AFNI
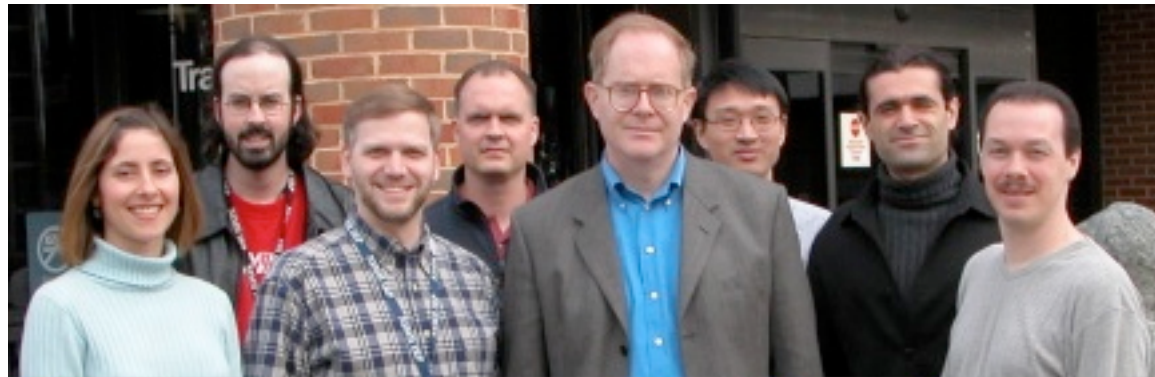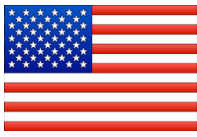## Introduction, Concepts, Principles

Analysis of Functional NeuroImages
by
Robert W Cox, PhD

Released under the GNU General Public License Version 2 (GPL)

AFNI is a research tool.
Clinical uses are *not* supported or advised.

Alison Sanders
AFNI User

http://afni.nimh.nih.gov/afni

AFNI = Analysis of Functional NeuroImages

- Developed to provide an environment for FMRI data analyses
  - ★ And a platform for development of new software
- **AFNI** refers to both the program of that name and the entire package of programs and plugins (more than 100)
- Important principles in the development of AFNI:
  - ★ Allow user to stay close to the data and view it in many different ways
  - ★ Give users the power to assemble pieces in different ways to make customized analyses
    - o "With great power comes great responsibility" — to understand
  - ★ "Provide mechanism, not policy"
  - ★ Allow other programmers to add features that can interact with the rest of the package
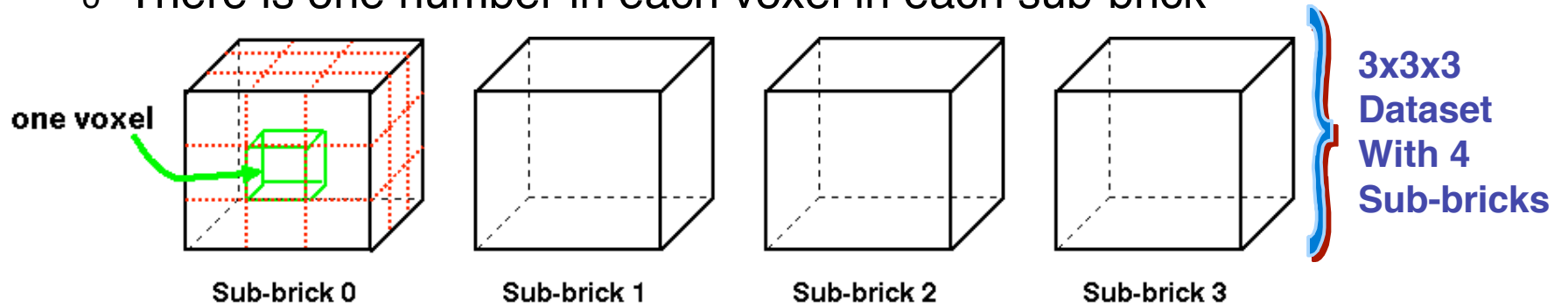
Principles We* Live By

- 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

  ★ 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 will need may not be what you actually end up needing

*

Fundamental AFNI Concepts

- Basic unit of data in AFNI is the dataset
  - ★ A collection of 1 or more 3D arrays of numbers
    - o Each entry in the array is in a particular spatial location in a 3D grid (a voxel = 3D pixel)
    - o Image datasets: each array holds a collection of slices from the scanner
      - ➥ Each number is the signal intensity for that particular voxel
    - o 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
   - o There is one number in each voxel in each sub-brick

one voxel

Sub-brick 0   Sub-brick 1   Sub-brick 2   Sub-brick 3

3x3x3 Dataset With 4 Sub-bricks

Dataset Contents: Numbers

- Different types of numbers can be stored in datasets

  ★ 8 bit bytes (e.g., from grayscale photos)

  ★ 16 bit short integers (e.g., from MRI scanners)

    ○ Each sub-brick may also have a floating point scale factor α attached, so that "true" value in each voxel is actually $\alpha\cdot$(value in dataset file)

  ★ 32 bit floats (e.g., calculated values; lets you avoid the α)

  ★ 24 bit RGB color triples (e.g., JPEGs from your digital camera!)

  ★ 64 bit complex numbers (e.g., for the physicists in the room)

- Different sub-bricks are allowed to have different numeric types

    and I mean this

  ★ But this is **_not_** recommended

  ★ Will occur if you "catenate" two dissimilar datasets together (e.g., using **3dTcat** or **3dbucket** commands)

    ○ Programs will display a warning to the screen if you try this

Dataset Contents: Header

- 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

  o Needed to overlay one dataset onto another

  o Very important to get right in FMRI, since we deal with many datasets

  ★ Time between sub-bricks, for 3D+time datasets

  o Such datasets are the basic unit of FMRI data (one per imaging run)

  ★ Statistical parameters associated with each sub-brick

  o e.g., a *t*-statistic sub-brick has degrees-of-freedom parameter stored

 o e.g., an *F*-statistic sub-brick has 2 DOF parameters stored
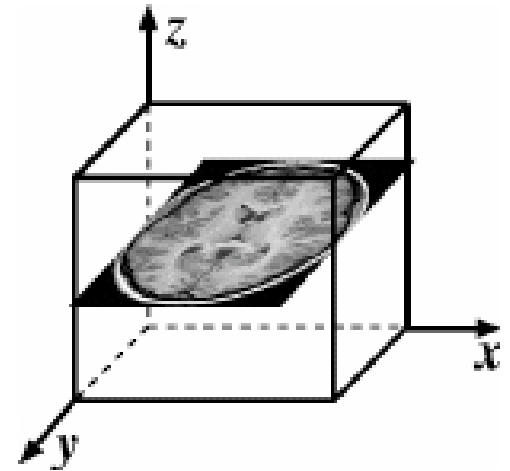
AFNI Dataset Files - I

- AFNI formatted datasets are stored in 2 files
  - ★ The .HEAD file holds all the auxiliary information
  - ★ The .BRIK file holds all the numbers in all the sub-bricks
- Datasets can be in one of 3 coordinate systems (AKA views)
  - ★ Original data or +orig view: from the scanner
  - ★ AC-PC aligned or +acpc view:
    - o Dataset rotated/shifted so that the anterior commissure and posterior commissure are horizontal (y-axis), the AC is at (x,y,z)=(0,0,0), and the hemispheric fissure is vertical (z-axis)
  - ★ Talairach or +tlrc view:
    - o Dataset has also been rescaled to conform to the Talairach-Tournoux atlas dimensions (R-L=136 mm; A-P=172 mm; I-S=116 mm)
    - o AKA Talairach or Stererotaxic coordinates
    - o Not quite the same as MNI coordinates, but very close

AFNI Dataset Files - II

- AFNI dataset filenames consist of 3 parts
  - ★ The user-selected prefix (almost anything)
  - ★ The view (one of +orig, +acpc, or +tlrc)
  - ★ The suffix (one of .HEAD or .BRIK)
  - ★ Example: **BillGates+tlrc.HEAD** and **BillGates+tlrc.BRIK**
  - ★ When creating a dataset with an AFNI program, you supply the prefix; the program supplies the rest
- AFNI programs can ***read*** datasets stored in several formats
  - ★ ANALYZE (.hdr/.img file pairs); i.e., from SPM, FSL
  - ★ MINC-1 (.mnc); i.e., from mnitools
  - ★ CTF (.mri, .svl) MEG analysis volumes
  - ★ ASCII text (.1D) — numbers arranged into columns
  - ★ Have conversion programs to write out MINC-1, ANALYZE, ASCII, and NIfTI-1.1 files from AFNI datasets, if desired

NIfTI Dataset Files
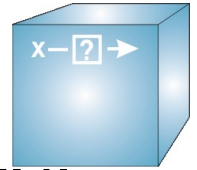
- NIfTI-1.1 (.nii or .nii.gz) is a new standard format that AFNI, SPM, FSL, BrainVoyager, et al., have agreed upon
  - ★ Adaptation and extension of the old ANALYZE 7.5 format
  - ★ Goal: easier interoperability of tools from various packages
- All data is stored in 1 file (cf. http://nifti.nimh.nih.gov/)
  - ★ 348 byte header (extensions allowed; AFNI uses this feature)
  - ★ Followed by the image numerical values
  - ★ Allows 1D-5D datasets of diverse numerical types
  - ★ .nii.gz suffix means file is compressed (with gzip)
- AFNI now reads and writes NIfTI-1.1 formatted datasets
  - ★ To write: when you give the prefix for the output filename, end it in ".nii" or ".nii.gz", and all 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"

Dataset Directories

- Datasets are stored in directories, also called <u>sessions</u>

  ★ All the datasets in the same session, in the same view, are presumed to be aligned in *xyz*-coordinates

   o 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 (in grayscale) from same session

   o Even if voxel sizes and orientations differ

  ★ Typical AFNI contents of a session directory are all data derived from a single scanning session for one subject

   o Anatomical reference (T1-weighted SPGR or MP-RAGE volume)

   o 10-20 3D+time datasets from FMRI EPI functional runs

   o Statistical datasets computed from 3D+time datasets, showing activation (you hope and pray)

   o Datasets transformed from +orig to +tlrc coordinates, for comparison and conglomeration with datasets from other subjects

Getting and Installing AFNI

- AFNI runs on <u>Unix</u> systems: Linux, Sun, SGI, Mac OS X
  - ★ Can run under Windows with Cygwin Unix emulator
    - o This option is really just for trying it out — not for production use
- **If you are at the NIH**: SSCC can install AFNI and update it on your system(s)
  - ★ You must give us an account with *ssh* access
- You can download precompiled binaries from our Website
  - ★ **http://afni.nimh.nih.gov/afni**
  - ★ Also: documentation, message board, humor, data, …
- You can download source code and compile it
- AFNI is updated fairly frequently, so it is important to update occasionally
  - ★ We won't help you with old versions*!*

AFNI at the NIH Scanners

- AFNI can take images in "realtime" from an external program and assemble them into 3D+time datasets slice-by-slice

- Recently (July 2005), Jerzy Bodurka (FMRIF) has set up the GE Excite-based scanners (3T-1, 1.5 T, NMRF 3 T, and 7 T) to start AFNI automagically when scanning, and send reconstructed images over as soon as they are available:

  - ★ For immediate display (images and graphs of time series)
  - ★ Plus graphs of estimate subject head movement

- Goal is to let you see data as it is acquired, so that if there are any big problems, you can fix them right away

  - ★ Sample problem: someone typed in the imaging field-of-view (FOV) size wrong (240 cm instead of 24 cm), and got garbage data, **but only realized this too late** (after subject had left the scanner and gone home) — *D'oh!*

A Quick Overview of AFNI

- Starting AFNI from the Unix command line
  - ★ **afni** reads datasets from the current directory
  - ★ **afni dir1 dir2** … reads datasets from directories listed
  - ★ **afni -R** reads datasets from current directory and from all directories below it
- AFNI also reads a file named .afnirc from your home directory
  - ★ Used to change many of the defaults
    - ○ Window layout and image/graph viewing setup; popup hints; whether to compress .BRIK files when writing
    - ○ cf. file README.environment in the AFNI documentation
- Also can read file .afni.startup_script to restore the window layout from a previous run
  - ★ Created from **Define Datamode->Misc->Save Layout** menu
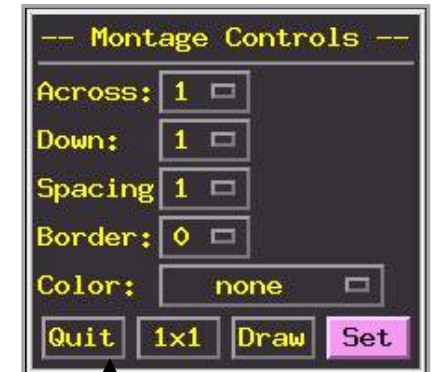   - ○ cf. file README.driver for what can be done with AFNI scripts
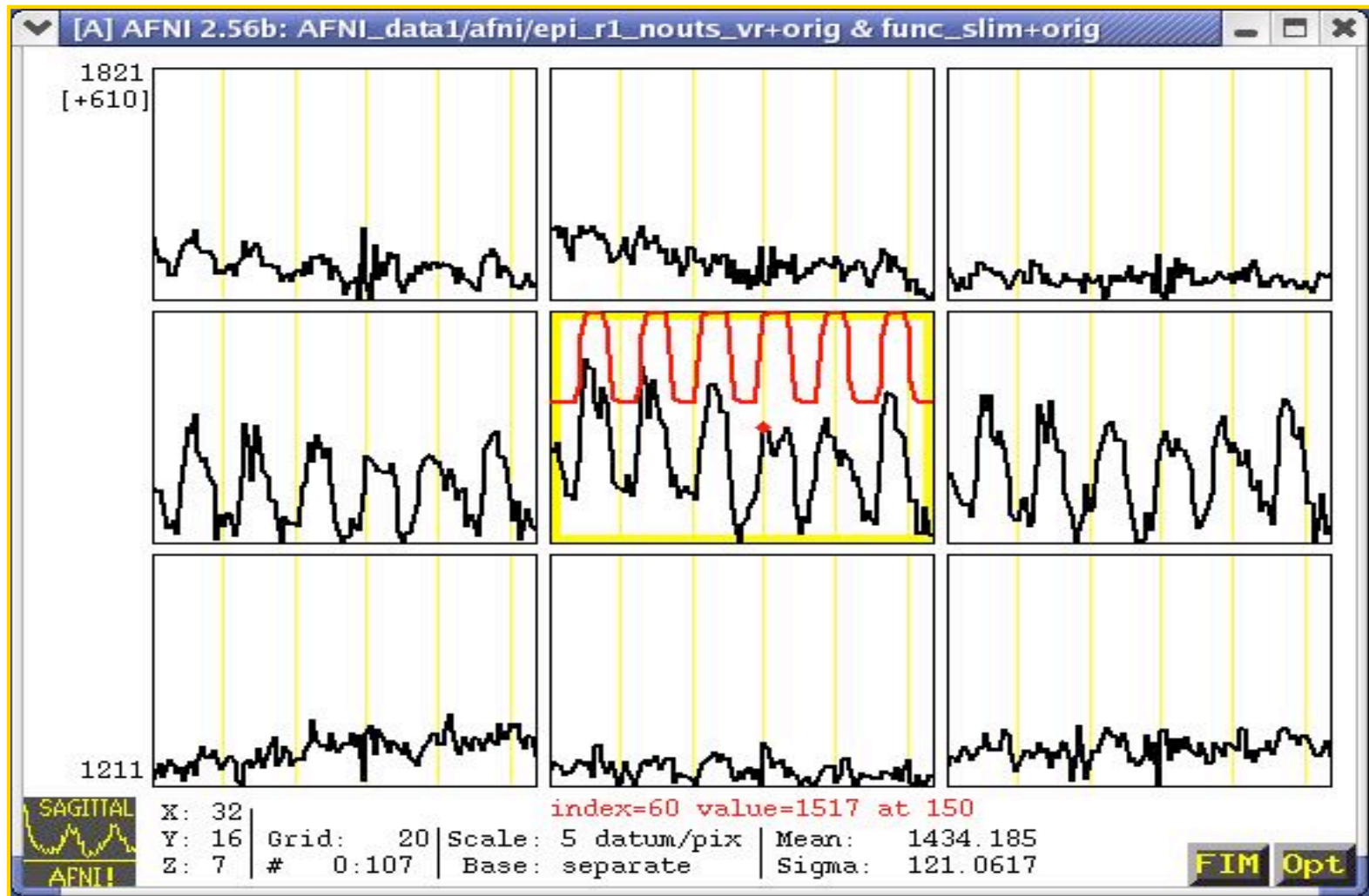
AFNI controller window at startup

Titlebar shows current datasets

Switch to different coordinate system

Coordinates of current focus point

Control crosshairs appearance

Time index

Open images and graphs of datasets

Open new AFNI controller

Help Button

Markers control transformation to +acpc and +tlrc coordinates

Controls color functional overlay

Miscellaneous menus

Switch between directories, underlay (anatomical) datasets, and overlay (functional) datasets

Controls display of overlaid surfaces

Close this controller

AFNI Image Viewer

Disp and **Mont** control panels
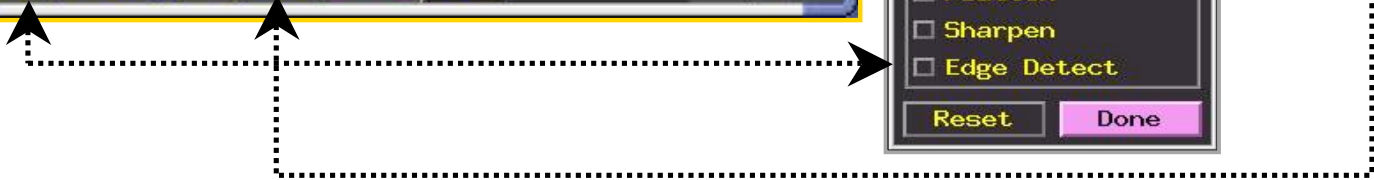
AFNI Time Series Graph Viewer

Data (black) and Reference waveforms (red)

Menus for controlling graph displays

Define Function: Color Overlay Panel

Color map

Hidden popup menu here

Choose which dataset makes the underlay image

Choose which sub-brick from Underlay dataset to display (usu. Anat - has only 1 sub-brick)

Threshold slider

p-value of current threshold

Choose which sub-brick of functional dataset makes the color

Choose which sub-brick of functional dataset is the Threshold

Shows ranges of data in Underlay and Overlay dataset

Choose range of threshold slider, in powers of 10

Shows automatic range for color scaling

Rotates color map

Positive-only or both signs of function?

Number of panes in color map

Shows voxel values at focus

Lets you choose range for color scaling

Volume Rendering: an AFNI plugin

Pick new underlay dataset

Name of underlay dataset

Sub-brick to display

Open color overlay controls

Range of values in underlay

Range of values to render

Change mapping from values in dataset to brightness in image

Histogram of values in underlay dataset

Mapping from values to opacity

Maximum voxel opacity

Cutout parts of 3D volume

Menu to control scripting (control rendering from a file)

Compute many images in a row

Render new image immediately when a control is changed

Show 2D crosshairs

Control viewing angles

Accumulate a history of rendered images (can later save to an animation)

Detailed instructions

Force a new image to be rendered

Reload values from the dataset

Close all rendering windows

Staying Close to Your Data!
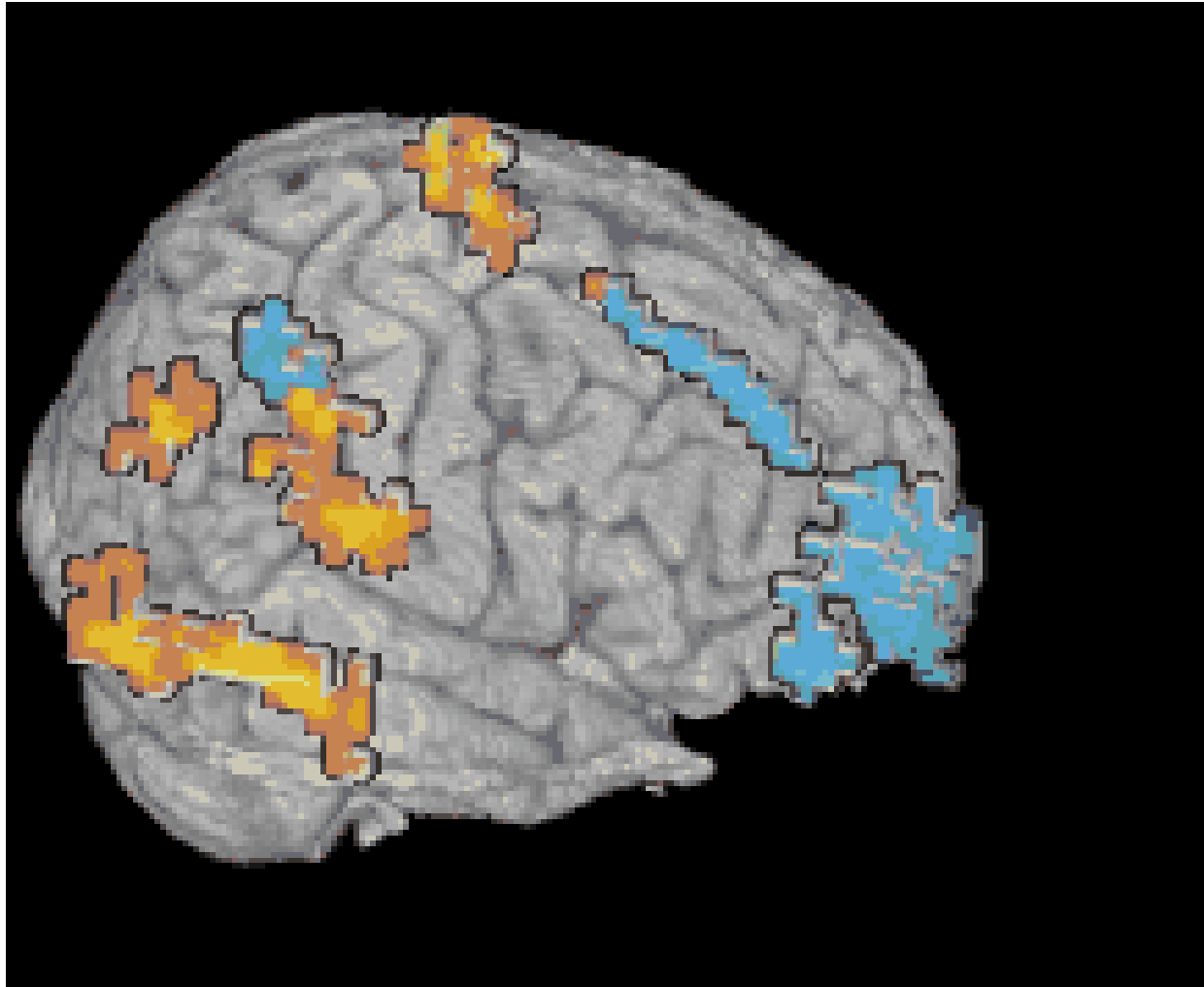
"ShowThru" rendering of functional activation:
animation created with Automate and Save:aGif controls

Other Parts of AFNI

- Batch mode programs
  - ★ Are run by typing commands directly to computer, or by putting commands into a text file (script) and later executing them
- Good points about batch mode
  - ★ Can process new datasets exactly the same as old ones
  - ★ Can link together a sequence of programs to make a customized analysis (a personalized pipeline)
  - ★ Some analyses take a long time
- Bad points about batch mode
  - ★ Learning curve is "all at once" rather than gradual
  - ★ If you are, like, under age 35, you may not know how to type commands into a computer
    - o At least we don't make you use punched cards (yet)

AFNI Batch Programs

- Many important capabilities in AFNI are **only** available in batch programs

  ★ A few examples (of more than 100, from trivial to complex)

- **3dDeconvolve** = multiple linear regression on 3D+time datasets, to fit each voxel's time series to an activation model and then test these fits for significance

- **3dvolreg** = 3D+time dataset registration, to correct for small subject head movements, and for inter-day head positioning

- **3dANOVA** = 1-, 2-, 3-, and 4- way ANOVA layouts, for combining & contrasting datasets in Talairach space

- **3dcalc** = general purpose voxel-wise calculator

- **3dclust** = find clusters of activated voxels

- **3dresample** = re-orient and/or re-size dataset voxel grid

- **3dSkullStrip** = remove "skull" from anatomical dataset

AFNI Plugins

- A plugin is an extension to AFNI that attaches itself to the interactive AFNI GUI

  ★ **Not** the same as a batch program

  ★ Offers a relatively easy way to add certain types of interactive functionality to AFNI

  ★ A few examples:

- **Draw Dataset** = ROI drawing (draws numbers into voxels)

- **Render [new]** = Volume renderer

- **Dataset#N** = Lets you plot multiple 3D+time datasets as overlays in an AFNI graph viewer (e.g., fitted model over data)

- **Histogram** = Plots a histogram of a dataset or piece of one

- **Edit Tagset** = Lets you attach labeled "tag points" to a dataset (e.g., as anatomical reference markers)

SUMA, et alii

- **SUMA** is the AFNI <u>su</u>rface <u>m</u>apper
  - ★ For displaying surface models of the cortex
    - o Surface models come from **FreeSurfer** (MGH) or **SureFit** (Wash U) or **BrainVoyager**
  - ★ Can display functional activations mapped from 3D volumes to the cortical surface
  - ★ Can draw ROIs directly on the cortical surface
    - o vs. AFNI: ROIs are drawn into the volume
- SUMA is a separate program from AFNI, but can "talk" to AFNI so that volume and surface viewing are linked
  - ★ Click in AFNI or SUMA to change focus point, and the other program jumps to that location at the same time
  - ★ Functional overlay in AFNI can be sent to SUMA for simultaneous display
- And much more — stayed tuned for the SUMA talks to come!

SUMA Teaser Movie
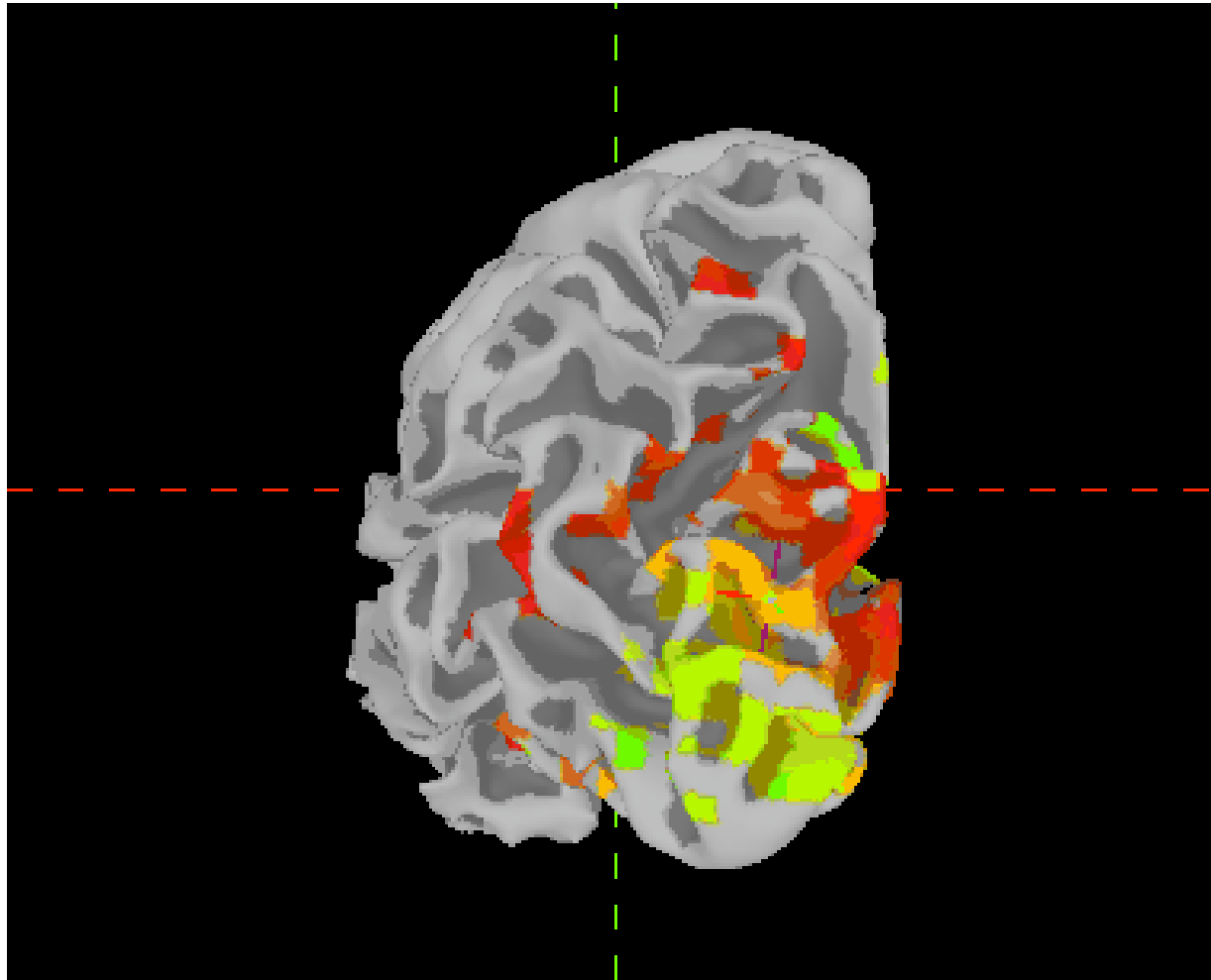
Color from AFNI, Images from SUMA
Images captured with the 'R' recorder function,
then saved as animation with Save:aGif control

Other Educational Presentations

- How to get images into AFNI or NIfTI format (program `to3d`)
- Detailed hands-on with using AFNI for data viewing (fun)
- Signal modeling & analysis: theory & hands-on (`3dDeconvolve`)
- Image registration (`3dvolreg`)
- Volume rendering hands-on (fun level=high)
- ROI drawing hands-on (fun level=extreme)
- Transformation to Talairach hands-on (fun level=low)
- Group analysis: theory and hands-on (`3dANOVAx`)
- SUMA hands-on (fun level=not-so-bad)
- Surface-based analysis
- Miscellaneous AFNI utility programs and plugins
- A tour of the AFNI Website

Ongoing AFNI+SUMA Projects

- Complex ANOVA models for group analyses

  ★ Unbalanced designs, missing data, continuous covariates, multi-nested designs, ….

- Diffusion 'tensor' analyses

  ★ Computation of **D** tensor, FA, tracts, ….

- Modeling of Gd-DTPA leakage rates into damaged tissue

- Finishing the changes to **3dDeconvolve** to simplify deconvolution analysis on paradigms with irregular timing

  ★ And to incorporate physiological noise cancellation, and correction for EPI time series autocorrelation, and …

- More surface-based analysis tools

- Automatic Talairach-ization + skull stripping (demo now working)

- Better ways to organize collections of datasets