AFNI & FMRI

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.

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 external 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 the analyses and the tools**
  - ★ "Provide mechanism, not policy"
  - ★ Allow other programmers to add features that can interact with the rest of the package

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
  - ⋆ 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

*

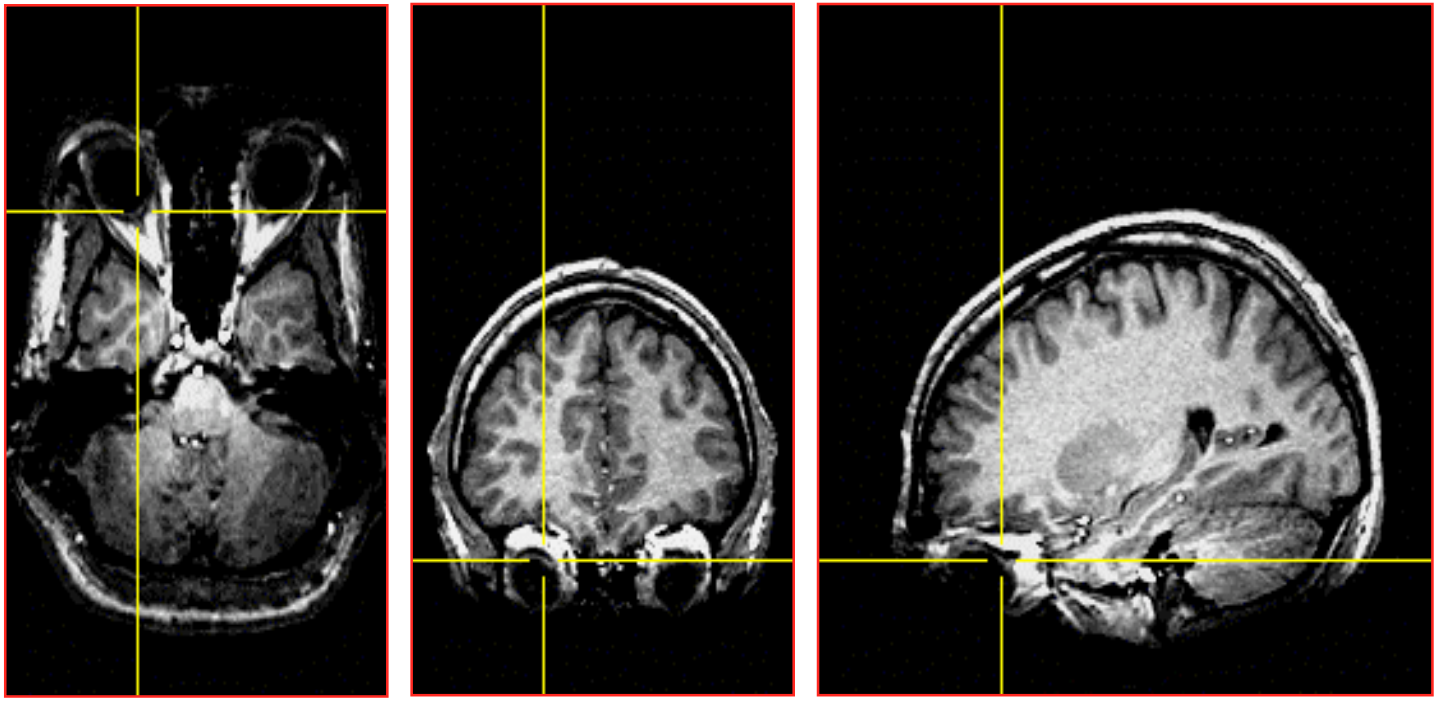
Outline of This Talk

- **Quick introduction to FMRI physics and physiology**
  - ⋆ So you have *some* idea of what is going on in the scanner and what is actually being measured
    - o Most of the slides for this talk are "hidden" — only visible in the download, not in the classroom
- **Overview of basic AFNI concepts**
  - ⋆ Datasets and file formats; Realtime input; Controller panels; SUMA; Batch programs and Plugins
- **Brief discussion of FMRI experimental designs**
  - ⋆ Block, Event-Related, Hybrid Event-Block
  - ⋆ But this is *not* a course in how to design your FMRI experimental paradigm
- **Outlines of standard FMRI processing pipeline (AFNI-ized)**
  - ⋆ Keep this in mind for the rest of the class!
  - ⋆ Many experiments require tweaking this "standard" collection of steps to fit the design of the paradigm and/or the inferential goals

Quick Intro to MRI and FMRI

Physics and Physiology

(in pretty small doses)



2D slices extracted from a 3D (volumetric) image
[resolution about 1×1×1 mm ; acquisition time about 10 min]

MRI = Cool (and useful) Pictures about anatomy (spatial structure)

FMRI = Cool (and useful) Pictures about function (temporal structure)

Synopsis of MRI

1) Put subject in big magnetic field (leave him there)

   Magnetizes the H nuclei in water (H_2O)

2) Transmit radio waves into subject [about 3 ms]

   Perturbs the magnetization of the water

3) Turn off radio wave transmitter

4) Receive radio waves re-transmitted by subject's H nuclei

   Manipulate re-transmission with magnetic fields during this *readout* interval [10-100 ms]

   Radio waves transmitted by H nuclei are sensitive to magnetic fields — both those imposed from outside and **those generated inside the body:**

   Magnetic fields generated by tissue components change the data and so will change the computed image

5) Store measured radio wave data vs. time

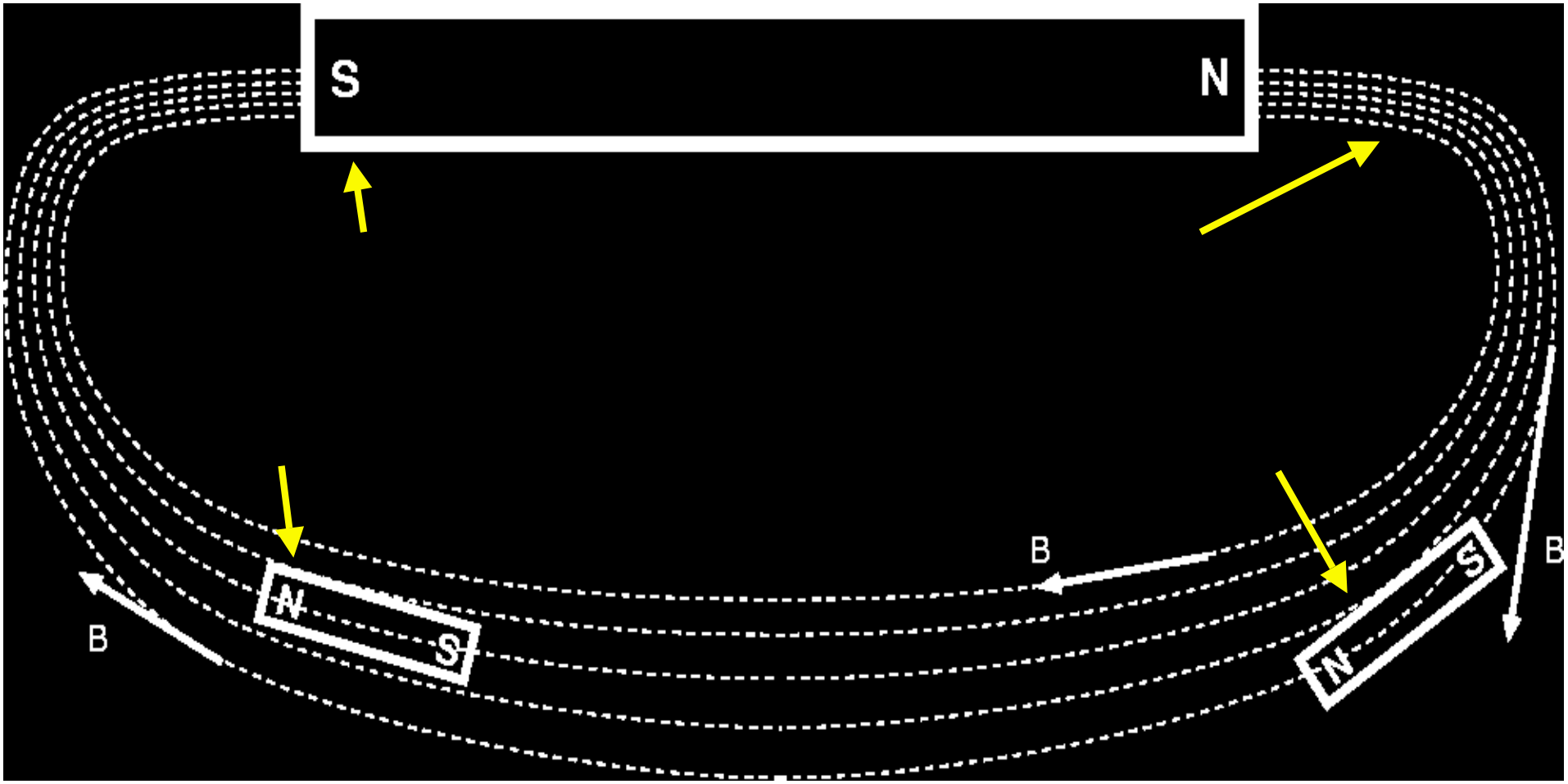
   Now go back to 2) to get some more data [many times]

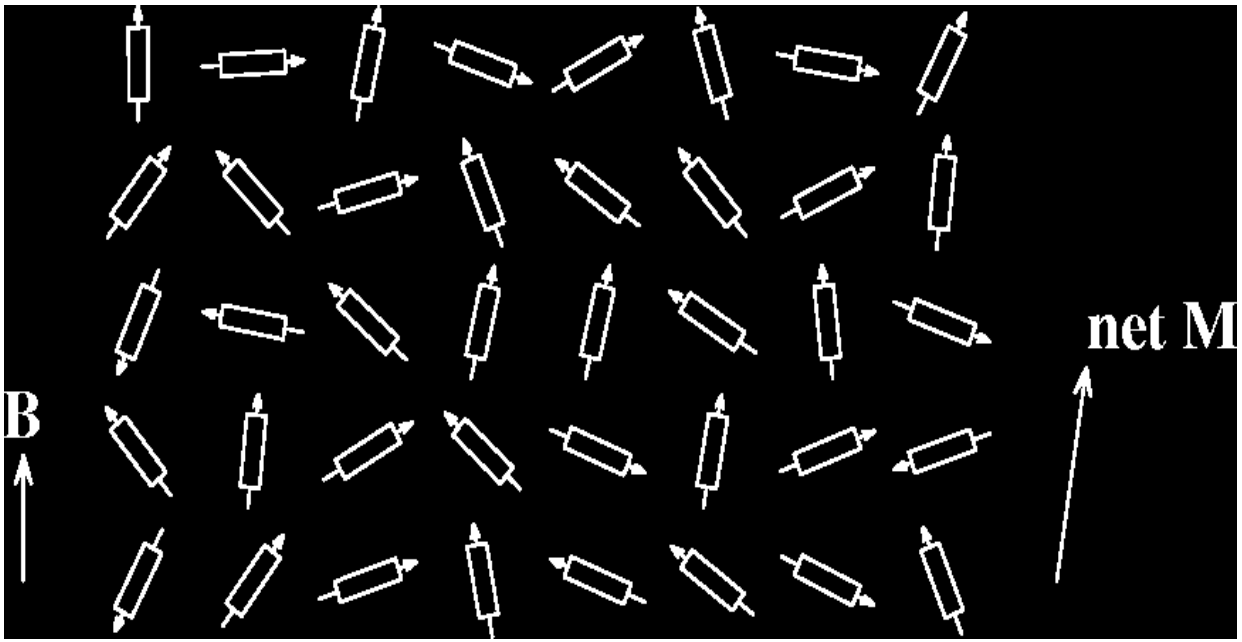
6) Process raw radio wave data to reconstruct images

   Allow subject to leave scanner (optional)

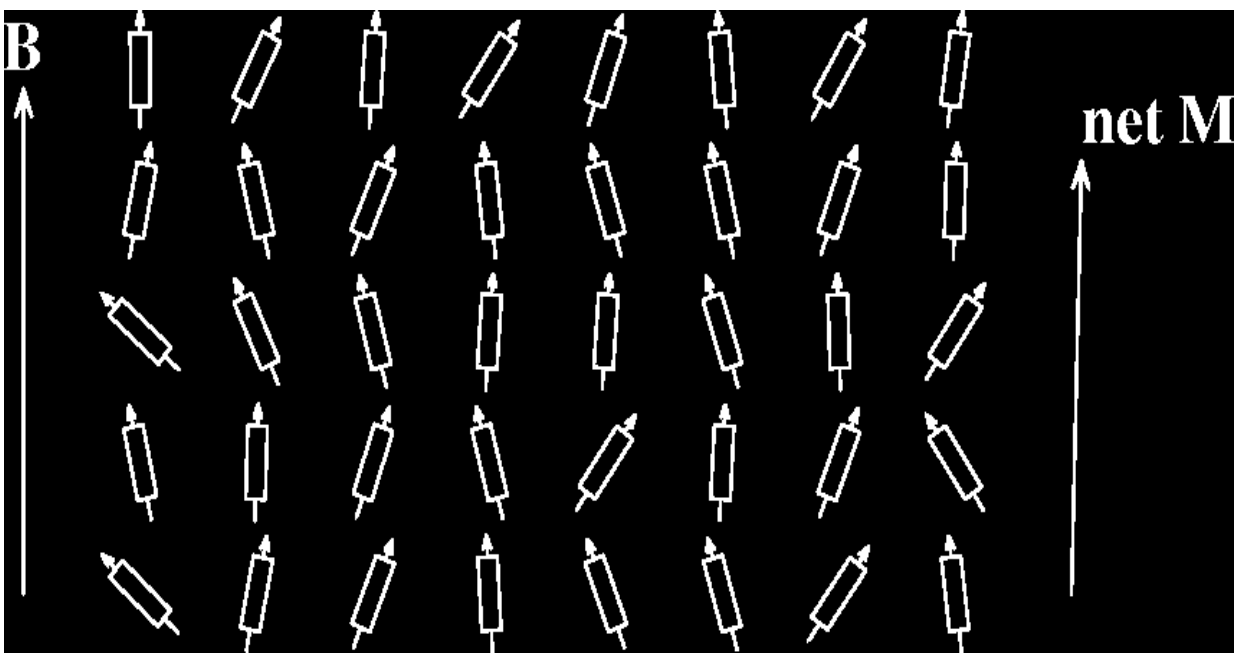
B_0 = Big Field Produced by Main Magnet

- Purpose is to align H protons in H_2O (little magnets)
- Units of *B* are ***Tesla*** (Earth's field is about 0.00005 Tesla)
  - ★ Typical field used in FMRI is 3 Tesla





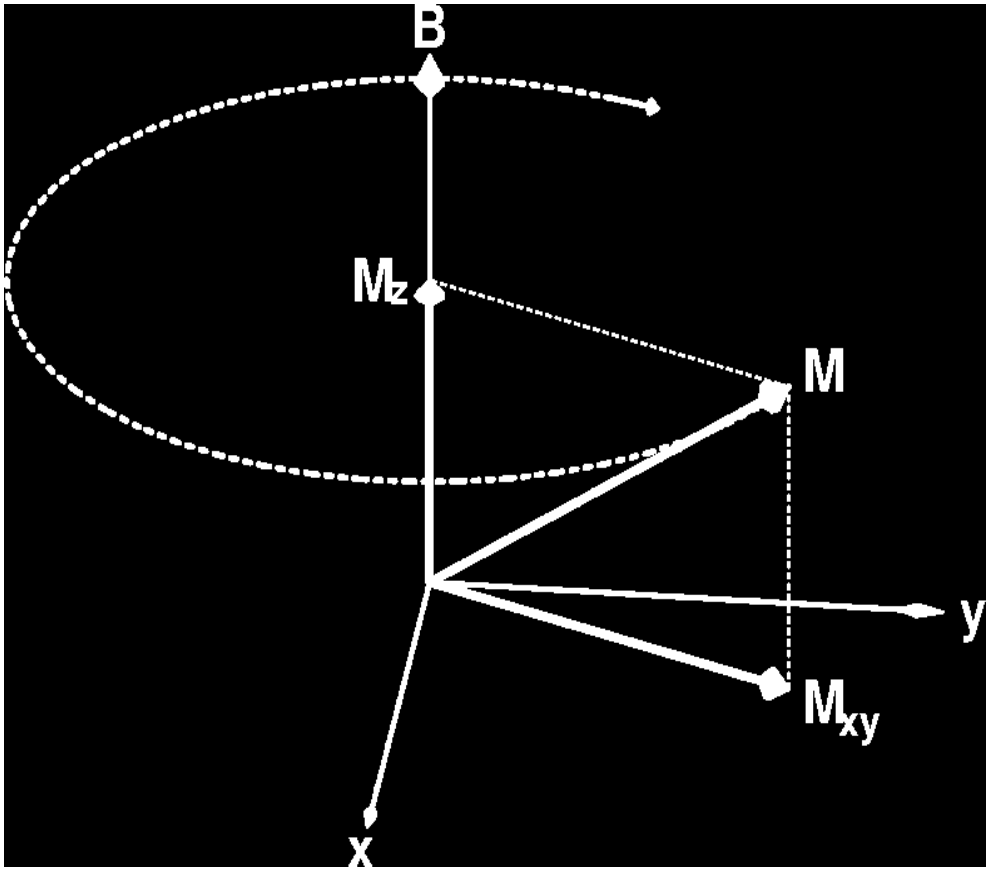
- Subject is <u>magnetized</u>
- Small B_0 produces small net <u>magnetization</u> M
- Thermal energy tries to randomize alignment of proton magnets

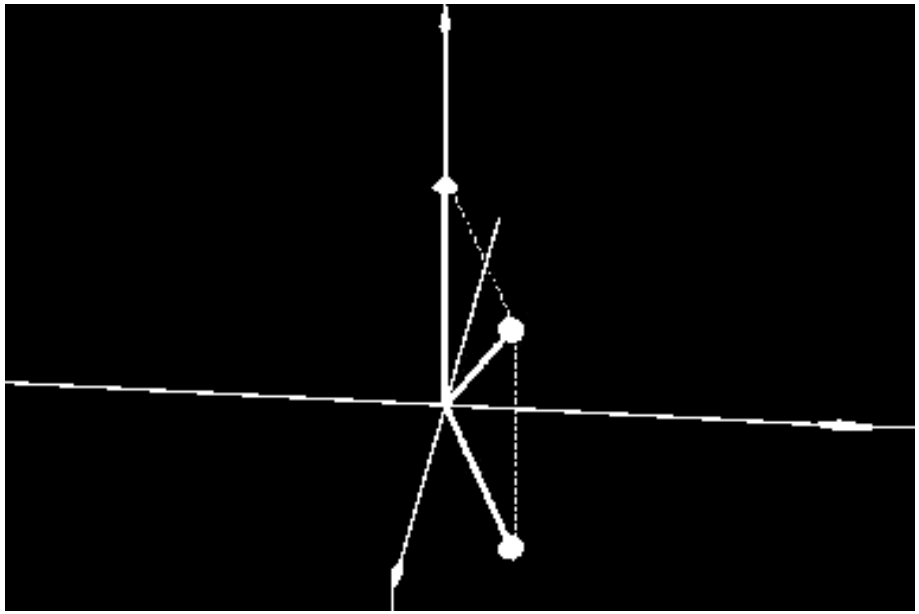


- Larger B_0 produces larger net magnetization M, lined up with B_0
- Reality check: **0.0003%** of protons aligned per Tesla of B_0

Precession of Magnetization *M*

- Magnetic field *B* causes *M* to rotate (“*precess*”) about the direction of *B* at a frequency proportional to the size of *B* — 42 million times per second (42 MHz), per Tesla of *B*
  - ★ 127 MHz at *B* = 3 Tesla — range of radio frequencies

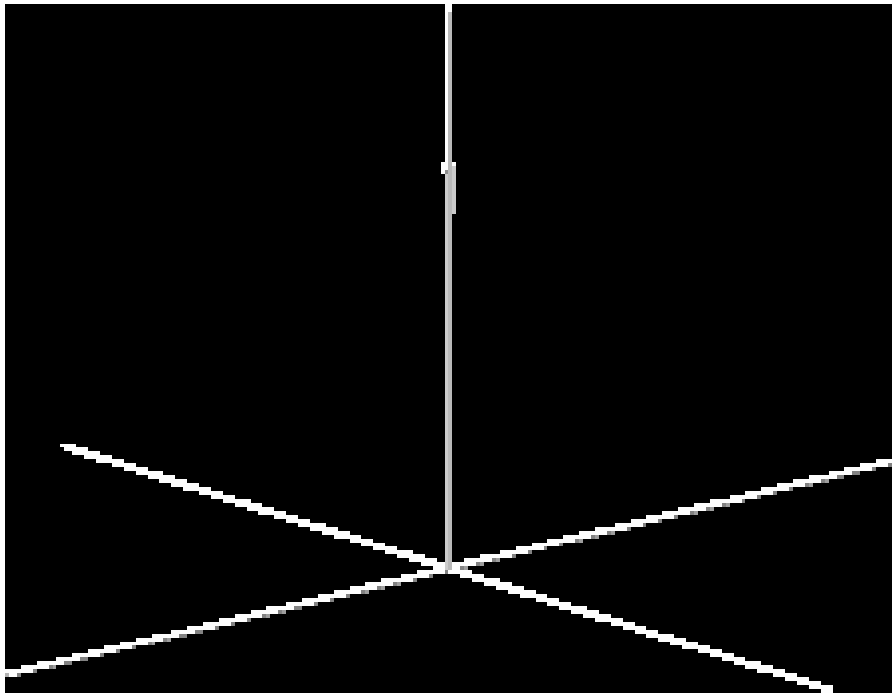


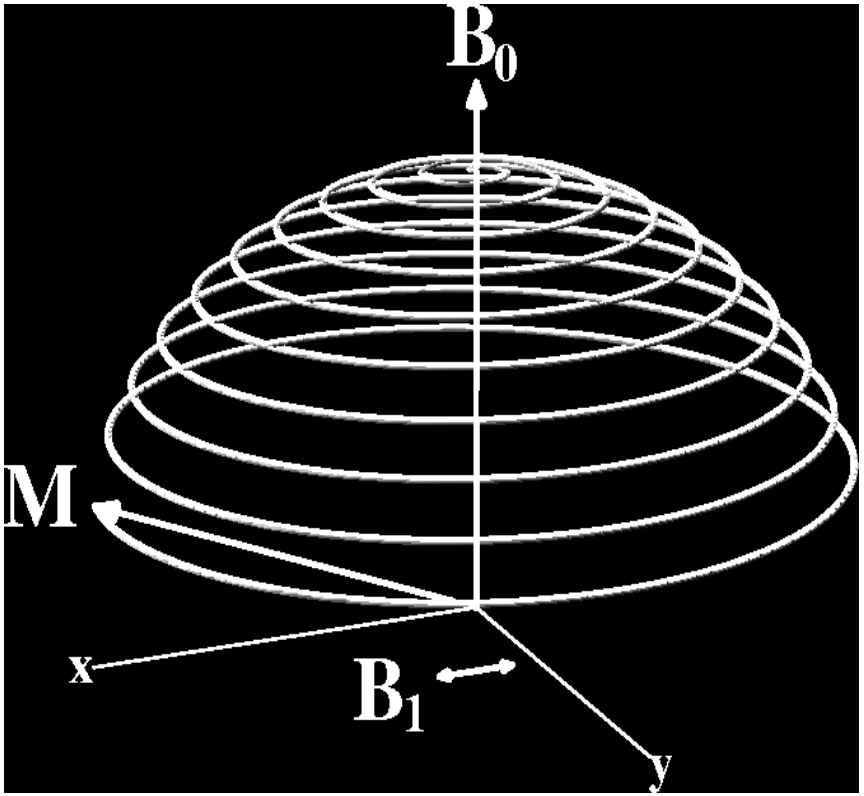


- N.B.: part of *M* parallel to *B* (M_z) does not precess

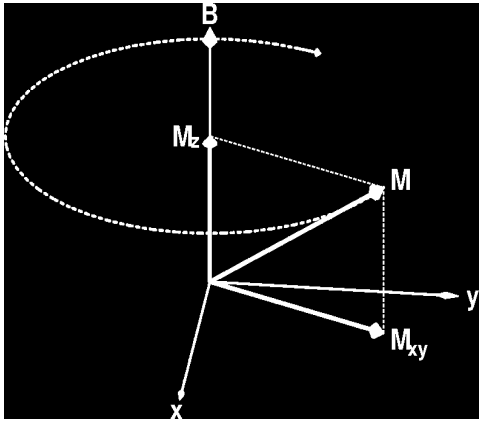
B_1 = Excitation (Transmitted) RF Field

- Left alone, M will align itself with B in about 2–3 s
  - ⋆ ⇒ No precession ⇒ no detectable signal
- <u>So don't leave it alone</u>: apply (transmit) a magnetic field B_1 that fluctuates at the precession frequency (radio frequency=*RF*) and that points perpendicularly to B_0



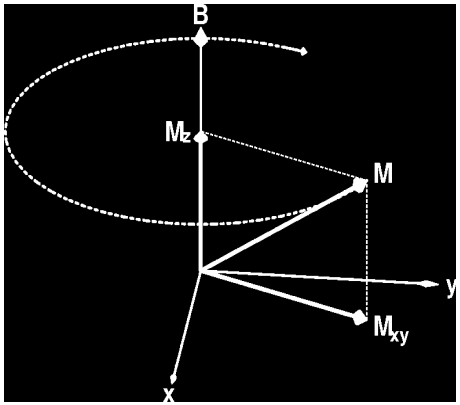


Readout RF



- When excitation RF is turned off, *M* is left pointed off at some angle to B_0 [*flip angle*]
- Precessing part of *M* [M_{xy}] is like having a magnet rotating around at very high speed (at RF speed: millions of revs/second)
- Will generate an oscillating voltage in a coil of wires placed around the subject — this is magnetic *induction*
- This voltage is the *RF signal* = the raw data for MRI
  - At each instant *t*, can measure one voltage $V(t)$, which is proportional to the sum of all transverse M_{xy} inside the coil
  - <u>Must separate signals originating from different regions</u>
    - By reading out data for 5-60 ms, manipulating *B* field, being clever …
    - Then have **image** of M_{xy} = map of how much signal from each ***voxel***

Relaxation: Nothing Lasts Forever



- In the absence of external B_1, M will go back to being aligned with static field B_0 = *relaxation*
- Part of M perpendicular to B_0 shrinks [M_{xy}]
  - ★ This part of M = *transverse magnetization*
  - ★ It generates the detectable RF signal
  - ★ The relaxation of M_{xy} during readout affects the image

Time scale for this relaxation is called T2 or T2* = 20-40 ms in brain

- Part of M parallel to B_0 grows back [M_z]
  - ★ This part of M = *longitudinal magnetization*
  - ★ Not directly detectable, but is converted into transverse magnetization by external B_1
    - o Therefore, M_z is the *ultimate* source of the NMR signal, but is not the *proximate* source of the signal

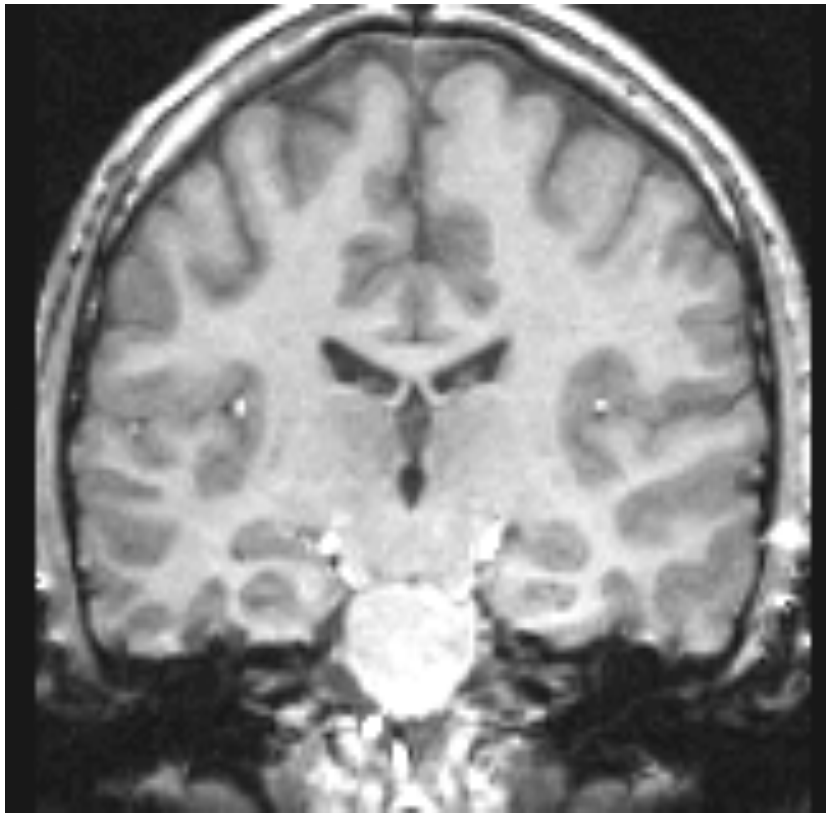
Time scale for this relaxation is called T1 = 500-2500 ms

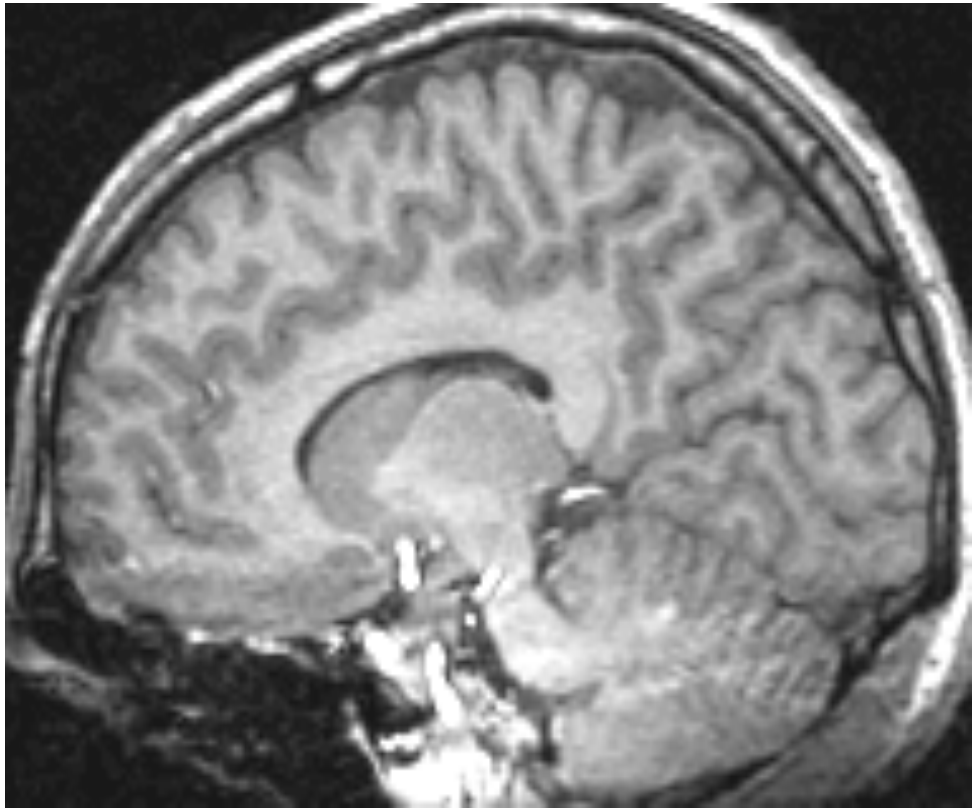
Material Induced Inhomogeneities in B

- Adding a nonuniform object (like a person) to B_0 will make the total magnetic field B nonuniform
  - ★ This is due to *susceptibility*: generation of extra magnetic fields in materials that are immersed in an external field
  - ★ *Diamagnetic* materials produce negative B fields [most tissue]
  - ★ *Paramagnetic* materials produce positive B fields [deoxyhemoglobin]
  - ★ Size of changes about $10^{-7} \cdot B_0$ = 1–100 Hz change in precession
- Makes the precession frequency nonuniform, which affects the image intensity and quality
  - ★ For large scale (100+ mm) inhomogeneities, scanner-supplied nonuniform magnetic fields can be adjusted to “even out” the ripples in B — this is called *shimming*
  - ★ Nonuniformities in B bigger than voxel size distort whole image
  - ★ Nonuniformities in B smaller than voxel size affect voxel “brightness”

The Concept of Contrast (or Weighting)

- ***Contrast*** = difference in RF signals — emitted by water protons — between different tissues
- Example: gray-white contrast is possible because T1 is different between these two types of tissue





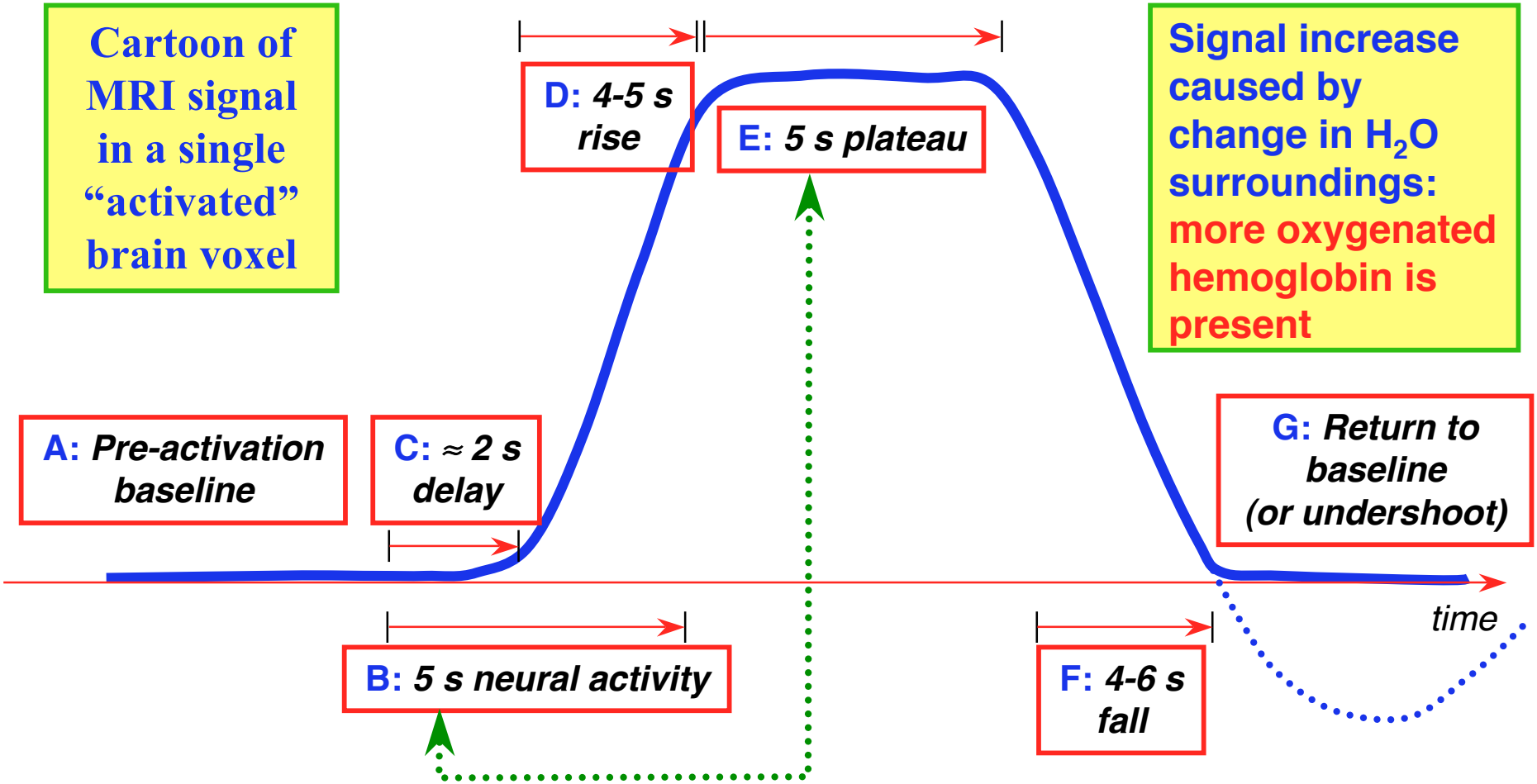
Types of Contrast Used in Brain FMRI

- T1 contrast at high spatial resolution
  - ⋆ Technique: use very short timing between RF shots (small *TR*) and use large flip angles
  - ⋆ Useful for anatomical reference scans
  - ⋆ 5-10 minutes to acquire 256×256×128 volume
  - ⋆ 1 mm resolution easily achievable
    - o finer voxels are possible, but acquisition time increases a lot
- T2 (spin-echo) and T2* (gradient-echo) contrast
  - ⋆ Useful for functional activation studies
  - ⋆ 100 ms per 64×64 2D slice ⇒ 2-3 s to acquire whole brain
  - ⋆ 4 mm resolution
    - o better is possible with better gradient system, and/or multiple RF readout coils

A

What is Functional MRI?

- 1991: Discovery that MRI-measurable signal increases a few % *locally* in the brain subsequent to 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 MR images repeatedly during this process

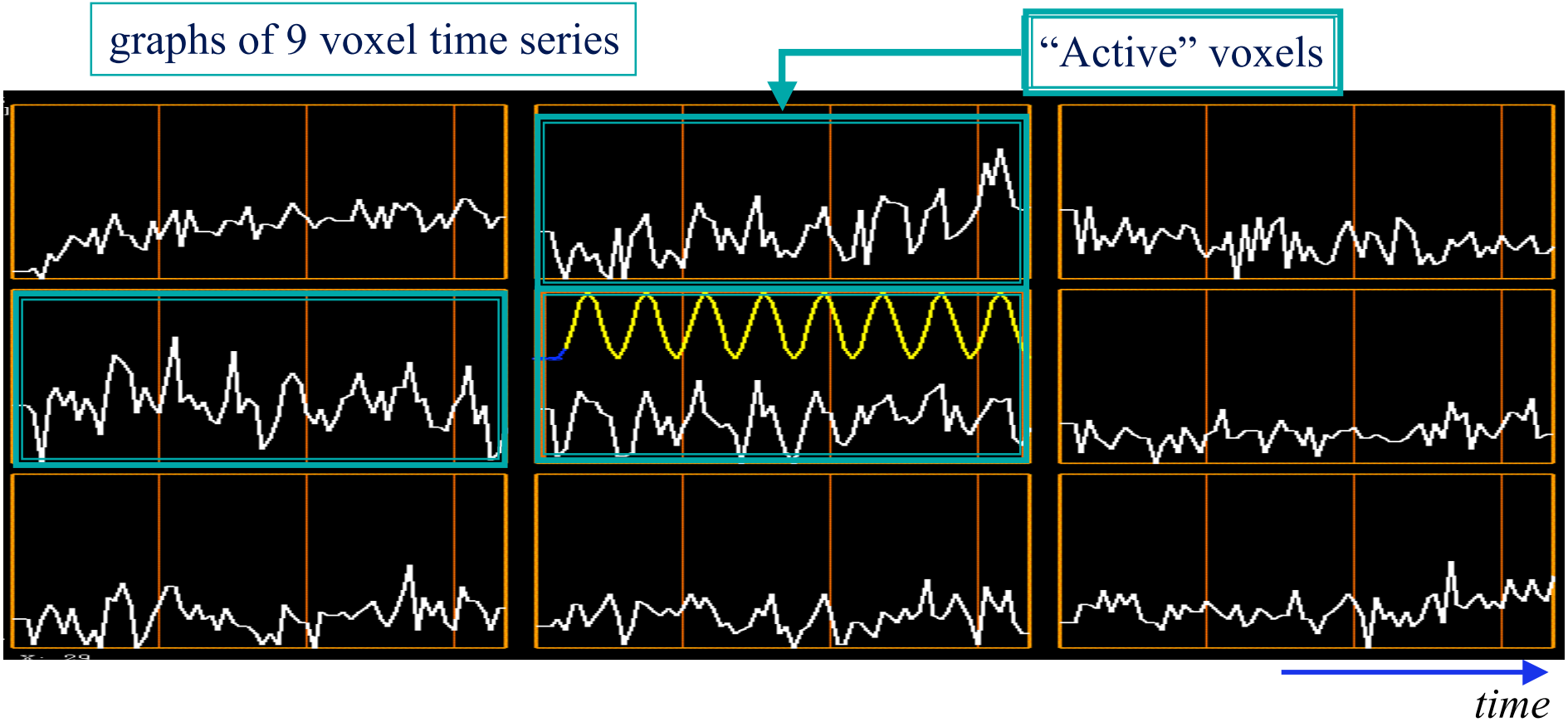
- Search for voxels whose NMR signal time series (up-and-down) matches the stimulus time series pattern (on-and-off)
  - → ★ FMRI data analysis is basically pattern matching *in time*

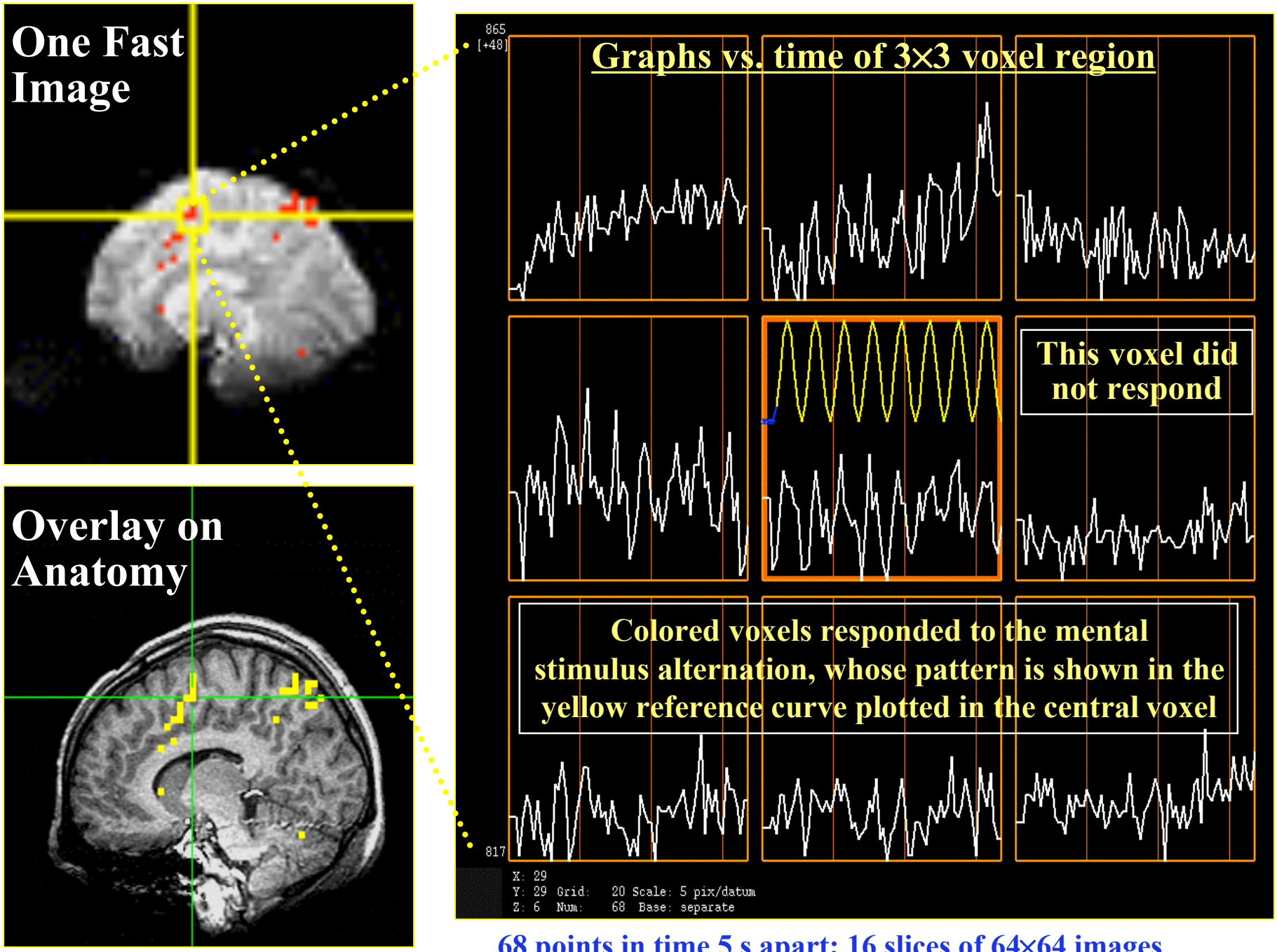
- Signal changes due to neural activity are small
  - Need 1000 or so images in time series (in each slice) ⇒ takes an hour or so to get reliable activation maps
    - Must break image acquisition into shorter "runs" to give the subject and scanner some break time
  - Other small effects can corrupt the results ⇒ postprocess the data to reduce these effects & *be careful*

- Lengthy computations for image recon and temporal pattern matching ⇒ data analysis usually done offline

Some Sample Data Time Series

- 16 slices, 64×64 matrix, 68 repetitions (TR=5 s)
- Task: phoneme discrimination: 20 s “on”, 20 s “rest”

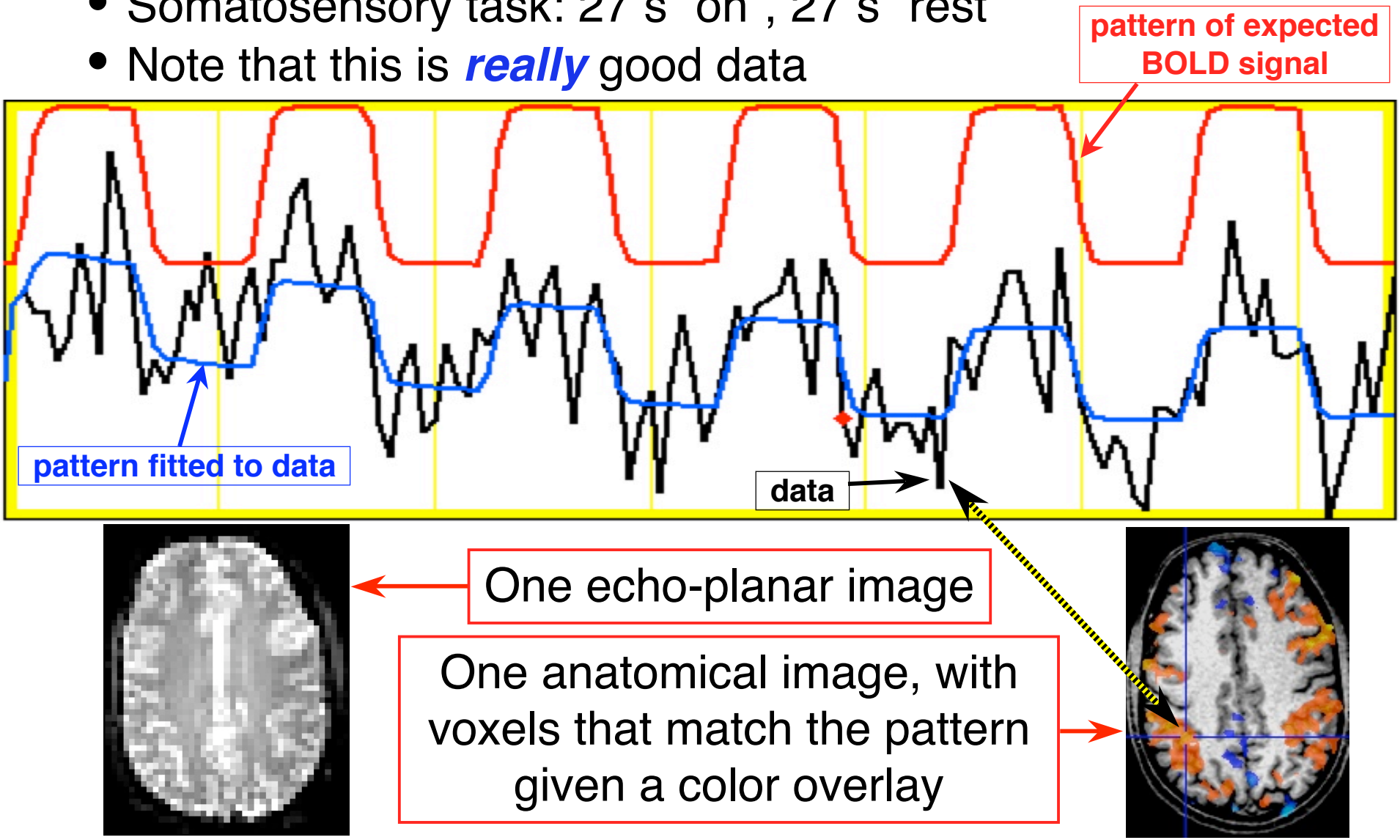




68 points in time 5 s apart; 16 slices of 64×64 images

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



Why (and How) Does NMR Signal Change With Neuronal Activity?

- There must be something that affects the water molecules *and/or* the magnetic field inside voxels that are "active"
  - ★ neural activity changes blood flow and oxygen usage
  - ★ blood flow changes which H_2O molecules are present
  - ★ and *also* changes the magnetic field locally because oxygenated hemoglobin and de-oxygenated hemoglobin have different magnetic properties
- FMRI is thus at least *doubly* indirect from physiology of interest (synaptic activity)
  - ★ also is much slower: 4-6 seconds after neurons
  - ★ also "smears out" neural activity: cannot resolve 10-100 ms timing of neural sequence of events

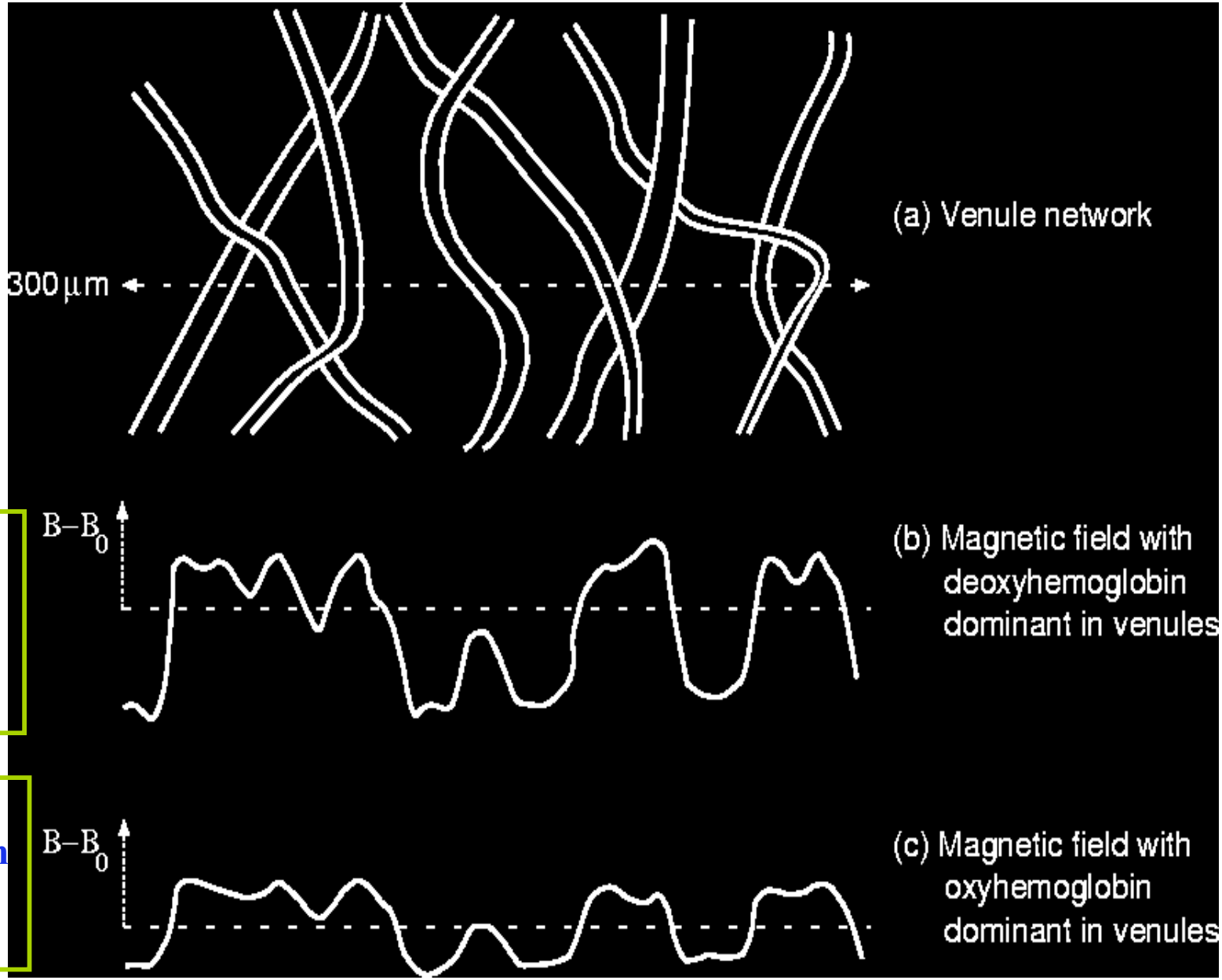
Neurophysiological Changes & FMRI

- There are 4 changes caused by neural activty that are currently observable using MRI:
- <u>Increased Blood Flow</u>
  - ★ New protons flow into slice from outside
  - ★ More protons are aligned with B_0
  - ★ Equivalent to a shorter T1 (as if protons are realigned faster)
  - ★ NMR signal goes up [mostly in arteries]
- <u>Increased Blood Volume (due to increased flow)</u>
  - ★ Total deoxyhemoglobin increases (as veins expand)
  - ★ Magnetic field randomness increases
    [more paramagnetic stuff in blood vessels]
  - ★ NMR signal goes down [near veins and capillaries]

- **BUT**: <u>"Oversupply" of oxyhemoglobin after activation</u>
  - ★ Total deoxyhemoglobin decreases
  - ★ Magnetic field randomness decreases [less paramag stuff]
  - ★ NMR signal goes up [near veins and capillaries]
  - ★ **<u>This is the important effect for FMRI as currently practiced</u>**

- <u>Increased capillary perfusion</u>
  - ★ Most inflowing water molecules exchange to parenchyma at capillaries
    - o i.e., the water that flows into a brain capillary is *not* the water that flows out!
  - ★ Can be detected with perfusion-weighted imaging methods
  - ★ This factoid is also the basis for ^{15}O water-based PET
  - ★ May someday be important in FMRI, but is hard to do now

Cartoon of Veins inside a Voxel



Deoxyhemo-globin is paramagnetic (increases *B*)

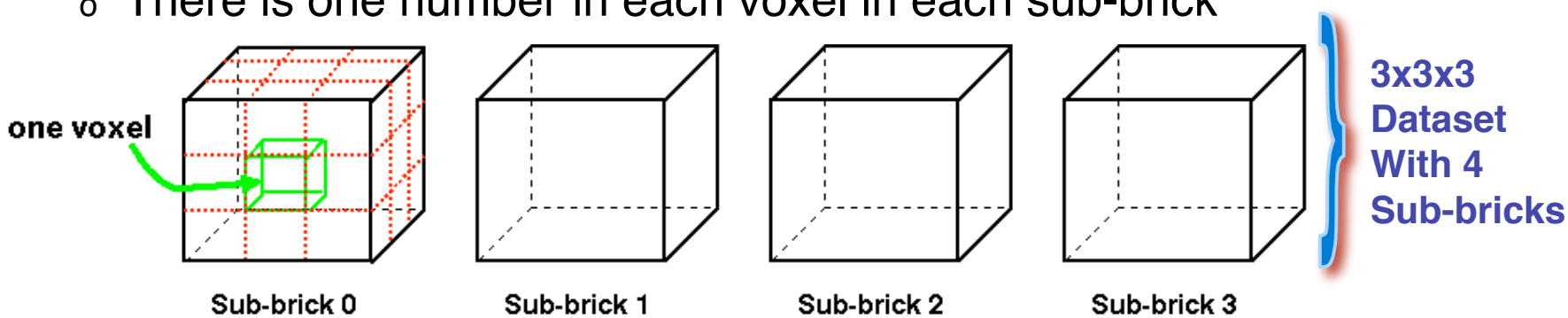
Rest of tissue +oxyhemoglobin is diamagnetic (decreases *B*)

BOLD Contrast

- **BOLD** = **B**lood **O**xygenation **L**evel **D**ependent
- Amount of deoxyhemoglobin in a voxel determines how inhomogeneous that voxel's magnetic field is at the scale of the blood vessels (and red blood cells)
- Increase in oxyhemoglobin in veins after neural activation means magnetic field becomes more uniform inside voxel
  - ★ So NMR signal goes up (T2 and T2* are larger), since it doesn't decay as much during data readout interval
  - ★ So MR image is brighter during "activation" (a little)
- **Summary**:
  - ★ NMR signal increases 4-6 s after "activation", due to hemodynamic (blood) response
  - ★ Increase is same size as noise, so need lots of data

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



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)
    - o Each sub-brick may also have a floating point scale factor α attached, so that "true" value in each voxel is actually α·(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
  - ★ But this is ***not*** recommended

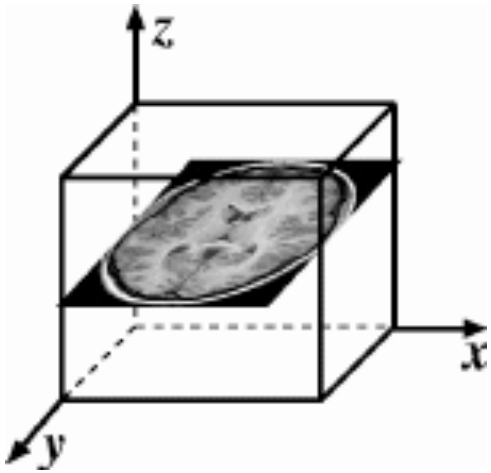
    and I mean this
  - ★ Will occur if you "catenate" two dissimilar datasets together (e.g., using **3dTcat** or **3dbucket** commands)
    - o Programs will display a warning to the screen if you try this

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 sessions
  - ★ 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 Unix systems: Linux, Sun, 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 can't help you with old versions!

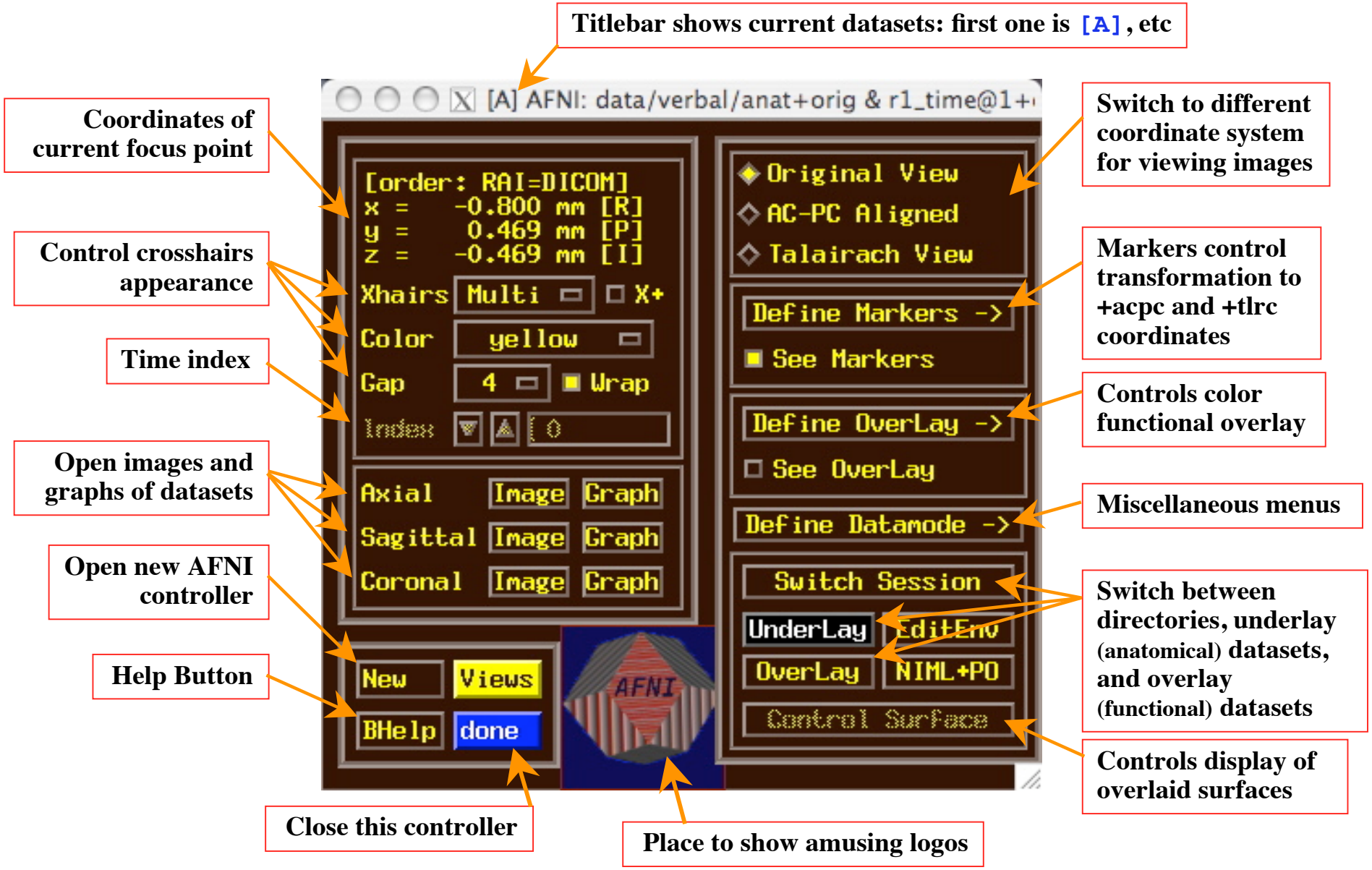
AFNI at the NIH Scanners

- AFNI can take 2D images in “realtime” from an external program and assemble them into 3D+time datasets slice-by-slice
- Jerzy Bodurka (FMRIF) has set up the GE Excite-based scanners (3 Ts, 1.5 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 estimated subject head movement
- Goal is to let you see image data as they are 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 so got garbage data, ***but only realized this too late*** (after scanning 8 subjects this way) — ***D’oh!***

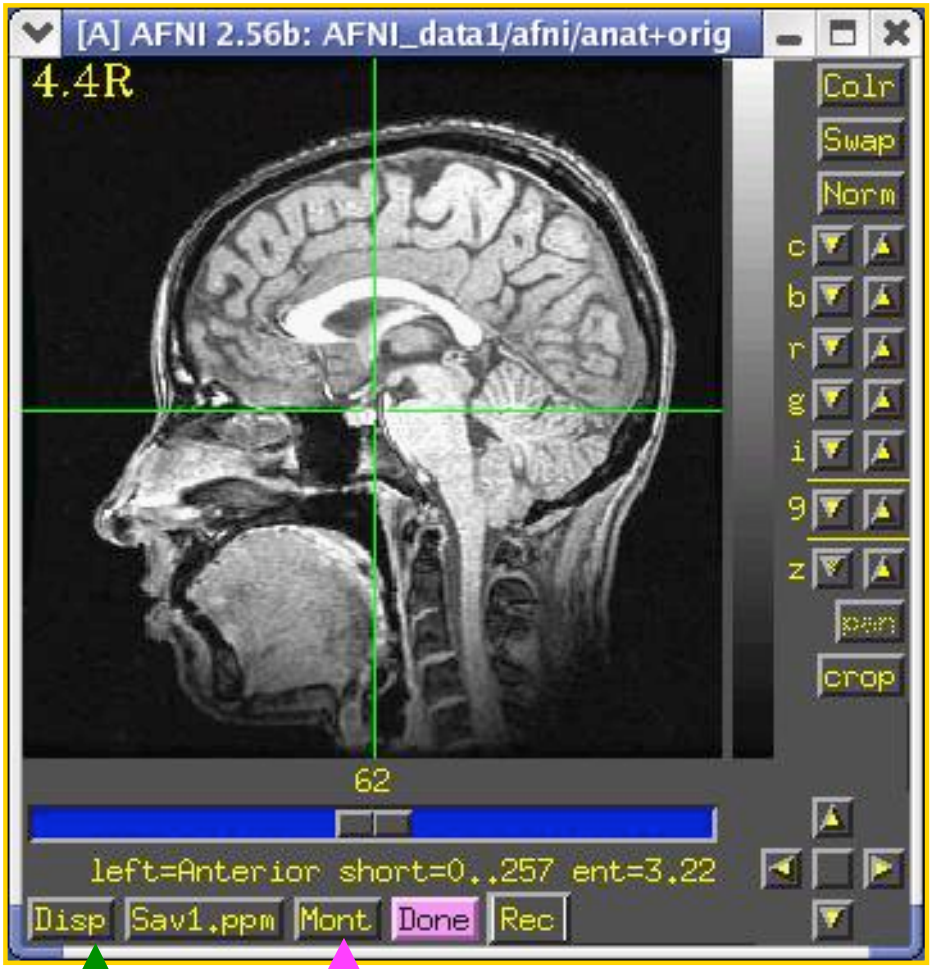
A Quick Overview of AFNI

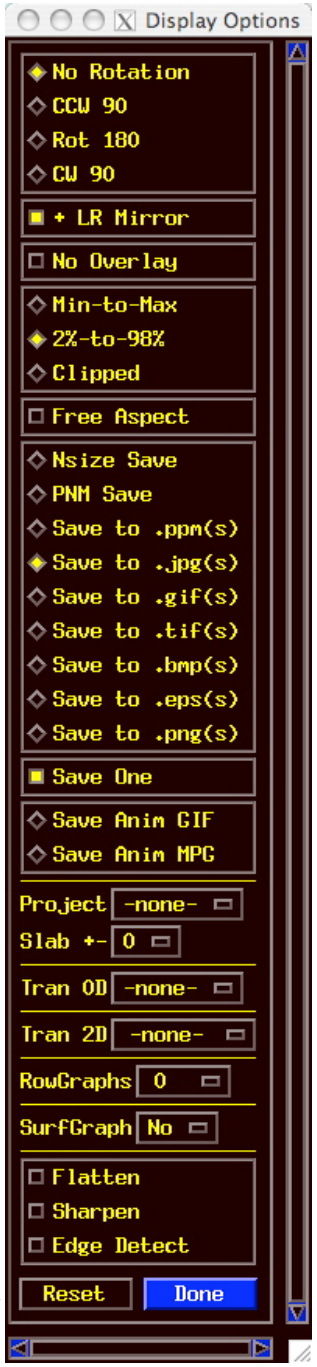
- Starting AFNI from the Unix command line
  - ★ **afni** reads datasets from the current directory
  - ★ **afni dir1 dir2** … reads datasets from directories listed
  - ★ **afni -R** reads datasets from current directory and from all directories below it
- AFNI also reads file named `.afnirc` from your home directory
  - ★ Used to change many of the defaults
    - o Window layout and image/graph viewing setup; popup hints; whether to compress .BRIK files when writing
    - o 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
    - o cf. file `README.driver` for what can be done with AFNI scripts

AFNI controller window at startup

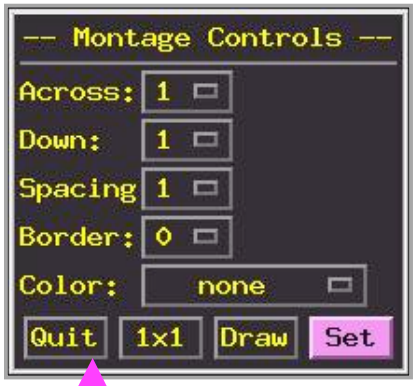


AFNI Image Viewer

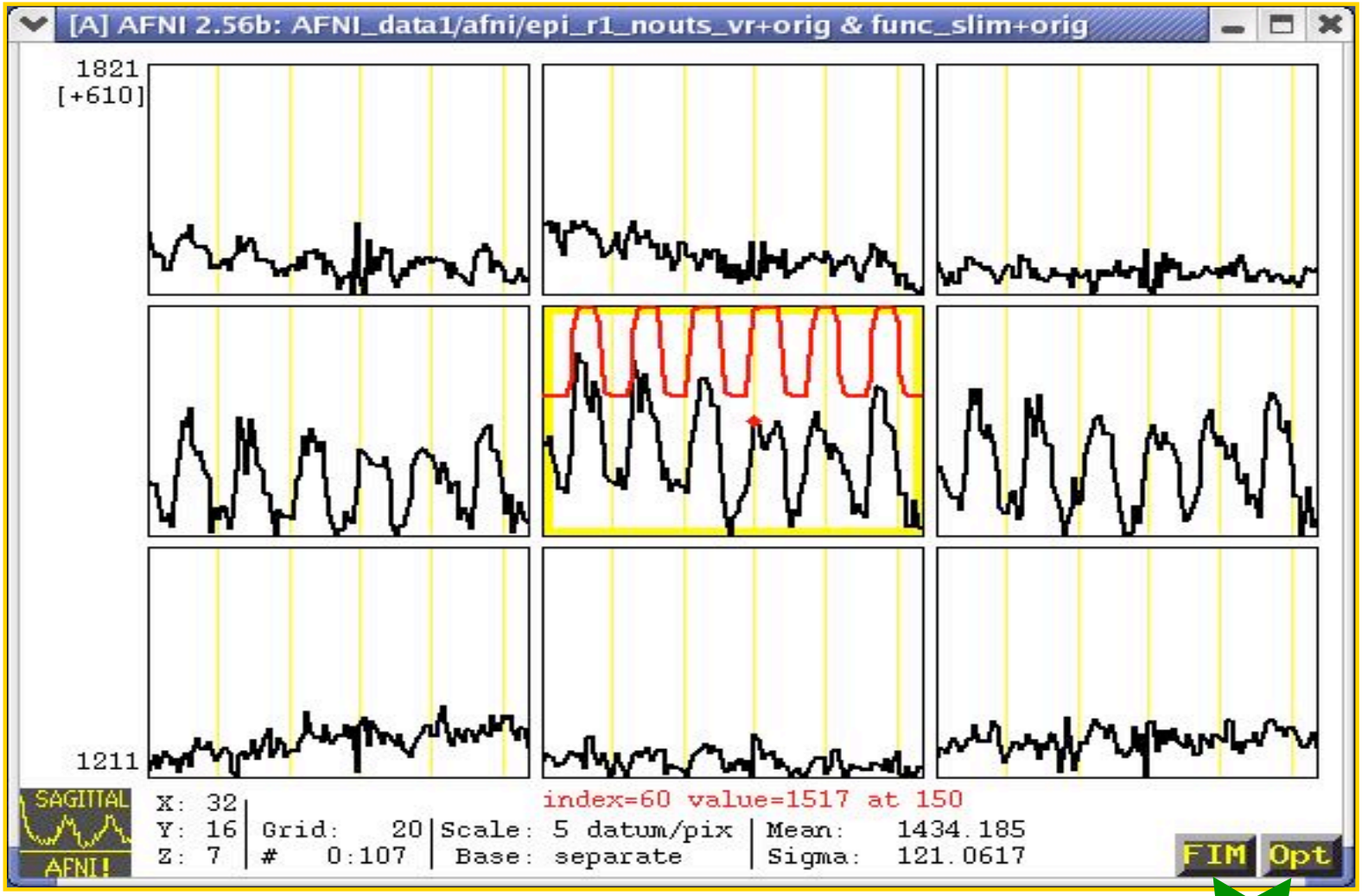




Disp and **Mont** control panels



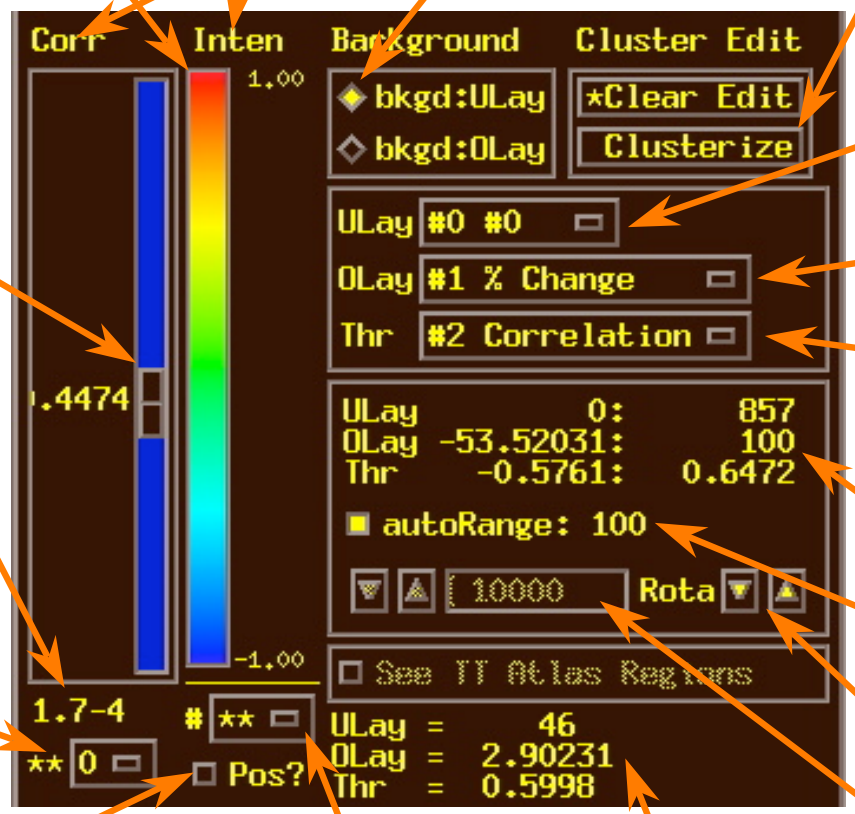
AFNI Time Series Graph Viewer



Data (black) and Reference waveforms (red)

Menus for controlling graph displays

Define Overlay: Colorizing Panel (etc)



Color map for overlay

Hidden popup menus here

Choose which dataset makes the underlay image

Cluster above-threshold voxels into contiguous "blobs" bigger than some given size

Threshold slider: voxels with Thr sub-brick above this get colorized from Olay sub-brick

Choose which sub-brick from Underlay dataset to display (usually an anatomical dataset)

Choose which sub-brick of functional dataset is colorized (after threshold)

p-value of current threshold value

Choose which sub-brick of functional dataset is the Threshold

Shows ranges of data in Underlay and Overlay dataset

Shows automatic range for color scaling

Choose range of threshold slider, in powers of 10

Rotates color map

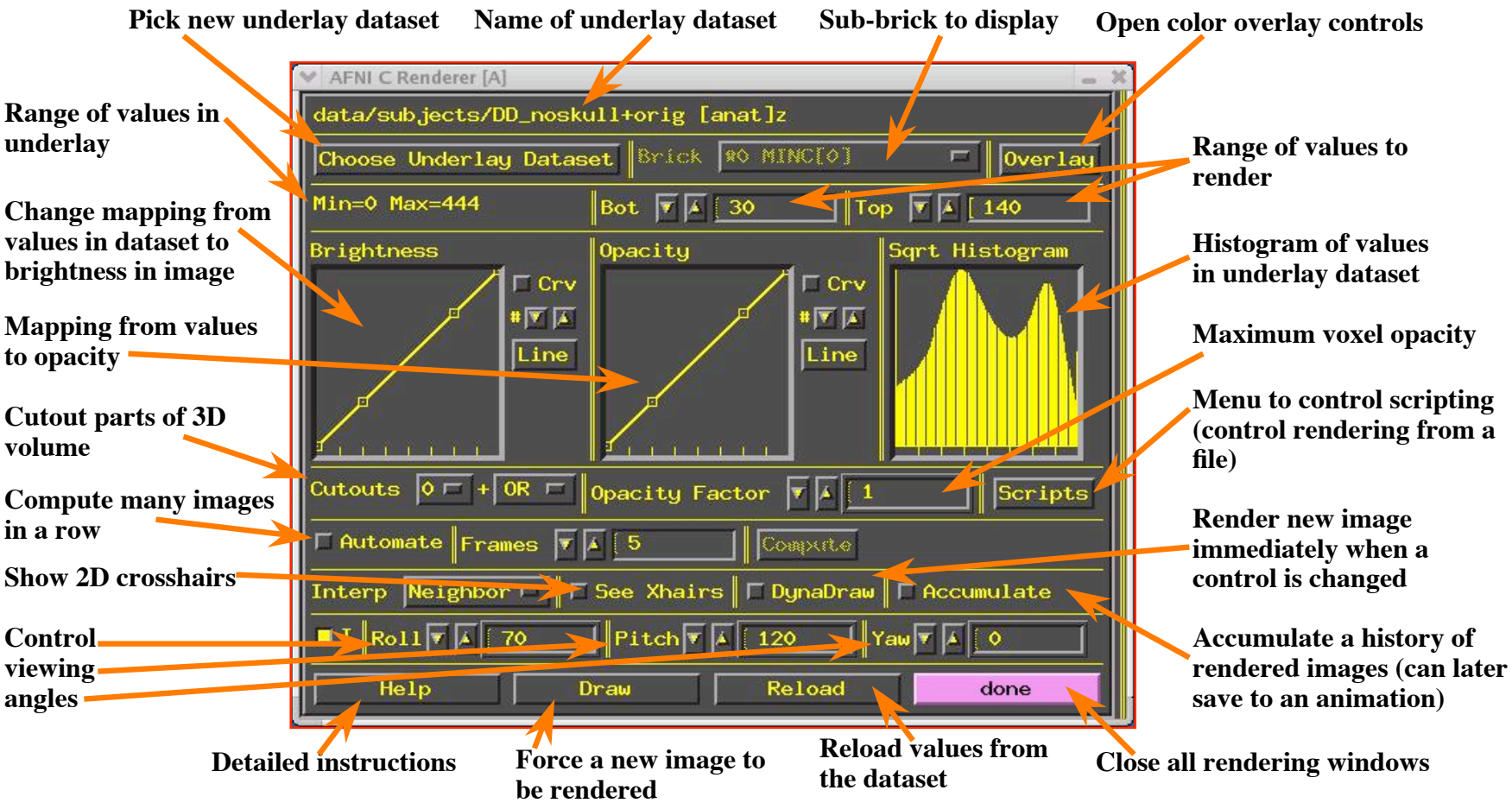
Positive-only or both signs of function?

Number of panes in color map (2-20 or **)

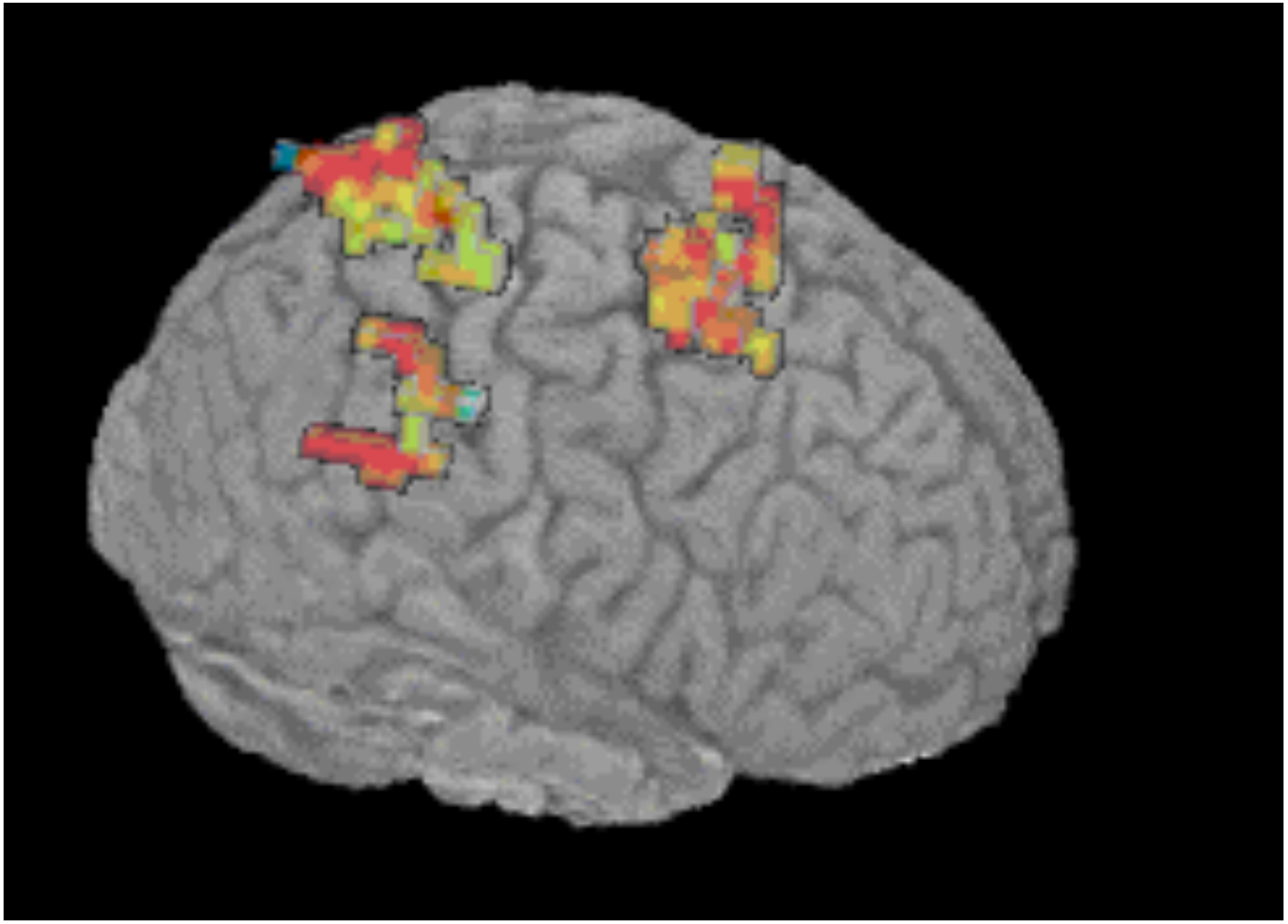
Shows voxel values at focus

Lets you choose range for color scaling (instead of autoRange)

Volume Rendering: an AFNI plugin



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 (are not interactive)
- 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, like, type commands into a computer to make it do things
    - o At least we don’t make you use punched cards or paper tape (yet)

AFNI Batch Programs

- Many 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 test these fits for significance (`3dNLfim` for nonlinear fitting)
- `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
- `3dDWItoDT` = compute diffusion tensor from DWI

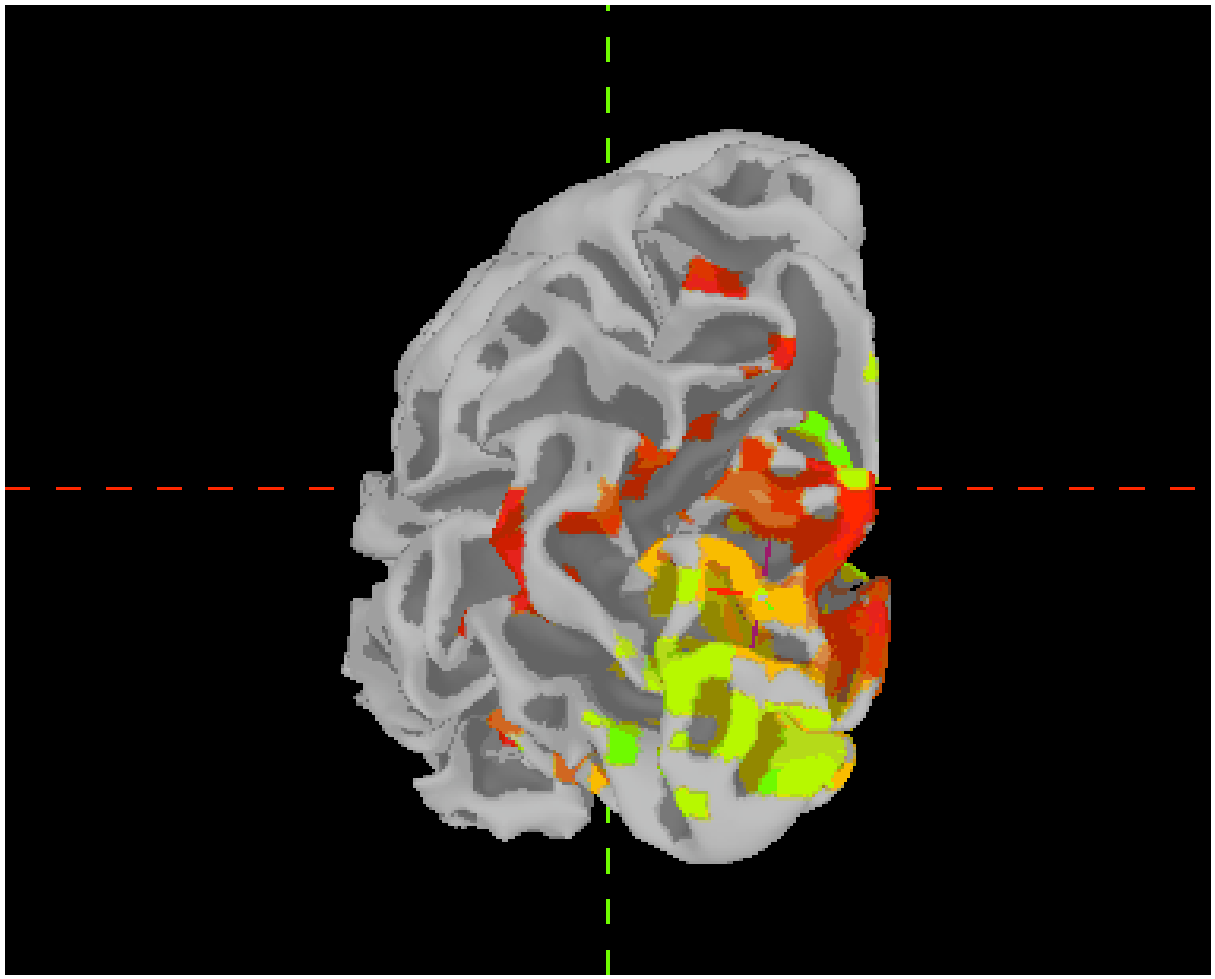
AFNI Plugins

- 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 for a programmer 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 models 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 surface mapper
  - ★ For displaying surface models of the cortex
    - o Surface models come from **FreeSurfer** (MGH) or **Caret** (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

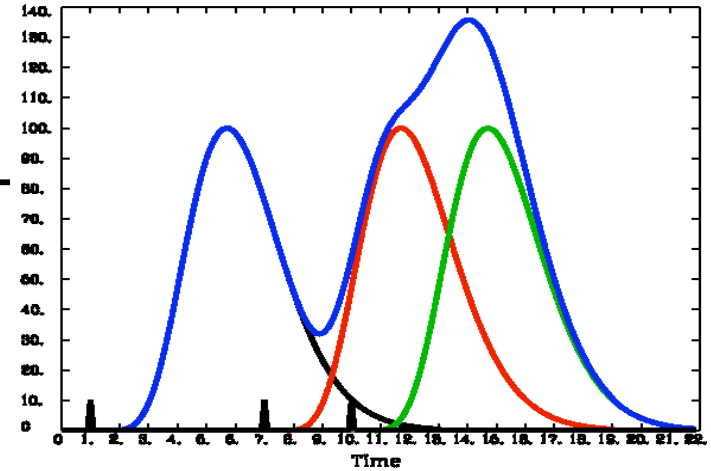
Images captured with the ‘R’ recorder function, then saved as animation with Save:aGif control

FMRI Experiment Design and Analysis

All on one slide!

- **FMRI experiment design**
  - ★ **Event-related, block, hybrid event-block?**
  - ★ **How many types of stimuli? How many of each type? Timing (intra- & inter-stim)?**
  - ★ **Will experiment show what you are looking for? (Hint: bench tests)**
  - ★ **How many subjects do you need? (Hint: the answer does not have 1 digit)**
- **Time series data analysis (individual subjects)**
  - ★ **Assembly of images into AFNI datasets; Visual & automated checks for bad data**
  - ★ **Registration of time series images (AKA motion correction)**
  - ★ **Smoothing & masking of images; Baseline normalization; Censoring bad data**
  - ★ **Catenation into one big dataset**
  - → ★ **Fit statistical model of stimulus timing+hemodynamic response to time series data**
    - o **Fixed-shape or variable-shape response models**
  - ★ **Segregation into differentially activated blobs (i.e., what got turned on – or off?)**
    - o **Threshold on statistic + clustering and/or Anatomically-defined ROI analysis**
  - ★ **Visual examination of maps and fitted time series for validity and meaning**
- **Group analysis (inter-subject)**
  - ★ **Spatial normalization to Talairach-Tournoux atlas (or something like it)**
  - ★ **Smoothing of fitted parameters**
    - o **Automatic global smoothing + voxel-wise analysis or ROI averaging**
  - ★ **ANOVA to combine and contrast activation magnitudes from the various subjects**
  - ★ **Visual examination of results (usually followed by confusion)**
  - ★ **Write paper, argue w/ mentor, submit paper, argue w/ referees, publish paper, …**

FMRI Experiment Design - 1



- Hemodynamic (FMRI) response
  - ★ peak is 4-6 s after neural activation
  - ★ width is 4-5 s for very brief (< 1 s) activation
  - ★ ⇒ two separate activations less than 12-15 s apart will have their responses overlap and add up (approximately — more on this in a later talk!)
- Block design experiments: Extended activation, or multiple closely-spaced (< 2-3 s) activations
  - ★ Multiple FMRI responses overlap and add up to something more impressive than a single brief blip
  - ★ <u>But</u> can't distinguish distinct but closely-spaced activations; example:
    - o Each brief activation is "**subject sees a face for 1 s, presses button #1 if male, #2 if female**" and faces come in every 2 s for a 20 s block, then 20 s of "**rest**", then a new faces block, etc.
    - o What to do about trials where the subject makes a mistake? These are presumably neurally different than correct trials, but there is no way to separate out the activations when the hemodynamics blurs so much in time.

FMRI Experiment Design - 2

- Event-related designs:
  - ★ Separate activations in time so can model the FMRI response from each separately, as needed (e.g., in the case of subject mistakes)
  - ★ Need to make inter-stimulus intervals vary ("jitter") if there is any potential time overlap in their FMRI response curves; e.g., if the events are closer than 12-15 s in time
    - o Otherwise, the tail of event #x always overlaps the head of event #x+1 in the same way, and as a result the amplitude of the response in the tail of #x can't be told from the response in the head of #x+1
  - ★ **Important note**!
    - o You cannot treat every single event as a distinct entity whose response amplitude is to be calculated separately!
    - o You must still group events into classes, and assume that all events in the same class evoke the same response.
      - ➥ Approximate rule: 25**+** events per class (with emphasis on the '**+**')
    - o There is just too much noise in FMRI to be able to get an accurate activation map from a single event!

FMRI Experiment Design - 3

- Hybrid Block/Event-related designs:
  - ★ The long “blocks” are situations where you set up some continuing condition for the subject
  - ★ Within this condition, multiple distinct events are given
  - ★ Example:
    - o Event stimulus is a picture of a face
    - o Block condition is instruction on what the subject is to do when he sees the face:
      - ➥ Condition A: press button #1 for male, #2 for female
      - ➥ Condition B: press button #1 if face is angry, #2 if face is happy
    - o Event stimuli in the two conditions may be identical, or at least fungible
    - o It is the instructional+attentional modulation between the two conditions that is the goal of such a study
      - ➥ Perhaps you have two groups of subjects (patients and controls) which respond differently in bench tests
      - ➥ You want to find some neural substrates for these differences

3D Individual Subject Analysis

Assemble images into AFNI-formatted datasets — to3d *OR* can do at NIH scanners

Check images for quality (visual & automatic) — afni + 3dToutcount

Register (realign) images — 3dvolreg *OR* 3dWarpDrive

Smooth images spatially — 3dmerge *OR* 3dBlurToFWHM (optional)

Mask out non-brain parts of images — 3dAutomask + 3dcalc (optional)

Normalize time series baseline to 100 (for %-izing) — 3dTstat + 3dcalc (optional: could be done post-fit)

Fit stimulus timing + hemodynamic model to time series
- catenates imaging runs, removes residual movement effects, computes response sizes & inter-stim contrasts

3dDeconvolve

Segregate into differentially "activated" blobs — Alphasim + 3dmerge *OR* Extraction from ROIs

Look at results, and *ponder* — afni *AND* your personal brain

… to group analysis (next page)

Group Analysis: in 3D or on folded 2D cortex models

Datasets of results from individual subject analyses

Construct cortical surface models

Normalize datasets to Talairach "space"

OR

Project 3D /results to cortical surface models

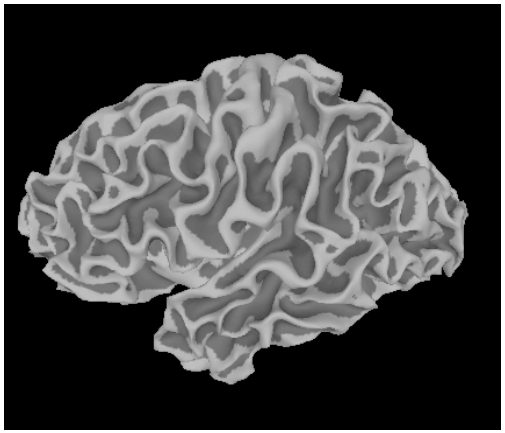
Smooth fitted response amplitudes

OR

Average fitted response amplitudes over ROIs

Use ANOVA to combine + contrast results

View and understand results;
Write paper;
Start all over



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`, et al.)
- 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`)
- Experiment design
- FMRI analysis from start to end (the “soup to nuts” hands-on)
- SUMA hands-on (fun level=pretty OK)
- Surface-based analysis
- AFNI “Jazzercise” (practice sessions & directed exercises)

Ongoing AFNI+SUMA Projects

- Complex ANOVA-like models for group analyses
  - ★ Unbalanced designs, missing data, continuous covariates, multi-nested designs, …. (the list and the project will never end)
- Changing **3dDeconvolve** to incorporate physiological noise cancellation, and correction for EPI time series autocorrelation, and …
- More surface-based analysis tools
  - ★ Especially for inter-subject (group) analyses
- Better EPI-anatomical registration tools
- Integrating some external diffusion tensor (DTI) tools with AFNI (e.g., *DTIquery*)
- Integrating more atlas datasets (animal and human) into AFNI
- Semi-linear global deconvolution analysis

This one is done!