



AFNI



Didactics and Demonstrations

Introduction, Concepts, Principles

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Analysis of Functional NeuroImages
by **Robert W Cox, PhD**
Released under the GNU General Public License Version 2 (GPL) [or any later GPL version]
AFNI is a research tool.
Clinical uses are not supported or advised.



Carol Myers
AFNI User



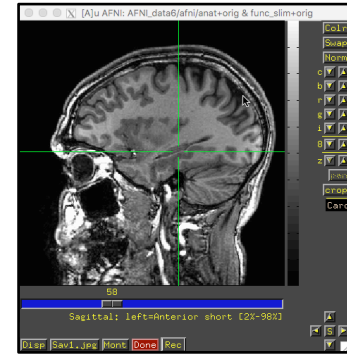
[A]u AFNI: AFNI_data6/afni/anat+orig & func_slim+orig

Order: LPI=SPM 1
x = 43.000 mm [R]
y = 17.038 mm [A]
z = 21.180 mm [S]

Xhairs Multi X+
Color green
Gap 5 Wrap
Index 0

Axial Image Graph
Sagittal Image Graph
Coronal Image Graph

New Etc-> AFNI News AFNI Forum
BHelp done AFNI Tips Prog Helps



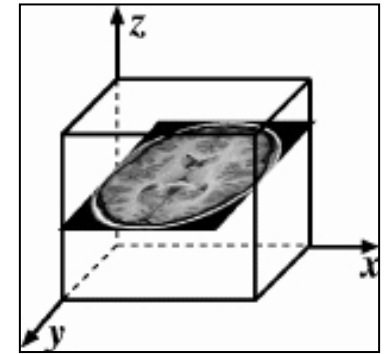
[A]u AFNI: AFNI_data6/afni/anat+orig & func_slim+orig

Original View
AC-PC Aligned
Talairach View

Define Overlay ->
See Overlay
Define Datamode ->
DataDir Switch Read
UnderLay EditEnv
OverLay NIML+PO
Control Surface

Sagittal: left=Anterior short [23-983]

File Edit View Window Help
File Save... Print Done Quit



Web Page to Bookmark

<https://afni.nimh.nih.gov/pub/dist/doc/html/doc/index.html>

== <https://bit.ly/AFNIstuff>

- Links to AFNI **Installation** instructions, Documentation, Data, **Tips**, *et cetera*
- To use hands-on videos: install AFNI and then the **Prep for Bootcamp** link

AFNI = **A**nalysis of **F**unctional **N**euro**I**mages

- Created to provide an environment for fMRI data analyses
- **AFNI** refers to both the program of that name and the entire package of external programs and plugins (several hundred)
- Important principles in the development of **AFNI**:
 - Allow user (you) to stay close to the data and view it in different ways
 - Give users the power to assemble computing pieces in different ways to make customized analyses
 - ***With great power comes great responsibility***
— ***to understand the analyses and the tools***
 - “Provide mechanism, not policy”
 - Allow other programmers to add features/components

Principles (and Caveats) We Live By

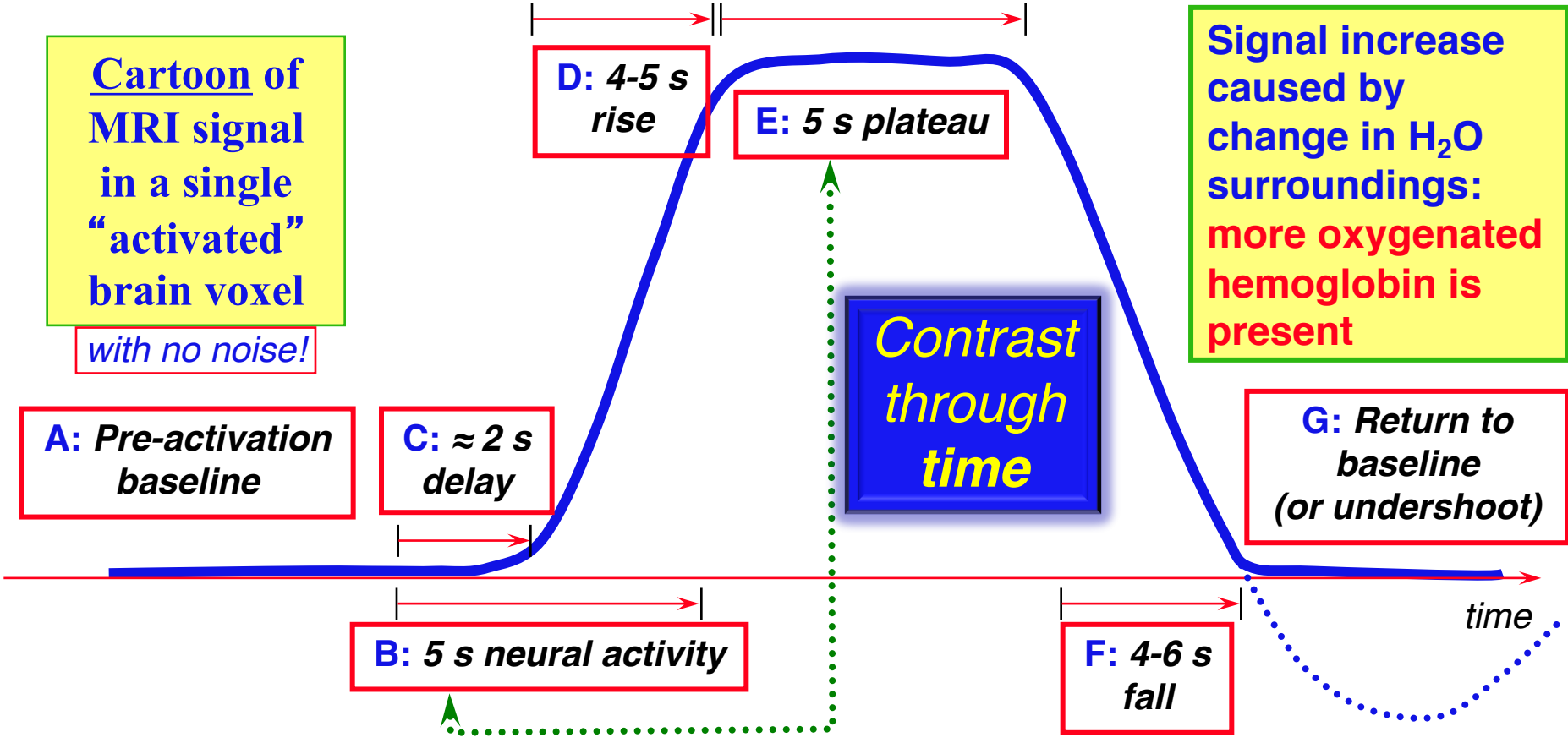
- Fix significant bugs as soon as possible
 - But, we define “significant”
- Nothing is secret or hidden (**AFNI** is open source)
 - But, possibly not very well documented or advertised
- Release early and often
 - So, All users are beta-testers for life
- Help the user (message board; consulting with NIH users)
 - Until our patience expires
- Try to anticipate users’ future needs
 - What we *think* you need may not be what you *really* need

Before We Really Start

- **AFNI** has many programs and each has many options
- Assembling the programs to do something useful *and* good seems confusing (OK, *is* confusing) when you start
- To help overcome this problem, we have “super-scripts” that carry out important tasks
 - Each script runs multiple **AFNI** programs
 - **We recommend** using these as the basis for FMRI work
- **afni_proc.py** = Single subject FMRI pre-processing and time series analysis for functional activation
- **align_epi_anat.py** = Image alignment (registration), including anatomical-EPI, anatomical-anatomical, EPI-EPI, and alignment to atlas space (Talairach/MNI)

What is Functional MRI?

- 1991 discovery: MRI-measurable signal increases few % *locally* in brain after increases in neuronal activity (Kwong, *et al.*)



How fMRI Experiments Are Done

- Alternate subject's neural state between 2 (or more) conditions using sensory stimuli, tasks to perform, ...
 - Can only measure relative signals, so must look for **changes** in the signal between the conditions

- Acquire lots of MR images repeatedly during this process

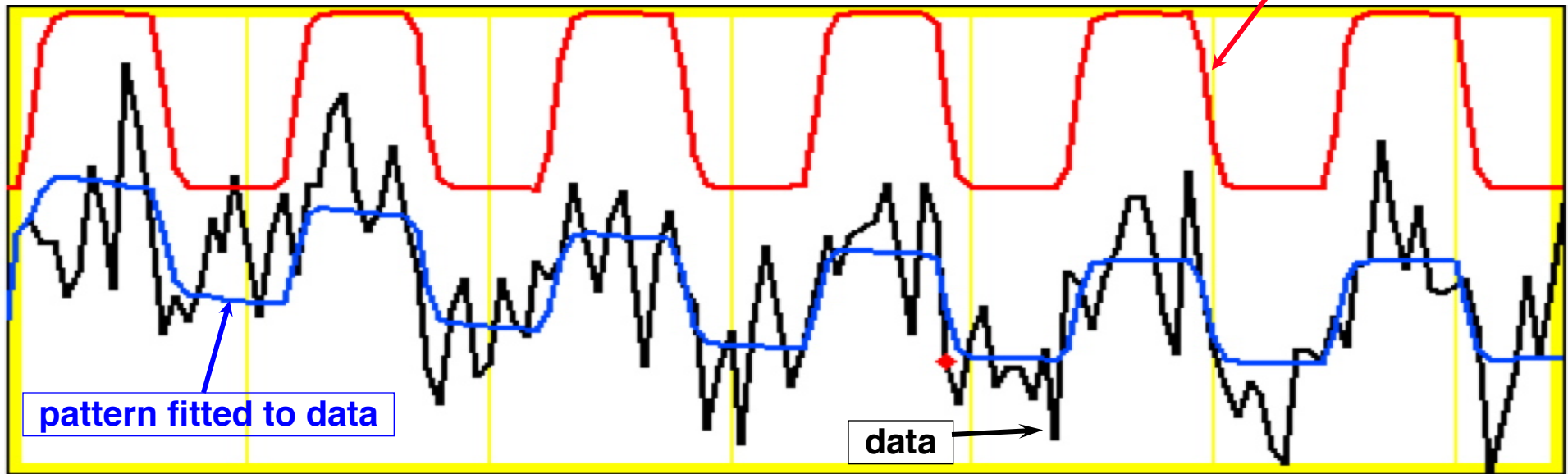
- Search for voxels whose NMR signal time series (up-and-down) matches stimulus time series pattern (on-and-off)
 - fMRI data analysis is basically pattern matching **in time**

- MRI signal changes due to neural activity are small
 - Need 500 or so images in time series (in each slice) → takes 30 min or so to get reliable activation maps
 - Other small effects can corrupt the results → post-process the data to reduce these effects & **be vigilant**

Sample Data Time Series

- 64×64 matrix (TR=2.5 s; 130 time points per imaging run)
- Somatosensory task: **27 s “on”**, **27 s “rest”**
- Note that this is *really* good data

pattern of expected BOLD signal

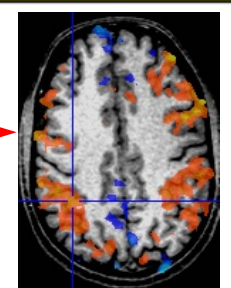
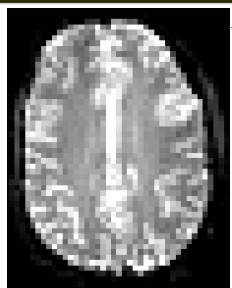


pattern fitted to data

data

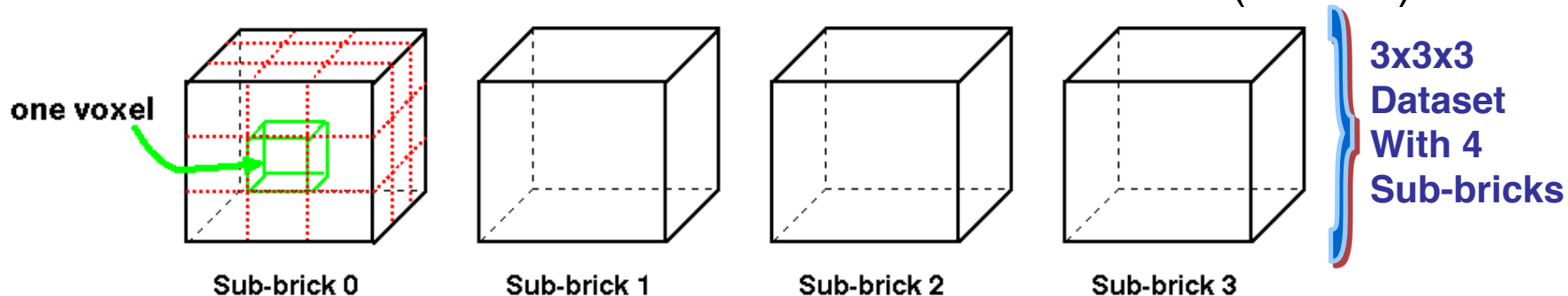
One echo-planar image

One anatomical image, with voxels that match the pattern given a color overlay



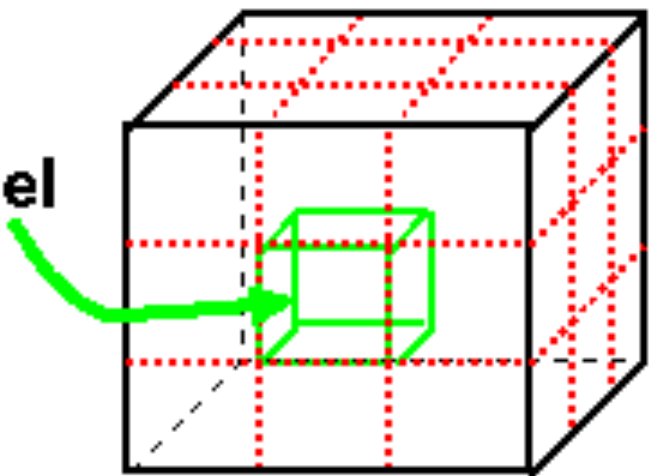
Fundamental AFNI Concepts

- Basic unit of data in **AFNI** is the dataset ← **Jargon!**
 - A collection of 1 or more 3D arrays of numbers
 - Each entry in array is in a particular spatial location in a 3D grid
 - Image datasets: each array holds collection of slices from scanner
 - Each number is the signal intensity for that particular voxel
 - Derived datasets: each number is computed from other dataset(s)
 - e.g., each voxel value is a *t*-statistic reporting “activation” significance from an fMRI time series dataset, for that voxel
 - Each 3D array in a dataset is called a sub-brick ← **Jargon!**
 - There is one number in each voxel in each sub-brick (volume)



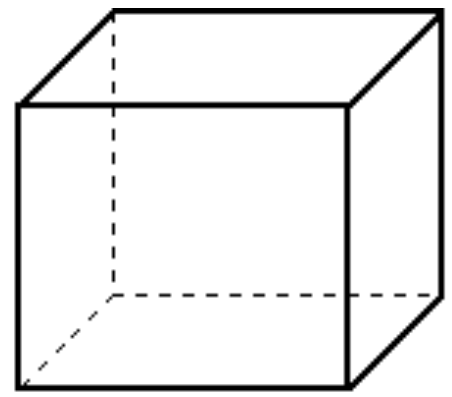
Dataset Cartoon: A Little Bit Bigger

one voxel

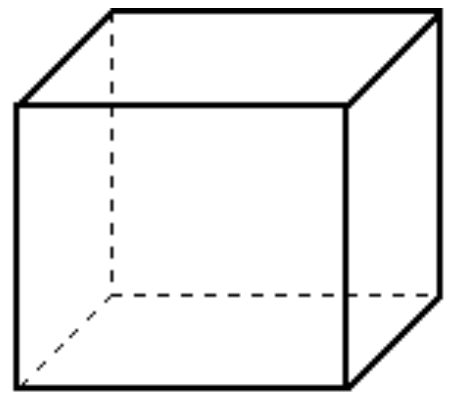


“sub-brick” = one 3D volume inside a multi-volume dataset

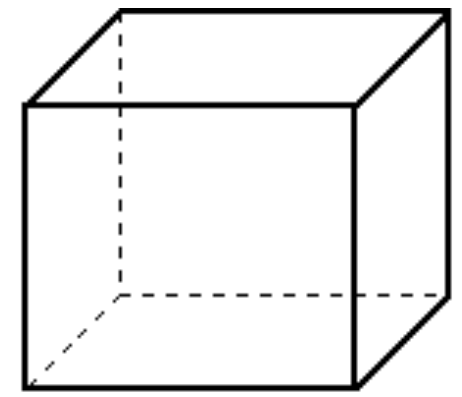
Sub-brick 0



Sub-brick 1



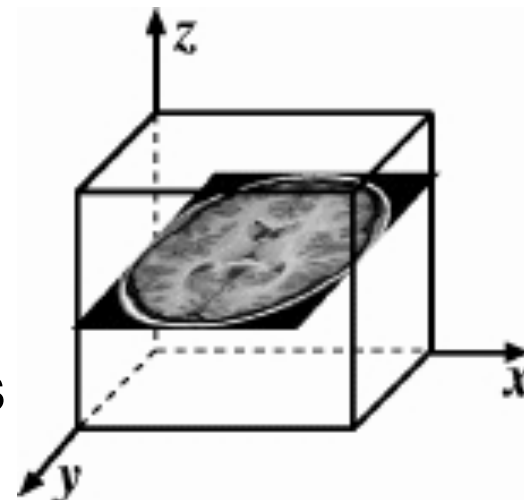
Sub-brick 2



Sub-brick 3

What's in a Dataset: Header Stuff


- Besides the voxel numerical values, a dataset also contains auxiliary information, including (some of which is optional):
 - xyz dimensions of each voxel (in mm)
 - Orientation of dataset axes;
for example, x-axis=R-L, y-axis=A-P, z-axis=I-S
= axial slices (we call this orientation “RAI”)
 - Location of dataset in scanner coordinates
 - Needed to overlay one dataset onto another
 - Important to get right in FMRI, since we deal with many datasets
 - Time (s) between sub-bricks, for 3D+time datasets ← **Jargon!**
 - Such datasets are basic unit of FMRI data (one per imaging run)
 - Statistical parameters associated with each sub-brick
 - e.g., a *t*-statistic sub-brick has degrees-of-freedom parameter stored



AFNI Formatted Dataset Files - 1

- **AFNI formatted** datasets are stored in 2 files
 - The .HEAD file holds all the auxiliary information (ASCII)
 - The .BRIK file holds all the numbers in all the sub-bricks
- Datasets can be in one of 2 coordinate systems (“views”)
 - Original data or +orig view = from the scanner
 - “Talairach” or +tlrc view =
 - Dataset has been rescaled to conform to the Talairach-Tournoux atlas dimensions *or* another atlas, such as MNI
 - AKA Stererotaxic coordinates
 - **All** datasets scaled+aligned to some atlas are labeled +tlrc
 - Header file holds name of actual atlas “space” (*e.g.*, “MNI”)
 - Alignment can be *linear* or *nonlinear* (**3dQwarp** program)

AFNI Formatted Dataset Files - 2

- **AFNI** dataset filenames consist of 3 parts
 - The user-selected prefix (almost anything) 
 - The view (one of +orig, or +tlrc)
 - The suffix (one of .HEAD or .BRIK)
 - **TonyFauci_epi+tlrc.HEAD & TonyFauci_epi+tlrc.BRIK**
 - You supply the prefix; the **AFNI** program supplies the rest

- **AFNI** programs can *read* datasets stored in several formats
 - ANALYZE (.hdr/.img file pairs); very old nowadays
 - MINC-1 (.mnc); i.e., from mnitools (also very old now)
 - CTF (.mri, .svl) MEG analysis volumes
 - ASCII text (.1D) — text numbers arranged into columns
 - Have conversion programs to write out MINC-1, ANALYZE, ASCII, and NIfTI-1.1 files, if desired

NIfTI Dataset Files

- NIfTI-1 ([.nii](#) or [.nii.gz](#)) is a standard format that **AFNI**, SPM, FSL, FreeSurfer, BrainVoyager, et al., agreed upon
 - Goal: easier interoperability of tools from various packages
- All data is stored in one file (*cf.* <http://nifti.nimh.nih.gov/>)
 - 352 byte header (extensions allowed; **AFNI uses this feature**)
 - Followed by the image binary numerical values
 - Allows 1D–5D datasets of diverse numerical types
 - [.nii.gz](#) suffix means file is compressed (using Unix program gzip)
- **AFNI** reads and writes NIfTI-1 (and NIfTI-2) datasets
 - **To write:** when you give the [prefix](#) for the output filename, end it in “[.nii](#)” or “[.nii.gz](#)”, and **AFNI** programs will automatically write NIfTI-1.1 format instead of [.HEAD/.BRIK](#)
 - **To read:** just give the full filename ending in “[.nii](#)” or “[.nii.gz](#)”

Creating Datasets from DICOM Files

- Program 1: Rick Reynolds' **AFNI** program **Dimon**
 - Was originally created for sending image data directly into **AFNI** for “realtime FMRI”
- Program 2: Chris Rorden's **dcm2niix_afni**
 - Can create a whole collection of datasets
 - Works with more DICOM formats than **Dimon** does
 - *Problem*: Standard NIFTI .nii format can't store complicated slice timings
 - So programs like **dcm2niix_afni** cannot store this information even if the program can find it in the DICOM files
- Solution: use **3drefit** to add the slice timing information to the header (inside **AFNI** extension for NIFTI .nii files)

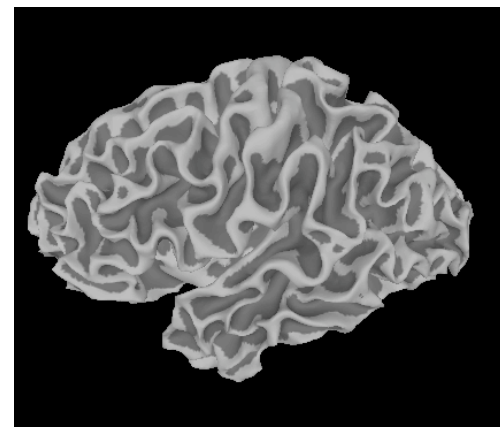
Dataset Directories

- Datasets are stored in directories (AKA “folders”)
 - All the datasets in the same directory, in the same view, are presumed to be aligned in *xyz*-coordinates
 - Voxels with same value of (x,y,z) correspond to same brain location
 - Can **overlay** (in color) any one dataset on top of any other one dataset (**underlay** in grayscale) from same directory
 - Even if voxel sizes and spatial orientations differ
 - Overlay of one dataset upon another is based on *xyz* coordinates
 - Typical **AFNI** contents of a directory are all data derived from a single scanning session for one subject
 - Anatomical reference (T1-weighted SPGR or MP-RAGE volume)
 - 10-20 3D+time datasets from FMRI EPI functional runs
 - Statistical datasets computed from 3D+time datasets
 - Datasets transformed from +orig to +tlrc coordinates

Getting and Installing AFNI

- **AFNI** runs on Unix systems: Linux, Sun, Mac OS X
 - Can also run under Windows Subsystem for Linux
 - Requires also installing X11 (Unix graphics display software)
- You can download precompiled binaries from our Website
 - <https://afni.nimh.nih.gov/pub/dist/doc/html/doc/index.html>
 - Documentation, message board, data, class materials,...
- You can download source code and compile it
 - And from GitHub: <https://github.com/afni/AFNI>
- **AFNI** is updated fairly frequently, so it is important to update occasionally = [@update.afni.binaries](#)
 - It's hard to help you with outdated versions!
 - ***Please check for updates every 6 months (or less)***

SUMA, et alii



- **SUMA** is the **AFNI** surface mapper
 - For displaying surface models of cortex
 - Surfaces from **FreeSurfer** (MGH) *et cetera*
 - Interactively display functional activations mapped from 3D volumes to the cortical surface representation
 - Draw ROIs directly on the cortical surface
 - vs. **AFNI**: ROIs are drawn into the 3D volume
- SUMA is separate from **AFNI**, but can “**t**alk” with **AFNI**
 - Click in **AFNI** or SUMA to change focus point, and the other program jumps to that location at the same time
 - Functional (color) overlay in **AFNI** can be sent to SUMA for simultaneous display
- And much more — stayed tuned for the SUMA talks to come!

That's All for Now



**KEEP
CALM
AND
CARRY
ON**