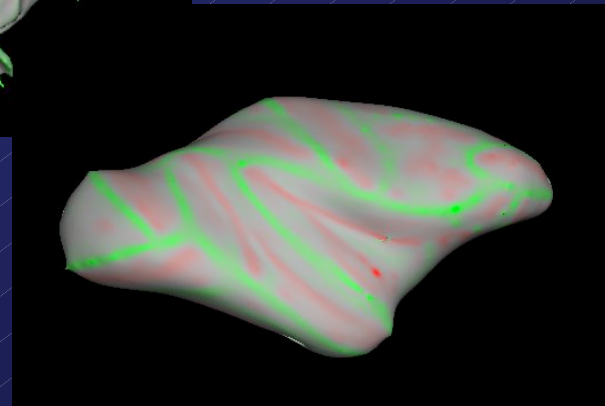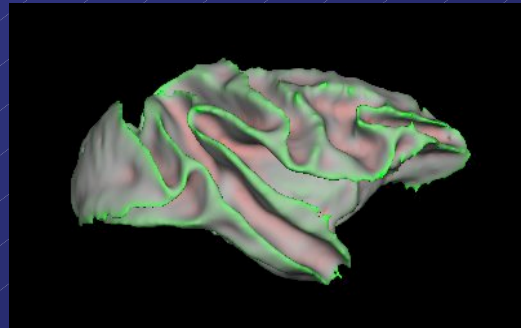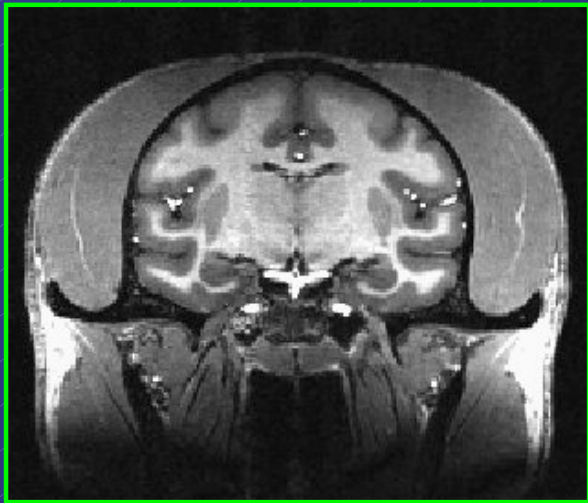# FREESURFER: RECONSTRUCTING THE NONHUMAN BRAIN

Peggy Christidis
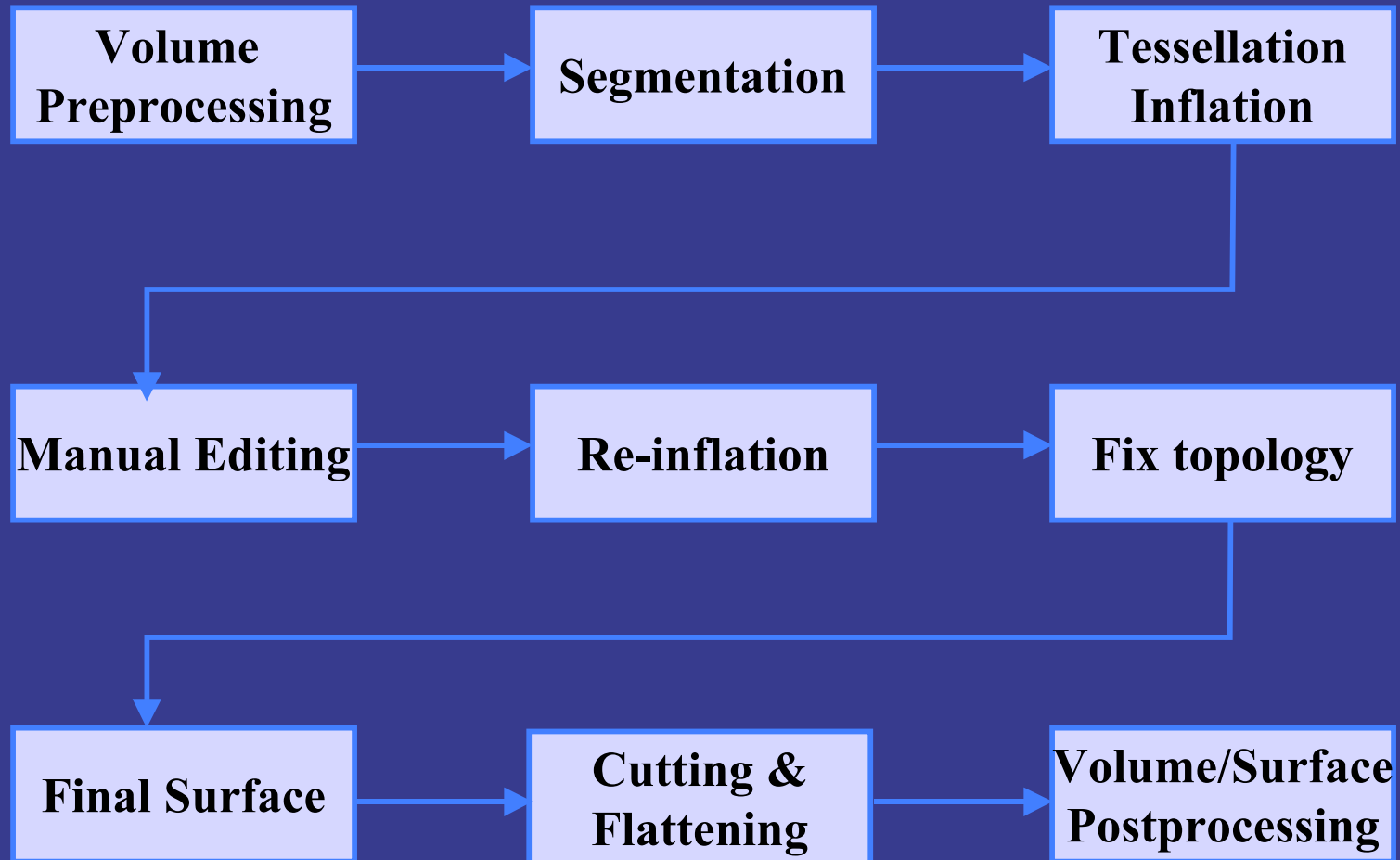
July 14, 2005

National Institutes of Health

GOAL:
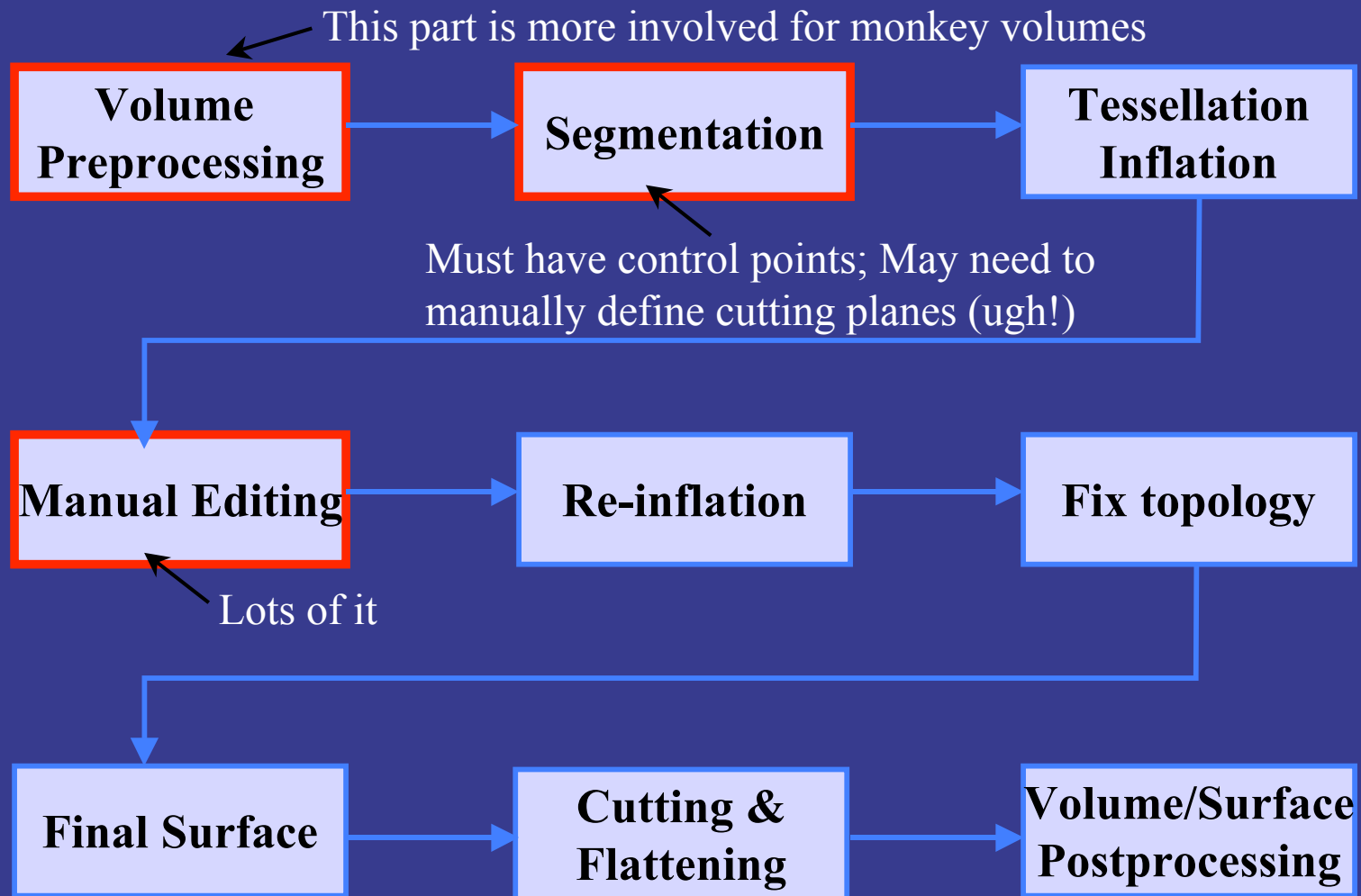
Learn to create monkey surfaces using FreeSurfer

- Primate cortical reconstruction is a more involved and complicated process than human cortical reconstruction
- FreeSurfer manual recommends starting with at least 3 anatomical scans

FreeSurfer Flowchart for Human Brains

Volume Preprocessing	→	Segmentation	→	Tessellation Inflation

Manual Editing	→	Re-inflation	→	Fix topology

Final Surface	→	Cutting & Flattening	→	Volume/Surface Postprocessing

FreeSurfer Flowchart for Primate Brains

This part is more involved for monkey volumes

Volume Preprocessing → **Segmentation** → **Tessellation Inflation**

Must have control points; May need to manually define cutting planes (ugh!)

Manual Editing → **Re-inflation** → **Fix topology**

Lots of it

Final Surface → **Cutting & Flattening** → **Volume/Surface Postprocessing**

Volume Preprocessing: Humans

Volume preprocessing → Segmentation → Tessellation Inflation → Manual Editing → Re-inflation → Fix topology → Final Surface → Cutting & Flattening → Volume/Surface postprocessing
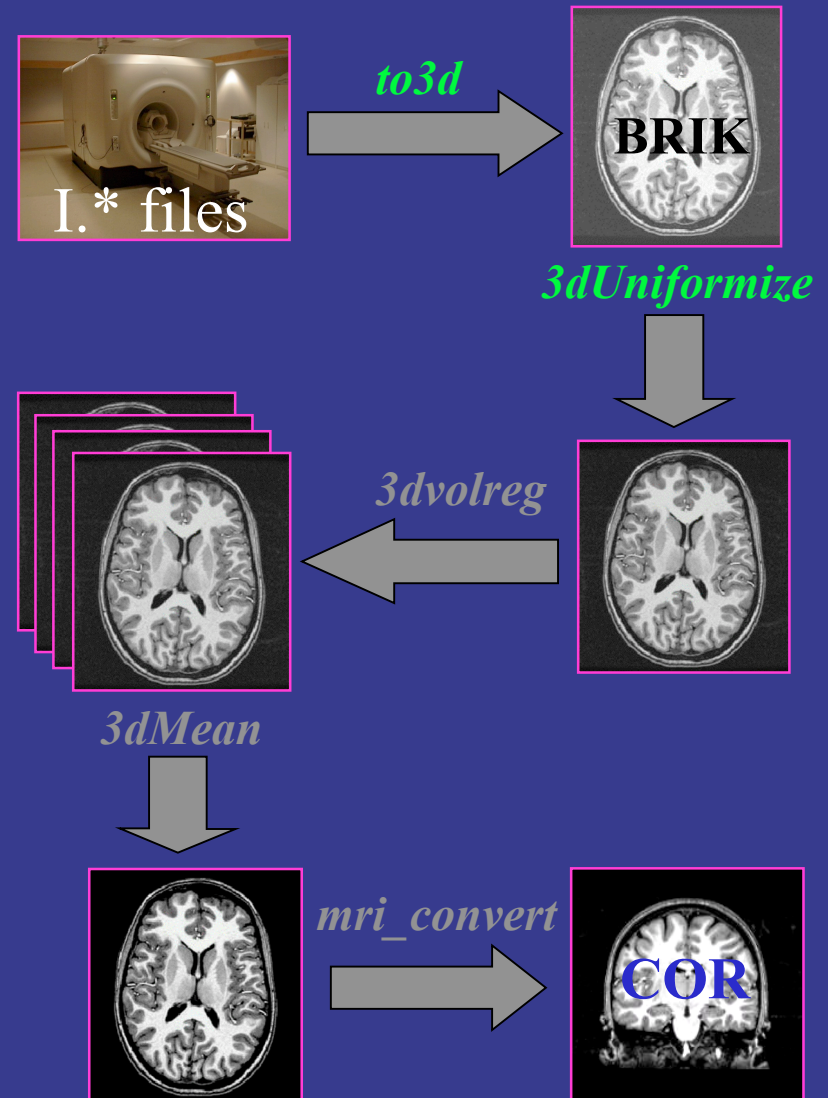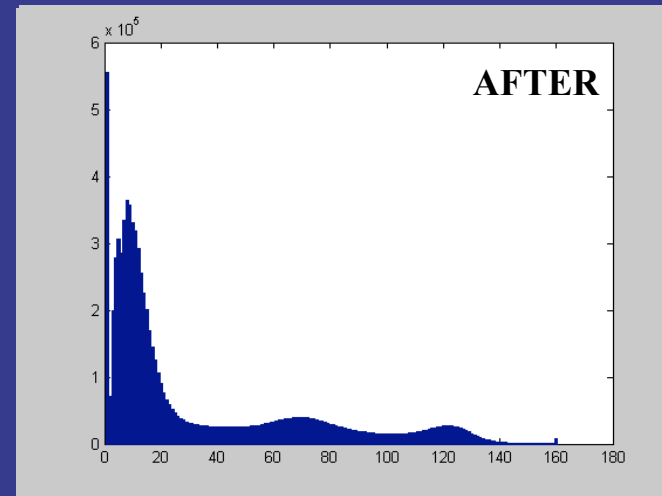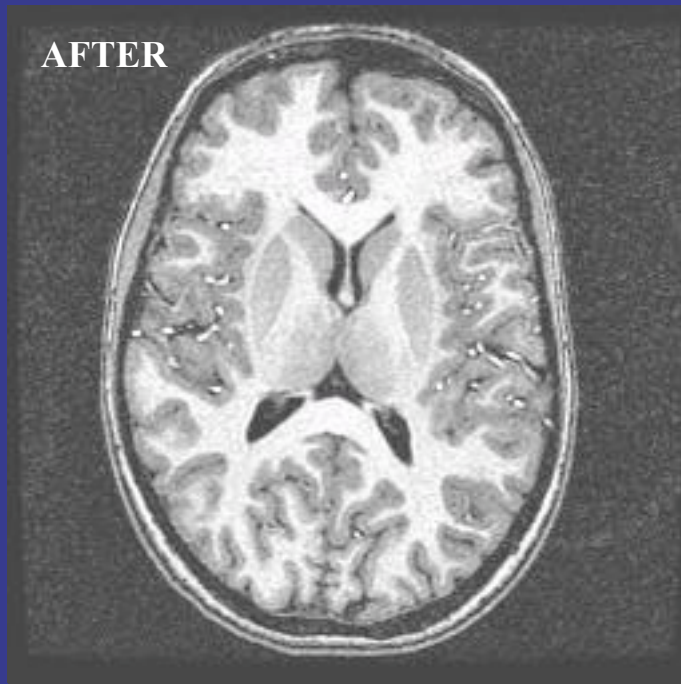
Volume Preprocessing: Humans

- Convert I.* files to BRIK using AFNI *to3d*

- Perform intensity normalization using AFNI *3dUniformize*

- Register multiple volumes using AFNI *3dvolreg*

- Average the registered volumes using AFNI *3dMean*

- Convert to FreeSurfer format using FreeSurfer *mri_convert*

I.* files → *to3d* → BRIK

3dUniformize

3dvolreg

3dMean

mri_convert → COR

Intensity normalization – critical for segmentation

- Inhomogeneities in scanner fields cause gray and white matter intensities to vary as a function of their spatial location.

- Removes residual non-uniformities in gray and white matter intensity values.
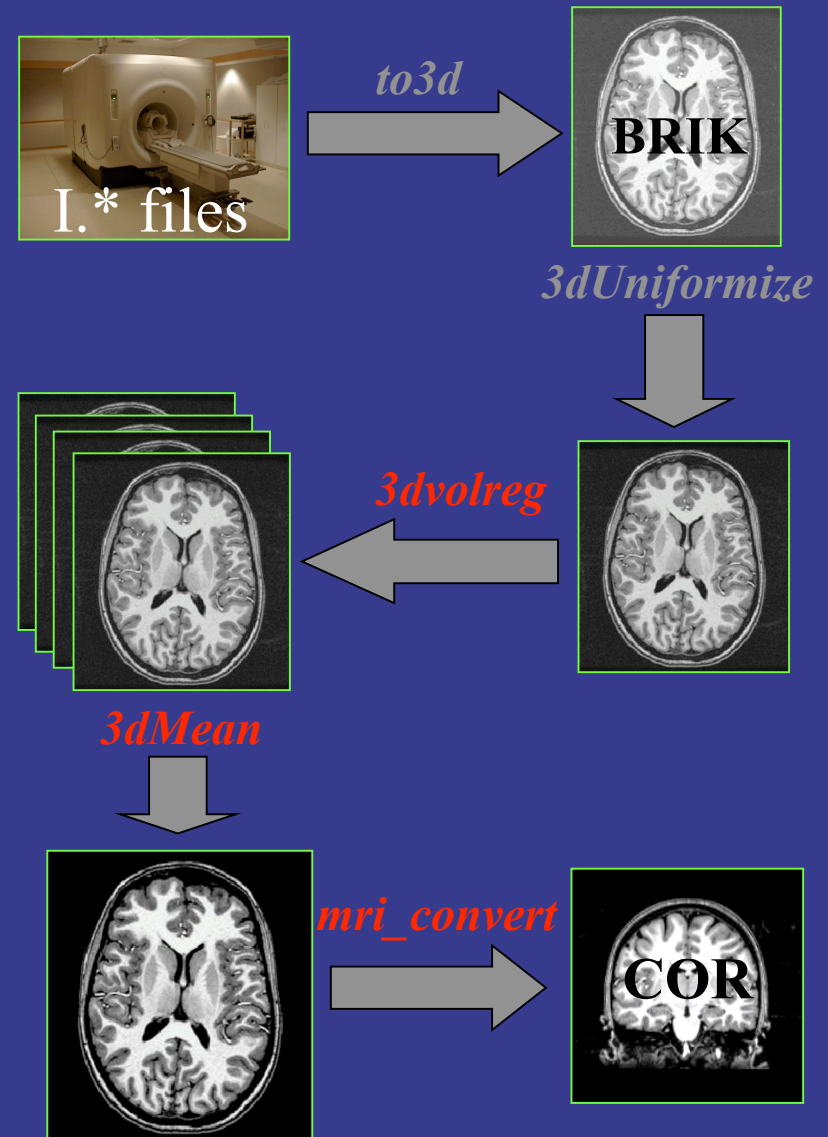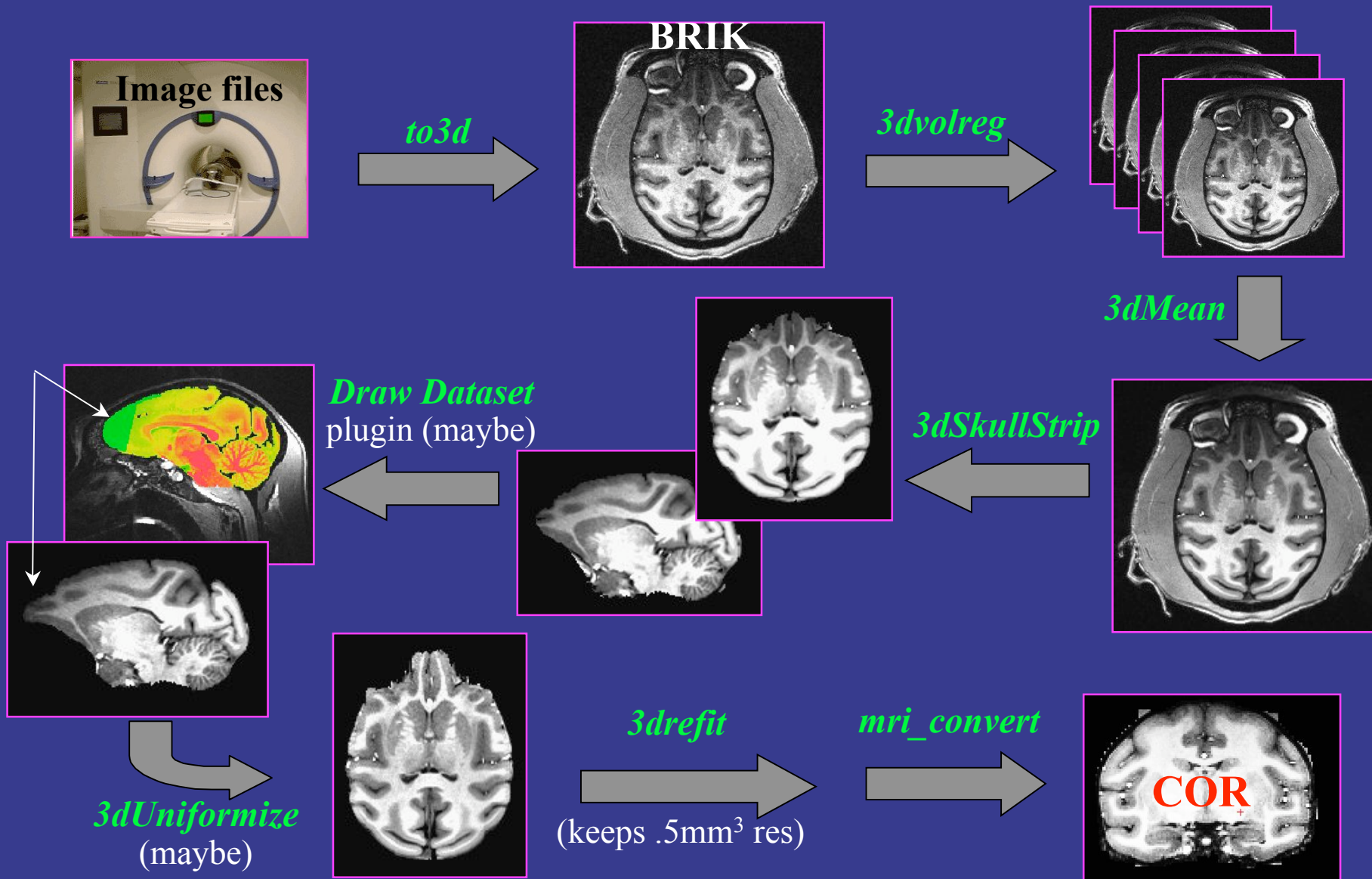

- Increases gray and white matter contrast.

- Sharpens the peaks of the two tissue classes.

- Makes the intensity distribution of gray and white matter spatially uniform.

Volume Preprocessing: Humans

- Convert I.* files to BRIK using AFNI *to3d*

- Perform intensity normalization using AFNI *3dUniformize*

- *Register multiple volumes using AFNI 3dvolreg*

- *Average the registered volumes using AFNI 3dMean*

- *Convert to FreeSurfer format using FreeSurfer mri_convert*

# Volume Preprocessing: Monkeys

Volume Preprocessing: <u>Monkeys</u>

Image files → *to3d* → **BRIK** → *3dvolreg* → *3dMean* → *3dSkullStrip* → *Draw Dataset* plugin (maybe) → *3dUniformize* (maybe) → *3drefit* (keeps .5mm³ res) → *mri_convert* → **COR**

STEP 1: Collect your data

|	
1st_scan+orig.HEAD	1st_scan+orig.BRIK
2nd_scan+orig.HEAD	2nd_scan+orig.BRIK
3rd_scan+orig.HEAD	3rd_scan+orig.BRIK
4th_scan+orig.HEAD	4th_scan+orig.BRIK

STEP 2: Volume registration

```
foreach num (1st  2nd  3rd  4th)
        3dvolreg -twopass -cubic -zpad 4  \
                -base 1st_scan+orig      \
                -prefix {$num}_scan_vr  \
                {$num}_scan+orig
end
```
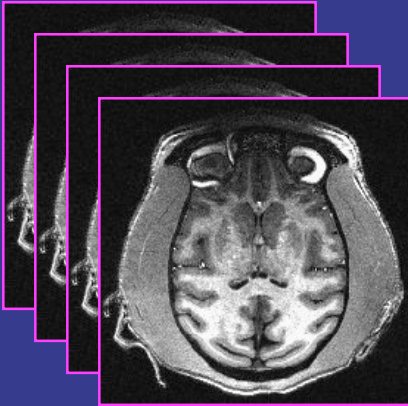

1st_scan_vr+orig.HEAD	1st_scan_vr+orig.BRIK
2nd_scan_vr+orig.HEAD	2nd_scan_vr+orig.BRIK
3rd_scan_vr+orig.HEAD	3rd_scan_vr+orig.BRIK
4th_scan_vr+orig.HEAD	4th_scan_vr+orig.BRIK

NOTE:

Check your datasets in AFNI or whatever fMRI sofware package you're comfortable with.

In this case, the datasets had really high intensity values. The nonuniformity correction program in AFNI (**3dUniformize**) has difficulty with high intensity values.

AFNI's **3dcalc** program was used to divide each voxel in each dataset by '20', resulting in smaller intensity values for each volume.

This is a temporary problem with 3dUniformize that will be fixed in the near future.
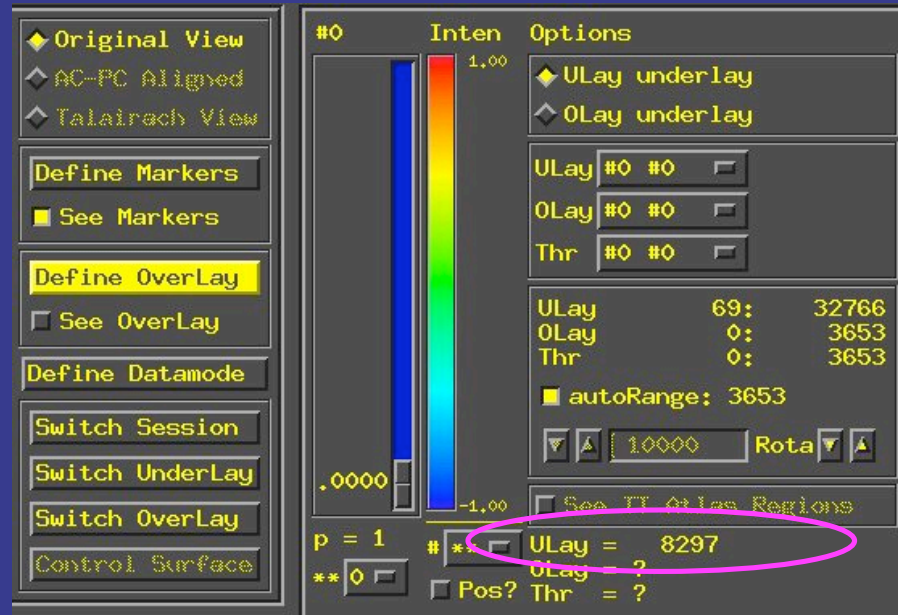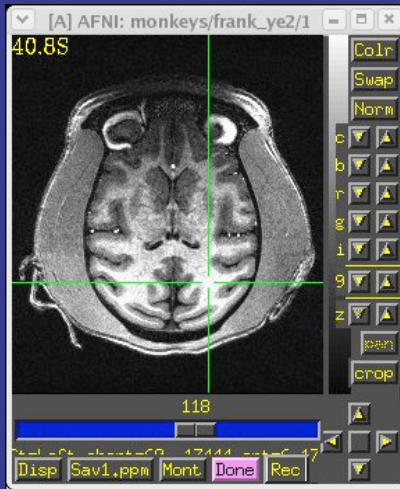
```
foreach num (1st 2nd 3rd 4th)

        3dcalc -a {$num}_scan_vr+orig  -expr 'a/20' \
               -nscale  -prefix {$num}_scan_vr20

end
```

1st_scan_vr20+orig.HEAD   1st_scan_vr20+orig.BRIK
2nd_scan_vr20+orig.HEAD   2nd_scan_vr20+orig.BRIK
3rd_scan_vr20+orig.HEAD   3rd_scan_vr20+orig.BRIK
4th_scan_vr20+orig.HEAD   4th_scan_vr20+orig.BRIK

STEP 3: <u>Average your volume-registered datsets together</u>

```
3dMean -prefix avg4                     \
        ???_scan_vr+orig.BRIK
```
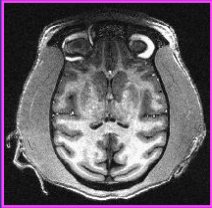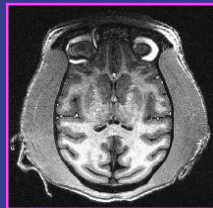

1st_scan_vr   2nd_scan_vr   3rd_scan_vr   4th_scan_vr
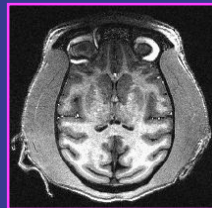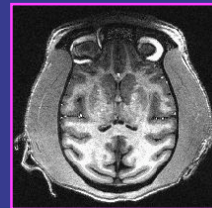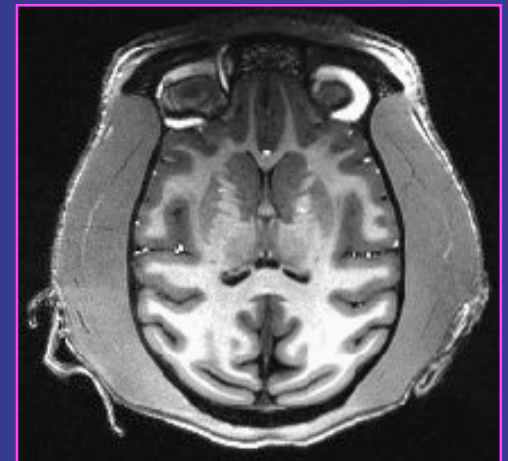
avg4+orig.HEAD
avg4+orig.BRIK

STEP 4: <u>Extract the brain from surrounding tissue and skull</u>

```
3dSkullStrip -prefix avg4_brain \
              -input avg4+orig -no_avoid_eyes
```
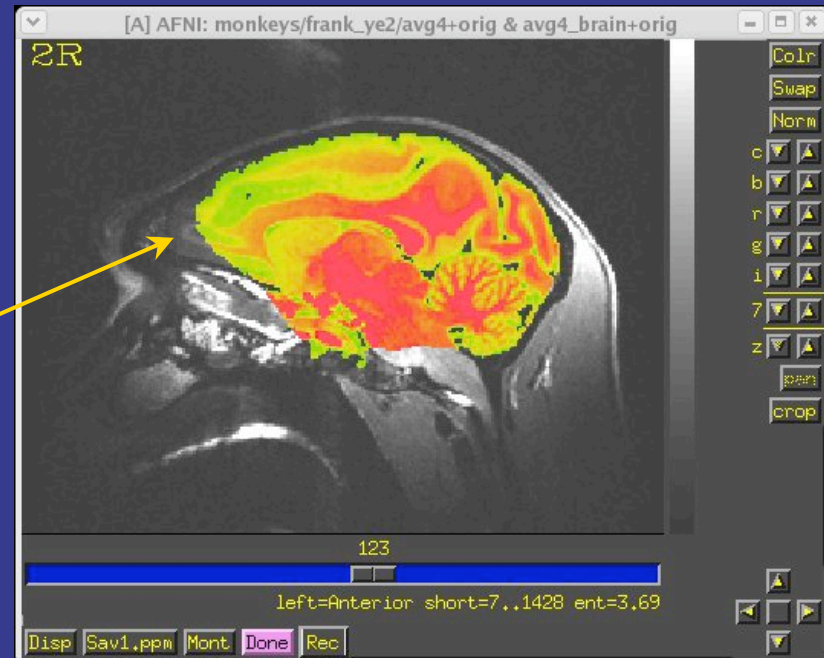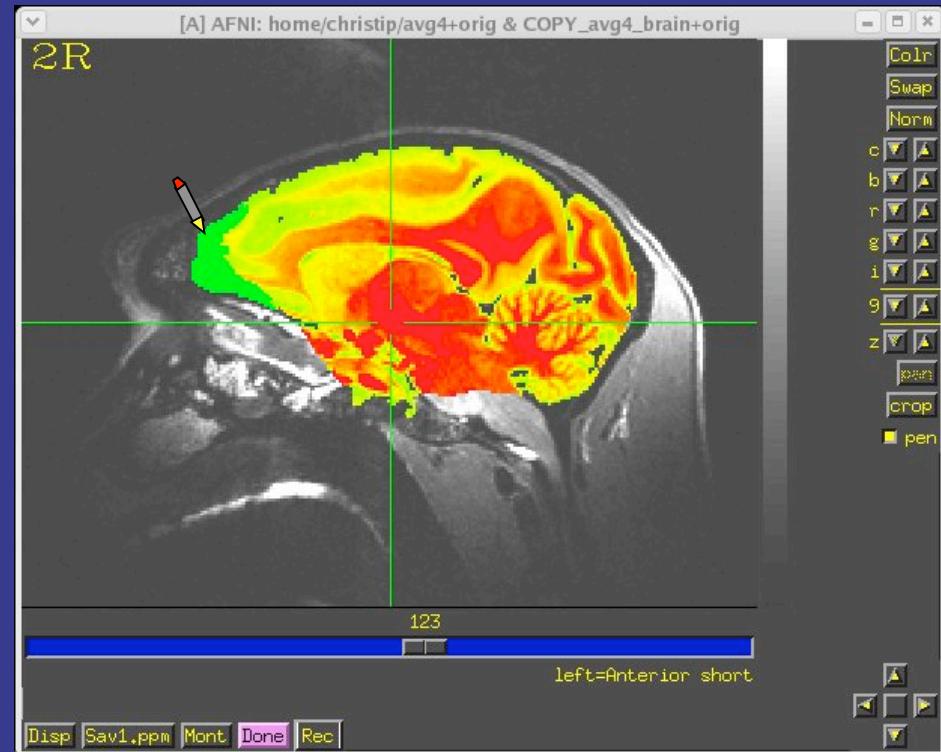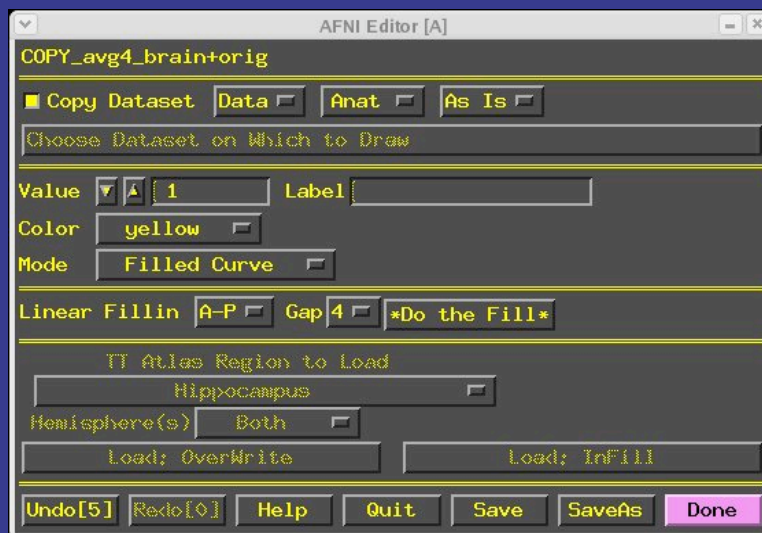
avg4_brain+orig.HEAD

avg4_brain+orig.BRIK
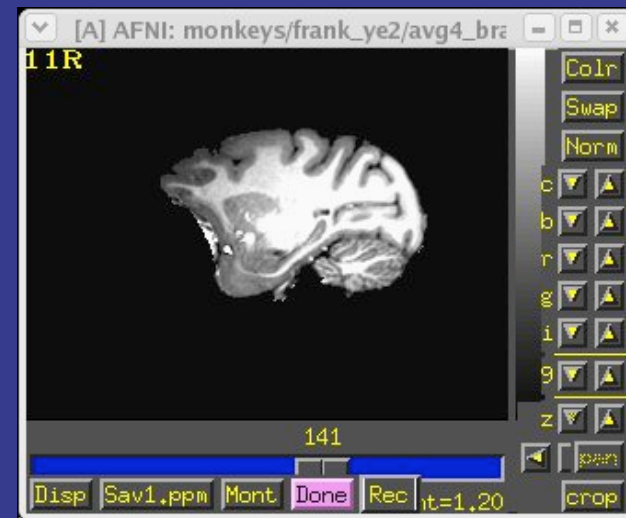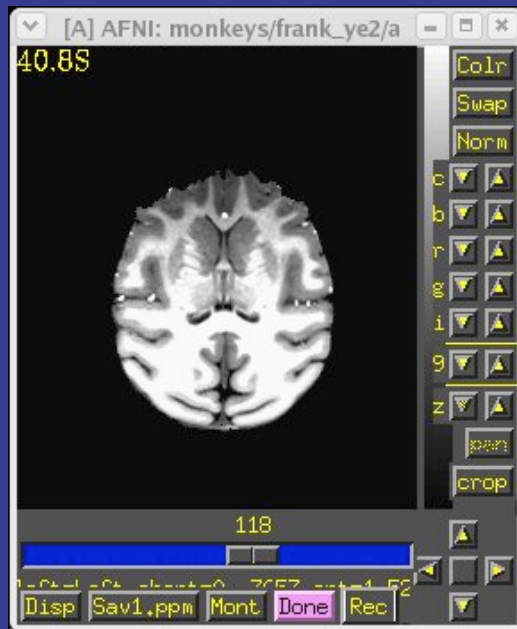
NOTE:

- Check your dataset in AFNI or whatever fMRI sofware package you're comfortable with.

- In this case, if we overlay the skull-stripped dataset on top of the un-skull-stripped dataset, we can see that a little too much brain was removed from the frontal lobes (that's because these volumes are really dark in the frontal lobe region).

- You may have to manually re-draw it back into the dataset.

- Try the AFNI plugin '**Draw Dataset**'

- Yes, this is labor intensive.

- And yes, you really should do it.

trouble area

STEP 5: If necessary, manually insert "brain" voxels that were removed when you did the skull stripping

COPY_avg4_brain+orig.HEAD

COPY_avg4_brain+orig.BRIK

STEP 5: continued…

```
3dcalc  -a avg4+orig  -b COPY_avg4_brain+orig
        -expr 'a*ispositive(b)'
        -prefix avg4_brain_edited
```
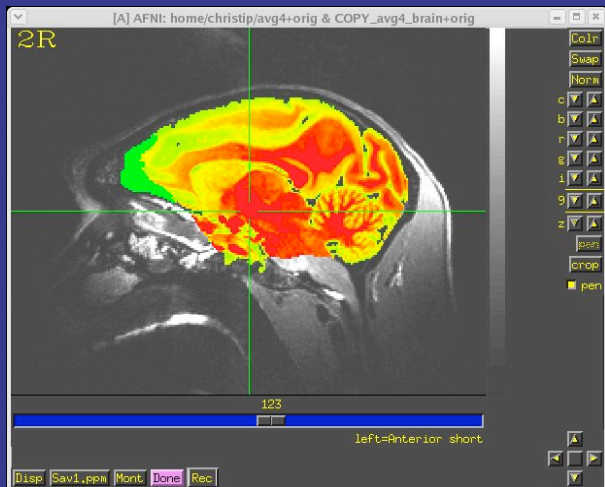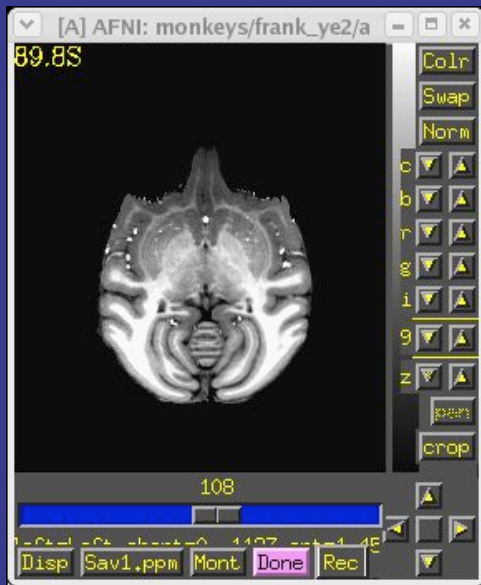

COPY_avg4_brain+orig.HEAD
COPY_avg4_brain+orig.BRIK

avg4_brain_edited+orig.HEAD
avg4_brain_edited+orig.BRIK
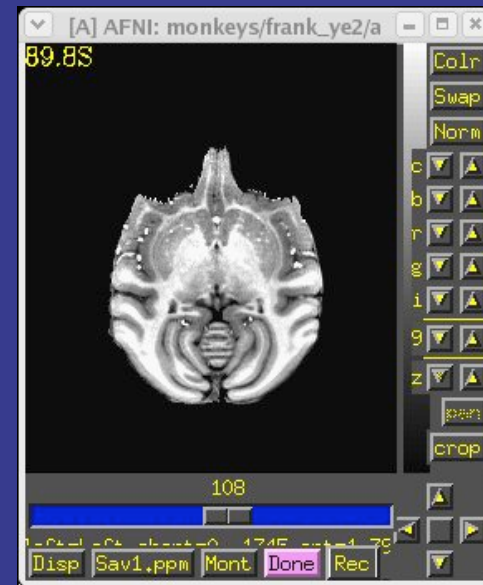
STEP 6: <u>Perform a nonuniformity correction</u>

```
3dUniformize  -prefix avg4_ready4FS  -quiet \
               -anat avg4_brain_edited.orig
```

Before 3dUniformize

After 3dUniformize

avg4_brain_edited+orig

avg4_ready4FS+orig

<u>Note</u>: This may or may not make the data look better. It really depends on the volume. Also, it may look great in AFNI, but not so great in FreeSurfer (I still can't figure that one out)

STEP 6: <u>Use 3drefit to make sure FreeSurfer doesn't resample your high-resolution volume to a lower resolution</u>

- When importing your volume into FreeSurfer, the program will resample it to its **COR** format, which is made up of 256 coronal slices, a 256 x 256 in-plane, and a $1mm^3$ voxel resolution.

- For human volumes, this is okay because a voxel resolution of $1 mm^3$ is great. For monkey volumes however, the voxel resolution is often better than $1mm^3$. In our example, the voxel resolution is $0.5mm^3$.

- If we allowed FreeSurfer to resample our dataset from a $0.5mm^3$ voxel resolution to a $1mm^3$ voxel resolution, the COR volume in FreeSurfer would look pixelated.

- Pixelated volume = unsuccessful white matter segmentation

Volume in FreeSurfer if **3drefit** isn't run first:
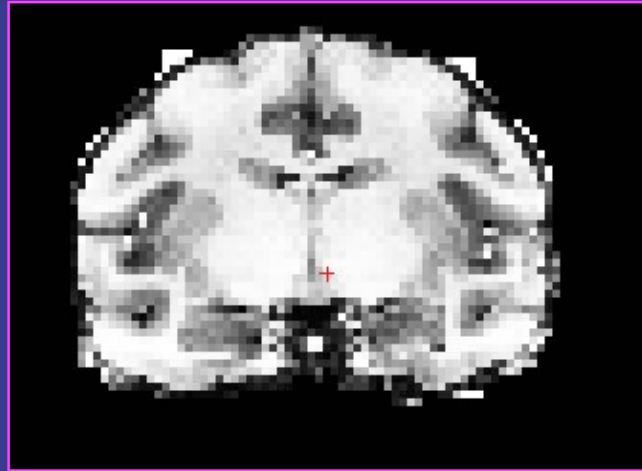
Segmentation will either fail or give you terrible results:
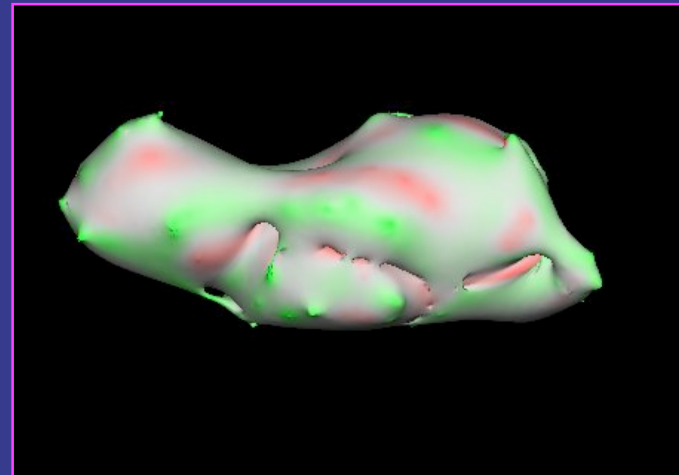
```
3drefit -xdel 1.0 -ydel 1.0 -zdel 1.0 \
     avg4_brain_edited+orig
```

3drefit will change the header information in the above dataset, but it won't change the actual data. This way, when FreeSurfer reads the header information of that dataset, it will see the 1mm^3 voxel-resolution and won't enlarge the voxels, thinking they're already at the resolution they need to be.

```
3dinfo avg4_brain_edited+orig
```



```
Dataset File:      avg4_brain_edited+orig
Identifier Code: PPC_LO5xhKvh_i1-vUR188eKYw  Creation Date: Fri Jul  8 16:18:17
2005
Dataset Type:      Spoiled GRASS (-spgr)
Byte Order:        LSB_FIRST [this CPU native = LSB_FIRST]
Storage Mode:      BRIK file
Data Axes Orientation:
  first  (x) = Right-to-Left
  second (y) = Anterior-to-Posterior
  third  (z) = Inferior-to-Superior    [-orient RAI]
R-to-L extent:    -52.000 [R] -to-   171.000 [L] -step-    1.000 mm [224 voxels]
A-to-P extent:    -83.183 [A] -to-   171.817 [P] -step-    1.000 mm [256 voxels]
I-to-S extent:    -18.199 [I] -to-   176.801 [S] -step-    1.000 mm [196 voxels]
Number of values stored at each pixel = 1
  -- At sub-brick #0 '#0' datum type is short:       0 to        1628
```

STEP 7a: <u>Create FreeSurfer directory tree using</u> <u>mksubjdirs</u>

NOTE: Now we're entering FreeSurfer country, so be sure to source your FreeSurfer login file first.

(e.g., my login file is called .fs_login)

```
source ~/.fs_login
```

Usage: *mksubjdirs <subject_name>*

Example: `mksubjdirs avg4_surf`

Result from **mksubjdirs avg4_surf**:

Peggy_home/

avg4_surf/

bem/ label/ morph/ mpg/ mri/ rgb/ scripts/ surf/ tiff/ tmp/

T1/ aparc/ brain/ filled/ orig/ tmp/ transforms/ wm/
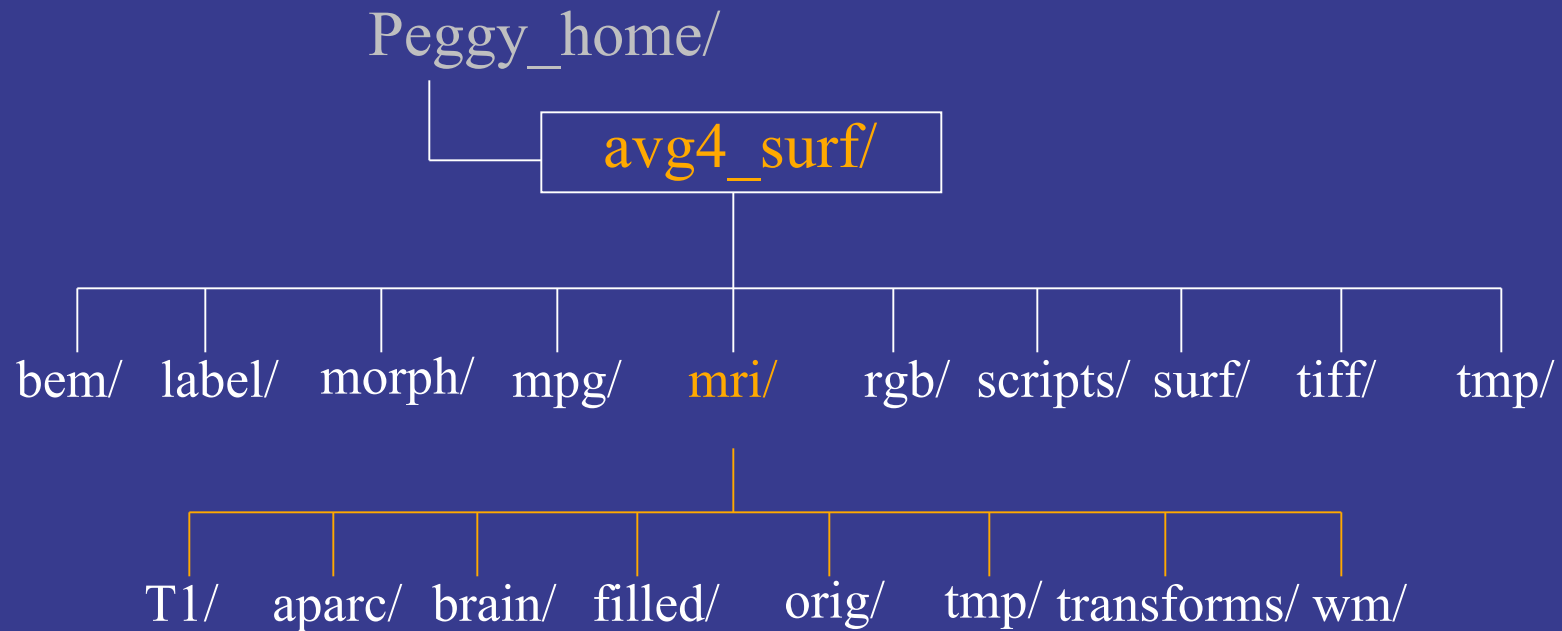
STEP 7b: <u>Convert volume dataset into **COR** format</u>

(i.e., 256 coronal slices, 256 x 256 in-plane, 1mm^3 voxel resolution)

Usage: *mri_convert <volume_dataset> <surf_directory/mri/orig>*

Example: (from within Peggy_home/ directory)

`mri_convert avg4_brain_edited+orig.BRIK  avg4_surf/mri/orig`

VOLUME PRE-PROCESSING DONE.

NEXT, SEGMENTING THE WHITE MATTER

This step is crucial because the quality of the surface creation is dependent on the quality of the white matter segmentation.

Segmenation Process in FreeSurfer

aka "Process Volume" or "segment_subject" or "recon-all -stage1"

Segmentation

- Intensity normalization
- Skull stripping
- White matter labeling

Segmentation

- Intensity normalization
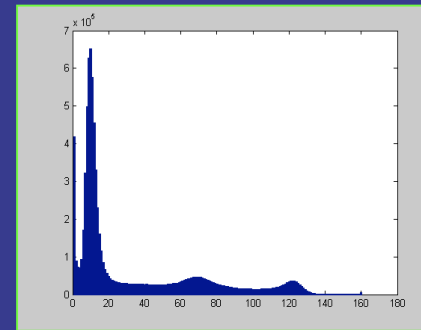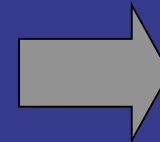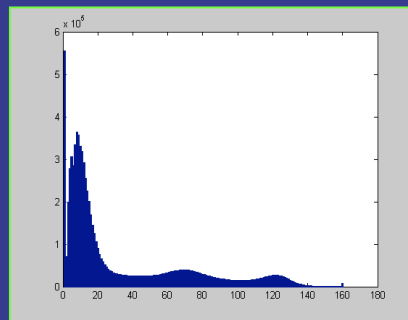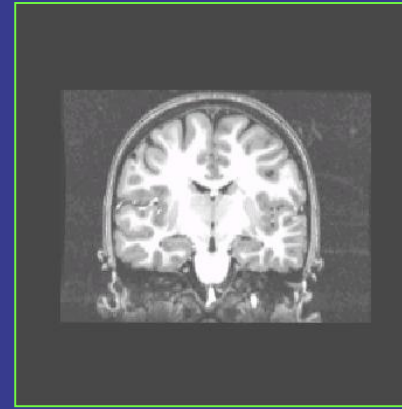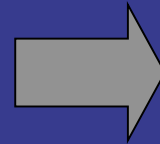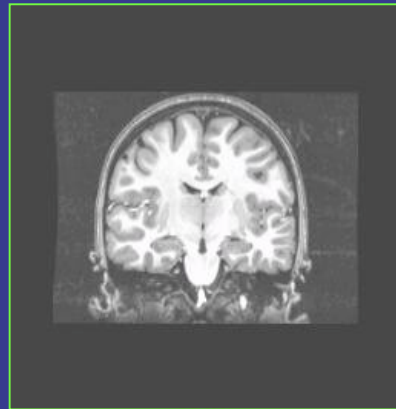
- Skull stripping
- White matter labeling
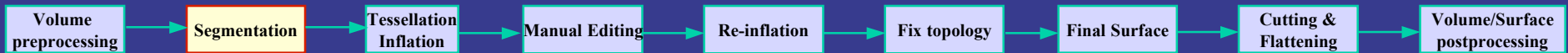
Segmentation

- ## Intensity normalization

- ## Skull stripping
  - Shrink-wrap algorithm
  - Start with ellipsoidal template
  - Minimize brain penetration and curvature

Skull stripping
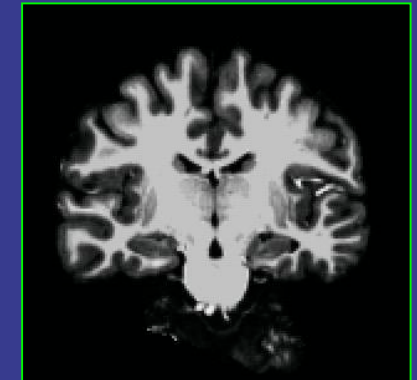
- ## White matter labeling

Skull Stripping
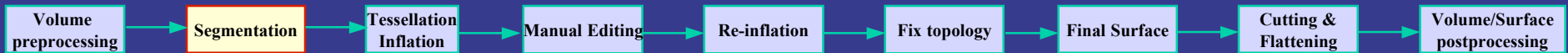
Segmentation

- Intensity normalization
- Skull stripping
- White Matter labeling
  - Preliminary classification solely intensity based
  - Relabeling of mislabeled voxels based on neighborhood information
  - Define cutting planes
  - Find connected components and fill

Can fail miserably with monkey volumes

segment → *define cutting planes* → *Connect components and fill*
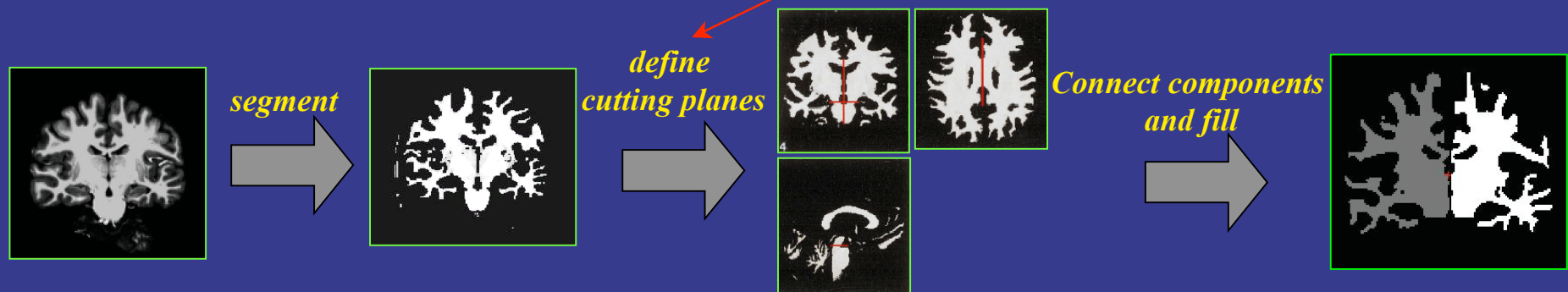
HUMANS
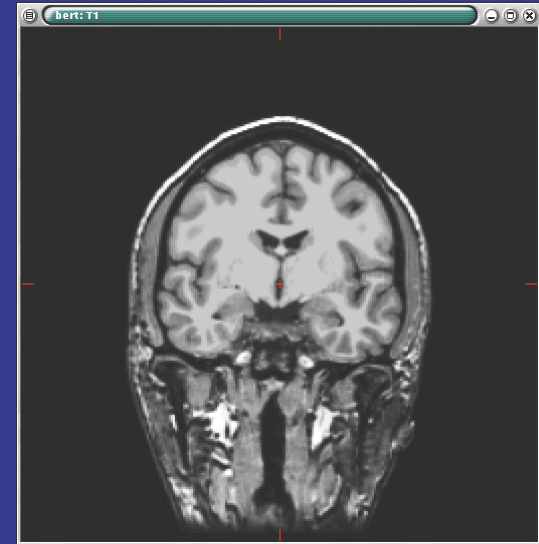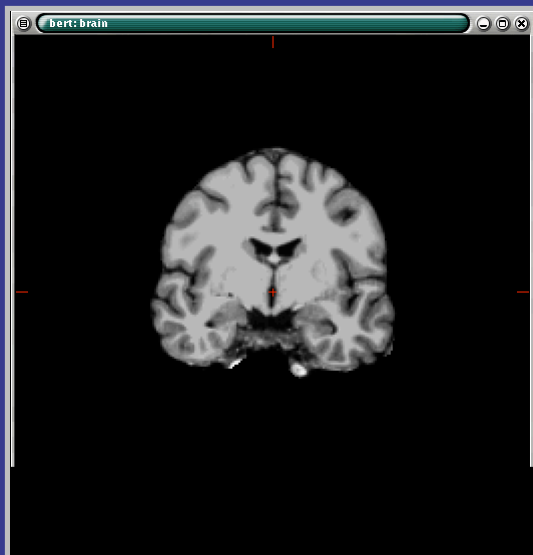
mri/orig
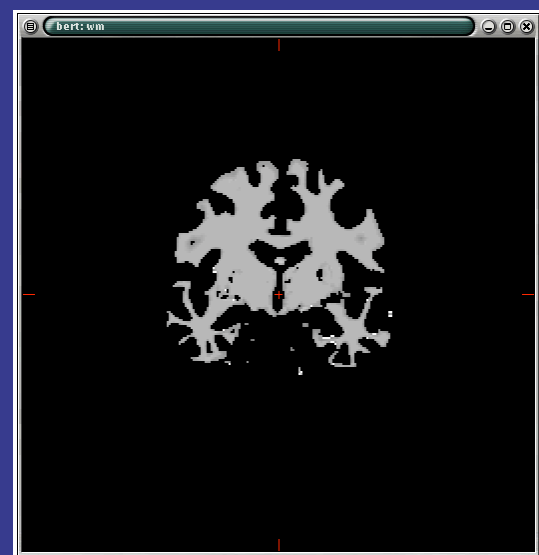
mri/T1

mri/brain

mri/wm

MONKEYS
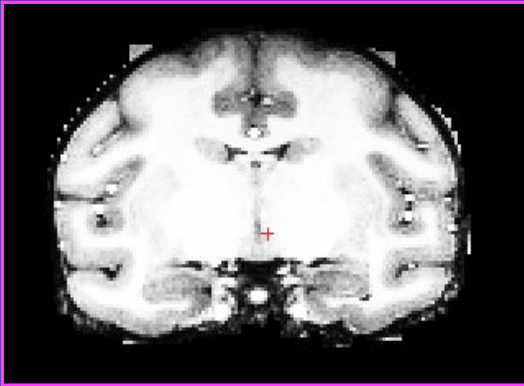
mri/orig
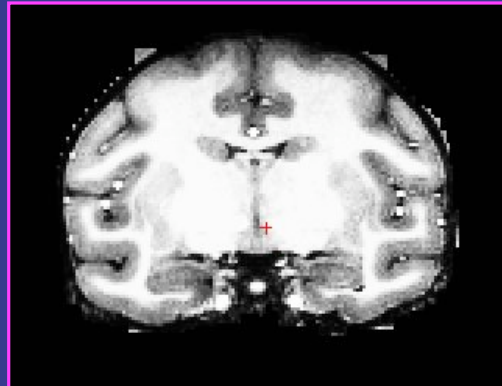
mri/T1
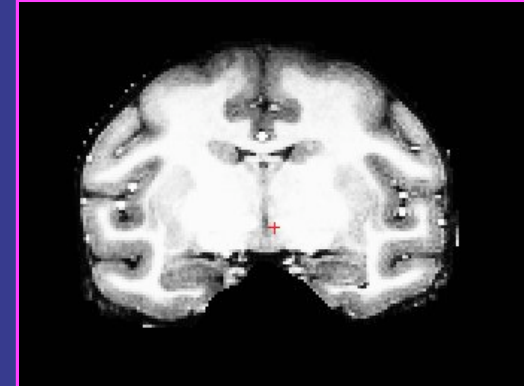
mri/brain
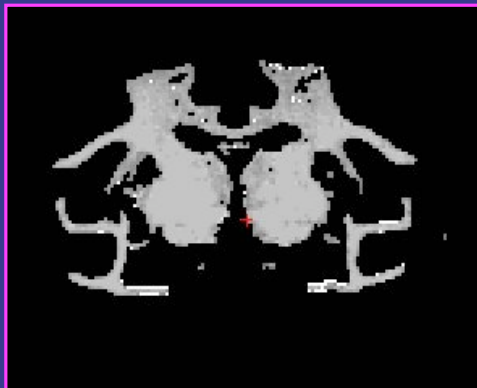
mri/wm
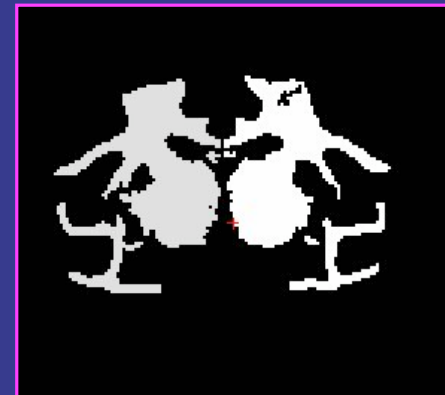
mri/filled

Tessellation and Inflation

- ## Surface Tessellation
  - Use two triangles to represent each face separating white matter voxels from other voxels
  - Smooth initial tessellation with a deformable surface algorithm

- ## Surface Inflation
 - Retain shape and metrics while making the interior of sulci visible

Tessellate and smooth

Inflate

Inflation

Courtesy: http://cogsci.ucsd.edu/~sereno/movies.html
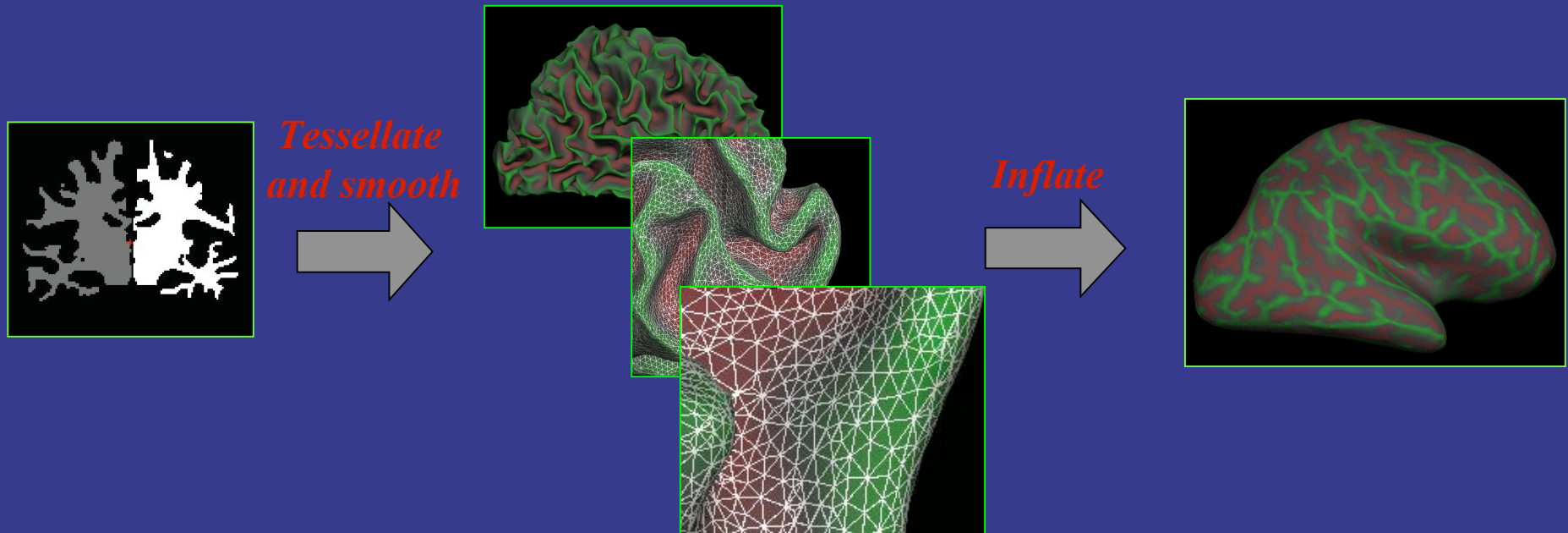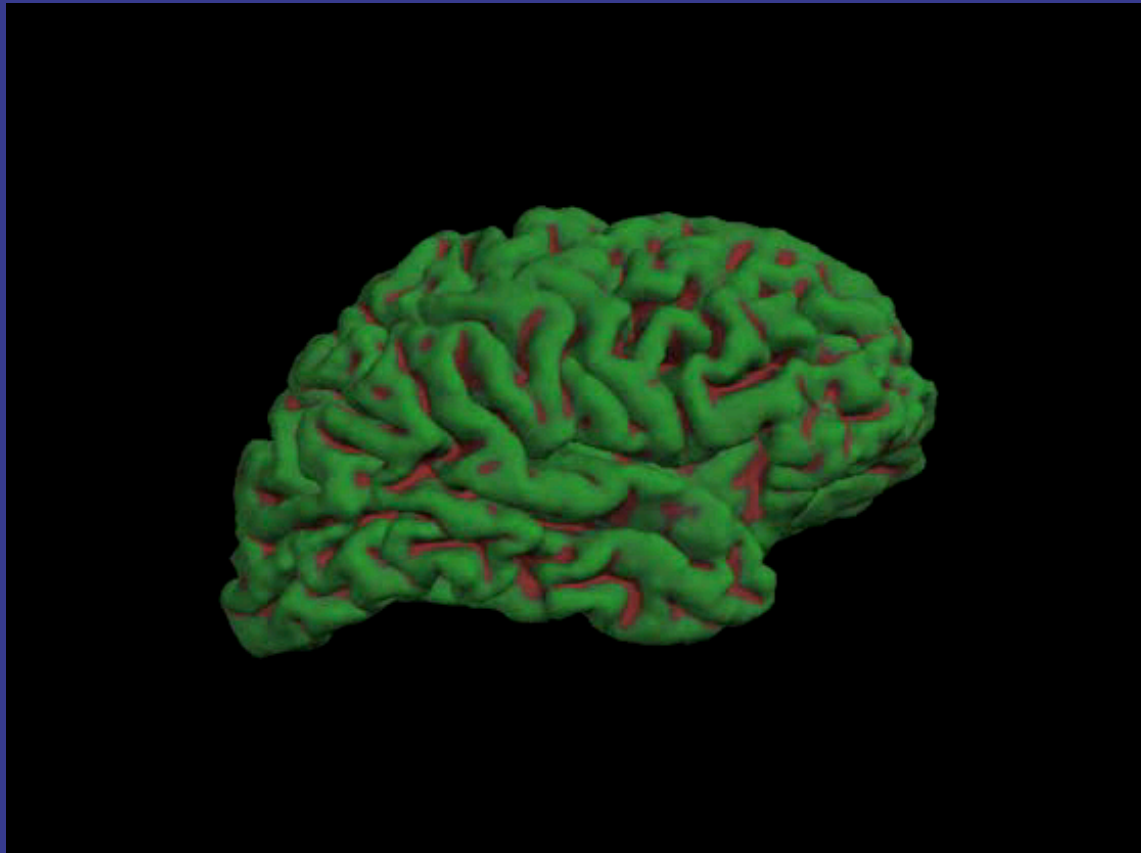
Troubleshooting

- OFTEN, things can go terribly wrong when dealing with monkey volumes/surfaces.

- Be prepared for this!!!

- Luckily, with some patience and skill, you can fix these problems.

- If you still can't fix the problem, join the FreeSurfer mailing list and ask for help!

# Troubleshooting: When segmentation fails

Ghastly
segmentation →

wm

T1
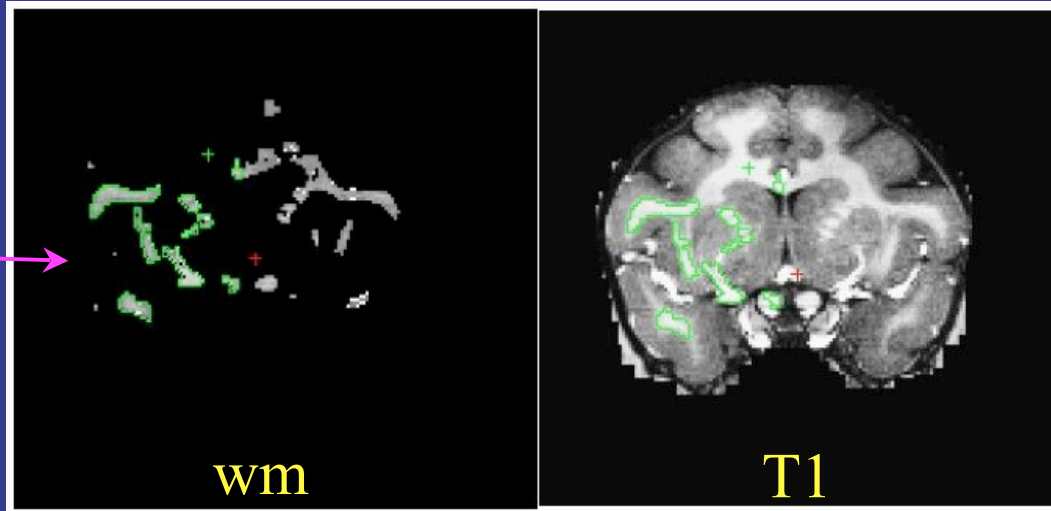
Lots of
missing
frontal lobe →
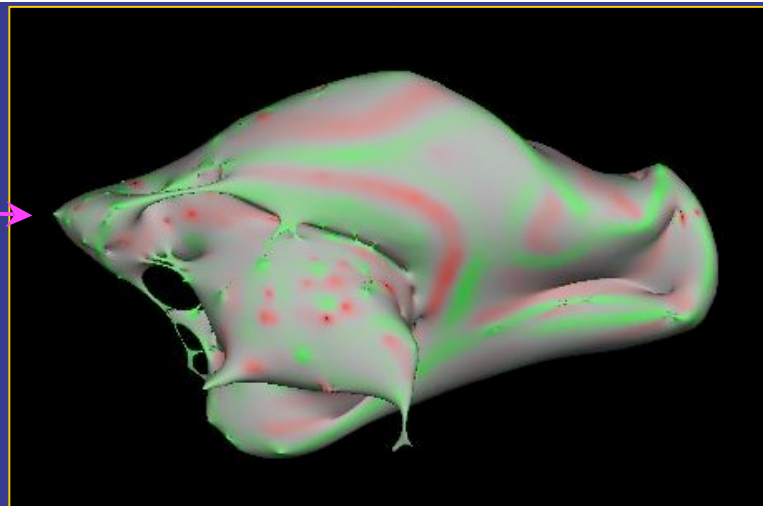
- Monkey brains have very fine lines of white matter that are difficult to segment.

- With monkey volumes, you must always add control points to the T1 volume (inside a clearly white matter area), then re-run the white matter segmentation.

- The FreeSurfer manual recommends using about 20 control points max.
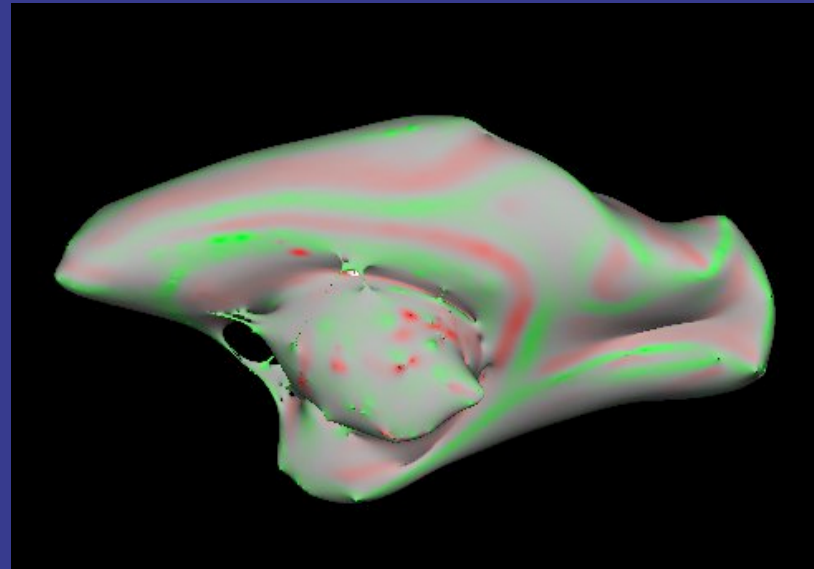
Add ctrl pts like this one throughout T1 volume and re-run the wm segmentation
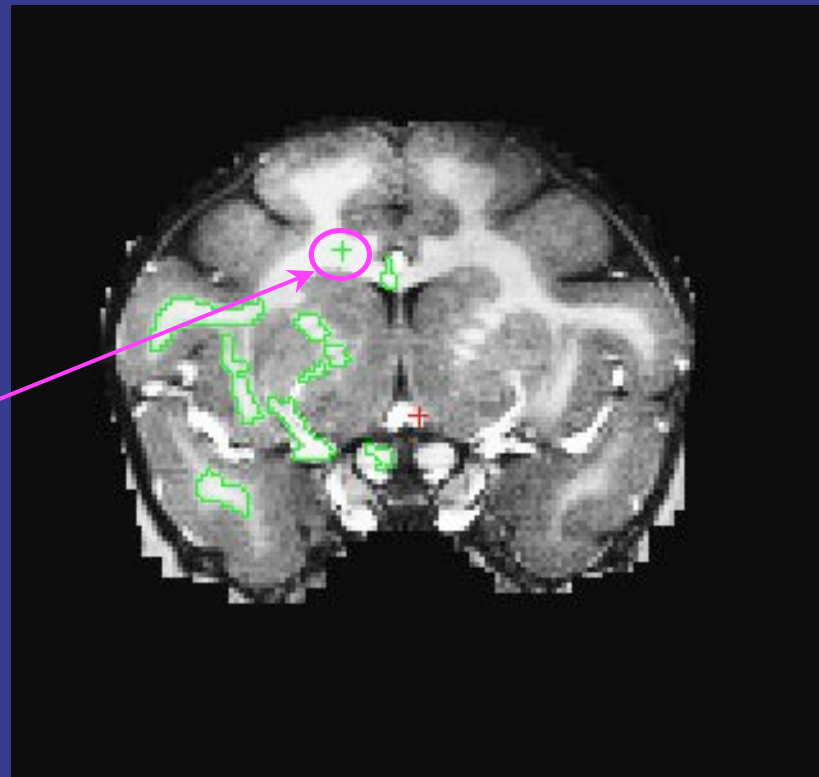
After adding control points:
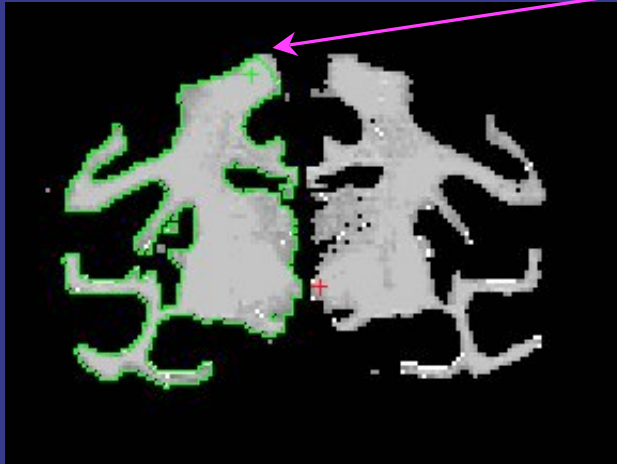
You may still have to hand draw the wm areas that the control points did not pick up

Much better surface

Occipital area also a little problematic. Some gray matter has been included in the white matter segmentation. You may have to manually remove it.
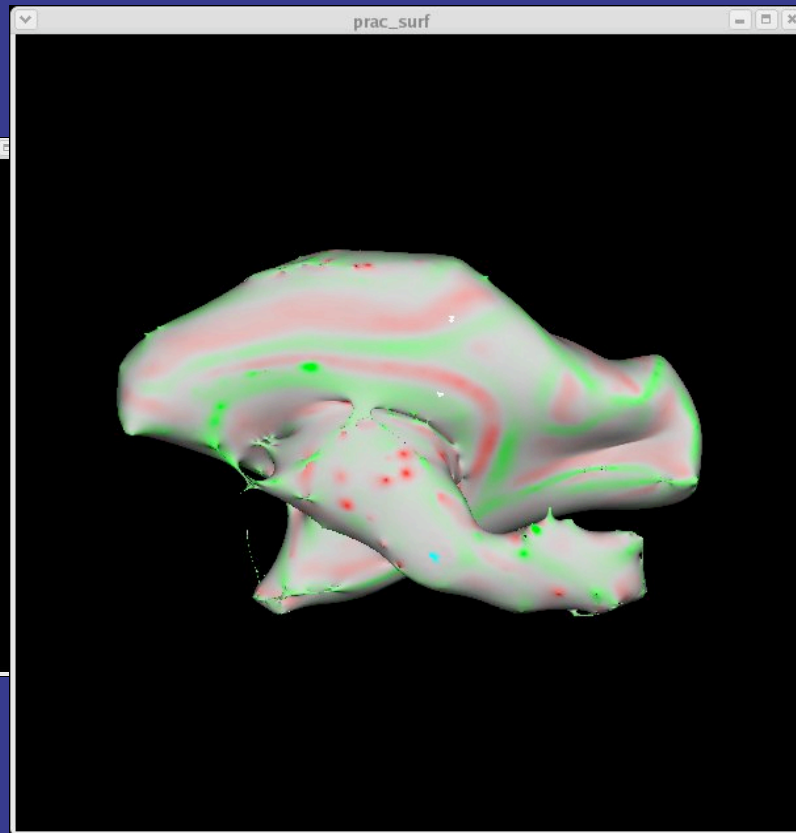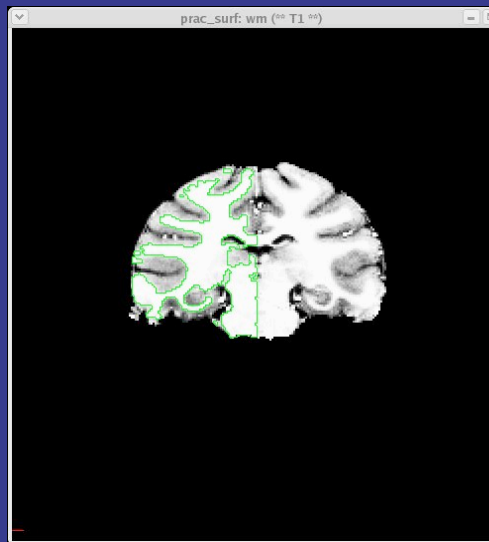

Gray matter in the wm segmentation. Must be manually removed.

T1

wm
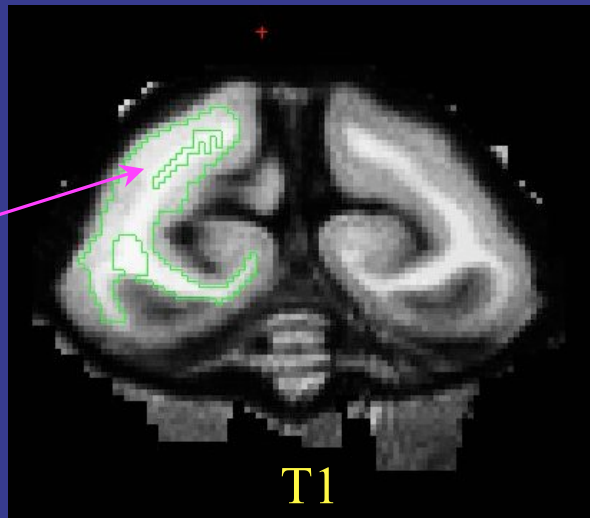
After manual editing. Much better

wm, edited

Troubleshooting: When cutting planes fail
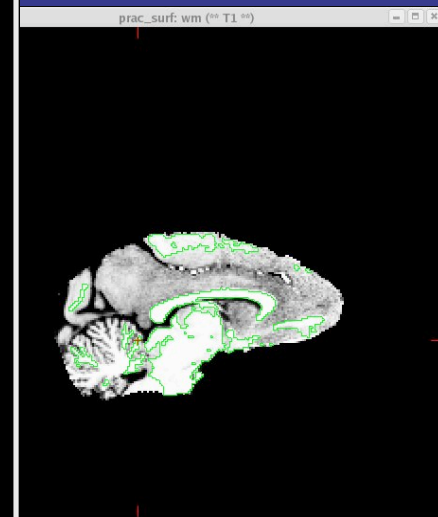
- According to FreeSurfer manual:

  "All nonhuman cortexes need to have their cutting planes defined manually after the initial segmentation."

- This is a royal pain. Not always the easiest thing to do.

- Manually defining the cutting planes must be done via the FreeSurfer interface. Select "Expert Preferences".

- Follow instructions in the manual.

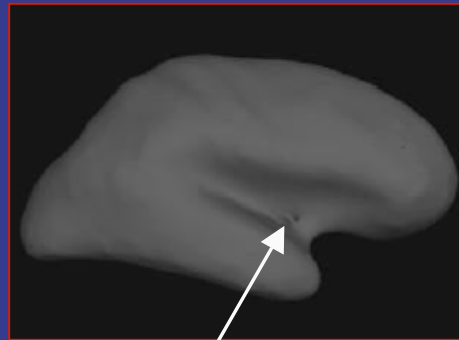Example of properly defined cutting planes
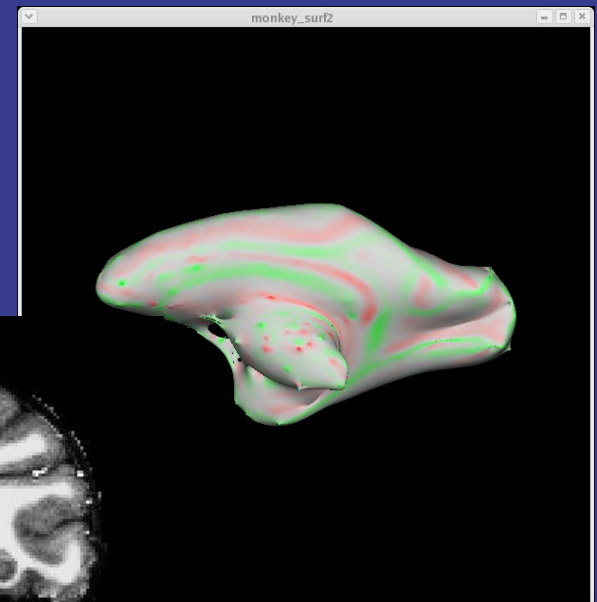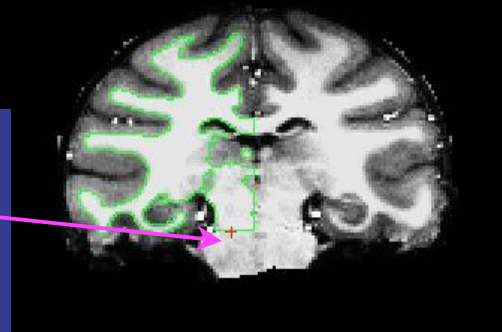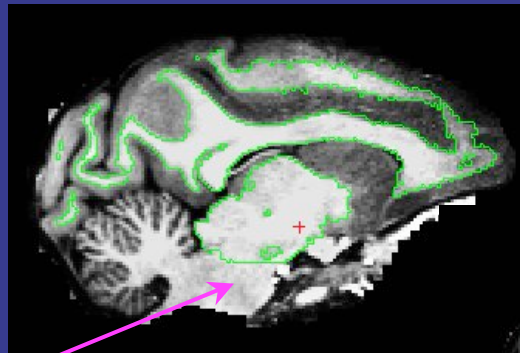
- Good news: FreeSurfer often defines the cutting planes of monkey volumes pretty well without any manual intervention.
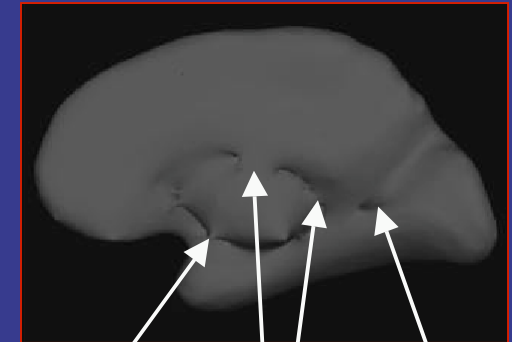
Manual editing

- Examine surface for defects
  - manually reclassify voxels in the following areas:
    - Lateral ventricle
    - Fornix
    - Optic nerve
    - Basal ganglia
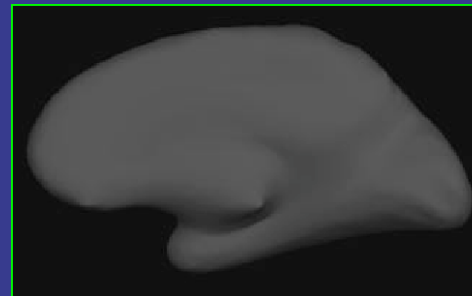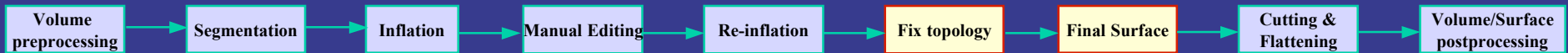   - Other defect areas

Basal Ganglia

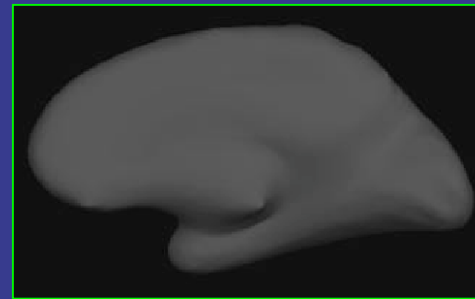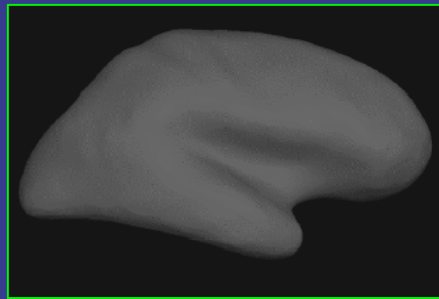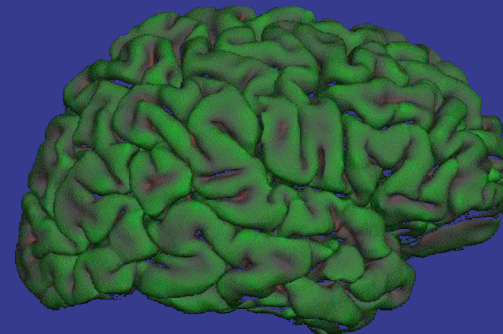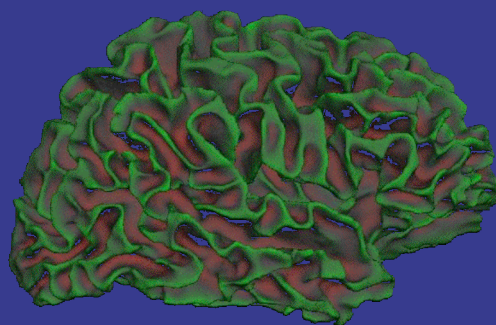*Optic Nerve*   *Fornix*   *Lateral Ventricle*

Re-inflation

Fix topology

- Automatic defect removal algorithm that removes minor defects ensuring that the surface is topologically correct.
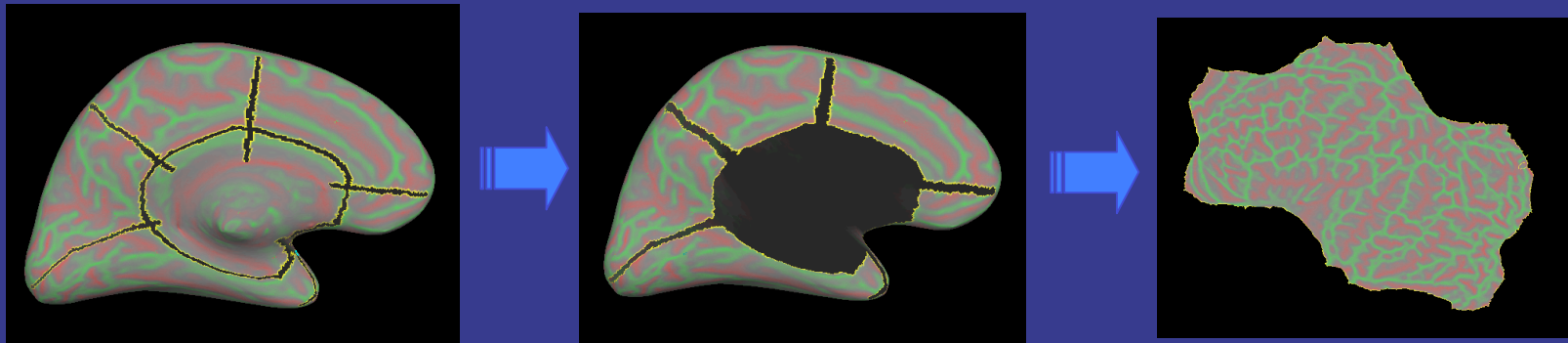

Make final surface

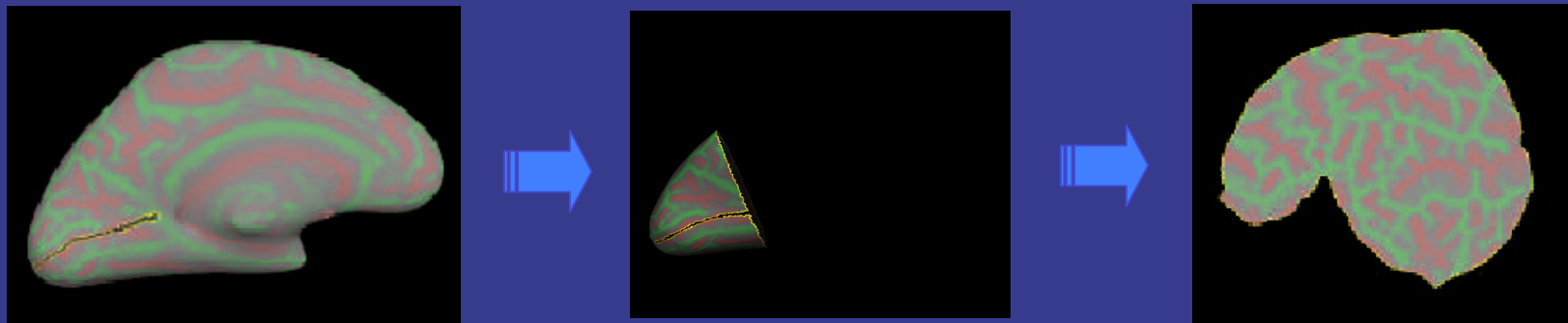- Final gray/white boundary (white)
- Final gray/csf boundary (pial)

Cutting and flattening
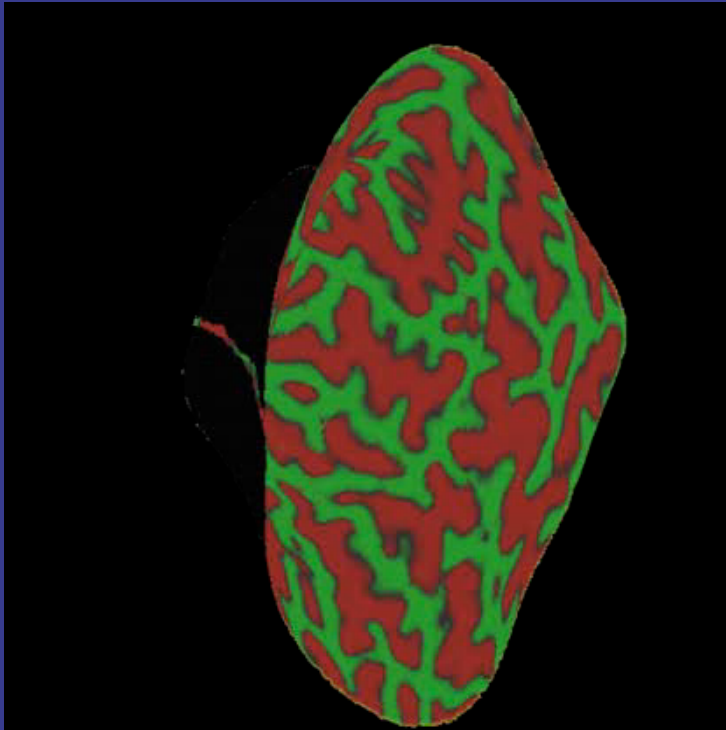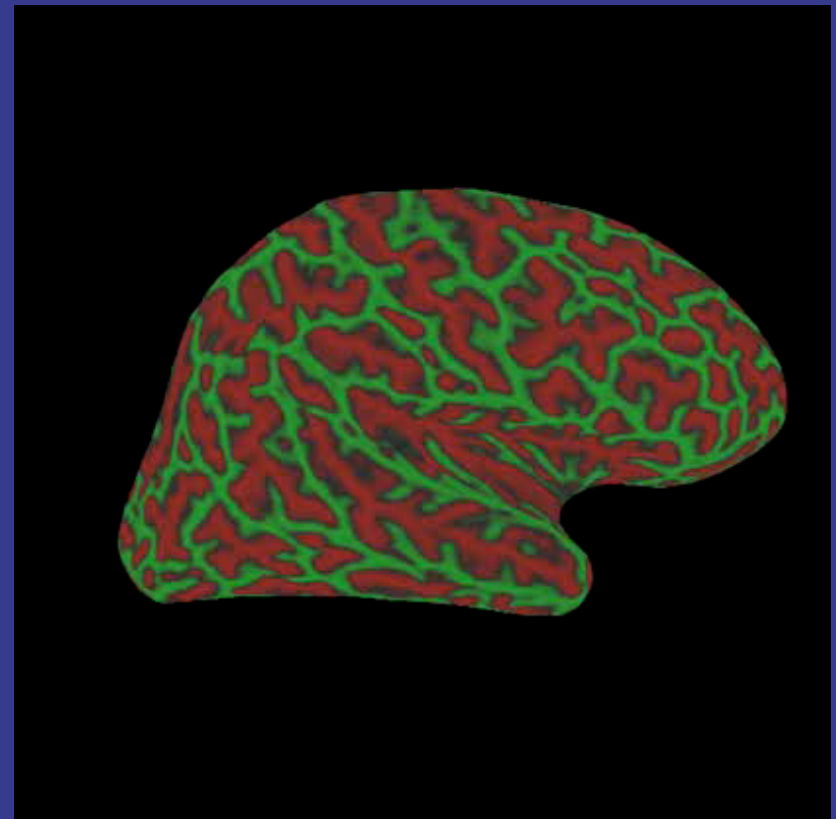
- ## For a full surface patch

- ## For occipital patch

Flattening of occipital patch

Flattening of full surface

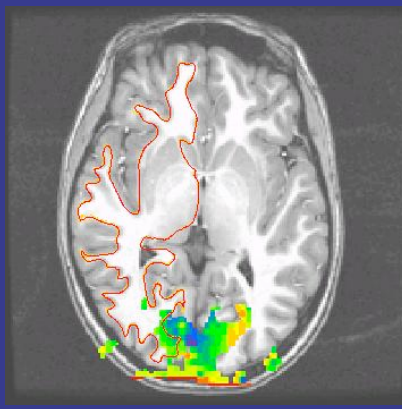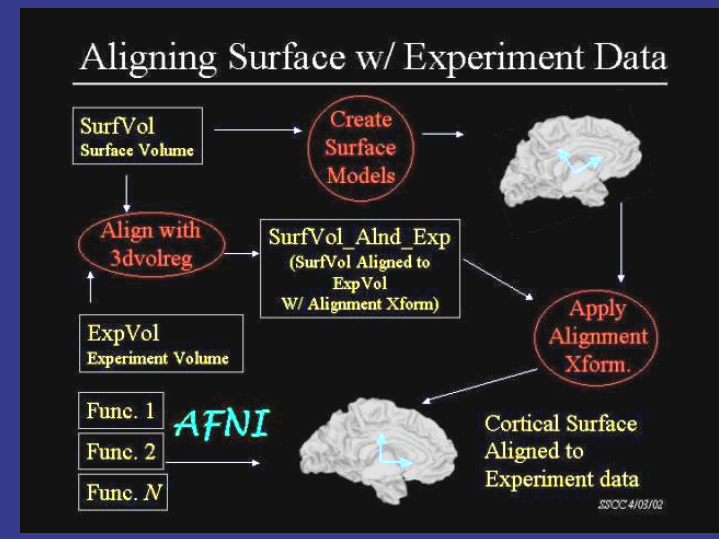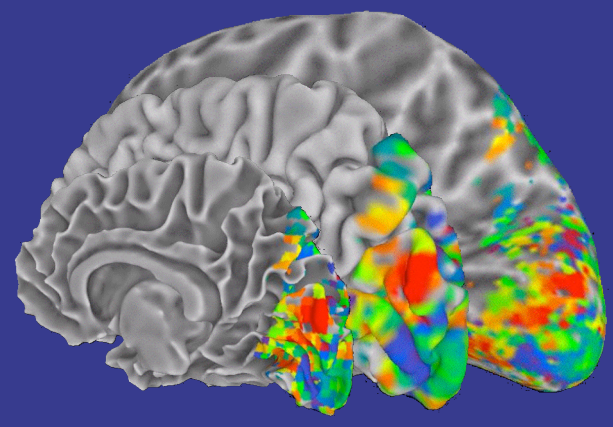| Volume preprocessing | → | Segmentation | → | Inflation | → | Manual Editing | → | Re-inflation | → | Fix topology | → | Final Surface | → | Cutting & Flattening | → | Volume/Surface Postprocessing |

SUMA

- Convert surfaces to ASCII format
- Align surface volume to experiment volume
- Overlay functional data onto surface
- Create link between AFNI and SUMA
- View function on volume and surface simultaneously
- Visit SUMA website for details:
  - http://afni.nimh.nih.gov/ssc/ziad/SUMA/

Aligning Surface w/ Experiment Data

SurfVol Surface Volume → Create Surface Models

Align with 3dvolreg → SurfVol_Alnd_Exp (SurfVol Aligned to ExpVol W/ Alignment Xform)

ExpVol Experiment Volume → Apply Alignment Xform.

Func. 1
Func. 2
Func. N
AFNI

Cortical Surface Aligned to Experiment data

SSCC 4/03/02

AFNI

SUMA

FreeSurfer Links

FreeSurfer Website (articles, download, docs, FAQ):

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

Mail Archives:

www.mail-archive.com/freesurfer@mail.nmr.mgh.harvard.edu