do groups differ in Intercept?
\(b_1 = b_2?\)
\(C = [+1\ -1\ 0\ 0], \ g = C^*b\)

Do groups differ in Slope?
\(m_1 = m_2?\)
\(C = [0\ 0\ +1\ -1], \ g = C^*b\)

Is average slope different from 0?
\((m_1 + m_2)/2 = 0?\)
\(C = [0\ 0\ 0.5\ 0.5], \ g = C^*b\)

Surface-based Group Analysis in FreeSurfer

- Create your own design and contrast matrices
- Create an FSGD File
- FreeSurfer creates design matrix
- You still have to specify contrasts
- QDEC
  - Limited to 2 discrete variables, 2 levels max
  - Limited to 2 continuous variables
Processing Stages
- Specify Subjects and Surface measures
- Assemble Data:
  - Resample into Common Space
  - Smooth
  - Concatenate into one file
- Model and Contrasts (GLM)
- Fit Model (Estimate)
- Correct for multiple comparisons
- Visualize

Command-line Processing Stages
- Assemble Data (\texttt{mris_preproc})
  - Resample into Common Space
  - Smooth
  - Concatenate into one file
- Model and Contrasts (GLM) (FSGD)
- Fit Model (Estimate) (\texttt{mri_glmfit})
- Correct for multiple comparisons
- Visualize (\texttt{freeview/ktsher})

Specifying Subjects

\texttt{SUBJECTS_DIR}

\texttt{bert} \hspace{1cm} \texttt{fred} \hspace{1cm} \texttt{jenny} \hspace{1cm} \texttt{margaret} \hspace{1cm} ...

FreeSurfer Directory Tree

\texttt{SUBJECTS_DIR}

\texttt{bert} \hspace{1cm} \texttt{fred} \hspace{1cm} \texttt{jenny} \hspace{1cm} \texttt{margaret} \hspace{1cm} ...

Example: Thickness Study

1. \texttt{$SUBJECTS_DIR/bert/surf/lh.thickness}
2. \texttt{$SUBJECTS_DIR/fred/surf/lh.thickness}
3. \texttt{$SUBJECTS_DIR/jenny/surf/lh.thickness}
4. \texttt{$SUBJECTS_DIR/margaret/surf/lh.thickness}
5. ...

FreeSurfer Group Descriptor (FSGD) File

- Simple text file
- List of all subjects in the study
- Accompanying demographics
- Automatic design matrix creation
- You must still specify the contrast matrices

Note: Can specify design matrix explicitly with \texttt{--design}
FSGD Format

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

Class = Group

Note: Can specify design matrix explicitly with --design

FSGDF \rightarrow X (Automatic)

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

Contrasts – You create

\[ C = \begin{bmatrix} 1 & 0 \end{bmatrix} \]
Tests for the difference in intercept/offset between groups

\[ C = \begin{bmatrix} 0 & 1 \end{bmatrix} \]
Tests for the difference in age slope between groups

Interaction Contrast

- Two Discrete Factors (no continuous, for now)
  - Gender: Two Levels (M&F)
  - Handedness: Two Levels (L&R)
- Four Regressors (Offsets)
  - MR (b_1), ML (b_2), FR (b_3), FL (b_4)

\[ g = D_1 \cdot D_2 = 0 \]
\[ g = (b_3 \cdot b_4) - (b_2 \cdot b_5) \]
\[ = -b_1 + b_2 + b_3 - b_4 \]
\[ C = \begin{bmatrix} 1 & 1 & 1 & 1 \end{bmatrix} \]

Factors, Levels, Groups

Usually each Group/Class:
- Has its own Intercept
- Has its own Slope (for each continuous variable)
- NRegressors = NClasses * (NVariables+1) \( \text{DODS} \)
- NRegressors = NClasses + NVariables \( \text{DOSS} \)

Why is this important? Because you will need to create contrast matrices, and the contrast matrix must have NRegressors elements.
Factors, Levels, Groups, Classes

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

Discrete Variables/Factors:
- Gender, Handedness, Diagnosis

Levels of Discrete Variables:
- Handedness: Left and Right
- Gender: Male and Female
- Diagnosis: Normal, MCI, AD

Group or Class: Specification of All Discrete Factors
- Left-handed Male MCI
- Right-handed Female Normal

Assemble Data: mris_preproc

mris_preproc --help

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

Lots of other options!

- Output: lh.thickness.mgh – file with stacked thickness maps for all subjects
- Input to Smoother or GLM

Surface Smoothing

- mri_surf2surf --help
- Loads stacked lh.thickness.mgh
- 2D surface-based smoothing
- Specify FWHM (eg, fwhm = 10 mm)
- Saves stacked lh.thickness.sm10.mgh
- recon-all -qcache (computes for each subject, run after you are finished editing subject)

mri_glmfit

- Reads in FSGD File and constructs X
- Reads in your contrasts (C1, C2, etc.)
- Loads data (lh.thickness.sm10.mgh)
- Fits GLM (ie, computes b)
- Computes contrasts (g=C*b)
- t or F ratios, significances
- Significance -log10(p) (.01 → 2, .001 → 3)

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

- 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
--fgd 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

- Contrast Matrices
- Simple text/ASCII files
- Test hypotheses
- You must create these by hand!

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

Output directory: lh.gender_age.glmdir/
beta.mgh – parameter estimates
rvar.mgh – residual error variance etc ...
sig.mgh – log10(p), uncorrected
gamma.mgh, F.mgh
gender/
sig.mgh – log10(p), uncorrected
gamma.mgh, F.mgh

glmfit
--y lh.thickness.sm10.mgh
--fgd gender_age.txt
--C age.mtx –C gender.mtx
--surf fsaverage lh
--cortex
--glmdir lh.gender_age.glmdir

GLM Analysis Using Aseg/Aparc Stats Files

mri_glmfit
--table aparc lh_vol_stats.txt
--fgd gender_age.txt
--C age.mtx –C gender.mtx
--glmdir roi.gender_age.glmdir

- Use "-table table.txt" instead of "--y" to specify input
- The rest of the command-line is the same as you would use for a group study (eg, FSGD file and contrasts).
- Output is text file sig.table.dat that lists the significances (-log10(p)) for each ROI and contrast.

Visualization with freeview

freeview -f $FREESURFER_HOME/subjects/fsaverage/surf/
lh.pial:overlay=sig.mgh

Use "Configure Overlay" tool to change thresholds for visualization (recall lower threshold of 1.3 will only display regions where p<0.05)
Tutorial

Command-line Stream

- Create an FSGD File and contrasts for a thickness study
- Age and Gender
- Run
  - mris_preproc
  - mri_surf2surf
  - mri_glmfit
Correction for multiple comparisons in FreeSurfer

**Problem of Multiple Comparisons**

- Threshold too liberal: many false positives
- Threshold too restrictive: lose activation (false negatives)

**Clusters**

- True signal tends to be clustered
- False Positives tend to be randomly distributed in space
- Cluster – set of spatially contiguous voxels that are above a given threshold.

**Cluster Table, Uncorrected**

<table>
<thead>
<tr>
<th>ClusterNo</th>
<th>Area(mm$^2$)</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>3738.82</td>
<td>-11.1</td>
<td>34.5</td>
<td>27.2</td>
<td>superiorfrontal</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>5194.19</td>
<td>-32.4</td>
<td>-23.3</td>
<td>15.7</td>
<td>insula</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>1271.30</td>
<td>-25.9</td>
<td>-75.0</td>
<td>19.0</td>
<td>superiorparietal</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>775.38</td>
<td>-44.4</td>
<td>-9.7</td>
<td>51.3</td>
<td>precentral</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>440.56</td>
<td>-33.0</td>
<td>-36.8</td>
<td>37.5</td>
<td>supramarginal</td>
</tr>
</tbody>
</table>

How likely is it to get a cluster of a certain size under the null hypothesis?

**Clusterwise Correction**

How likely is it to get a cluster 440.56mm$^2$ or bigger by chance?

How likely is it to get a cluster of a certain size under the null hypothesis?
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.)
2. Analyze, threshold, cluster, get MaxClusterSizeNull
3. Repeat 10,000 times – gives a list of 10000 MaxClusterSizeNulls under the null
4. Analyze real data, get ClusterSize (eg, 440.56 mm²)
5. Count number of times MaxClusterSizeNull > ClusterSize
   \[ P(\text{cluster}) = \#(\text{MaxClusterSizeNull} > \text{ClusterSize}) / 10000 \]

Cluster Table, Corrected

<table>
<thead>
<tr>
<th>Cluster No</th>
<th>Area (mm²)</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Structure</th>
<th>Cluster P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>3738.82</td>
<td>-11.1</td>
<td>34.5</td>
<td>27.2</td>
<td>superiorfrontal</td>
<td>.0001</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>5194.19</td>
<td>-32.4</td>
<td>-23.3</td>
<td>15.7</td>
<td>insula</td>
<td>.0003</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>1271.30</td>
<td>-25.9</td>
<td>-75.0</td>
<td>19.0</td>
<td>superiorparietal</td>
<td>.0050</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>775.38</td>
<td>-44.4</td>
<td>-9.7</td>
<td>51.3</td>
<td>precentral</td>
<td>.0100</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>440.56</td>
<td>-33.0</td>
<td>-36.8</td>
<td>37.5</td>
<td>supramarginal</td>
<td>.0400</td>
</tr>
</tbody>
</table>

22 clusters out of 38 have cluster p-value < .05

Note the difference between the Cluster Forming Threshold (p<.0001) and the Clusterwise p-value (.05).

Surface-based Correction for Multiple Comparisons

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

Original mri_glmfit command:

```bash
mri_glmfit
--y lh.thickness.sm10.mgh
--fsgd gender_age.txt
--C age.mtx –C gender.mtx
--surf Average lh
--glmdir lh.gender_age.glmdir
lh.gender_age.glmdir/
beta.mgh – parameter estimates
rvar.mgh – residual error variance
age/ sig.mgh – log10(p), uncorrected
gender/ sig.mgh – log10(p), uncorrected
gender_age.glmdir/ sig.mgh – log10(p), uncorrected
gender_age.glmdir/ beta.mgh – parameter estimates
```

• Use pre-computed simulation results
• positive contrast
• voxelwise threshold thres = 2 (p<.01)
• Can do another simulation or permutation
• Not related to recon-all -qcache

Surface-based Correction for Multiple Comparisons

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

```bash
mri_glmfit-sim
--glmdir lh.gender_age.glmdir
--cache 2 pos
--2spaces
--cwpvalthresh .05
```
Surface-based Correction for Multiple Comparisons

• 2D Cluster-based Correction at p < .05
  mri_glmfit-sim
  --glmdir lh.gender_age.glmdir
  --cache 2 pos
  --cwpvalthresh .05
  --2spaces

Cluster-wise threshold p<.05
cw = cluster-wise
pval = p value
thresh = threshold
Doing analysis with left hemi but right hemi will be done separately. Need to correct for full search space.

Correction for Multiple Comparisons Output

mri_glmfit-sim
--glmdir lh.gender_age.glmdir
--cache 2 pos
--cwpvalthresh .05
--2spaces

sig.mgh – pre-existing uncorrected p-values
cache.th20.pos.sig.cluster.mgh – map of significance of clusters
cache.th20.pos.sig.occ.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<.05, change –cwpvalthresh to a larger value to get more (ie, less sig) clusters

Corrected Outputs

cache.th20.pos.sig.occ.annot – annotation of significant clusters

Corrected Outputs

Output files are the same (prepended with “perm.”)

Eklund, et al. 2016 PNAS

• High false positive rates using resting state fMRI analyzed as task data
• Caused by non-Gaussian smoothness
• Applies to parametric (random field theory) and Monte Carlo simulations
• Effect present in thickness analysis, but not as bad

Example command:
mri_glmfit-sim -perm 10000 2 pos

Permutation

mri_glmfit-sim -perm <nsim> <vthresh> <sign>

• If there is no effect of group, then group membership can be randomly changed.
• Repeat this many times to get NULL distribution
• Makes no assumptions about smoothness or Gaussianity of the data.
• Requires designs without nuisance vars (age)

Example command:
mri_glmfit-sim -perm 10000 2 pos

Output files are the same (prepended with “perm.”)
False Discover Correction Possible

- False Discovery Rate (FDR)
- Built into tksurfer & QDEC (Genovese, et al, NI 2002)
- mri_fdr --help

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 – can be huge!

<table>
<thead>
<tr>
<th>Id</th>
<th>Subject</th>
<th>Gender</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Left-Cerebral-White-Matter-Vol</th>
</tr>
</thead>
<tbody>
<tr>
<td>011121_vx8048</td>
<td>Female</td>
<td>70</td>
<td>Demented</td>
<td>202291</td>
<td></td>
</tr>
<tr>
<td>011121_62313</td>
<td>Female</td>
<td>71</td>
<td>Demented</td>
<td>230198</td>
<td></td>
</tr>
<tr>
<td>016067_vx7607</td>
<td>Female</td>
<td>73</td>
<td>Demented</td>
<td>170653</td>
<td></td>
</tr>
<tr>
<td>021121_vx1557</td>
<td>Male</td>
<td>75</td>
<td>Demented</td>
<td>142029</td>
<td></td>
</tr>
<tr>
<td>020718_62545</td>
<td>Male</td>
<td>76</td>
<td>Demented</td>
<td>186087</td>
<td></td>
</tr>
<tr>
<td>020322_vx817</td>
<td>Male</td>
<td>77</td>
<td>Non-demented</td>
<td>149810</td>
<td></td>
</tr>
</tbody>
</table>

QDEC GUI

- Load QDEC Table File
- List of Subjects
- List of Factors (Discrete and Cont)
- Choose Factors
- Choose Input (cached):
  - Hemisphere
  - Measure (eg, thickness)
  - Smoothing Level
- "Analyze":
  - Builds Design Matrix
  - Builds Contrast Matrices
  - Constructs Human-Readable Questions
  - Analyzes
  - Displays Results

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

2. QDEC – same data set

-qcache

For QDEC to work interactively, you need to run:

```
recon-all -s <sid> -qcache
```

(or as additional flag in your regular processing)

This will map and smooth thickness maps to fsaverage, use --target <id> to specify your own target and --measure <surfmeas> to specify curv, area, sulc etc.
What can we do with FreeSurfer?

- measure volume of cortical or subcortical structures
- compute thickness (locally) of the cortical sheet
- study differences of populations (diseased, control)

Neurodegenerative disease:

14 time points, 6 years, Huntington’s Disease

We’d like to:

- exploit longitudinal information (same subject, different time points)
- to reduce variability on intra-individual morph estimates
- to detect small changes, or use less subjects (power)
- for marker of disease progression (atrophy)
- to better estimate time to onset of symptoms
- to study effects of drug treatment

Why longitudinal?

Example 1

Example 2

...
Challenges in Longitudinal Designs

1. Over-Regularization:
   - Temporal smoothing
   - Non-linear warps
   - Potentially underestimating change

2. Bias
   - Interpolation Asymmetries \cite{Yushkevich2010}
   - Asymmetric Information Transfer
   - Often overestimating change

3. Limited designs:
   - Only 2 time points
   - Special purposes (e.g. only surfaces, WM/GM)

How can it be done?

- Stay \textit{unbiased} with respect to any specific time point by treating all the same
- Create a within subject template (base) as an \textit{initial guess} for segmentation and reconstruction
- Initialize each time point with the template to reduce variability in the optimization process
- For this we need a \textbf{robust registration} (rigid) and \textbf{template estimation}

Robust Registration

\textbf{Goal}: Highly accurate inverse consistent registrations

- In the \textit{presence} of:
  - Noise
  - Gradient non-linearities
  - Movement: jaw, tongue, neck, eye, scalp ...
  - Cropping
  - Atrophy (or other longitudinal change)

\textbf{We need}:
- \textit{Inverse consistency} keep registration \textit{unbiased}
- \textit{Robust statistics} to reduce influence of outliers

\textbf{Robust Registration}

- \textit{Limited contribution of outliers} \cite{NestaresHeeger2000}

Registered Src FSL FLIRT
Registered Src Robust

\textbf{Robust Registration}

\textbf{Robust Registration}

\begin{tabular}{c c}
\textbf{Square} & \textbf{Tukey’s Biweight} \\
\end{tabular}
Robust Registration

Image 1: Tumor data with significant intensity differences in the brain, registered to first time point (left).

Inverse consistency:
- a symmetric displacement model:
  \[ r(p) = \frac{1}{2} \left( \| \hat{T} \|_2^2 + \| R - 1 \|_F^2 \right) \]
- resample both source and target to an unbiased half-way space in intermediate steps (matrix square root)

Robust Registration

Inversion consistency of different methods on original (orig), intensity normalized (T1) and skull stripped (norm) images.
- LS and Robust: nearly perfect symmetry (worst case RMS < 0.02)
- Other methods: several alignments with RMS errors > 0.1

Robust Template Estimation

- Minimization problem for N images:
  \( \{ \hat{I}, \hat{\phi}_i \} := \arg \min_{I, \phi_i} \sum_{i=1}^{N} E(\hat{I} \circ \phi_i, I) + D(\phi_i)^2 \)
- Image Dissimilarity:
  \( E(\hat{I}, I) = \int_{\Omega} |I(x) - \hat{I}(x)| \ dx \)
- Metric of Transformations:
  \( D(\hat{T}, r)^2 = \| \hat{T} \|_2^2 + \| R - 1 \|_F^2 \)

Challenges

1. Over-regularization (limited flexibility):
   - Will avoid by only initializing processing
2. Bias (Reuter and Fischl 2011), (Reuter et al. 2012)
   - Will avoid by treating time points the same
3. Limited designs:
   - Allow n time points
   - Reliably estimate all of FS measurements

mri_robust_register is part of FreeSurfer
- can be used for pair-wise registration (optimally within subject, within modality)
- can output results in half-way space
- can output ‘outlier-weights’
- see also Reuter et al., NeuroImage 2010 for comparison with FLIRT (FSL) and SPM coreg
- for more than 2 images use: mri_robust_template
(i) Interpolation Asymmetries (Bias)

Mapping follow-up to baseline:
• Keeps baseline image fixed (crisp)
• Causes interpolation artefacts in follow-up (smoothing)
• Often leads to overestimating change

MIRIAD dataset: 65 subjects
First session first scan compared to twice interpolated image.
Regional: not finding it does not mean it is not there.

http://miriad.drc.imi.ucl.ac.uk
(ii) Asymmetric Information Transfer

Example:
1. Process baseline
2. Transfer results from baseline to follow-up
3. Let procedures evolve in follow-up
   (or construct skullstrip in baseline, or Talairach transform ...)
   Can introduce bias!

Robust Unbiased Subject Template

1. Create subject template (iterative registration to median)
2. Process template
3. Transfer to time points
4. Let it evolve there
   · All time points are treated the same
   · Minimize over-regularization by letting tps evolve freely

Review the central ideas

Idea: Would like to include some information that much of the anatomy is the same over time, but don’t want to lose sensitivity to disease effects.

How to minimize over-regularization:
   ✓ Only initialize processing, evolve freely

How to avoid processing bias:
   ✓ Treat all time points the same

Why not simply do independent processing then?
   ➢ Sharing information across time points increases reliability, statistical power!

Improved Surface Placement

Test-Retest Reliability

[LONG] significantly improves reliability
115 subjects, MEMPRAGE, 2 scans, same session
**Test-Retest Reliability**

- Diff. ([CROSS]:[LONG])
- of Abs. Thick. Change:
- Significance Map
- [LONG] significantly improves reliability
- 115 subjects, ME MPRAGE, 2 scans, same session

**Increased Power**

- Sample Size Reduction when using [LONG]
- (based on test-retest 14 subjects, 2 weeks)

**Huntington’s Disease (3 visits)**

- (with D. Rosas)
- Independent Processing
- Longitudinal Processing
- [LONG] shows higher precision and better discrimination power between groups (specificity and sensitivity).

**Huntington’s Disease (3 visits)**

- (with D. Rosas)
- Rate of Atrophy
- Baseline Vol. (normalized)
- Putamen Atrophy Rate can is significantly different between CN and PHD far, but baseline volume is not.

**Robust Template for Initialization**

- • Unbiased
- • Reduces Variability
- • Common space for:
  - TIV estimation
  - Skullstrip
  - Affine Talairach Registration
- • Basis for:
  - Intensity Normalization
  - Non-linear Registration
  - Surfaces / Parcellation

**FreeSurfer Commands (recon-all)**

1. **CROSS** (independently for each time point tpNid):
   ```bash
   recon-all -subjid tpNid -all
   ```
2. **BASE** (creates template, one for each subject):
   ```bash
   recon-all -base baseid -tp tp1id \ -tp tp2id ... -all
   ```
3. **LONG** (for each time point tpNid, passing baseid):
   ```bash
   recon-all -long tpNid baseid -all
   ```

This creates the final directories tpNid.long.baseid
Directory Structure

Contains all CROSS, BASE and LONG data:

• me1
• me2
• me3
• me_base
• me1.long.me_base
• me2.long.me_base
• me3.long.me_base
• you1
• ...

Single time point

Since FS5.2 you can run subjects with a single time point through the longitudinal stream!

• Mixed effects models can use single time point subjects to estimate variance (increased power)
• This assures identical processing steps as in a subject with several time points
• Commands same as above:
  
  + recon-all -subjid tp1id -all
  + recon-all -base baseid -tp tp1id -all
  + recon-all -long tp1id baseid -all

Final Remarks ...

Sources of Bias during Acquisition

BAD: influence images directly and cannot be easily removed!

• Different Scanner Hardware (Headcoil, Pillow?)
• Different Scanner Software (Shimming Algorithm)
• Scanner Drift and Calibration
• Different Motion Levels Across Groups
• Different Hydration Levels (season, time of day)

Hydration Levels

14 subjects, 12h dehydration (over night)

rehydration 1L/h


Motion Biases GM Estimates

• 12 volunteers
• 5 motion types:
  • Still
  • Nod
  • Shake
  • Free
• Duration:
  • 5-15 s/min

Effect:
roughly 0.7-1% volume loss per 1mm/min increase in motion

Reuter, et al., Neuroimage 2014
Still to come …

- Common warps (non-linear)
- Optimized intracranial volume estimation
- Joint intensity normalization
- New thickness computation
- Joint spherical registration

http://freesurfer.net/fswiki/LongitudinalProcessing

Longitudinal Tutorial

1. How to process longitudinal data
   - Three stages: CROSS, BASE, LONG

2. Post-processing (statistical analysis):
   - (i) compute atrophy rate within each subject
   - (ii) group analysis (average rates, compare)
   - here: two time points, rate or percent change

3. Manual Edits
   - Start in CROSS, do BASE, then LONGs should be fixed automatically
   - Often it is enough to just edit the BASE
   - See http://freesurfer.net/fswiki/LongitudinalEdits

Longitudinal Tutorial

- Temporal Average
- Rate of Change
- Percent Change (w.r.t. time 1)
- Symmetrized Percent Change (w.r.t. temp. avg.)
FreeSurfer: Troubleshooting
surfer.nmr.mgh.harvard.edu

Hard and Soft Failures

Categories of errors: Hard & Soft Failures

• Hard = recon-all quits before it finishes
• Soft = recon-all finishes but results need modification
  (i.e. surface or segmentation inaccuracy)

- recon-all takes a long time (3-20 hours) to run & some part of the process may need modification
  (e.g. cerebellum removed in skull stripping)

Troubleshooting: Soft Failures

• Some Examples of soft failures:
  – Skull Strip Errors
  – WM/ASEG Segmentation Errors
  – Intensity Normalization Errors
  – Pial Surface misplacement
  – Topological Defect incorrectly fixed

Upon Completion of recon-all…

scripts   mri      surf     label     stats
recon-all.log
recon-all.done

Just because it finishes "without error" does not mean that everything is ok!
Could be a "soft" failure.

Troubleshooting: Hard Failures

bert

scripts   mri      surf     label     stats
recon-all.log
recon-all.error

• Ran out of disk space?
• Ran out of RAM?
• Unix file permissions?
• Pathological conditions (brain, artifact)
• Sunspots???????

Hard Failure: What to do

• Check recon-all.log for error message
• Examine data quality
• Rerun step that failed
• Verify output from last successful step
• Search FreeSurfer mailing list for this problem
• Run modified version of command if needed
• Email the mailing list

Send us recon-all.log
freesurfer@nmr.mgh.harvard.edu
Hard Failure: Help Us Help You!

- Report version currently using
  - see top of recon-all.log
  - cat $FREESURFER_HOME/build-stamp.txt
- Operating System/hardware
- Exact command-line tried to run
- Send recon-all.log
- Output from terminal window if appropriate

(Nick even has a command to help – bugr)

Soft Failures

- recon-all finishes but surfaces or aseg not accurate
- It is not possible to directly edit the location of a surface.
- When the surfaces are inaccurate, you have to (manually) change the information in a volume and regenerate the surface.

Check Your Recon for Accuracy

- Do your surfaces follow gm/wm borders?
- Does the subcortical segmentation follow intensity boundaries?

Unfortunately we almost never have access to ground truth in imaging.

(editorial note: ALL morphometry packages make errors. FS allows you to correct these errors. This feature is not available in other packages.)

Manual Interventions

1. Erase voxels
2. Fill voxels
3. Clone voxels (ie, copy from one volume to another)
4. Add “Control Points”

Manual interventions should take less than 30min
After manual intervention, re-run parts of recon-all

It is also possible to re-run recon-all with different parameters which is good for systematic or large errors

Processing Stream Overview

T1 Weighted Input → Skull Stripping → Volumetric Labeling → Intensity Normalization

Gyral Labeling → Surface Extraction → Surface Atlas Registration → White Matter Segmentation

Stats!
Reconstruction Stages

recon-all is broken into three stages

– autorecon1
– autorecon2
– autorecon3

these 3 stages are equivalent to -all

Processing Stream Overview

Processing Stream Order

http://surfer.nmr.mgh.harvard.edu/fswiki/ReconAllDevTable

Or Make Life Easier

recon-all -make all -s subj

Skull Strip Failure: Too Much Removed

Use “clone” tool to manually correct, or adjust watershed parameters and run (default wthresh is 25, higher means strip less):

recon-all -skullstrip -wthresh 35 -clean-bm -no-wsgcaatlas -s <subj>
recon-all -s <subject> -autorecon2 -autorecon3

Skull Strip Failure: Not enough Removed

Dura and GM have extremely similar intensity characteristics on most T1-weighted sequences (but different T2*!). Typical fix: edit the brainmask.mgz to erase dura/blood vessels, and run:

recon-all -s <subject> -autorecon-pial
Eye Socket classified as WM due to Skull Strip Failure. Erase in wm.mgz then run:
recon-all -s <subject> -autorecon2-wm -autorecon3

This is NOT a Skull Strip Error
It appears that the skull strip left a lot of dura. It did, but it does not affect the surface, so leave it!

Skull Strip Failure: Not Enough Removed

Segmentation Errors
- White Matter classified as non-White Matter
- Gray Matter classified as White Matter
- Causes:
  - Intensity Normalization Failures
  - Partial voluming

Even if it does not affect the surface, leave it!

Segmentation Error
“Hypo-Intensities”
White Matter Lesions Misclassified as gray matter
Fill in wm.mgz then run:
recon-all -s <subject> -autorecon2-wm -autorecon3

This is NOT an error.
Make sure to look at all 3 views before deciding!
**Intensity Bias**

- One side of the image much brighter than the other side
- Worse with many coils
- Makes gray/white segmentation difficult

**Intensity Normalization**

- Removes B1 bias field
- NU (MNI) nu.mgz
- Presegmentation (T1.mgz)
  - Most WM = 110 intensity
  - Pre- and Post-Skull Strip

**Troubleshooting: Intensity Normalization**

Intensity Normalization Failure. Most WM in T1 volume (T1.mgz) should be close to 110. Can fix by editing wm.mgz or adding “Control Points” (+). Beware partial voluming!

```
recon-all -s <subject> -autorecon2-cp -autorecon3
```

**Control Points: Summary**

- Used to rescale intensity near the control point
- Must go in voxels that are fully WM but not 110 !!!
- Use sparingly
- Can be created viewing any volume
- Saved in a separate text file (e.g., bert/tmp/control.dat)

**Segmentation Errors: Topological Defects**

- Holes, Handles
- Automatically Fixed
- Not always fixed correctly
- Edit wm.mgz

**Topology Correction**

BEFORE

AFTER
Segmentation Errors: Topological Defects

- **Hole**: Partial Voluming: WM + GM looks like non-WM, it is segmented as non-WM and creates a hole.
- **Handle**: Something bright in a sulcus that gets classified as WM.

**Holes**: fill voxels in the wm.mgz  
**Handles**: erase voxels in the wm.mgz

Noncortical Regions: These are not errors

Amygdala, Putamen, Hippocampus, Caudate, Ventricles, CC

Noncortical Regions: These are not errors

These are NOT errors

It appears that the aseg cortical ribbon is inaccurate. It is, but the aseg cortical ribbon is not used for anything!

Surfaces are not valid in subcortical regions along the medial wall.

It is possible to edit the segmentation.

How Do You Know What to Edit?

- If pial surface includes too much:
  - edit brainmask.mgz

- If it affects the white surface (too much/little) or if pial surface includes too little:
  - edit the wm.mgz *(if segmentation error)*
  - add control points *(if normalization error)*

Pial surf grows from white surf

Errors in pial surface placement are typically caused by underlying errors in the white surface placement, and can be corrected by interventions that fix the white surface.
Which Volumes to Edit & When…

<table>
<thead>
<tr>
<th>Volume</th>
<th>Non-gm in pial surf</th>
<th>Non-wm in white surf</th>
<th>wm excluded from surf &amp; intensity &lt; 110</th>
<th>wm excluded from surf &amp; intensity &gt; 110</th>
<th>Cerebellum in pial surf</th>
</tr>
</thead>
<tbody>
<tr>
<td>brainmask.mgz</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>brain.finalsurfs.mgz</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wm.mgz</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>add control points</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

FreeSurfer Remembers!

- When edits are made, the changes are saved in a way that indicates manual changes were made (e.g. voxels that are erased are set to 1, not 0, so we can detect them)
- Re-running with a new version preserves these changes.
- To rerun without edits, use -clean flags or start from scratch

Summary

- Hard Errors (recon-all.log file)
- Soft Errors – surfaces not accurate
- Change volumes, regenerate surfaces
  - Manual touch ups (erase, fill, clone, control points)
  - Volumes: brainmask.mgz, wm.mgz
- Skull strip: too much, not enough
- Segmentation: WM classified as non-WM, or reverse
- FreeSurfer keeps track of edits
- Look at all 3 views and scroll back and forth a few slices

- Should take less than 30 min (or even 15min)
- If you don’t know, leave it alone

Troubleshooting – Advice (Bruce)

- Always look at the data in multiple views and scroll back and forth a few slices – 3D structure is difficult to discern!
- If large regions of white matter are significantly darker than 110 (the target white matter intensity for normalization) then try adding control points, but make sure they are in the interior of the white matter.
- If the ‘h.orig surface misses white matter that is accurately labeled in the wm.mgz or extends into regions where there is no wm in the wm.mgz, then there is an incorrectly fixed topological defect.
- Even one or two missing voxels can cause large-scale defects, so very minor editing (e.g. filling in white matter voxels that are holes, or erasing handles) may fix the problem.
- Don’t edit too much! This will reduce reliability and is almost never needed. Usually this means you need to start over as you’ve done something wrong (e.g. put control points in the wrong place).

Troubleshooting – Advice (Allison)

FLOW

AMBIGUITY
- Edit consistently within and across subjects.

CONSISTENCY

SPEED
- You will get faster with time; certain sections go faster.
Multimodal Integration

Outline
- Spatial Transformation
- Motion Correction
- Registration, Automatic and Manual
- MultiModal Integration
  - DTI Integration
  - fMRI Integration
  - Viewing on Volume and Surface
  - ROI analyses
  - Surface-based group analysis

Spatial Transformations
Anatomical (1x1x1.1mm, 256x256x128, Sag)

Scanner Acquisition
fMRI/DTI/PET (3x3x5mm, 64x64x30, Axial)

Conformed Anatomical Space 1x1x1mm, 256x256x256, Cor

Spatial Transformations

fMRI/DTI/PET
Have Multiple Frames/Time Points

Movement!
Motion Correction

- Adjust translation and rotation of input time point to reduce absolute difference.

Motion Correction

- Motion correction reduces motion
- All frames/time points should be in alignment
- Not perfect

fMRI/DTI/PET “Reference”

- Functional Template
- Template + fMRI Map

Usually template/reference/target used for motion correction

Registration

Note: Registering the reference functional volume to the anatomical volume is sufficient to register the reference to the surface.

FreeSurfer Registration

- Anatomical and Reference Volume

FreeSurfer Subject:
- Specific
  - Volumes
  - Surfaces
  - Thickness
  - ROIs

Reference/Template Volume:
- In voxel-for-voxel registration with parameter map
- Best gray-white contrast

Automatic Registration

- Command name
  - bbregister
  - --s bert
  - --mov mmtemplate.nii
  - --bold
  - --init-fsl
  - --lta register.lta
- FreeSurfer subject name
- Multimodal template volume
- Multimodal contrast
- Initialize with FSL-FLIRT
- Output registration file

• BB = Boundary-based
• Registers reference/template to conformed anatomical of given subject (bert)
• Registration is initialized with FSL-FLIRT, (or --init-spm, --init-header)
• 6 DOF, runtime about 5 min

FreeSurfer Registration Matrix

- Simple text file
- Default format: .lta (still supporting .dat)
- 4x4 Matrix to encode the transformation
- As many as 12 DOF (usually 6 = rigid)
- Also source / target file information
- Coordinate system not easy to explain

Manual Registration

- Rigid = 6 DOF = No stretching
- Use CSF to get a sense of where the folds are
- Avoid using ventricles
- Avoid using B0 distortion regions
- Warning about "edge" of the brain
- Same Subject, Left-Right Flips

Freeview — template.nii

Save As

button to save the registration matrix

LTA Transform File

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<th>Volume</th>
<th>Type of transform</th>
<th>Matrix</th>
<th>Subject volume information</th>
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Manual Registration

- Turn the orig volume on/off or change opacity of top volume to see current quality of alignment
- Select volume to move, then "Tools" and "Transform Volume"
- Explore the Translate and Rotate tabs
- To restart the process, use "Restore to Original"
- Use the "Save Reg" button to save the registration matrix
- Use the "Save As" button to save the resampled volume in the new coordinate system (will also save a registration file automatically)
- Default registration matrix file format: .lta
## Command-line Tools

### Automatic Registration:
- `bbregister --help`
- `fslregister --help`
- `spmregister --help`
- `reg-feat2anat --help`

### Manual Registration:
- `freeview --help`

### Transformations:
- `mri_vol2surf --help`
- `mri_vol2vol --help`
- `mri_label2vol --help`
- `mri_surf2vol --help`

### FreeSurfer Scripts
A Non-Physicist’s Intro to Diffusion MRI

Dylan Tisdall
Water molecules diffuse (move) inside of all tissues.

At 37 °C, water has a diffusion rate of $3 \times 10^{-3}$ mm²/s.

We expect a displacement of about 17 µm in 50 ms.
Diffusion

no gradient

Diffusion

opposite gradient

stationary = re-focused

diffused = not re-focused

signal has cancelled out because of diffusion parallel to the gradients

Diffusion

opposite gradient

Diffusion

opposite gradient

Diffusion

no gradient

Diffusion

opposite gradient

Diffusion

stationary = re-focused
What happens with diffusion perpendicular to the gradients?
Diffusion imaging uses gradients to cancel out signal in water that moves in one direction.

Diffusion imaging uses gradients to cancel out signal in water that moves in one direction.

Diffusion imaging uses gradients to cancel out signal in water that moves in one direction.

Diffusion imaging uses gradients to cancel out signal in water that moves in one direction.

Diffusion imaging uses gradients to cancel out signal in water that moves in one direction.

Diffusion imaging uses gradients to cancel out signal in water that moves in one direction.
Diffusion imaging uses gradients to cancel out signal in water that moves in one direction.

Repeating the experiment, each time using gradient in a different direction, creates a map of how freely water diffuses in each voxel.

questions?
Introduction to diffusion MRI

Anastasia Yendiki
HMS/MGH/MIT Athinoula A. Martinos Center for Biomedical Imaging

White-matter imaging

- Axons measure ~ 1 μm in width
- They group together in bundles that traverse the white matter
- We cannot image individual axons but we can image bundles with diffusion MRI
- Useful in studying neurodegenerative diseases, stroke, aging, development...

From Gray's Anatomy: IX. Neurology
From the National Institute on Aging

Diffusion in brain tissue

- Differentiate between tissues based on the diffusion (random motion) of water molecules within them
- Gray matter: Diffusion is unrestricted → isotropic
- White matter: Diffusion is restricted → anisotropic

Diffusion MRI

- Magnetic resonance imaging can provide "diffusion encoding"
- Magnetic field strength is varied by gradients in different directions
- Image intensity is attenuated depending on water diffusion in each direction
- Compare with baseline images to infer on diffusion process

How to represent diffusion

- At every voxel we want to know:
  - Is this in white matter?
  - If yes, what pathway(s) is it part of?
    - What is the orientation of diffusion?
    - What is the magnitude of diffusion?
  - A grayscale image cannot capture all this!

Tensors

- One way to express the notion of direction is a tensor D
- A tensor is a 3x3 symmetric, positive-definite matrix:
  \[
  D = \begin{bmatrix}
  d_{11} & d_{12} & d_{13} \\
  d_{12} & d_{22} & d_{23} \\
  d_{13} & d_{23} & d_{33}
  \end{bmatrix}
  \]
- D is symmetric 3x3 → It has 6 unique elements
- Suffices to estimate the upper (lower) triangular part
Introduction to diffusion MRI

**Eigenvalues & eigenvectors**

- The matrix $D$ is positive-definite $\Rightarrow$
  - It has 3 real, positive eigenvalues $\lambda_1, \lambda_2, \lambda_3 > 0$.
  - It has 3 orthogonal eigenvectors $e_1, e_2, e_3$.

$$D = \lambda_1 e_1 \cdot e_1' + \lambda_2 e_2 \cdot e_2' + \lambda_3 e_3 \cdot e_3'$$

**Physical interpretation**

- Eigenvectors express diffusion direction
- Eigenvalues express diffusion magnitude

**Physical interpretation**

- Isotropic diffusion: $\lambda_1 \approx \lambda_2 \approx \lambda_3$
- Anisotropic diffusion: $\lambda_1 \gg \lambda_2 = \lambda_3$

One such ellipsoid at each voxel: Likelihood of water molecule displacements at that voxel

**Diffusion tensor imaging (DTI)**

- Image: An intensity value at each voxel
- Tensor map: A tensor at each voxel

**Diffusion tensor imaging (DTI)**

- Image: An intensity value at each voxel
- Tensor map: A tensor at each voxel

**Summary measures**

- Mean diffusivity (MD): Mean of the 3 eigenvalues
  $$\text{MD}(j) = \frac{(\lambda_1(j) + \lambda_2(j) + \lambda_3(j))}{3}$$

- Fractional anisotropy (FA): Variance of the 3 eigenvalues, normalized so that $0 \leq \text{FA} < 1$
  $$\text{FA}(j) = \frac{3}{2} \left( \frac{[\lambda_1(j) - \text{MD}(j)]^2 + [\lambda_2(j) - \text{MD}(j)]^2 + [\lambda_3(j) - \text{MD}(j)]^2}{\lambda_1(j)^2 + \lambda_2(j)^2 + \lambda_3(j)^2} \right)^{1/2}$$

**More summary measures**

- Axial diffusivity: Greatest of the 3 eigenvalues
  $$\text{AD}(j) = \lambda_1(j)$$

- Radial diffusivity: Average of 2 lesser eigenvalues
  $$\text{RD}(j) = \frac{(\lambda_2(j) + \lambda_3(j))}{2}$$

- Inter-voxel coherence: Average angle b/w the major eigenvector at some voxel and the major eigenvector at the voxels around it
Beyond the tensor

• The tensor is an imperfect model: What if more than one major diffusion direction in the same voxel?

• High angular resolution diffusion imaging (HARDI): More complex models to capture more complex microarchitecture
  - [Tuch’02]
  - [Frank’03, Özarslan’03]
  - [Behrens’03]
  - [Tuch’04]
  - [Wedeen’05]

Models of diffusion

Diffusion spectrum (DSI):
- Full distribution of orientation and magnitude

Orientation distribution function (Q-ball):
- No magnitude info, only orientation

Ball-and-stick:
- Orientation and magnitude for up to N anisotropic compartments

Tensor (DTI):
- Single orientation and magnitude

Example: DTI vs. DSI

From Wedeen et al., Mapping complex tissue architecture with diffusion spectrum magnetic resonance imaging, MRM 2005

Data acquisition

• Remember: A tensor has six unique parameters

\[ D = \begin{bmatrix} d_{11} & d_{12} & d_{13} \\ d_{12} & d_{22} & d_{23} \\ d_{13} & d_{23} & d_{33} \end{bmatrix} \]

• To estimate six parameters at each voxel, must acquire at least six diffusion-weighted images

• HARDI models have more parameters per voxel, so more images must be acquired

Choice 1: Gradient directions

• True diffusion direction || Applied gradient direction
  - Maximum attenuation

• True diffusion direction ⊥ Applied gradient direction
  - No attenuation

• To capture all diffusion directions well, gradient directions should cover 3D space uniformly

How many directions?

• Acquiring data with more gradient directions leads to:
  - +susceptible to artifacts due to motion, respiration, etc.

• DTI:
  - Six directions is the minimum
  - Usually a few 10’s of directions
  - Diminishing returns after a certain number (Jawe’s, Friman)

• HARDI/DSI:
  - Usually a few 100’s of directions
Choice 2: The b-value

- The b-value depends on acquisition parameters:
  \[ b = \gamma G^2 \delta^2 (\Delta - \delta/3) \]
  - \( \gamma \) the gyromagnetic ratio
  - \( G \) the strength of the diffusion-encoding gradient
  - \( \delta \) the duration of each diffusion-encoding pulse
  - \( \Delta \) the interval b/w diffusion-encoding pulses

How high b-value?

- Increasing the b-value leads to:
  - Increased contrast b/w areas of higher and lower diffusivity in principle
  - Decreased signal-to-noise ratio ⇒ Less reliable estimation of diffusion measures in practice

  - DTI: \( b \approx 1000 \text{ sec/mm}^2 \)
  - HARDI/DSI: \( b \approx 10,000 \text{ sec/mm}^2 \)

  - Data can be acquired at multiple b-values for trade-off
  - Repeat acquisition and average to increase signal-to-noise ratio

Looking at the data

- A diffusion data set consists of:
  - A set of non-diffusion-weighted a.k.a. “baseline” a.k.a. “low-b” images (b-value = 0)
  - A set of diffusion-weighted (DW) images acquired with different gradient directions \( g_1, g_2, \ldots \) and b-value >0
  - The diffusion-weighted images have lower intensity values

Distortions: Field inhomogeneities

- Causes:
  - Scanner-dependent (imperfections of main magnetic field)
  - Subject-dependent (changes in magnetic susceptibility in tissue/air interfaces)

- Results:
  - Signal loss in interface areas
  - Geometric distortions (warping) of the entire image

Distortions: Eddy currents

- Cause: Fast switching of diffusion-encoding gradients induces eddy currents in conducting components
- Eddy currents lead to residual gradients that shift the diffusion gradients
- The shifts are direction-dependent, i.e., different for each DW image
- Result: Geometric distortions

Data analysis steps

- Pre-process images to reduce distortions
  - Either register distorted DW images to an undistorted (non-DW) image
  - Or use information on distortions from separate scans (field map, residual gradients)
- Fit a diffusion model at every voxel
  - DTI, DSI, Q-ball, ...
- Do tractography to reconstruct pathways and/or
- Compute measures of anisotropy/diffusivity and compare them between populations
  - Voxel-based, ROI-based, or tract-based statistical analysis
**Caution!**

- The FA map or color map is not enough to check if your gradient table is correct - display the tensor eigenvectors as lines
- Corpus callosum on a coronal slice, cingulum on a sagittal slice

**Tutorial**

- Use `dt_recon` to prepare DWI data for a simple voxel-based analysis:
  - Calculate and display FA/MD/... maps
  - Intra-subject registration (individual DWI to individual T1)
  - Inter-subject registration (individual T1 to common template)
  - Use anatomical segmentation (aparc+aseg) as a brain mask for DWIs
  - Map all FA/MD/... volumes to common template to perform voxel-based group comparison

**Multimodal Integration**

- View FA, etc, on subject’s anatomical volume
- Intensity ROI Study: Average FA, etc, inside of White Matter Parcellation ROIs (wmparc.mgz)
Data analysis steps

- Pre-process images to reduce distortions
  - Either register distorted DW images to an undistorted (non-DW) image
  - Or use information on distortions from separate scans (field map, residual gradients)
- Fit a diffusion model at every voxel
  - DTI, DSI, Q-ball, ...
- Do tractography to reconstruct pathways and/or
- Compute measures of anisotropy/diffusivity and compare them between populations
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Tractography studies

- Exploratory tractography:
  - Example: "Show me all regions that the motor cortex is connected to."
  - Seed region can be anatomically defined (motor cortex) or functionally defined (region activated in an fMRI finger-tapping task)
- Tractography of known pathways:
  - Example: "Show me the corticospinal tract."
  - Use prior anatomical knowledge of the pathway’s terminations and trajectory (connects motor cortex and brainstem through capsule)

Tractography takes time

- Get whole-brain tract solutions, edit manually
- Use knowledge of anatomy to isolate specific pathways

Tractography methods

- Use local diffusion orientation at each voxel to determine pathway between distant brain regions
- Local orientation comes from diffusion model fit (tensor, ball-and-stick, etc.)
- Deterministic vs. probabilistic tractography:
  - Deterministic assumes a single orientation at each voxel
  - Probabilistic assumes a distribution of orientations
- Local vs. global tractography:
  - Local fits the pathway to the data one step at a time
  - Global fits the entire pathway at once

Deterministic vs. probabilistic

- Deterministic methods give you an estimate of model parameters
- Probabilistic methods give you the uncertainty (probability distribution) of the estimate
**Deterministic vs. probabilistic**

- **Deterministic tractography:** One streamline per seed voxel
- **Probabilistic tractography:** Multiple streamline samples per seed voxel (drawn from probability distribution)

**Local vs. global**

- **Local tractography:** Fits pathway step-by-step, using local diffusion orientation at each step
- **Global tractography:** Fits the entire pathway, using diffusion orientation at all voxels along pathway length

**Global tractography**

- Best suited for reconstruction of known white-matter pathways
- Constrained to connection of two specific end regions
- Not sensitive to areas of high local uncertainty in orientation, integrates over entire pathway
- Symmetric between “seed” and “target” regions
- Need to search through a large solution space of all possible connections between two regions:
  - Computationally expensive
  - Sensitive to initialization

**TRACULA**

- TRAct Constrained by Underlying Anatomy
- Global probabilistic tractography with prior information on tract anatomy from training subjects
- Learn from training subjects which anatomical regions each pathway typically goes through/next to
- Constrain pathway in new subject based on this prior anatomical knowledge
- Reconstruct 18 major white-matter pathways
  - No manual intervention in new subjects
  - Robustness with respect to pathway initialization
  - Anatomically plausible solutions
- Ad-hoc anatomical constraints are often used by other methods: constraints on path bending angle or length, WM masks, ...
White-matter pathway atlas

- Labeling based on an established protocol (Wakana '07)
- Corticospinal tract
- Inferior longitudinal fasciculus
- Uncinate fasciculus
- Corpus callosum
  - Forceps major
  - Forceps minor
- Anterior thalamic radiation
- Cingulum
  - Cingulate (superior)
  - Angular (inferior)
- Superior longitudinal fasciculus
  - Parietal
  - Temporal

Intra/inter-rater errors: 1mm/2mm on average

Automated pathway reconstruction

Have image data $Y$
Want most probable path $F$

- Determine the most probable path based on:
  - What the images tell us about the path
  - What we already know about the path
- Estimate posterior probability of path $F$ given images $Y$
  - $p(F | Y)$ \( \neq \frac{p(Y | F)}{p(F)} \)
  - $p(Y | F)$: Uncertainty due to imaging noise
  - $p(F)$: Uncertainty due to anatomical variability
  - Fit of pathway to prior anatomical knowledge from training set

Tract-based measures

- Reconstruction outputs:
  - Posterior probability distribution of pathway given data ($\mathcal{D}$)
  - Maximum a posteriori pathway ($\mathcal{D}$)
- Tract-based diffusion measures (FA, MD, RD, AD, etc):
  - Average over pathway distribution
  - Weighted average over pathway distribution
  - Average over MAP pathway
  - As a function of arc length along MAP pathway

Schizophrenia study

Pathway distributions reconstructed automatically in a SZ patient using 30 healthy training subjects

- Reconstruct pathways in 34 SZ patients and 23 healthy controls with
  - No training subjects
  - 30 healthy training subjects
  - 15 healthy / 15 SZ training subjects
  - 30 SZ training subjects
- Evaluate distance h/w automatically reconstructed and manually labeled pathways

Yendiki et al., Frontiers 2011
Head motion in diffusion MRI

- Head motion during a dMRI scan can lead to:
  - Misalignment between consecutive DWI volumes in the series
  - Attenuation in the intensities of a single DWI volume/slice, if the motion occurred during the diffusion-encoding gradient pulse
  - The former can be corrected with rigid registration, the latter cannot

- Conventional EPI sequences for dMRI ignore the problem
  - If motion in several directions ⇒ underestimation of anisotropy
  - False positives in group studies where one group moves more
  - Effects more severe when higher $b$-values, more directions acquired

Motion in a dMRI group study

- 57 children with autism spectrum disorder (ASD)
- 73 typically developing children (TD)
- Ages 5-12
- 193 total scans (some retest)
- $b=700$ s/mm$^2$
- Translation, rotation, intensity drop-out due to motion assessed
- Outlier data sets excluded
- Pathways reconstructed automatically with TRACULA

Data courtesy of Dr. Nancy Kanwisher and Ellison autism study

Yendiki et al., Neuroimage 2014

ASD vs. TD

Differences in dMRI measures between groups with low differences in head motion

Differences in dMRI measures between groups with high differences in head motion

TD vs. TD

Differences in dMRI measures between groups with low differences in head motion

Differences in dMRI measures between groups with high differences in head motion

Head motion, in summary

- Differences in head motion between groups can induce spurious group differences in diffusivity and anisotropy
- General trend: Head motion $\uparrow \rightarrow$ RD$\uparrow$, AD$\downarrow$, MD$\downarrow$, FA$\downarrow$
- This is after registration-based motion correction
- Match motion between groups and/or use a motion score as a nuisance regressor
- Note that all this will address false positives, but not false negatives due to head motion in the data
- Methods for tackling the problem during data acquisition are needed

TRACULA usage

- All processing options are defined in a configuration file, `dmrirc`
- Step 1: Pre-processing (distortion compensation, registration, etc.)
  `trac-all -prep -c dmrirc`
- Step 2: Fitting of ball-and-stick model (FSL’s `bedpostx`)
  `trac-all -bedp -c dmrirc`
- Step 3: Reconstruct pathways
  `trac-all -path -c dmrirc`

Yendiki et al., Neuroimage 2014
### Configuration file

- Example configuration file:
  ```bash
  $FREESURFER_HOME/bin/dmrirc.example
  ```
- The simplest configuration file possible, using all default options and only defining inputs:
  ```bash
  setenv SUBJECTS_DIR /path/to/fs/output/directory
  set subjlist = (subjA subjB ...
  set dcmlist = (/path/to/A/1.dcm /path/to/B/011-1.dcm ...)
  set bvecfile = /path/to/bvecs.txt
  set bvalfile = /path/to/bvals.txt
  ```
- Same gradient vectors and b-values assumed for all scans
- Can specify trac-all output directory different from recon-all:
  ```bash
  set droot = /path/to/tracula/output/directory
  ```

### Pre-processing

- `trac-all -prep -c dmrirc`
  - Includes the following steps:
    - Image corrections: `corr`
    - NEW: Quality assessment (motion scores): `-qa`
    - Intra-subject registration (DWI to T1): `-intra`
    - Inter-subject registration (T1 to template): `-inter`
    - Anatomical masks and labels: `-mask`
    - Tensor fit: `-tensor`
    - Anatomical priors: `-prior`
  - Can do some of the steps only (assuming previous steps have been done):
    - `trac-all -corr -qa -c dmrirc`
  - Or exclude some of the steps (assuming they have been done previously):
    - `trac-all -prep -nocorr -noqa -c dmrirc`

### Ball-and-stick model fit

- `trac-all -bedp -c dmrirc`
  - This step simply runs FSL bedpostX to fit the ball-and-stick model of diffusion to every voxel in the brain mask
  - This can take a while, but it’s possible to run every slice in parallel
  - Set `nstick = 3`

### Pathway reconstruction

- `trac-all -path -c dmrirc`
  - Reconstruct the 18 pathways (or a subset) using a random sampling algorithm:
    - Pick an initial guess for the path from the training subjects in the atlas (the only step that requires decent alignment between individual and atlas)
  - At every iteration, perturb control points of path and compute its fit to diffusion data and to anatomical priors from atlas
  - Set `nsample = 10000`

### Visualization with freeview

- There is a 4D volume where all the pathway distributions that were estimated have been merged
- Opening this file in freeview will display all distributions as isosurfaces, thresholded at 20% of their maximum value.

### Visualization: 3D view

- Use `freeview dmri/dtifit_FA.nii.gz`
  - Change threshold for display
Visualization: 3D view
- `freeview dmri/dtifit_FA.nii.gz`
  - `tv dpath/merged_avg33_mni_bbr.mgz`

Visualization: Slice view
- `freeview dmri/dtifit_FA.nii.gz`
  - `tv dpath/merged_avg33_mni_bbr.mgz`

Tract-based measures
- Reconstruction outputs
  - Posterior probability distribution of pathway given data (3D):
    - `paths.pd.nii.gz`
  - Maximum a posteriori pathway (1D):
    - `path.map.nii.gz`
- Tract-based diffusion measures (FA, MD, RD, AD)
  - Averaged over the entire pathway distribution: `pathstats_overall.txt`
  - As a function of position along the pathway: `pathstats_byvoxel.txt`

Path stats (average values)
- `pathstats_overall.txt`
  - *Avg*: Average values of every voxel with probability > 20% of the maximum
  - *Avg_Weight*: Multiply value at voxel with the probability at that voxel, sum over every voxel with probability > 20% of the maximum
  - *Center*: Average values only on the 1-D path with the highest probability

Path stats (values along the path)
- `pathstats_byvoxel.txt`
  - At each position along the path
    - Value on 1-D path with the highest probability
  - *Avg*: Average value over nearest points from all sampled paths
- Coordinates are given in native diffusion space
- Paths from different subjects generally have different number of positions along path

Along-the-path analysis
- Compute average FA/MD/RD/AD at each cross-section of the pathway
- Plot as a function of position along the pathway
- Correspondence of points between subjects based on Euclidean distance in MNI space
New: Assemble group stats

`trac-all -stat -o dmrirc`

- Combine files of stats along the path from multiple subjects:
  - Interpolate values of FA/MD/... at the same arc lengths for all paths
  - Find mean path for visualizing group results

- Outputs can be used for group studies on FA, MD, RD, AD along the pathway
  - One text file per pathway per measure (FA, MD, RD, AD)
  - Coordinates of mean path for visualization in freeview
  - Log file shows which subjects are outliers (shape-wise)

Example: p-values along each tract

- Save p-values in a simple text file, load it as a "scalar map"

Tutorial

- How to run TRACULA and view outputs:
  - Set up configuration file (input images, gradient directions, b-values, registration method, etc.)
  - "Run" trac-all (don’t actually run it!)
  - Look at pathways in freeview
  - Look at FA, MD, and other stats for each pathway

New: Longitudinal tractography

- Goal: Reconstruct a white-matter pathway consistently among all time points of a subject
- Challenging to do when processing each time point independently, as if it were a cross-sectional data point
- Different parts of the pathway may be reconstructed in each time point, due to noise or white matter degeneration
  - Changes in average anisotropy/diffusivity may be underestimated
  - Point-to-point correspondence difficult to establish for along-the-path analysis of anisotropy/diffusivity

Longitudinal TRACULA

- Reconstruct a subject’s pathways simultaneously in all time points:
  - Perturb path in the space of the base template
  - Map to each time point
  - Compute likelihood of DWI data at all time points
  - Compute anatomical prior based on segmentations of all time points
- Ensures point-to-point correspondence along path between time points
- Unbiased, treats all time points the same way

Longitudinal TRACULA: Sensitivity

- Improved sensitivity to longitudinal changes in FA in Huntington’s disease with longitudinal TRACULA

\[ p \approx 0.05 \]
Longitudinal TRACULA : Usage

- Example configuration file:
  `$FREESURFER_HOME/bin/dmrirc.long.example`

- List all time points and their corresponding base templates:
  ```
  set subjlist = (subjA-tp1 subjA-tp2 ... subjB-tp1 subjB-tp2 ...)
  set baselist = (subjA-base subjB-base ... subjA-base subjB-base ...)
  ```

- If `baselist` is not specified, data will be processed cross-sectionally

- The same 3 steps of `true-all` must be run for either cross-sectional or longitudinal stream (the only difference is in the configuration file)
Basics of fMRI Analysis: Preprocessing, First-Level Analysis, and Group Analysis

Overview
- Neuroanatomy 101 and fMRI Contrast Mechanism
- Preprocessing
- Hemodynamic Response
- “Univariate” GLM Analysis
- Hypothesis Testing
- Group Analysis

Visual Activation Paradigm
- Flickering Checkerboard
- Visual, Auditory, Motor, Tactile, Pain, Perceptual, Recognition, Memory, Emotion, Reward/Punishment, Olfactory, Taste, Gambling, Economic, Acupuncture, Meditation, The Pepsi Challenge, …

Magnetic Resonance Imaging
- T1-weighted Contrast
- BOLD-weighted Contrast

Blood Oxygen Level Dependent (BOLD)
- Oxygenated Hemoglobin (DiaMagnetic)
- Deoxygenated Hemoglobin (ParaMagnetic)
- Oxygen
- CO₂

4D Volume
- 64×64×35
- 85×1
Preprocessing

- Assures that assumptions of the analysis are met
  - Time course comes from a single location
  - Uniformly spaced in time
  - Spatial “smoothness”
  - vs Analysis – separating signal from noise

Motion

- Analysis assumes that time course represents a value from a single location
- Subjects move
- Shifts can cause noise, uncertainty
  - Edge of the brain and tissue boundaries

Motion and Motion Correction

- Motion correction reduces motion
- Not perfect

Motion Correction

- Motion correction parameters
- Six for each time point
- Sometimes used as nuisance regressors
- How much motion is too much?
Slice Timing

- Volume not acquired all at one time
- Acquired slice-by-slice
- Each slice has a different delay

Effect of Slice Delay on Time Course

- Volume = 30 slices
- TR = 2 sec
- Time for each slice = 2/30 = 66.7 ms

Can be corrected, but you must know the slice timing!

B₀ Distortion

- Metric (stretching or compressing)
- Intensity Dropout
- A result of a long readout needed to get an entire slice in a single shot.
- Caused by B₀ Inhomogeneity

B₀ Map: Voxel Shift Map

- Units are voxels (3.5 mm)
- Shift is in-plane
- Blue = P → A, Red A → P
- Regions affected near air/tissue boundaries (e.g., sinuses)

B₀ Distortion Correction

- Can only fix metric distortion
- Dropout is lost forever

Spatial Normalization

- Transform volume into another volume (or surface)
- New volume is an “atlas” space
- Align brains of different subjects so that a given voxel represents the “same” location.
- Preparation for comparing across subjects
- Not always done in preprocessing (FSL)
- More in Group Analysis later in this talk
Spatial Smoothing

- Replace voxel value with a weighted average of nearby voxels (spatial convolution)
- 3D (volume), 2D (surface)
- Improves SNR
- Improves intersubject registration
- Can have a dramatic effect on your results

Spatial Smoothing

- Spatially convolve image with Gaussian kernel.
- Kernel sums to 1
- Full-Width/Half-max: FWHM = $\sigma / \sqrt{\log(256)}$
- $\sigma$ = standard deviation of the Gaussian

Preprocessing

- Start with a 4D data set
  1. Motion Correction - Interpolation
  2. Slice-Timing Correction
  3. $B_0$ Distortion Correction - Interpolation
  4. Spatial Normalization - Interpolation
  5. Spatial Smoothing – Interpolation-like
- End with a 4D data set
- Can be done in other orders
- Not all are done

Effect of Smoothing on Activation

- Working memory paradigm
- FWHM: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 mm

fMRI Time-Series Analysis
fMRI Analysis Overview

Block Design: 15s Off, 15s On

Hypotheses and Contrasts

Contrasts and Inference
**Statistical Parametric Map (SPM)**

- Contrast Amplitude: $\beta_{\text{ON}} - \beta_{\text{OFF}}$ (CON, COPE, CES)
- Variance of Contrast (Error Bars): VARCOPE, CESVAR
- $H_0$ Significance $t$-Map $(p,z,F)$ (Thresholded $p<0.01$)

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

- Massive Univariate Analysis: Analyze each voxel separately

**Hemodynamics**

- Delay
- Dispersion
- Grouping by simple time point inaccurate

**Hemodynamic Response Function (HRF)**

- Time-to-Peak (~6 sec)
- Delay (~1-2 sec)
- Dispersion
- Undershoot (~16-32 sec)
- Equilibrium (~16-32 sec)

Amplitude $\beta$ related to the amount of neural firing

**Convolution Stimulus with HRF**

- Shifts, rolls off; more accurate
- Loose ability to simply group time points
- More complicated analysis
- General Linear Model (GLM)

**GLM**

- Data from one voxel
- Task
- Baseline Offset (Nuisance)

$$ y = \beta_{\text{Task}} + \beta_{\text{base}} $$

- $\beta_{\text{base}} = \beta_{\text{off}}$
- $\beta_{\text{Task}} = \beta_{\text{on}} - \beta_{\text{off}}$

- Implicit Contrast
- HRF Amplitude

**Matrix Model**

- Observations
- Design Matrix

$$ y = X \beta $$

- Vector of Regression Coefficients ("Betas")
- Design Matrix Regressors
Two Task Conditions

\[ y = X \beta \]

Odd

Even

\[ \beta_{\text{base}} \]

Observations

Data from one voxel

Design Matrix

Regressors

First Level Design

- Every stimulus type gets a column in the design matrix
- Every stimulus type gets a regression coefficient (\( \beta \))
- \( \beta \) related to neural firing
- Contrasts created by adding/subtracting \( \beta \)s
- “Nuisance” factors can be added as more columns
  - Low frequency drifts (DCT, polynomial)
  - Motion correction regressors
  - Physiological (e.g., pulse and respiration)
  - CSF or WM waveforms
- Functional Connectivity waveforms added as a column

All factors get a column and a regression coefficient

For those interested in the math …

\[ y = X\beta + n, \quad y = s + n, \quad n \sim N(0, \sigma_n^2) \]

\[ \hat{\beta} = (X'X)^{-1}X'y \quad \text{Parameter Estimates} \]

\[ \hat{\sigma}_n^2 = \frac{\hat{n} - \hat{\beta}^T X \hat{\beta}}{\text{DOF}} \quad \text{Residual Variance,} \quad \hat{n} = y - X\hat{\beta} \]

\[ \hat{\gamma} = C\hat{\beta} \quad \text{Contrast} \]

\[ \hat{\sigma}_C^2 = \hat{\sigma}_n^2 \hat{\gamma}^T (C'(X'X)^{-1}C)^{-1} \hat{\gamma} \quad \text{Contrast Variance Estimate} \]

\[ J = \text{rows in } C \]

\[ t_{\text{test}} = \frac{\hat{\gamma}}{\hat{\sigma}_C} = \frac{\hat{\gamma}}{\sqrt{(C'(X'X)^{-1}C)^{-1}}\hat{\gamma}} \quad \text{t-Test (univariate)} \]

\[ F_{\text{test}} = \frac{\hat{\gamma}^2 \hat{\sigma}_C^2}{\hat{\sigma}_C^2} \quad \text{F-Test (multivariate)} \]

Time Series Analysis Summary

- Correlational
- Design Matrix (HRF shape)
- Estimate HRF amplitude (Parameters)
- Contrasts to test hypotheses
- Results at each voxel:
  - Contrast Value
  - Contrast Value Variance
  - \( p \)-value
- Pass Contrast Value and Variance up to higher level analyses

fMRI Analysis Overview
Overview

- Spatial Normalization
- Goal of Group Analysis
- Types of Group Analysis
  - Random Effects, Mixed Effects, Fixed Effects
- Multi-Level General Linear Model (GLM)

Spatial Normalization

- Transform fMRI data to an Atlas Space where it can be compared voxel-by-voxel across subjects
- Multi-step procedure:
  1. Register fMRI to anatomical
  2. Register anatomical to atlas space
  3. Transform fMRI to atlas space
  4. Merge data
- Volume and/or Surface atlas spaces

Step 1 (vol and surf):
Register fMRI with Anatomical

Step 2 (vol): Register Anatomical with MNI305 (Talairach)

Step 3 (vol): Combine Steps 1 and 2

Step 4 (vol): Merge Subjects

Can be compared voxel-by-voxel

Native Anatomical Space (vol and surf)

Native Anatomical Space

MNI305 Space

fMRI in Anatomical Space

R

bbregister

fMRI in MNI305 Space

Native Anatomical Space

MNI305 Space

Subject 1
Subject 2
Subject 3
Step 2 (surf): Register Anatomical with Surface Atlas (fsaverage)

Native Anatomical Surface Space → Surface-based Registration → fsaverage Space → Anatomical Surface in fsaverage Space

Step 3 (surf): Combine Steps 1 and 2

Native Functional Space → Native Anatomical Space → fsaverage Space

Step 4 (surf): Merge Subjects

Subject 1 → Subject 2 → Subject 3 → Subject 4 → Subject 5 (Can be compared voxel-for-voxel)

Group Analysis

“Random Effects (RFx)” Analysis

\[
\frac{\beta_i - \beta}{\sigma^2} = \frac{\sigma^2}{N_e - 1} \\
\text{DOF} = N_e - 1
\]
“Random Effects (RFx)” Analysis

• Model Subjects as a Random Effect
• Variance comes from a single source:
  – Mean at the population mean
  – Variance of the population variance
• Does not take first-level noise into account
  (assumes 0)
• “Ordinary” Least Squares (OLS)
• Usually less activation than individuals

Higher Level GLM Analysis

\[ y = X \beta \]

Observations

\begin{bmatrix}
\text{Data from one voxel}
\end{bmatrix}

Design Matrix

\begin{bmatrix}
1 & 1 & 1 & 1 & 1
\end{bmatrix}

Vector of Regression Coefficients

\( \beta \)

Contrast Matrix:

\( C = \begin{bmatrix} 1 \end{bmatrix} \)

Contrast = \( C^T \beta = \beta \)

Summary

• Preprocessing – MC, STC, B\text{0}, Normalize, Smooth
• First Level GLM Analysis – Design matrix, HRF, Nuisance
• Contrasts, Hypothesis Testing – contrast matrix
• Group Analysis
  – Random Effects (Mixed and Fixed also possible)
  – Multi-level GLM (Design and Contrast Matrices)
fMRI Analysis with the FreeSurfer Functional Analysis Stream (FS-FAST) Preprocessing, First Level Analysis, and Group Analysis

Overview
- Atlas Spaces
- Directory Structure
- Preprocessing
- Setting up First-Level Analysis and Contrasts
- Group Analysis
  - Setting up
  - Correction for multiple comparisons

FSFAST
- Time-series functional analysis
  - Event-related, Blocked, Retinotopy, Functional Connectivity
- Built on FreeSurfer
- Surface-, Volume-, ROI-based
- Group Analysis
- Highly Automated
- Command-line driven
- Matlab/Octave, AFNI, and FSL used in the background

Philosophy
- Respect the inherent geometry of the brain structures (Smoothing and Clustering)
- Cortex – 2D
- Subcortical – 3D
- Requires that analysis be done in three spaces:
  - Left Hemisphere
  - Right Hemisphere
  - Subcortical Areas
- Not simple volumetric-based for all voxels!

FS-FAST Preprocessing

FS-FAST Analysis

FS-FAST Preprocessing

FS-FAST Analysis
**Surface Masking**

- Remove medial wall
- Intersect with functional brain mask
- 2D Smoothing only inside mask
- Later individual subjects masks merged (intersection).

**Volume (Subcortical) Masking**

- Remove most of cortex
- Remove some WM and CSF
- Intersect with functional brain mask
- 3D Smoothing only inside mask
- Later individual subjects masks merged (intersection).

Tip: use compressed NIFTI files (nii.gz)

**Typical Volume-based Analysis**

Single map, activation in both cortical and subcortical GM.

fBIRN Group n=18, distractor-vs-fix

**FSFAST Analysis**

Left Hemi  Right Hemi

Three mutually exclusive maps

**Recombining Cortical and Subcortical**

Visualization only!!

**Correction for Multiple Comparisons**

- Cluster-based
- Performed separately in each space
  - 2D clustering for Left and Right Hemispheres
  - 3D clustering for MNI305
  - Cluster table for each individual space
- Final cluster table is union of individual spaces
FSFAST Pipeline Summary
1. Analyze anatomicals in FreeSurfer
2. Unpack each subject (dcmunpack, unpacksdcmdir)
3. Create subjectname file.
4. Copy paradigm files into run directories
5. Configure analyses (mkanalysis-sess, mkcontrast-sess)
6. Preprocess (preproc-sess)
7. First Level Analysis (selxavg3-sess)
8. Higher Level Analysis (isxconcat-sess, mri_glmfit)
9. Correction for Multiple Comparisons (mri_glmfit-sim)

FSFAST Directory Structure
1. Project
   - Session
     - Functional Subdirectory (FSD, "bold")
     - Run
       - Raw Time-Series Data

Project Directory
- Folder where all/most of your data reside (can use symbolic links to data too)
- Directory where you will run most commands
- NOT the same as $SUBJECTS_DIR

Session Directory
- All the data collected between the time you put a subject into the scanner until you take him/her out.
  - May include data across “breaks”
- All one subject
- Data from one subject may be spread over different sessions (eg, longitudinal study)
- Session does not necessarily equal Subject
- Folder name can be anything.

Functional Subdirectory (FSD, “bold”)
- All the data associated with a given paradigm
- Most people just have one paradigm and so only one FSD
- Usually called “bold”
- Default is “bold”

Run Folder/Directory
- All the data collected between pressing the “Apply” button and the end of the scan.
  - Eg, 150 time points (TPs)
  - Raw functional data stored in this folder
  - Usually called “f.nii” or “f.nii.gz”
  - Raw data will be in “native functional space”, eg, 64x64x30, 3.125mm x 3.125mm x 6mm
  - Folder name will be 3-digit, zero-padded number, eg, “002”, “014”
Setting Up the Directory Structure

Things you need to do before running automated commands:

1. Unpack raw data from DICOM
2. Add paradigm files
3. Add subjectname file

1. Unpacking: Creating the Directory Structure from DICOM Files
   - unpackdcmmdir – Siemens only
   - dcmunpack – Siemens or GE (not sure about Philips)
   - Manually

   Getting help:
   dcmunpack -help

   Unpack:
   cd ProjectDir
   dcmunpack –src dicomdir -martinos
   –trg sess01
   –run 3 bold nii f.nii
   –run 5 bold nii f.nii
   –run 6 bold nii f.nii

2. Add “Paradigm” File(s)
   - Codes Stimulus Schedule
   - Simple Text File
   - Manually copy into Run Folder

   Paradigm File
   - Codes Stimulus Schedule (and Weight)
   - Four Columns
     1. Onset Time (Since Acq of 1st Saved Volume)
     2. Stimulus Code (0, 1, 2, 3 …)
     3. Stimulus Duration
     4. Stimulus Weight (default is 1)
     5. Any other columns ignored
   - Simple Text File
   - Code 0 Always Fixation/NULL
   - Weight for parametric modulation

3. Add “subjectname” file
   - Integration with FreeSurfer anatomical analysis
   - Subject name is name passed to recon-all, eg,
     – recon-all –all –subject bert
     – $SUBJECTS_DIR/bert
   - Create a text file called “sess01/subjectname”, the content of the file will be, eg, “bert” (no quotes)
Congratulations: You are now ready to start running the “automated” commands … but before you do …

Session Id File (“SessId”):
• Text file with a list of sessions to process
• Easy way to keep track of groups
• Can have more than one
• A good way to parallelize

FS-FAST Commands will often take a SessId file as input:
```
 selxavg3-sess –sf sessid …
```
Will run for all sessions found in sessid
Alternatively, selxavg3-sess –s Sess01 –s Sess02 –s Sess03

First-Level Analysis:
• Time-series analysis
• Everything inside of a functional subdir (all runs)
• Preprocessing
• GLM Analysis

Preprocessing:
1. Registration Template Creation
2. Motion Correction
3. Slice-timing correction (if using)
4. Functional-Anatomical Registration
5. Mask creation
6. Intensity normalization, Part 1
7. Resampling raw time series to mni305, lh, and rh
8. Spatial smoothing
• B0 distortion correction not documented yet
**Preprocessing Command**

```shell
preproc-sess
   -sf sessids
   --surface fsaverage lh rh
   --mni305
   --fwhm 5
   --per-run

preproc-sess -help
```

- Preprocess all runs of all sessions
- Can take a long time!

**Directory Structure after Preprocessing**

- Final data in atlas space:
  - fmcpr.sm5.fsaverage...
- Lots of other intermediate files
- Lots more boring details

```
<table>
<thead>
<tr>
<th>Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sess01</td>
</tr>
<tr>
<td>bold</td>
</tr>
<tr>
<td>100S</td>
</tr>
</tbody>
</table>
```

**First Level GLM Analysis**

- Specify Task Model
  - Event-related or Blocked
  - AB-Blocked (Periodic two condition)
  - Retinotopy
  - Task timing (Paradigm file)
  - Hemodynamic Response Function (HRF)
- Contrasts
  - Specify Nuisance and Noise Models
    - Low frequency drifts
    - Time point exclusion
    - Motion Regressors
    - Other (Physiology, RETROICOR)
  - Temporal Whitening

**Example: Odd Even Blocks**

\[
y = X \beta
\]

\[
\beta_Odd = \begin{bmatrix} \beta_{Odd} \\ \beta_{Even} \\ \beta_{base} \end{bmatrix}
\]

**First Level GLM Analysis: Workflow**

- Do these two steps once regardless of number of sessions:
  1. Configure “Analysis” – collection of parameters, mkanalysis-sess
  2. Create Contrasts (mkcontrast-sess)
     - Don’t even need data to do this
     - Do this for each session:
       - Perform Analysis (selxavg3-sess)

```
cd ProjectDir
mkanalysis-sess
   -analysis oddeven.sm5.lh
   --surface fsaverage lh
   --fwhm 5
   --paradigm oddeven.par
   --event-related
   --spmhrf 0
   --reCommandEvent 4
   --polyfit 2
   --mcexreg 4
   --nskip 4
   --TR 2
   --nconditions 2
   --per-run
```
Configuration: Analysis Name

```
mkanalysis-sess
-analysos oddeven.sm5.lh -analysos oddeven.sm5.mni305 -surface fsaverage lh
-fwhm 5 -paradigm oddeven.par -event-related -spnhrf 0 -refeventdur 4
-polyfit 2 -mcextreg -nskip 4 -TR 2 -nconditions 2 -per-run
```

Analysis Name – name used to reference this collection of parameters. Use a different name for a different set of parameters.

Configuration: Preprocessing

```
mkanalysis-sess
-analysos oddeven.sm5.lh
-ssession oddeven.sm5.lh
-ssession oddeven.sm5.mni305 -surface fsaverage lh
-fwhm 5 -paradigm oddeven.par -event-related -spnhrf 0 -refeventdur 4
-polyfit 2 -mcextreg -nskip 4 -TR 2 -nconditions 2 -per-run
```

Preprocessing options indicate what the source time-series file name will be.

Configuration: Stimulus Timing

```
mkanalysis-sess
-analysos oddeven.sm5.lh
-ssession oddeven.sm5.mni305 -surface fsaverage lh
-fwhm 5 -paradigm oddeven.par -event-related -spnhrf 0 -refeventdur 4
-polyfit 2 -mcextreg -nskip 4 -TR 2 -nconditions 2 -per-run
```

A different analysis is needed for each space (lh, rh, and mni305)

Configuration: Task Type

```
mkanalysis-sess
-analysos oddeven.sm5.lh
-ssession oddeven.sm5.mni305 -surface fsaverage lh
-fwhm 5 -paradigm oddeven.par -event-related -spnhrf 0 -refeventdur 4
-polyfit 2 -mcextreg -nskip 4 -TR 2 -nconditions 2 -per-run
```

Event-related and blocked are the same. Other possibilities are: -abblocked -retinotopy

Configuration: HRF Model

```
mkanalysis-sess
-analysos oddeven.sm5.lh
-ssession oddeven.sm5.mni305 -surface fsaverage lh
-fwhm 5 -paradigm oddeven.par -event-related -spnhrf 0 -refeventdur 4
-polyfit 2 -mcextreg -nskip 4 -TR 2 -nconditions 2 -per-run
```

• SPM Canonical HRF
  • FSL FIFF
  • FSFAST
  • FSLfMR NonDerivatives
  • fsfMRI NDerivatives
  • fMRI PreStim ToTimeWindow
  • gammafit 2.25 1.25
Configuration: Reference Event Duration

mkanalysis-sess
-analysis oddeven.sm5.lh
-surface fsaverage lh
-fwhm 5
-paradigm oddeven.par
-event-related
-spmhrf 0
-refeventdur 4
-polyfit 2
-mcextreg
-nskip 4
-TR 2 -nconditions 2
-per-run

Just set this to the duration of your event in seconds.

Configuration: Nuisance Drift Modeling

mkanalysis-sess
-analysis oddeven.sm5.lh
-surface fsaverage lh
-fwhm 5
-paradigm oddeven.par
-event-related
-spmhrf 0
-refeventdur 4
-polyfit 2
-mcextreg
-nskip 4
-TR 2 -nconditions 2
-per-run

2nd Order Polynomial: This is the default.
0: mean offset
1: temporal trend
2: quadratic trend
Can also specify a high-pass filter with
-hpf CutOffHz
where CutOffHz is the cut-off frequency in Hz (e.g., 0.1). Careful with this.

Configuration: Nuisance Motion

mkanalysis-sess
-analysis oddeven.sm5.lh
-surface fsaverage lh
-fwhm 5
-paradigm oddeven.par
-event-related
-spmhrf 0
-refeventdur 4
-polyfit 2
-mcextreg
-nskip 4
-TR 2 -nconditions 2
-per-run

Use Motion Correction parameters as nuisance regressors (good idea?). Can specify arbitrary regressor files with
-nuisreg file N:

Configuration: Excluding Time Points

mkanalysis-sess
-analysis oddeven.sm5.lh
-surface fsaverage lh
-fwhm 5
-paradigm oddeven.par
-event-related
-spmhrf 0
-refeventdur 4
-polyfit 2
-mcextreg
-nskip 4
-TR 2 -nconditions 2
-per-run

Skip the 1st 4 time points. Do not need to adjust stimulus timing. Alternative:
-tpexclude tpexclude.dat to remove any TP. Good for motion.

Configuration: Why TR and NCond?

mkanalysis-sess
-analysis oddeven.sm5.lh
-surface fsaverage lh
-fwhm 5
-paradigm oddeven.par
-event-related
-spmhrf 0
-refeventdur 4
-polyfit 2
-mcextreg
-nskip 4
-TR 2 -nconditions 2
-per-run

It could get this from the data and paradigm files, but this command is set up to run without the need of any data, so it needs to know the TR and number of conditions.

Number of conditions is the number of Non-Fixation/Non-NULL conditions.
2 = Odd + Even

Contrasts: Odd Even Blocks

x = X * \beta

\begin{bmatrix}
\beta_{\text{Odd}} \\
\beta_{\text{Even}} \\
\beta_{\text{base}}
\end{bmatrix}

- Two task conditions
- One nuisance regressor
- Need weight for each condition

Does the hemodynamic response amplitude to the Odd stimulus differ from that of Even?

y = 1^T \beta_{\text{Odd}} - 1^T \beta_{\text{Even}}

C = [+1 -1] Contrast Matrix
Configuration: Contrasts

- Linear combination of regression coefficients (COPE, CON)
- Weight for each condition
- Embodies a hypothesis: Does the hemodynamic response amplitude to the Odd stimulus differ from that of Even?

C = [+1 -1]

parafile
mkcontrast-sess
-analysis oddeven.sm5.lh
-contrast odd-vs-even
-a 1
-c 2

Configuration: Three Conditions

1. Happy
2. Sad
3. Mad

Hypothesis: response to Happy is different than that to Mad

parafile
mkcontrast-sess
-analysis faces.sm5.lh
-contrast happy-vs-mad
-a 1
-c 3

Note: Condition 2 (Sad) not represented (set to 0)
C = [1 0 -1]
Configuration: Summary

- mkanalysis-sess, mkcontrast-sess
- Need configuration for lh, rh, and mni305
- Specify: Preproc, Task, Nuisance, Noise, Contrasts
- Does not do analysis, just creates configuration
- Do once for each parameter set (space)
- Do once regardless of number of sessions
- Should take a few seconds to run

First-Level GLM Analysis

```
cd ProjectDir
selxavg3-sess –sf sessidfile –analysis oddeven.sm5.lh
```
- Finds raw data, paradigm file, external regressors, etc
- Constructs design and contrast matrices
- Combines runs together using “smart” concatenation (1st and 2nd level)
- Performs GLM fit at each voxel
- Tests contrasts at each voxel
- All sessions specified in sessid file
- May take a few hours, depending on how many sessions
- Does not re-run if data are “up-to-date”
- Will run preprocessing if not done already
- Requires matlab or octave

After First Level Analysis…

Project
  | 1. Project
  |   | 2. Session
  |   |   | 3. Functional Subdirectory “bold”
  |   |   | ces - contrast effect size, COPE (FSL), CON (SPM)
  |   |   | cesvar - contrast variance VARCOPE (FSL)
  |   | 4. Analysis Folder
  |   | 5. Contrast Folder
  |   | 6. Contrast Values
  | odd-even.sm5.lh
  | odd-even.sm5.rh
  | ces.nii
  | cesvar.nii
  | sig.nii

First Level Analysis: Visualization

Surface-based analyses:
```
tksurfer-sess –s session –analysis oddeven.sm5.lh –c odd-vs-fix
tksurfer-sess –s session –a oddeven.sm5.rh –c odd-vs-fix
```
- One session at a time (-s session, NOT –sf sessidfile)
- Can specify multiple contrasts, eg, -c odd-vs-fix –c even-vs-fix –c odd-vs-even
- Or all contrasts with “-call”
- Note Shortcut: “-a” instead of “analysis” and “-c” instead of “contrast”

No activation in cortex
No activation in medial wall

Individual subject shown on fsaverage anatomy
Can show/analyze on individual anatomy.
FS-FAST Analysis

Atlas Space
Masked, Smoothed

First Level
GLM
Higher Level GLM

First Level
GLM
Higher Level GLM

2D +T Left Hemi
2D+T Right Hemi

2D Multiple Comparisons
Correction
2D Multiple Comparisons
Correction

3D+T MNI305

First Level
GLM
Higher Level GLM

X1, C1
XG, CG

First Level
GLM
Higher Level GLM

3D Multiple Comparisons
Correction
3D Multiple Comparisons
Correction

Group/Higher Level Analysis

cd ProjectDir
isxconcat-sess -analysis oddeven.sm5.lh
-contrast odd-vs-even
-sf group1.sessid
-o group1

Like mris_preproc in anatomical stream

Group/Higher Level Analysis: Consolidation

cd ProjectDir
isxconcat-sess -help

mri_glmfit
--surf fsaverage lh
--y ces.nii
--wls cesvar.nii
--fsgd group1.fsgd
--C group.con1.mtx
--C group.con2.mtx
--glmdir glm.group

See FreeSurfer Group Analysis, including correction for multiple comparisons.
http://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/GroupAnalysis
mri_glmfit --help

Group/Higher Level Analysis

mri_glmfit
--surf fsaverage lh
--y ces.nii
--wls cesvar.nii
--fsgd group1.fsgd
--C group.con1.mtx
--C group.con2.mtx
--glmdir glm.group

• Surface-based analysis on the left hemisphere of fsaverage.
• For right hemisphere, use --surf fsaverage rh.
• For mni305, so not specify --surf.

Group/Higher Level Analysis

mri_glmfit
--surf fsaverage lh
--y ces.nii
--wls cesvar.nii
--fsgd group1.fsgd
--C group.con1.mtx
--C group.con2.mtx
--glmdir glm.group

Lower-level contrast input data, one frame/time point for each subject.
mri_glmfit
--surf fsaverage lh
--y ces.nii
--wls cesvar.nii
--fsgd group1.fsgd
--C group.con1 mtx
--C group.con2 mtx
--glmndir glm.group

Lower-level contrast variances, one frame/time point for each subject. Performs weighted least squares (Pseudo-Mixed Effects)

FSGD file must have same order of sessions as sessidfile used when running isxconcat-sess

• Higher Level/Group contrasts.
• Eg, Normal vs Schizophrenia
• Easily confused with lower level contrasts (eg, odd-vs-even).

• Use pre-cached simulation results
• positive group 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
--glmndir glm.group
--cache pos 2
--cwpvalthresh .05
--3spaces
Surface-based Correction for Multiple Comparisons

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

```bash
mri_glmfit-sim
--glm职能部门 glm.group
--cache pos 2
--cwpvalthresh .05
--3space
```

Cluster-wise threshold p < .05

Correction for Multiple Comparisons Output (Surface)

```bash
mri_glmfit-sim
--glm职能部门 glm.group
--cache pos 2
--cwpvalthresh .05
--3spaces
```

Cache th20 pos sig cluster.nii – 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)

Group MNI305 Analysis

```bash
isxconcat-sess
-analysis odd-even.sm5.mni305
-contrast odd-vs-even
-of group1 sessid
-o group1
```

Group Subcortical (MNI305) Analysis

```bash
mri_glmfit
--y ces.nii
--wls cesvar.nii
--fsgd group1.fsgd
--C group.con1.mtx
--C group.con2.mtx
```

- Command-line is very similar to surface
- No "--surf fsaverage lh"

Volume-based Correction for Multiple Comparisons

- 3D Cluster-based Correction at p < .05

```bash
cd ProjectDir
mri_glmfit-sim
--glm职能部门 glm.group
--cache pos 2
--cwpvalthresh .05
--3spaces
```

Bonferroni correction across 3 spaces: lh, rh, and subcort

Group MNI305 Analysis

- Data structures:
  - Odd
  - Even
  - Odd vs. Even

- Statistical analysis:
  - Odd vs. Even
  - Group comparison

- Output:
  - Maps of significance
  - Annotations of significant clusters
  - Summary of clusters with details like significant atlases
FSFAST Pipeline Summary
1. Analyze anatomicals in FreeSurfer
2. Unpack each subject (dcmunpack,unpacksdcmdir)
3. Create subjectname file.
4. Copy paradigm files into run directories
5. Configure analyses (mkanalysis-sess, mkcontrast-sess)
6. Preprocess (preproc-sess)
7. First Level Analysis (selxavg3-sess)
8. Higher Level Analysis (isxconcat-sess, mri_glmfit)
9. Correction for Multiple Comparisons (mri_glmfit-sim)
10. Publish (publish-sess)

Tutorial: Working Memory Task
0. "Scrambled" – low-level baseline, no response
1. Encode – series of passively viewed stick figures
2. Emotional
3. Neutral
4. Following Emotional Distractor
5. Following Neutral Distractor
Multimodal Integration: 
DTI/fMRI Integration, Surface Analysis

Quick Review: DTI Integration
• View FA, etc., on subject’s anatomical volume
• Intensity ROI Study: Average FA, etc., inside of White Matter Parcellation ROIs (wmparc.mgz)

Quick Review: DTI Integration
• Motion/Eddy Current Correction (MC Template)
• Usually a low-b volume
• Use for registration template
  \texttt{bbregister --mov mctemplate.nii --s subject --init-fsl --lta register.lta}
  \texttt{freeview -v mctemplate.nii:reg=register.lta -f $SUBJECTS_DIR/subject/surf/?h.white}
• First-Level (Individual) Analysis
• Fit Tensor Model
• Maps: FA (0-1), ADC, Eigenvectors, etc
• All in alignment with MC Template!!!

fMRI Integration
• Visualize individual fMRI results on
  • surface
  • volume
• ROI Volume Study:
  • Count number of voxels above threshold in an anatomical ROI
• ROI Intensity Study:
  • Average HRF inside of an ROI
• Surface-based fMRI group analysis

Hemodynamic Response (BOLD)

Multiple Presentations/Averaging

Individual Output: HRF Amp, HRF Var, p/z/t/F
fMRI Preprocessing Overview

- Motion Correction (MC Template)
- Use reference/template for registration

- timereg -mv template.nii --old --subject --irls --fix --init -fsl
- FreeSurfer --template nuis -reg -register.lta -f SUBJECTS_DIR/subject/lta

- Do not use nonlinear resampling to Talairach/MNI space. Best work in native space!
- Do not spatially smooth (3D) (set fwhm=0 in SPM...)
  we do not smooth in volume, rather on surface later!

fMRI Analysis Overview

- First-Level (Individual) Analysis
  - HRF Amplitude (or Contrast of Amplitudes)
    - cope (FSL),
    - CON (SPM),
    - ces (FSFAST)
  - Variance of Amplitude
    - varcope (FSL), var (SPM), cesvar (FSFAST)
  - Activation/Significance Maps:
    - z, t, F
    - sig (-log10(p))
  - All in alignment with MC Template!!!!

Reference and Map

Volume Viewing

sig.nii – significance map in native functional space.
Could have been z, t, or F map as well.

register.lta – FreeSurfer registration file

fthresh – lower threshold (value depends on map).
You can change this in the interface.
fmax – saturation threshold. (value depends on map).
You can change this in the interface.
aparc+aaseg – display aparc+aaseg.mgz.
You can load this from the interface, too.
Volume Viewing

- Red/Yellow +
- Blue/Cyan -
- Seg Opacity
- ROI Average
- ROI Count

Sampling onto the Surface

- White/Gray
- Pial
- Half Way
- Average
  Projection Fraction
  --projfrac 0.5

Sampling onto the Surface

Surface Viewing

Resample HRF Contrast Significance to left hemisphere

```bash
mri_vol2surf
--mov sig.nii
--reg register.lta
--hemi lh
--projfrac 0.5
--o lh.sig.mgh
```

- Map in native functional space
- FreeSurfer registration file
- Hemisphere
- Projection fraction (half)
- Output (Nvertices x 1 mgh format)

Note similarity to bbregister command!

Load HRF Contrast Significance as overlay

```bash
freeview -f SUBJECTS_DIR lh.inflated:annot
= aparc.annot:overlay
= lh.sig.mgh:overlay_threshold=2,5
```

- -viewport 3d
Surface Viewing

- Red/Yellow +, Blue/Cyan -
- Parcellation Outline
- ROI Average
- ROI Count

Surface-based Group Analysis

```bash
mris_preproc
  --hemi lh
  --o lh.fsaverage.ces.mgh
  --iv subject1/ces.nii
  subject1func/register.lta
  --iv subject2/ces.nii
  subject2func/register.lta
  --iv subject3/ces.nii
  subject3func/register.lta
```

After that, everything else is the same as a thickness study...

```bash
mris_fwhm
  --i lh.fsaverage.ces.mgh
  --fwhm 10
  --o lh.fsaverage.ces.sm10.mgh
cortex
mri_glmfit
  --surf fsaverage lh
cortex
  --y lh.fsaverage.ces.sm10.mgh ...
```

fMRI ROI Analysis

- HRF Amplitude
  - Full Anatomical ROI
  - Functionally Constrained ROI
- Volume

E.g., average functional HRF amplitudes from voxels inside of superior temporal gyrus (light blue) regardless of significance.

Step 1. Resample HRF Contrast to anatomical space

```bash
mri_vol2vol
  --mov ces.nii
  --reg register.lta
  --interp nearest
  --fstarg 
  --o ces.anat.mgh
```

Note similarity to bbregister and mri_vol2surf commands!

Step 2: Average HRF Contrast within ROIs

```bash
mri_segstats
  --seg $SUBJECTS_DIR/subject/mri/aseg.mgz
  --ctab $FREESURFER_HOME/FreeSurferColorLUT.txt
  --i ces.anat.mgh
  --sum ces.aseg.stats
```

Notes:
- `seg` is the segmentation (e.g., aseg.mgz, aparc+aseg.mgz, etc.)
- `ctab` is matching color lookup table

Output File: ces.aseg.stats
- simple text file with same format aseg.stats
- multiple subjects can be combined with asegstats2table
Average HRF within a Functionally Active area inside of an Anatomical ROI

Contrast Amplitude

E.g., average functional HRF amplitudes from voxels inside of superior temporal gyrus (light blue) for voxels that have:
1. p<.01 (sig>2) regardless of sign (yellow or blue), or
2. p<.01 (sig>2) for positive activation (yellow only), or
3. p<.01 (sig>2) for negative activation (blue only)

Masked Average HRF within a Functionally Active Area inside of an Anatomical ROI

Resample HRF Contrast Significance to anatomical space
mri_vol2vol

mov sig.nii

reg register.lta

interp nearest

fstarg

--o sig.anat.mgh

Masked average HRF contrast within functionally constrained ROIs (sign independent):
mri_segstats

seg $SUBJECTS_DIR/subject/mri/aseg.mgz

ctab $FREESURFER_HOME/FreeSurferColorLUT.txt

ices.anat.mgh

--sum ces.aseg.mask.stats

--mask sig.anat.mgh

--mask-thresh 2

--mask-sign abs

• Volume in stats file is vol. above threshold (may be 0)
• Sign is important for Average!
  • abs, pos, or neg
  • pos will always result in positive HRF average
  • neg will always result in negative HRF average
  • abs ???

• Careful to avoid circularity

Summary

• Multi/Cross-modal map (HRF Amplitude, FA)
• Multimodal Integration requires a Reference
• A Reference/Template is:
  • Same size as multimodal map
  • In Voxel-to-voxel alignment with map
  • Has better anatomical contrast
  • Baseline functional
  • Low-B DTI
• Usually a motion corrected template
• Volume and Intensity ROI Analyses
• Functionally-constrained ROI

Tutorial

1. Registration – manual and automatic registration
2. fMRI Integration (Sensorimotor Paradigm)
   a) Individual
      i. Volume view sig
      ii. Surface view sig
      iii. ROI analysis with & without functional constraint
   b) Group
      i. mris_preproc
      ii. ROI analysis (asegstats2table)
More Registration Techniques

registration tool summary

- mris_register
- fsrRegister: bet + flirt
- bbRegister
- mri_robust_register
- mri_cvs_register
  - mris_register
  - mri_nl_align

registration morph summary

- .dat, .lta, .xfm, .fslmat: encode rigid and affine transformations
  - mri_vol2vol
- sphere.reg: encodes spherical morph
  - mris_resample
- .m3z: encode nonlinear volumetric morphs
  - mri_vol2vol

A new registration solution?

- Surface-based (2D) registration does an excellent job of aligning cortical folds, but does not apply to non-cortical structures (e.g., basal ganglia).
- Volumetric (3D) registration applies to the entire brain but does not, in general, align folding patterns.
- Goal: combined their strength

Why aligning folds in the volume is hard...

Affine transform of surfaces from one subject mapped to another.
Combined volumetric and surface-based registration (CVS)

- Spherical alignment
- Elastic propagation of cortical registration results in the 3D volume
- Volumetric alignment of sub-cortical regions

mri_cvs_register --mov subjid

- registering the subject to, by default, the CVS atlas space
- make sure that the SUBJECTS_DIR for subjid is correctly set

Optional Arguments
--template subjid : subjid for template subject
--template dir : recon directory for template
   (default is SUBJECTS_DIR)
--outdir dir : output directory for all the results
   (default is SUBJECTS_DIR/subjid/cvs)

... and many more: use --help

mri_cvs_register

Optional Arguments (cont)
--step1 : Only do step 1 (spherical registration).
--step2 : Only do step 2 (elastic registration).
--step3 : Only do step 3 (volumetric registration).
--noaseg : Do not use aseg volumes in the volumetric registration pipeline (default is 0). Setting this option could shorten significantly the time of registration, however, might also take away from the accuracy of the final results.
mri_cvs_register

Optional Arguments (cont)

--nocleanup
Do not delete temporary files (default is 0).

--keepelreg
Do not delete elastic registration (default is 0).

--cleanelreg
Recompute the CVS-related elastic registration morphs that might have been computed prior to the current CVS run (default is 0).

--cleansurfreg
Recompute CVS-related surface registration morphs that might have been computed prior to the current CVS run (default is 0).

--cleanvolreg
Overwrite /recompute CVS-related volumetric morphs that might have been computed prior to the current CVS run (default is 0).

CVS atlas

![CVS atlas](path: $FREESURFER_HOME/subjects/cvs_avg35)

CVS atlas in MNI152 space

![CVS atlas in MNI152 space](path: $FREESURFER_HOME/subjects/cvs_avg35_inMNI152/)

related commands

- mri_cvs_check
  - checking whether all files needed for a successful CVS registration are present

- mri_cvs_data_copy
  - copying the CVS-relevant recon directories over to a new location

- mri_vol2vol
  - applying the CVS registration morph to files corresponding to the moving subject

Applying CVS morphs

mri_vol2vol

1. applying CVS morph to asegreg file

   mri_vol2vol --tag templateid --m3z morph.m3z
   --noDefM3zPath --mov aseggvol
   --o aseggvol2CVS
   --interp nearest
   --no-save-reg

2. applying morph to corresponding diffusion file

   mri_vol2vol --tag templateid --m3z morph.m3z
   --noDefM3zPath --mov 2anat.register.dat
   --mov diffvol --o diffvol2CVS
   --no-save-reg

Application of CVS to tractography

- Goal: fiber bundle alignment
- Study: compare CVS to methods directly aligning DWI-derived scalar volumes
- Conclusion: high accuracy cross-subject registration based on structural MRI images can provide improved alignment

Average tracts after registration mapped to the template displayed with iso-surfaces

Mean Hausdorff distance measures for three fiber bundles

Functional MRI analysis in CVS space

Collaboration with Kami Koldewyn, Joshua Julien and Nancy Kanwisher at MIT

Ongoing development

- Improve CVS capability to register ex-vivo to in-vivo acquisitions
- Implemented MI-based volumetric registration (for CVS step 3) to accommodate intensity profile differences
- Qualitative preliminary results on 4 subjects

- L. Zöllei, Allison Stevens, Bruce Fischl: Non-linear Registration of Intra-subject Ex-vivo and In-vivo Brain Acquisitions, Human Brain Mapping, June 2010

Subject 1
FreeSurfer Tutorial and Workshop Quiz

Discuss the answers to these questions with your partner.

What is the difference between a volume and a surface?

- A volume stores information about 3D space, whereas a surface stores 2D space.

Do I have to use two MPRAGEs to run recon-all? Explain.

- No, you do not have to use two structural scans as input to recon-all. If a multi-channel head coil is being used, then generally the SNR will be high enough where averaging two scans is no longer beneficial (and can even worsen a perfectly good scan).

Can FreeSurfer help me select a region of interest and measure certain quantities within? How?

- You can draw a region of interest either in the volume (using tkmedit and the Select Voxels tool) or on the surface (using tkmedit or qdec).

What is fsaverage made of?

- It is constructed of MRI scans of 40 subjects that have been manually segmented. 10 are young adults, 10 are middle-ages, 10 are older cognitively normal, and 10 are older demented patients.

What measures will FreeSurfer give me?

- Volume, Mean Intensity (plus standard deviation, min, and max) of the Subcortical structures, Cortical gray matter, CSF, Cerebellum, White Matter, Ventrices, and Brainstern. Number of vertices, Surface Area, Volume, Thickness, Mean Curvature of the parcellated cortex. Also, Total Gray Matter Volume (cortical + subcortical gray), Supratentorial Volume (everything above the tentorium), Total Cortical Gray Volume (difference between pial and white surface), Subcortical Gray volume (total of all subcortical gm volumes), Total White Matter volume (volume within white surface excluding subcortical gray matter), estimated ICV.

How long does it take for recon-all to finish processing one subject?

- 20-24 hours.

How long would it take you to do this manually?

- To manually label the wm, gm, and subcortical structures of one case will likely take a month.
Why do I have to set so many variables before using FreeSurfer?

- The variables indicate the location of code and data sets. Setting them once at the beginning of your data processing ensures that the right version of the code is executed, all the binaries will be found and that the data files are also correctly located.

Where do I find all those fantastic stats files FreeSurfer created for me?

- The stats output for each subject can be found in <subjid>/stats where subjid is the the name of one particular subject. To grab the stats files for several subjects to put them in spreadsheet-ready format, use the commands asegstats2table or aparcstats2table.

When mailing the FreeSurfer list about a problem, what information should I include?

- You should include the version of FreeSurfer you are using, the command line you tried to run, the error message you got either in the shell window or the recon-all.log, and the Operating System you are running FreeSurfer on. You can find out which version you are running by typing:

```bash
more $FREESURFER_HOME/build-stamp.txt
```

Why is spherical averaging better than current volume-based methods out there?

- Current volume-based registration methods cannot achieve high accuracy in aligning the cortical regions because of their high inter-subject variability. When we are only interested in finding correspondences between these areas (for example, in the case of functional studies), it is sufficient and also more accurate to register only the cortical areas with the spherical averaging method.

What is a limitation of this procedure?

- The alignment does not take into account the subcortical structures.

When you do all that crazy morph stuff, are you changing the data?

- When computing the deformation fields between a subject and another or an atlas, the data itself is not changed. When the deformation field is computed, however, it can be applied to the subject, which means resampling its scan(s) in the target coordinate space.

Do I have to remove every bit of skull, dura, etc. that I see in the brainmask? Why or why not?

- You only need to remove skull or dura if it affects the surfaces since we get our measurements from the surfaces.

Oh no! I made all these edits to a subject but now I want to rerun recon-all on the subject again with a new version of FreeSurfer. Will I lose all my work?

- If you have already made edits to a dataset, you can rerun it with a new version of
FreeSurfer and it will keep all of the edits you have already made as long as you run it on the existing dataset. In other words, you would just want to do "recon-all -all -s existing_subject".

Where do all those atlases come from that FreeSurfer uses?

- The different FreeSurfer atlases were generated from manually segmented data sets. Given each definition of the manual segmentation procedure and its labels, we constructed an atlas.

If a ran my subjects with version 4.0, can I run the rest with the newest version?

- For population studies and large scale comparisons, the best is to process all your data sets with the same version of FreeSurfer.
MORPHOMETRY PROTOCOLS (December 2011)

The following protocols are recommended for use with FreeSurfer. They have been tested on Siemens 1.5 T and 3 T MRI scanners (Sonata, Avanto, Allegra, Trio, TIM Trio). The 32-channel coil is recommended if available, otherwise the sequences will work with the 12-channel head matrix coil (also with 2x acceleration). 1 mm\(^3\) isotropic resolution is recommended but 1.3 x 1 x 1.3 mm\(^3\) is acceptable and can be used to save time or improve SNR.

**MPRAGE**

The following MPRAGE protocol was developed for good contrast between gray matter, white matter and CSF per unit of acquisition time. CSF appears dark and gray matter intensity is somewhere between CSF and white matter. Since the MPRAGE is not a steady-state sequence, different spatial frequencies have different contrasts, and gray matter intensity is not exactly midway between white matter and CSF for all spatial frequencies.

This protocol evolved together with FreeSurfer and is the basic acquisition protocol for brain morphometry studies. In cases where cortical thickness is the main interest, or cortical thickness and segmentation of other brain structures is required but time is limited, the MPRAGE should be used. If more time is available, the multiecho FLASH protocol is the preferred protocol for whole-brain segmentation. Listed below is the multiecho MPRAGE (MEMPR) protocol which has better B1 distortion properties than the single echo MPRAGE (as described in the section below on bandwidth matched imaging). The multiecho MPRAGE also contains T2\(^*\) information that can be used to distinguish dura from cortical gray matter, adjacent tissues that are isointense in the standard MPRAGE protocol. If the MEMPRAGE sequence is not available, a single echo with a lower bandwidth of around 195 Hz/px is recommended.

<table>
<thead>
<tr>
<th><strong>Sequence:</strong></th>
<th>tfl_mgh_multiecho or tfl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acquisition time:</strong></td>
<td>6:03</td>
</tr>
<tr>
<td><strong>Voxel size:</strong></td>
<td>1.0 x 1.0 x 1.0 mm(^3)</td>
</tr>
<tr>
<td><strong>Geometry:</strong></td>
<td>FoV 256 mm (256 x 256 matrix), 176 sagittal slices, slice thickness 1 mm, phase encoding anterior-posterior (readout superior-inferior) (3D encoding)</td>
</tr>
<tr>
<td><strong>Timing:</strong></td>
<td>TR 2530 ms (3T)/2730 ms (1.5T), TI 1100 ms (3T)/1000 ms (1.5T), TE 1.64/3.5/5.36/7.22 ms, bandwidth 651 Hz/px for all echoes (bipolar readout trajectory)</td>
</tr>
<tr>
<td><strong>RF:</strong></td>
<td>Non-selective IR, non-selective excitation at 7°</td>
</tr>
<tr>
<td><strong>Acceleration:</strong></td>
<td>2x GRAPPA (32 ref. lines)</td>
</tr>
<tr>
<td>(fast RF and gradient mode, no oversampling, no partial Fourier encoding, no fat suppression, no partial phase encoding, use &quot;Prescan Normalize&quot; but no other filters and avoid regular &quot;Normalize&quot;, use &quot;Adaptive Combine&quot;, increase image intensity (Fourier) scaling factor to 4.0 if possible, enable RMS averaging for MEMPR if available)</td>
<td></td>
</tr>
<tr>
<td><strong>Exceptions:</strong></td>
<td>if multiple echo sequence is not available, choose bandwidth of 195 Hz/px and TE will be 3.31 ms. If acceleration is disabled and resolution is 1 mm(^3) isotropic, increase TI to 1200 ms.</td>
</tr>
</tbody>
</table>

**FLASH**

The FLASH protocol is preferred for whole-brain segmentation where all brain structures are labeled by FreeSurfer. The acquisition requires more time – at least two separate acquisitions at two different flip angles are needed. However, the acquisition provides the data needed to calculate true quantitative T1 tissue parameters (measured in units of time) rather than the arbitrary T1-weighting that the MPRAGE provides. Proton density can also be calculated using two or more FLASH acquisitions. Listed below is the multiecho FLASH (MEF) protocol which delivers less distortion due to B0 inhomogeneities than single echo FLASH (as described in more detail in the following section). The multiecho FLASH sequence also provides T2\(^*\) information, albeit quite noisy given the short TR. If this sequence is not available, a single echo with a lower bandwidth such as 130 Hz/px can be used.
**Bandwidth matched imaging**

For multispectral morphometry (where more than one contrast is used to assess structures), it is important that the images align properly so that voxels match across images of different contrasts. Although the amount of B0 related distortion is small, differences between structural scans with different bandwidths are nevertheless sufficient that the borders of structures and the cortical ribbon may not be properly aligned everywhere especially in areas of higher susceptibility change.

To solve the differential distortion problem, we assembled a set of high bandwidth protocols. The higher bandwidth results in lower SNR but the SNR is recovered by acquiring and combining the multiple echoes. The chosen bandwidth of 651 Hz/px is also convenient for the T2-SPACE (T2 weighted) sequence, therefore all of these sequences can be matched to a high bandwidth and residual distortions are matched.

Listed below is the T2-SPACE protocol to accompany the above-listed protocol. Together with the MEMPR and MEF, this protocol provides a T1-weighted volume, T2-weighted volume and the data needed to estimate quantitative PD, T1 and approximate T2*.

**Sequence:** tse_vfl  
**Acquisition time:** 5:00  
**Voxel size:** 1.0 x 1.0 x 1.0 mm³  
**Geometry:** FoV 256 mm (256 x 256 matrix), FoV phase 79.7%, 176 sagittal slices, slice thickness 1 mm, phase encoding anterior-posterior (readout superior-inferior) (3D encoding)  
**Timing:** TR 3390 ms, TE 388 ms, bandwidth 651 Hz/px, turbo factor 115, slice turbo factor 2, echo train duration 769, echo spacing will be 3.36 ms  
**RF:** non-selective excitation (flip angle mode “T2 var”)  
**Acceleration:** 2x GRAPPA (24 ref. lines)  
(normal RF and fast gradient mode, no oversampling, no partial Fourier encoding, no partial phase encoding, no flow compensation, use “Prescan Normalize” and avoid regular “Normalize”, use “Adaptive Combine”, different Siemens software baselines may vary w.r.t. timing parameters and exact FoV phase)

For 1.3 x 1 x 1.3 mm³ resolution, change “Phase resolution” to 75%, slice thickness to 1.33 mm and number of slices to 128 for all of the above sequences (for T2-SPACE the phase resolution may not be exactly 75%). Since this decreases acquisition time, parallel acceleration may be switched off to further increase SNR.

Custom multiecho sequences for Siemens scanners are available from the Martinos Center. The sequences and protocols are provided for free, but an indemnification document must be signed.
MPRAGE equivalent protocol for Philips and GE scanners

The following protocols were developed by the INTRuST NLC\(^1\) for Philips and GE 3 T scanners to match the Siemens recommended T1 structural (MPRAGE) protocol for morphometry.

**GE T1 structural protocol**

| Sequence: SPGR-BRAVO (IR-FSPGR with ASSET) |
| Acquisition time: 5:15 |
| Voxel size: 1.0 x 1.0 x 1.0 mm\(^3\) |
| Geometry: FoV 25.6 cm (256 x 256 matrix), 176 sagittal slices, slice thickness 1 mm, (3D encoding) |
| Timing: TR min (9150 ms), TI 600 ms, TE min (3.7 ms), bandwidth 25 kHz (195 Hz/px) |
| RF: flip angle 10° |
| Acceleration: 2x Asset |

**Philips T1 structural protocol**

| Sequence: T1W_3D_TFE_SENSE |
| Acquisition time: 5:13 |
| Voxel size: 1.0 x 1.0 x 1.0 mm\(^3\) |
| Geometry: FoV 256 mm (256 x 256 matrix), 176 sagittal slices, slice thickness 1 mm, (3D encoding) |
| Timing: TR shortest (7600 ms), TI 1100 ms, TE shortest (3.5 ms), bandwidth 191.5 Hz/px |
| RF: flip angle 7° |
| Acceleration: 2x SENSE |

**MPRAGE variants from MGH**

The MEMPR and slightly modified MEF sequences are available from MGH by C2P agreement. The legal process, although straightforward in principle, may take a couple of months. Email: André van der Kouwe (andre at nmr.mgh.harvard.edu).

The motion corrected MEMPR with vNavs is available from Siemens as a works-in-progress package (WiP 711) through your local Siemens representative (created by M. Dylan Tisdall, MGH, and Himanshu Bhat, Siemens).

**OTHER EXAMPLE PROTOCOLS**

This document and example generic morphometry, DTI and fMRI protocols for Siemens scanners are available at [http://www.nmr.mgh.harvard.edu/~andre/](http://www.nmr.mgh.harvard.edu/~andre/).

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\(^1\) INTRUST (Posttraumatic Stress Disorder and Traumatic Brain Injury Clinical Consortium) NLC (Neuroimaging Leadership Core): Shenton, Kikinis, Rosen (PIs), Helmer, van der Kouwe, Kubicki, Pasternak (http://intrust.spl.harvard.edu)