AFNI & FMRI

Introduction, Concepts, Principles

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 600)
- 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
    - "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

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2.6. Classified program list

All AFNI programs, great and small, are listed here and classified based on functionality. That is, they are grouped into some general categories that we made up and given short bios.

The ranking of each program is to highlight ones that we think are particularly useful in general processing ('5' being the most directly useful, and '1' being something that might just be a low-level, supplementary tool). Note that a given program may appear in more than one group.

This page might be most useful by using your browser to search through the text for keywords of interest, such as "ROI", "mask", "diffusion", "align", "model", etc. Clicking on the name of the program will bring you its online help documentation, referenced from this page of all AFNI "helps".

- Interactive viewer GUIs
- Voxelwise calcs, esp. stats and tests
- Get info/stats within ROIs
- Build FMRI pipelines
- Align/register/warp/axialize spatially
- SUMA surface calculations, formats and viewing
- Mask/skull-strip/segment
- Make/edit/evaluate stimulus timing files
- Edit dset headers
- Compute various numbers from datasets
- Blur and smooth dsets
- Volume editing/image processing
- Update AFNI, install software (not demos)

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

*

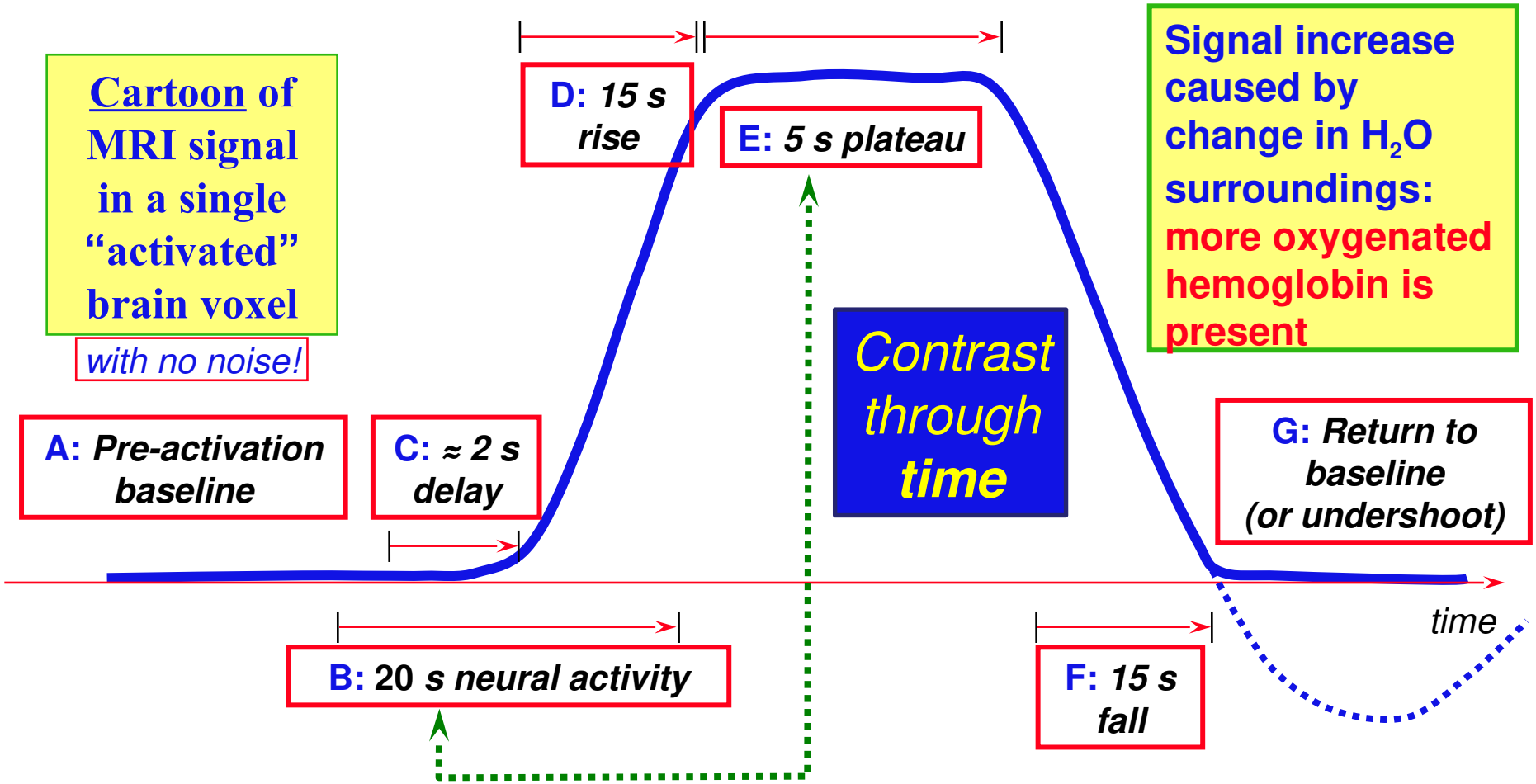
Before We Really Start

- AFNI has many programs and they have 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
    - When you need help, it will make things simpler for us *and* for you if you are using these scripts
- **`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)

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.*)

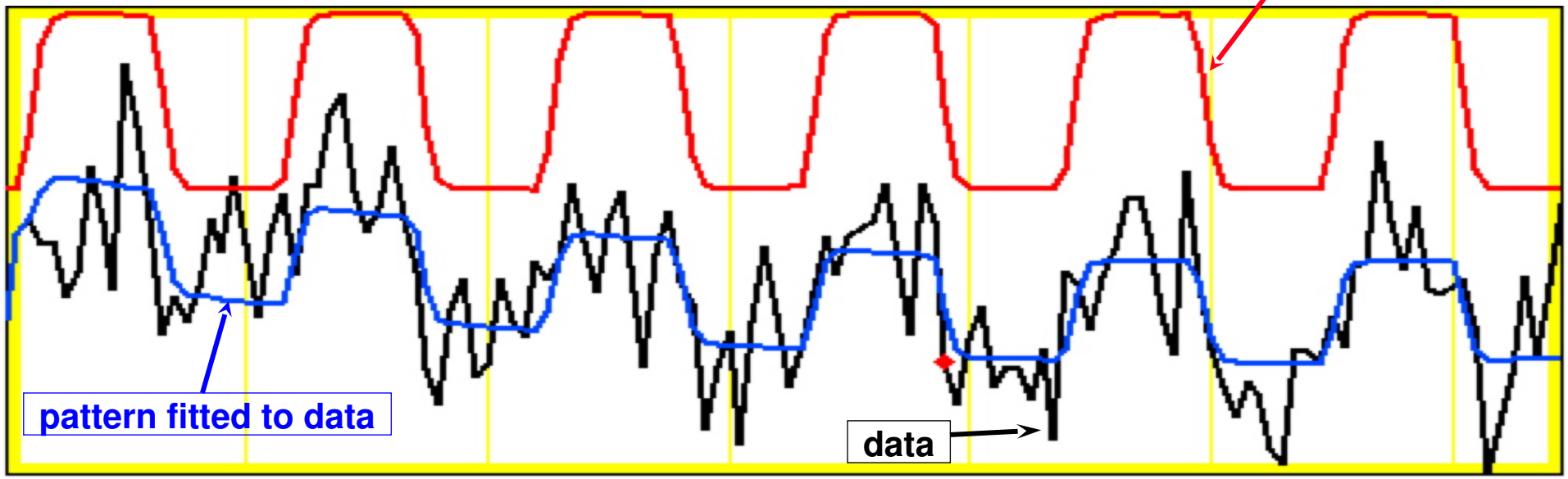


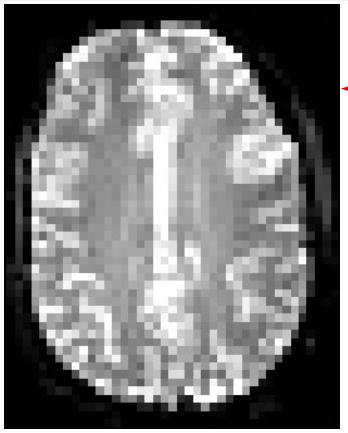
How FMRI Experiments Are Done

- 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 hundreds or more points in time series
    - Usually break image acquisition into shorter "runs" to give the subject and scanner some break time
  - Other small effects can corrupt the results post-process the data to reduce these effects & ***be vigilant***
- Lengthy computations for image recon and temporal pattern matching data analysis usually done offline

Sample Data Time Series

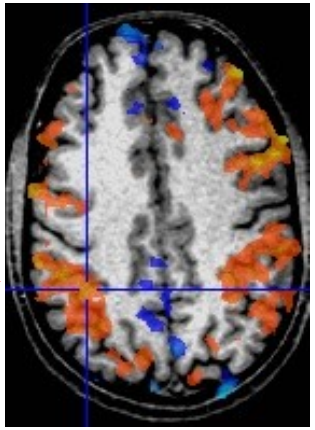
- 64×64 matrix (TR=2.5 s; 130 time points per imaging run)
- Somatosensory task: 27 s “on”, 27 s “rest”
- Note that this is ***really*** good data





One echo-planar image

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



What is a volumetric data set?
How do I get one?

Abbrevs used here

abbrev	= abbreviation
AKA	= also known as
anat	= anatomical
diff	= difference
dset	= dataset
e.g.	= exempli gratia (= “for example”)
EPI	= echo planar image
Ex	= example
FOV	= field of view
i.e.	= id est (= “that is”)
ijk	= coordinate indices (integer)
NB	= nota bene (= “note well”)
phys	= physics or physical
ref	= reference
subj	= subject
vol	= volume
vox	= voxel(s)
xyz	= physical coordinates (units of mm)

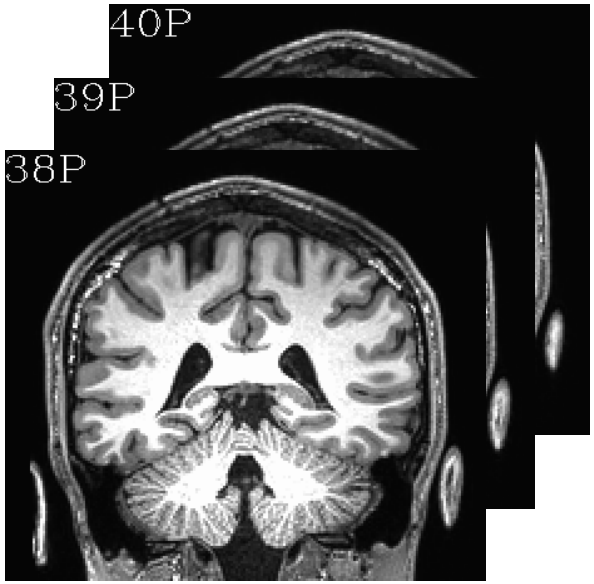
Creating dsets from DICOM files

- Data are often aquired as DICOM files
- AFNI has several programs for creating BRIK-HEAD and NIFTI files from DICOMs
- One has to be careful with DICOMs- not really standardized (booo!), fields/structure can change across scanner vendor, across version numbers, across acquisition sequences, and on the 3rd Tuesday after a blue moon.
- Some AFNI programs:
  + ***dcm2niix_afni***: Chris Rorden's popular program, distributed in AFNI (thx, Chris!)
    - very general use, can create whole collection of dsets
    - NB: NIFTI does *not* store complicated slice timings, so even if dcm2niix_afni can find it, it can't be stored
    - AFNI's 3drefit can be used to add slice timing info to the AFNI header extension
  + ***Dimon***: R Reynold's creation, originally for sending "realtime FMRI" direct to AFNI
  + ***fat_proc_convert_dcm_{anat,dwis}***: wrappers of dcm2niix_afni for DWI proc
  + and: https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/educational/classified_progs.html#dicom-info-and-conversion

- ****Always* check your results carefully (left-right flips!) when converting from DICOM!***

Volumetric data structure

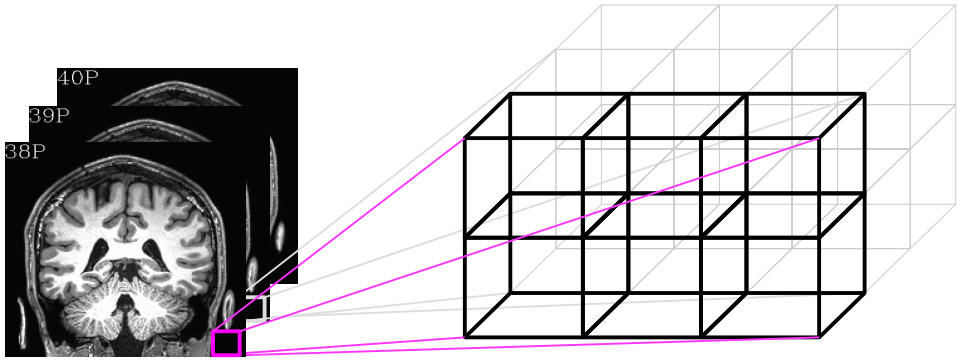
- What is a volumetric data set?



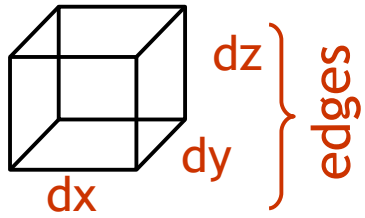
Volumetric data structure

- What is a volumetric data set?
  → *It a **grid** made up of voxels (basic case: 3D).*

3D grid



voxel



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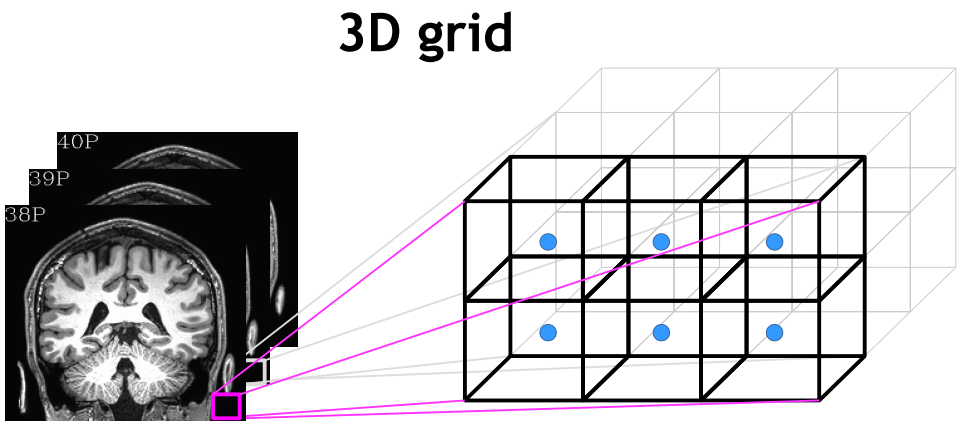

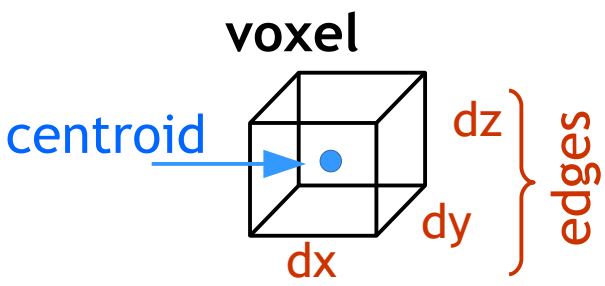

2 ways to describe each voxel location

+ "*i, j, k*": integer indices, counting voxels from one corner
  - independent of voxel size (just storage indices on disk)
+ "*x, y, z*": units of mm, physical location of voxel *centroid*
  - depends on voxel size *(dx, dy, dz)*

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- What is a volumetric data set?
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  Each voxel contains a number, which is represented by a color (gray, RGB, etc.).

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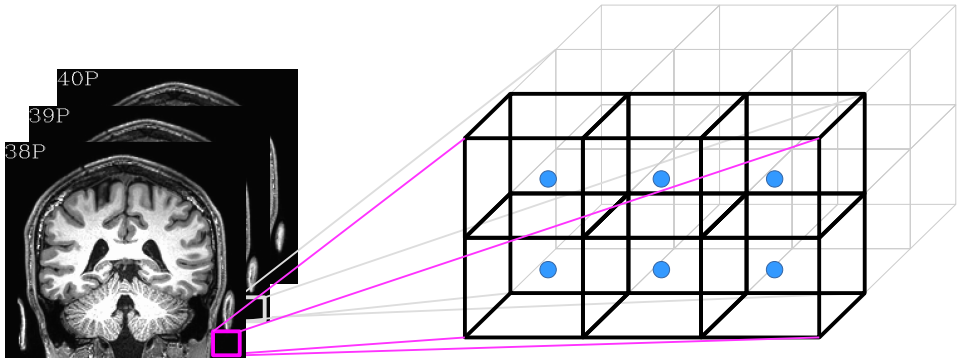
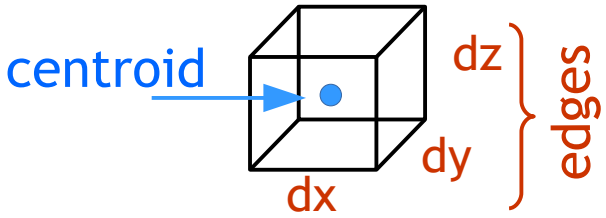
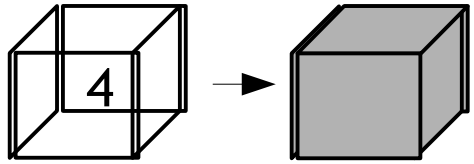


voxel



color mapping



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  A time series data set is an ordered set of 3D vols (→ a '4D data set').

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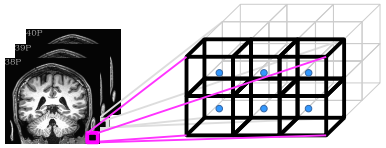
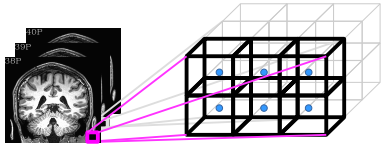
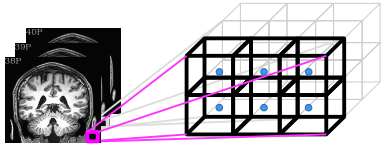


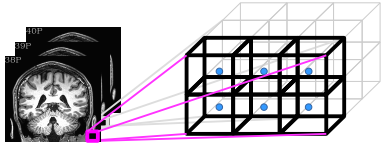
… at *t*=0



… at *t*= 1TR



… at *t*= 2TR



… at *t*= 3TR

…

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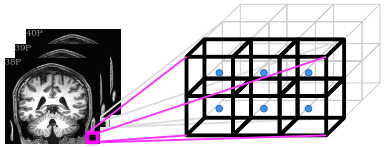
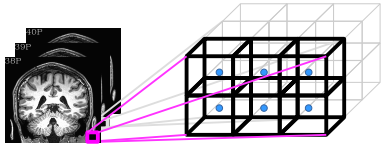
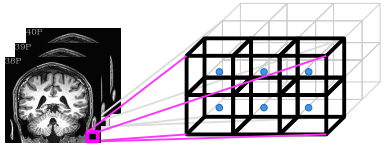


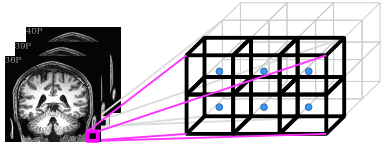
… at *t*=0



… at *t*= 1TR



… at *t*= 2TR



… at *t*= 3TR

…

3D grid + time dimension
→ "4D data set"
+ Can talk about time as "*t*" in physical units of seconds, or as "*n*" in index units of simple counting.

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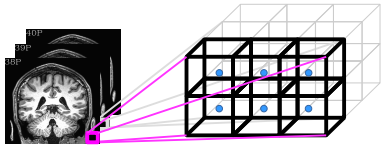
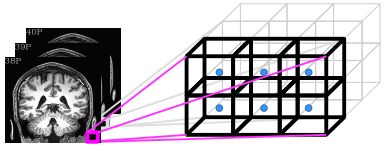


… at *t*=0



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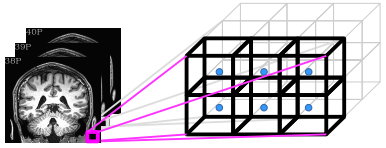

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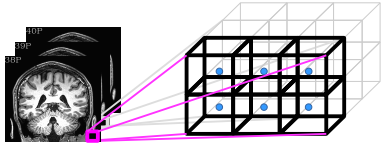
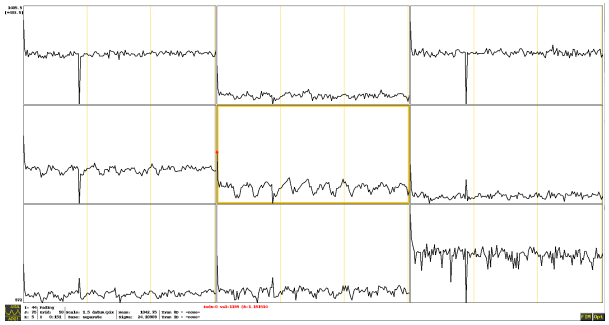


… at *t*= 3TR

…

3D grid + time dimension
→ "4D data set"
+ Can talk about time as "*t*" in physical units of seconds, or as "*n*" in index units of simple counting.
+ Also say that each voxel contains a "time series", e.g.:



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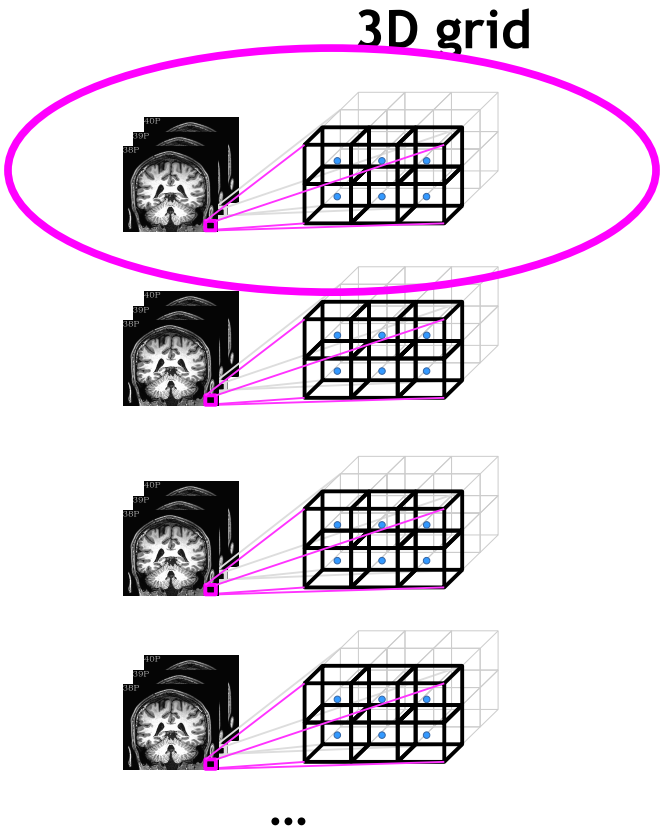

We often refer to a 3D volume as a **brick**, because, well, it is an example of a solid, similar-looking 3D shape.

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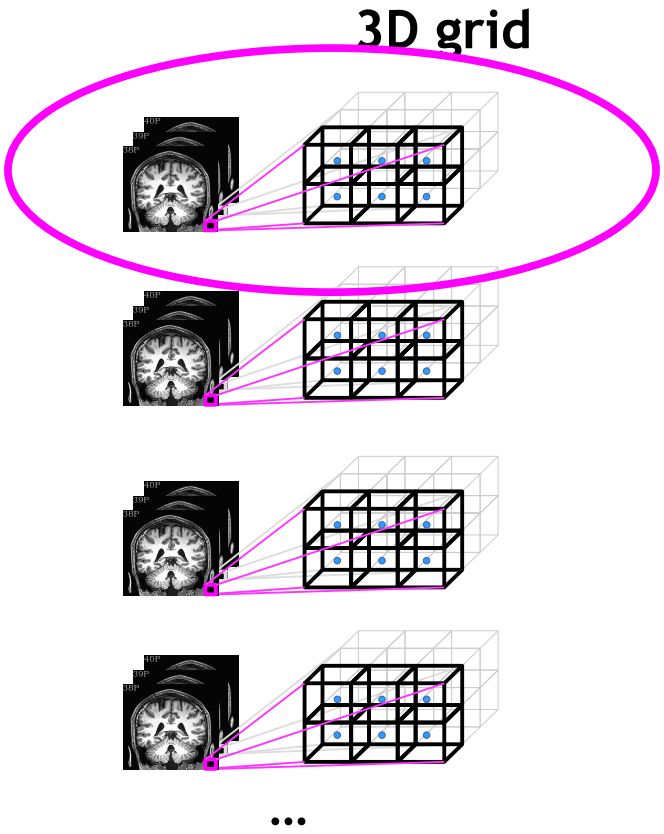

We often refer to a 3D volume as a **brick**, because, well, it is an example of a solid, similar-looking 3D shape.

Particularly in the context of 4D data sets, we also call a 3D volume a **sub-brick**.

This is an odd lingual quirk. But to date, this appears to be the only quirk in the AFNI software (or its developers).

AFNI terminology sidenote

- Sub-brick selection by volume index

  AFNI has a convenience feature of being able to select subset(s) of volumes for copying, calculation, etc. from a 4D set.

  This works by putting the index or index range in square brackets ***and*** quotation marks "[ ]" ("" keep the terminal from interpreting the square brackets specially).

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  This works by putting the index or index range in square brackets ***and*** quotation marks "[ ]" ("" keep the terminal from interpreting the square brackets specially).

  A comma separates indices, and two dots .. specifies an (inclusive) range; $ means final volume. *Ex.*:

```
DSET"[0]"           # initial subbrick (NB: count from 0!)
DSET"[0..5]"        # subbricks 0,1,2,3,4,5
DSET"[3,5..8,19]"   # subbricks 3,5,6,7,8,19
DSET"[1,14,29..$]"  # subbricks 1,14,29-to-the-last
DSET[0,4,5,15]      # ERROR in tcsh (no quotes); OK in bash
```

  Ex. application, to copy out subset:

```
3dcalc -a DSET "[3,5..8,19]" -expr 'a' -prefix DSET_NEW
```

  Fun fact: there are other forms of subbrick selection (brik label, voxel value...).

Grids

- What are the grid's properties?

Grids

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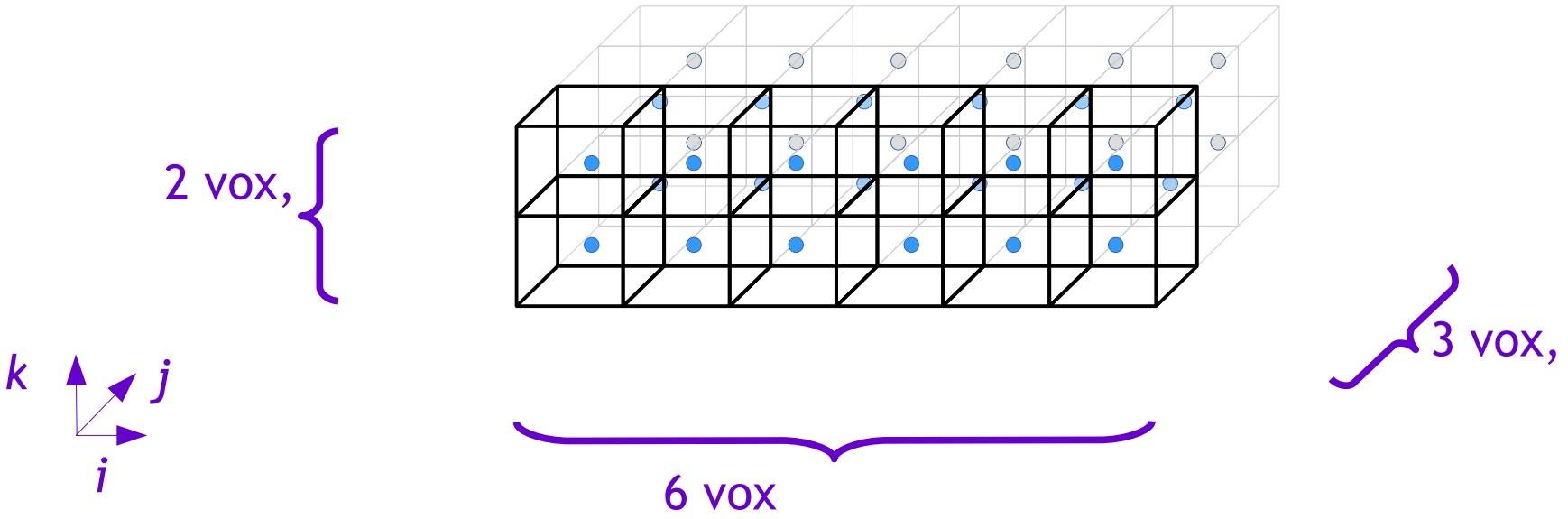
  → *One is "size." Three ways to describe it:*

1) *matrix size*: count voxels in each dimension (n_i rows, n_j cols, n_k slices)

   - independent of voxel size



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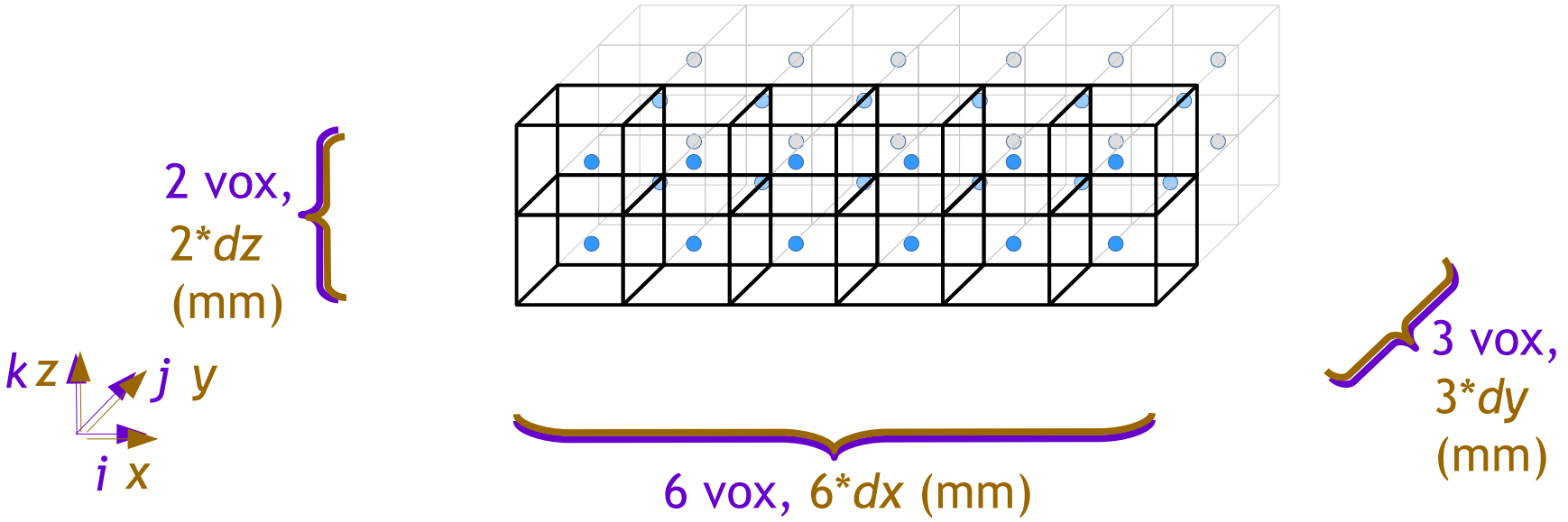
2) *field of view (FOV)*: units of mm, 3D phys vol of all voxels

   - depends on voxel size *(dx, dy, dz)*



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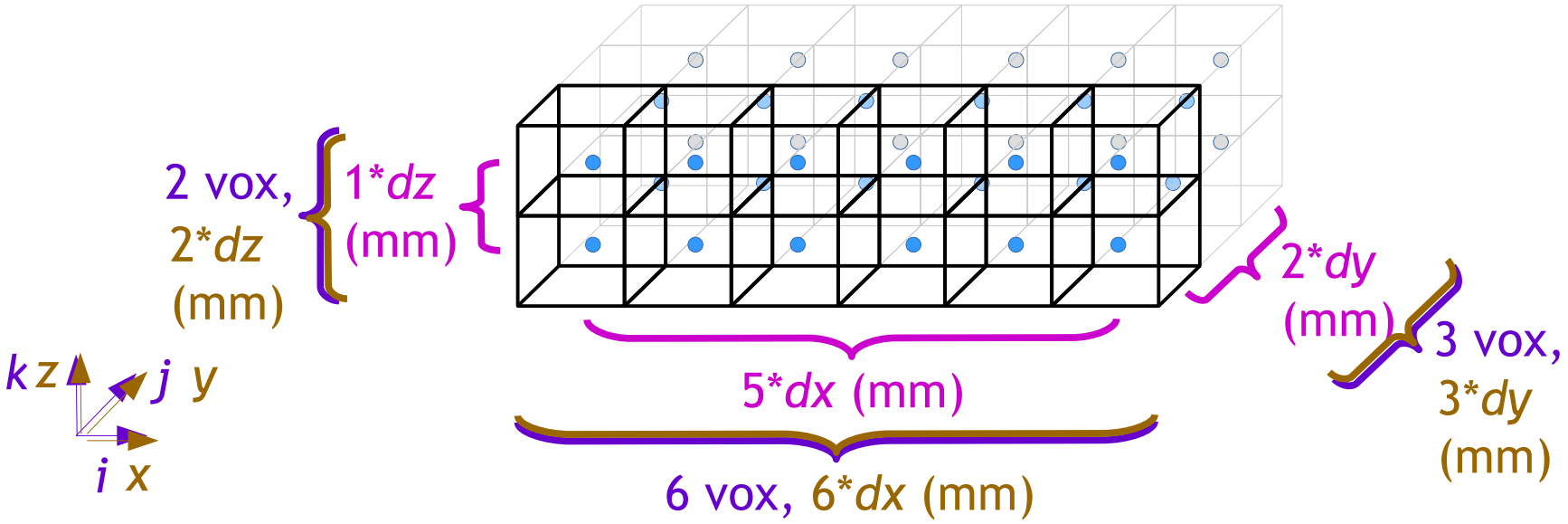
   - independent of voxel size
2) *field of view (FOV)*: units of mm, 3D phys vol of all voxels
   - depends on voxel size *(dx, dy, dz)*
3) *slab*: *units of mm, phys dist between first & last centroids*
   - e.g., dist between [0]th and [n_i-1]th centroid



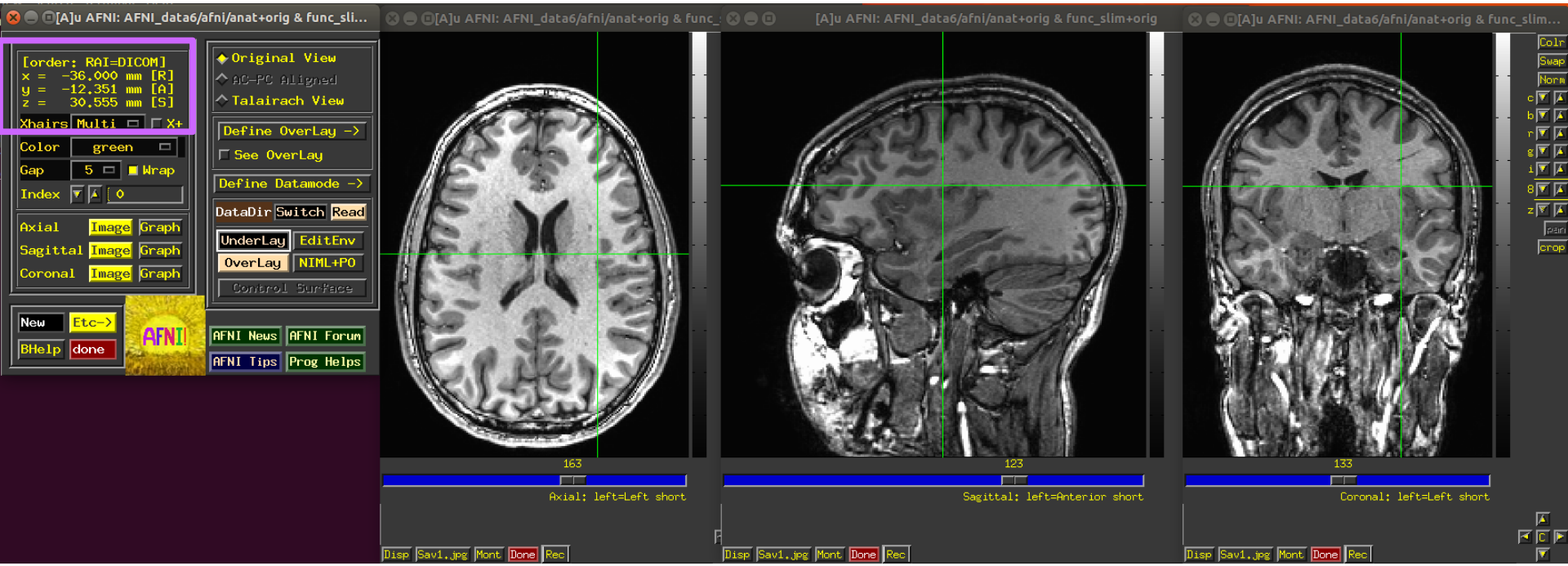
Describing location in dsets

- When reporting location of interest-- e.g., cluster peak-- one can't just provide *(x, y, z)* values. One also has to provide the ***coordinate order*** being used.
- Two main families of coordinate order in the literature:

  RAI (DICOM): negative numbers are to the right, anterior and inferior

  LPI (SPM): negative numbers are to the left, posterior and inferior

  e.g.: **in RAI order**, cross-hair coordinates are **(-36, -12, 31)**



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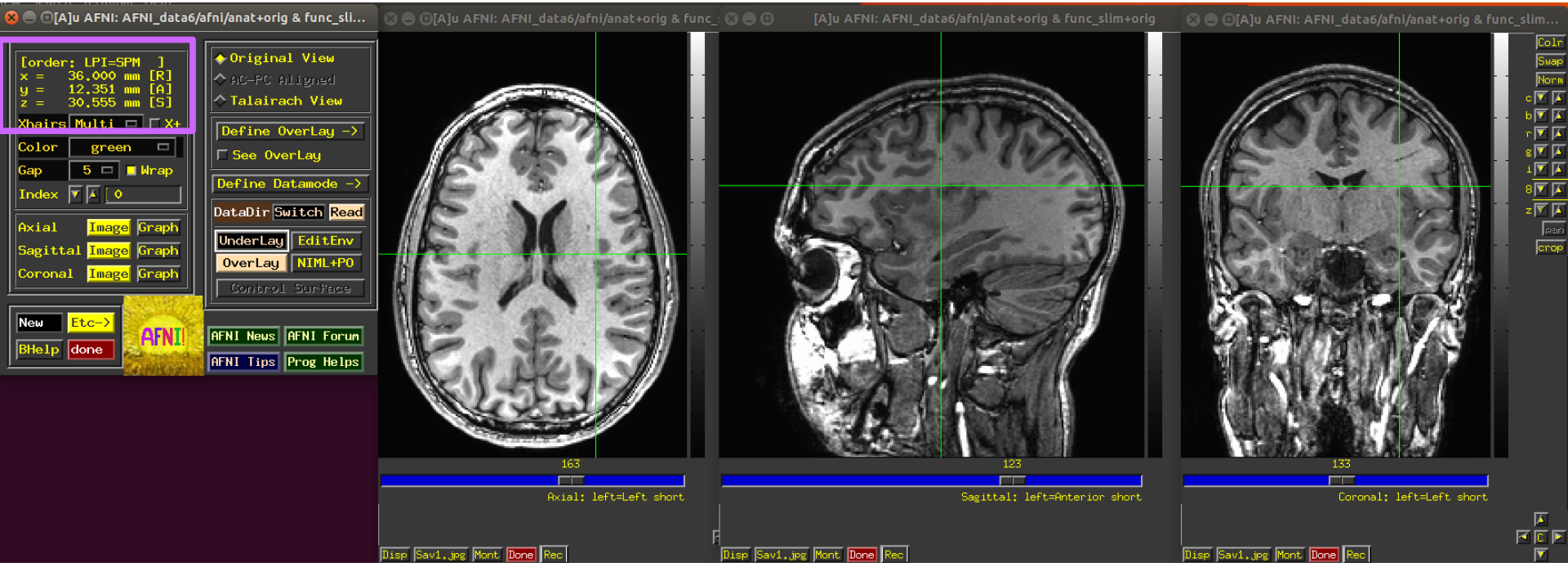
  e.g.: **in LPI order**, cross-hair coordinates are (**36, 12, 31**)



Describing location in dsets

- When reporting location of interest-- e.g., cluster peak-- one can't just provide *(x, y, z)* values. One also has to provide the ***coordinate order*** being used.
- Two main families of coordinate order in the literature:

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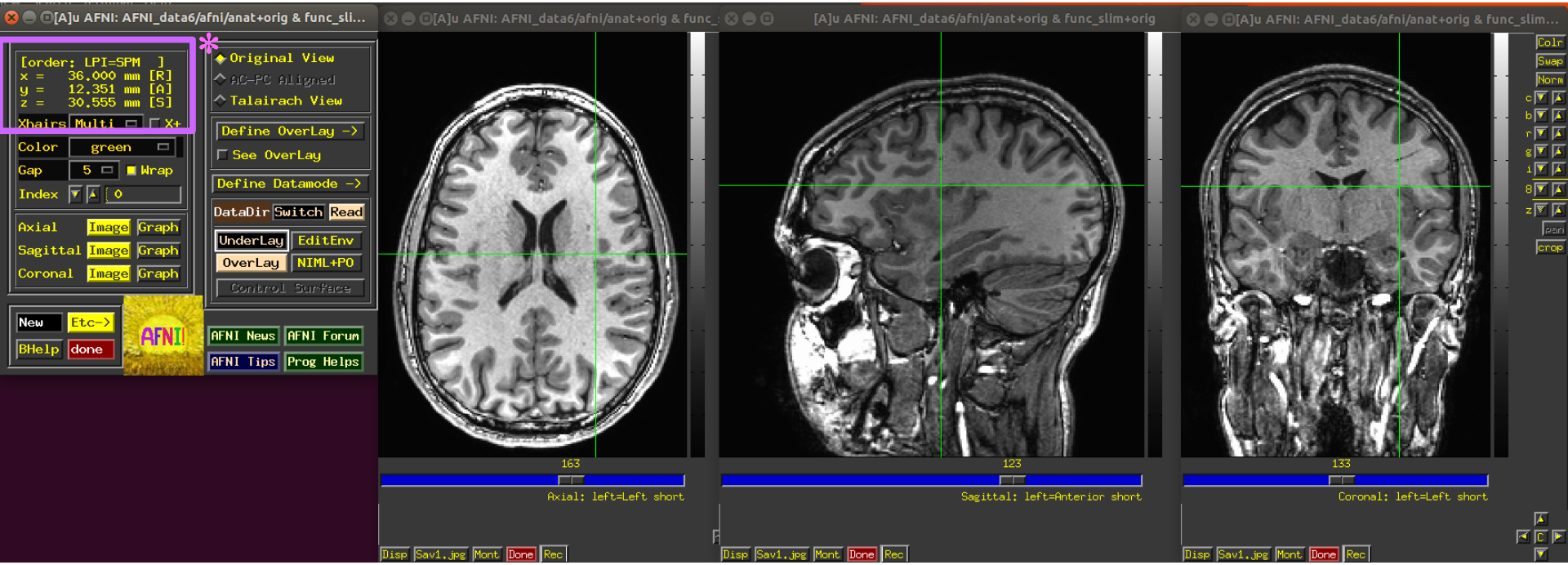

* The GUI default coordinate order can be set in the ~/.afnirc file.

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  e.g.: **in LPI order**, cross-hair coordinates are (**36, 12, 31**)

- **A better way to report coordinates**, which avoids this hassle (and chance for error), is to report *directionalized* coordinates, e.g., (**36R, 12A, 31S**). That way, there is not ambiguity/mental calculation.

Volumetric data sets: files

- Where is information stored in files?

  → *A file contains two categories of information:*

  data block: the numbers stored at each voxel

  header: organizational information about the dset, like:

  origin, orient, dimensions, voxel size, TR, labeltables, etc.

  → use **3dinfo** to see all header info, or individual parts

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  → *There are multiple volumetric file formats. In AFNI, we mostly use two*:

  BRIK-HEAD: pair of files, e.g., DSET+orig.HEAD and DSET+orig.BRIK

  - BRIK file contains data block (only); is binary format
  - HEAD file contains header info (only); is text format

  NIFTI: single file, e.g., DSET.nii, or (compressed) DSET.nii.gz

  - both header and data block in the same file; is binary format

AFNI terminology sidenote

- VIEW and SPACE properties in a header
- BRIK/HEAD file names contain more info than just names (for NII, just in header):

  DSET+**orig**.HEAD and DSET+**orig**.BRIK, or

  DSET+**tlrc**.HEAD and DSET+**tlrc**.BRIK, etc.
- This is the (AFNI) **view**. It describes if the dset is in original/native/acquired coordinates, or if it has been aligned to a template space (or AC-PC aligned).

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- The **space** property carries the specific name of *which* space (MNI, etc.) it is in.

  ```
  3dinfo -space -av_space DSET
  ```

  - **Hands-on time!**

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- In the GUI, tlrc dsets have special features like "whereami" and atlas access.
- Dsets of different spaces cannot be overlayed in the GUI.

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  `3dinfo -space -av_space DSET`
- In the GUI, tlrc dsets have special features like "whereami" and atlas access.
- Dsets of different spaces cannot be overlayed in the GUI.
- *Ex.*: VIEW SPACE

  orig *ORIG* # some original space

  orig *TLRC* # mapped to a template space, called TLRC

  orig *MNI* # mapped to a template space, called MNI

  orig *HaskinsPeds* # mapped to a template space, called HaskinsPeds
- Note: *tlrc* is generic view, while *TLRC* name is specific to a template space.

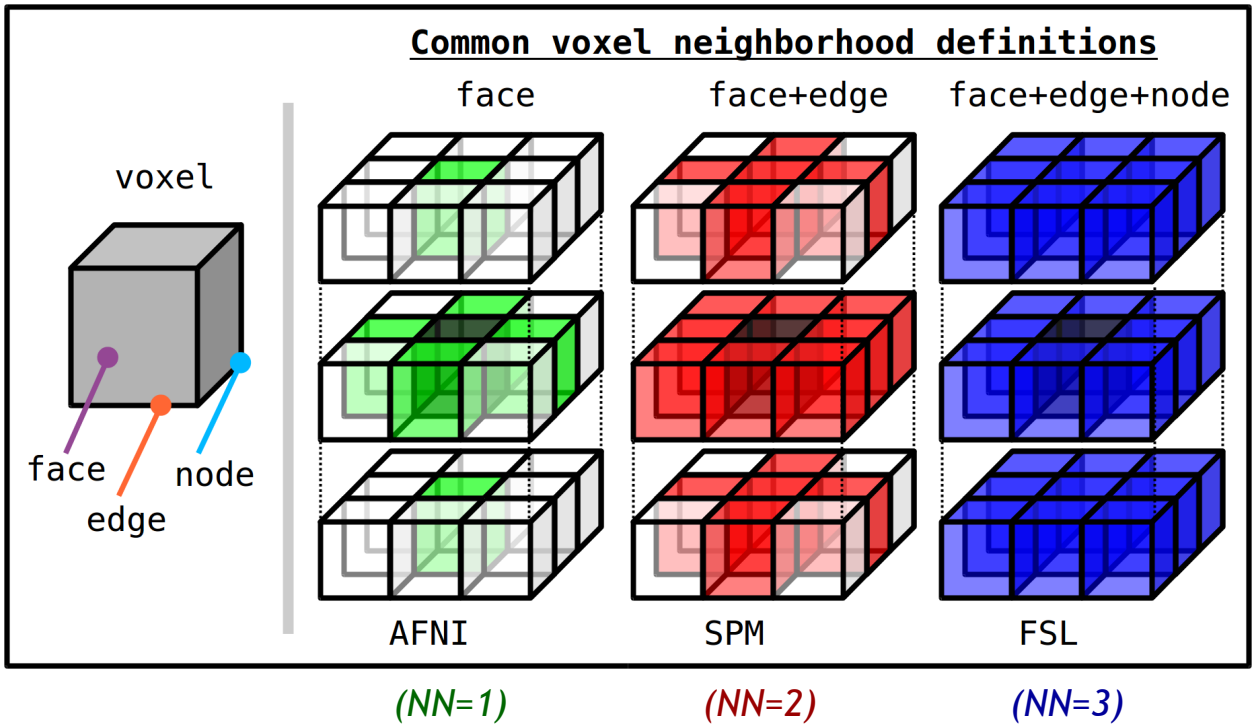
  Fun fact: these properties also map onto NIFTI sform and qform codes directly.

Sidenote: Getting to know your neighbors

- Who are a voxel's neighbors?

Sidenote: Getting to know your neighbors

- Who are a voxel's neighbors?
  → *This is used for blurring, dilating, clustering and several other steps.*
  → *Different softwares define this differently by default (o-o-o-of course…).*



NB: in AFNI, one can **choose** any of these three definitions, typically with the "NN" specification. Just be consistent.

Other data set formats

- AFNI can **read/transform** other data set formats
  + ANALYZE (.hdr/.img file pairs), such as from SPM, FSL; e.g., *3dcopy*
  + MINC-1 (.mnc), such as from mnitools [but not MINC-2]; e.g., *3dMINCtoAFNI*
  + CTF (.mri, .svl), from MEG analysis volumes
  + BrainVoyager (.vmr), from BrainVoyager; e.g., *3dBRAIN_VOYAGERtoAFNI*
  + ASCII text (.1D): just numbers arranged into columns; e.g., *3dUndump*
- Note: these other formats may be missing some standard header information, which may need to be borrowed/used from other known files in NII or BRIK/HEAD format (e.g., 3dUndump to get grid)

- AFNI can **convert** volumes to MINC-1, ANALYZE, text file of coordinates (3dAFNItoMINC, 3dAFNItoANALYZE, 3dmaskdump, etc.)

- For fuller related program list, see:
  https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/educational/classified_progs.html#copy-convert-manipulate-dsets

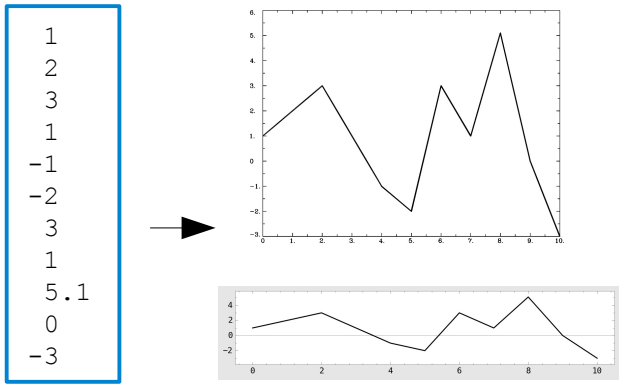
- ***Always check your results carefully when converting to other format/software!***

Appendix 1: non-volumetric files

*.1D files

1D files: text file, columns and/or rows of numbers
+ can represent time series, alignment/motion parameters, voxel locations, etc.
+ might include "# commented regions" at the top

Ways to view
+ open in text editor: "afni_open -t FILE.1D"
+ view in terminal: less, cat
+ plot: AFNI's 1dplot or 1dplot.py
  - each column is one time series
  - *Ex.:* `1dplot file.1D`




```
1dplot.py -infiles file.1D -prefix OUT.jpg
```

Useful programs for these types of dsets
+ 1dcat, 1dtranspose, 1d_tool.py, cat_matvec, 1deval, …
+ see: https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/educational/classified_progs.html#deal-with-1d-time-series

*.txt and/or *.dat files

TXT/DAT files: text file, could be numbers, could be words/strings
+ e.g., stimulus timing files with numbers and symbols
+ might include "# commented regions" at the top

Ways to view
+ open in text editor: "afni_open -t FILE.1D"
+ view in terminal: less, cat

*.json files

JSON files: text file, stores dictionaries and lists of information
+ general/standard file format, stands for **JavaScript Object Notation**
+ increasingly commonly used in neuroimaging to include extra/meta information about datasets

Ways to view
+ open in text editor: "afni_open -t FILE.1D"
+ view in terminal: less, cat
++ but to read/write/use: very common to use Python functionality

*.niml.dset and *.gii files

GII (GifTI): surface equivalent of NifTI files; standard format
NIML-DSET: surface data file format used in AFNI
→ for both, will deal more with these in the SUMA talks
(SUMA also has other intermediate/useful files *.niml*)

Useful programs for these types of dsets
+ See:

https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/educational/classified_progs.html#suma-surface-calculations-formats-and-viewing

*.niml.lt files

"labeltable" files made by and used in AFNI
+ files to associate a label (text string) with an ROI value (integer)
+ e.g., store names of ROIs in an atlas, such as FS parcellation
+ discussed more in the ROI talks

<u>If you can't wait to read more</u>
+ See *@MakeLabelTable*'s help:

  https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/programs/@MakeLabelTable_sphx.html#ahelp-makelabeltable

+ See ROI demo examples in AFNI doc tutorials:

  https://afni.nimh.nih.gov/pub/dist/doc/htmldoc/tutorials/rois_corr_vis/main_toc.html

Appendix 2: data storage on disk (+ set origin/orient)

Dataset storage: origin and orientation

- How is a 3D vol stored on the computer?

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  → *Row by row (as a flattened matrix), starting from one corner called the* ***origin****.*

  Orientation *states* which *corner, and* in which order *the rows are read (e.g., RPI).*

  At dset origin (i, j, k) = (0, 0, 0); *at coordinate origin (x, y, z) = (0, 0, 0)!*

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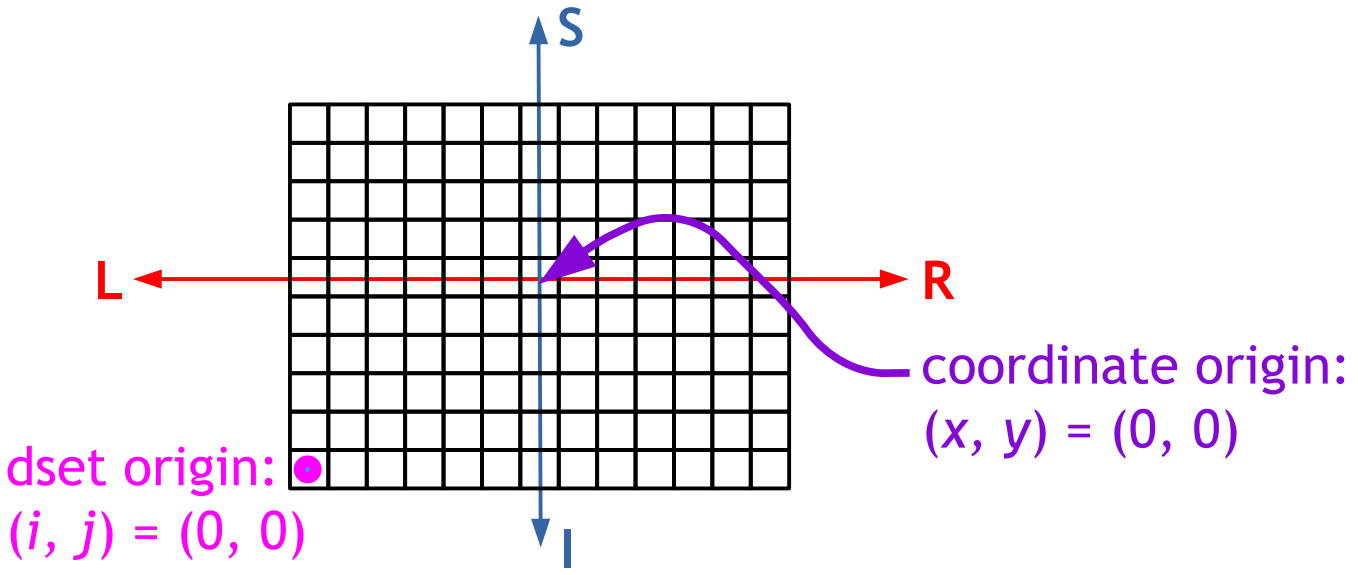
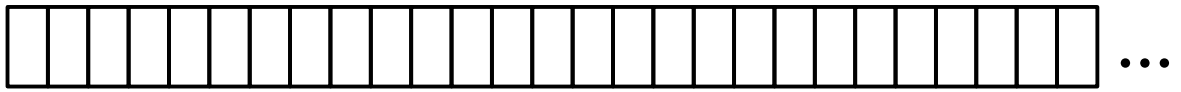
Example,
2D grid



→ stored on comp:



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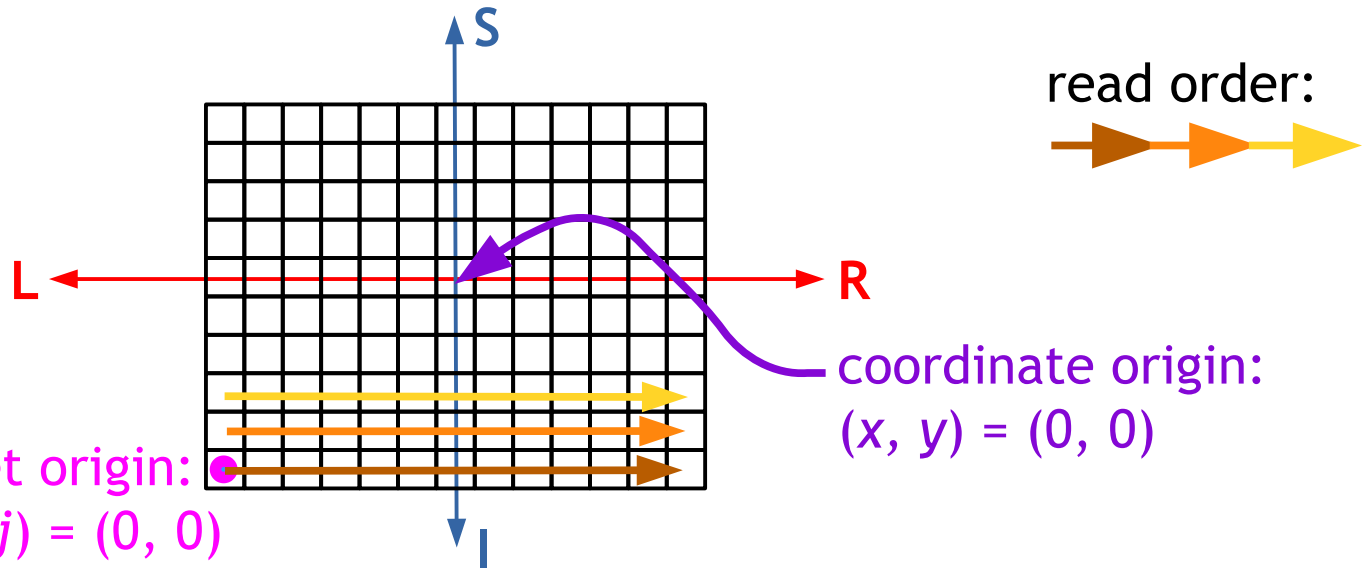

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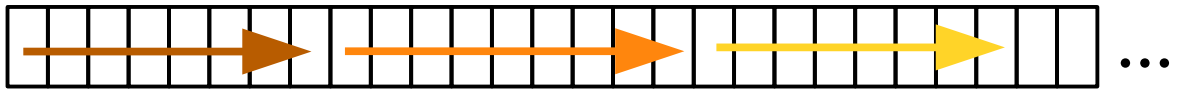

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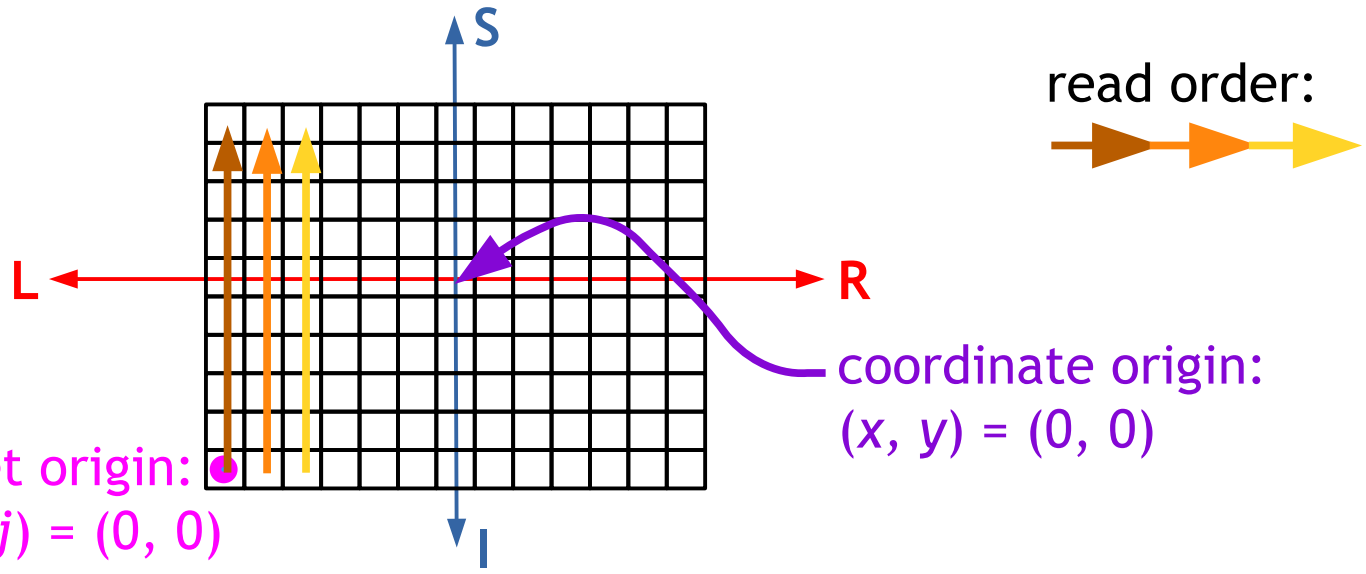

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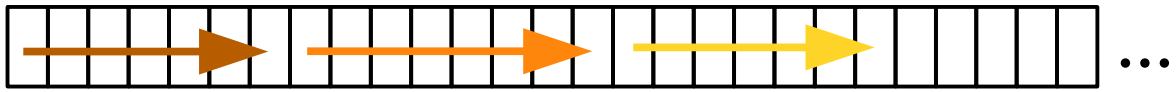

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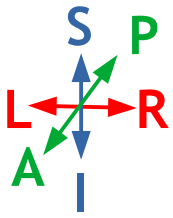
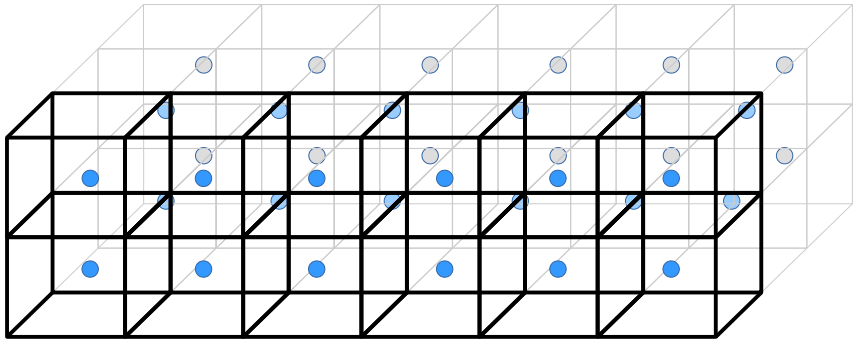
Ex: orientation = LAI

Q1: So where is the origin here?

read order:

3D vol grid:





→ stored on comp:

...

See this in a vol with: *3dinfo -orient -origin DSET*

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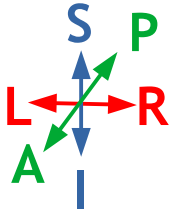
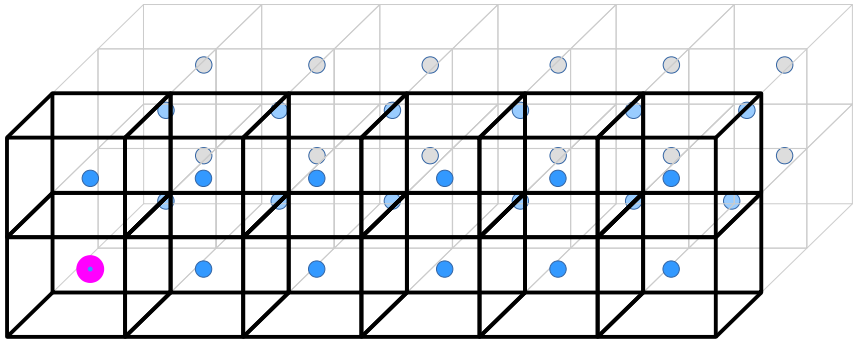


origin



Q2: So how is the data read into storage?

→ stored on comp:

...

See this in a vol with: *3dinfo -orient -origin DSET*

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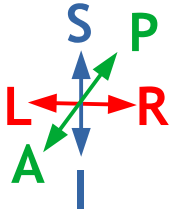
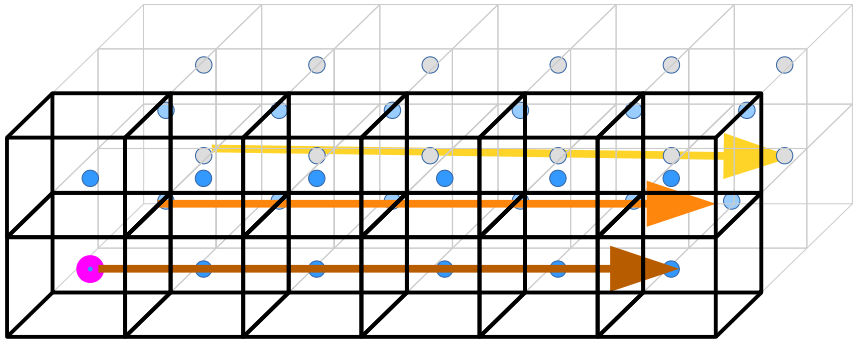
read order:

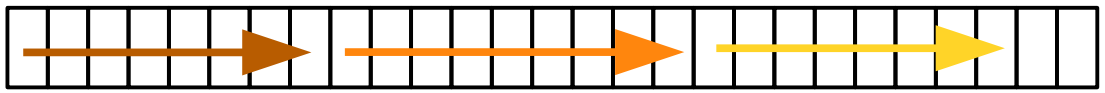
3D vol grid:



origin



→ stored on comp:



...

See this in a vol with: *3dinfo -orient -origin DSET*

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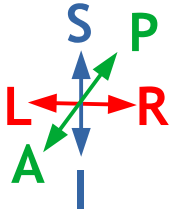
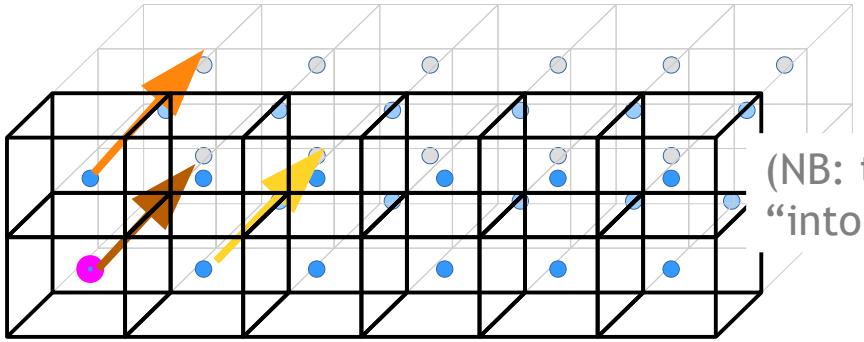
Ex: orientation = AIL

read order:

3D vol grid:

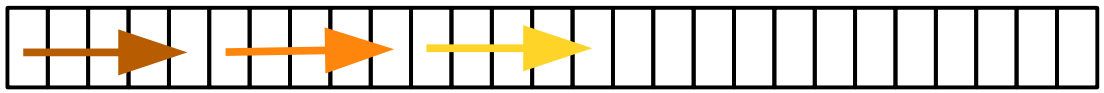


origin



(NB: the arrows point "into" slide here...)

→ stored on comp:



...

See this in a vol with: *3dinfo -orient -origin DSET*

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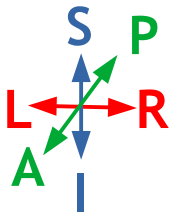
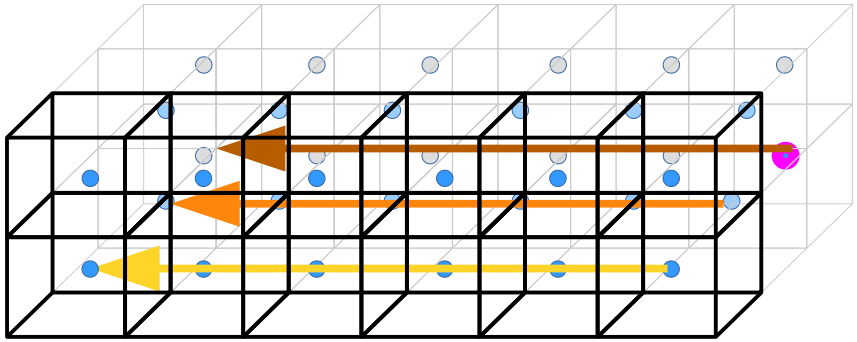
Ex: orientation = RPI

read order:

3D vol grid:





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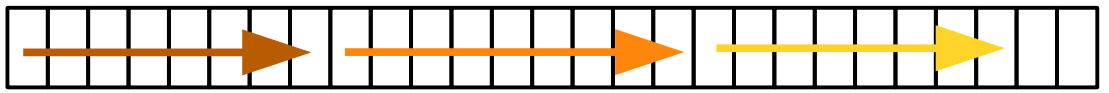

See this in a vol with: *3dinfo -orient -origin DSET*

AFNI program note on dset and grid properties

To simply find out what the dset's grid, orientation, origin, etc. properties are, ***3dinfo*** is the way to go. *Ex.:*

```
3dinfo -orient -o3 DSET
```

AFNI program note on dset and grid properties

To simply find out what the dset's grid, orientation, origin, etc. properties are, ***3dinfo*** is the way to go. *Ex.:*

```
3dinfo -orient -o3 DSET
```

To alter dset/grid properties:

In AFNI, the program ***3dresample*** is useful for starting with one input and making a dset with a new grid, orientation, origin, etc. The program assumes that the starting information (both header **and** brick info) are *correct*. *Ex.:*

```
3dresample -orient RAI -prefix DSET_NEW -inset DSET
```

To change grid, orientation, origin, etc. properties when the header information is *incorrect*, then the program ***3drefit*** is useful. *Ex.:*

```
3drefit -orient RAI -inset DSET
```

Note the different purposes of ***3dresample*** and ***3drefit***.