Tutorial 2:
Segmentation and Tissue Metrics

SNSS 2025

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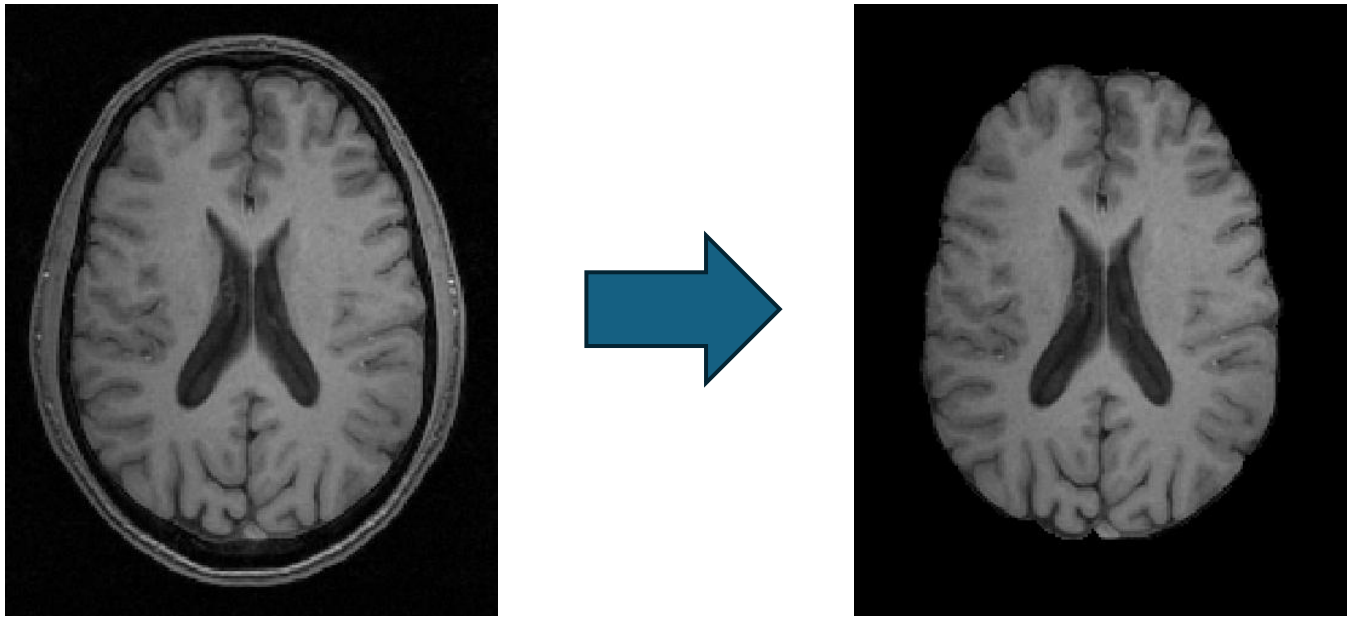
Images are aligned, now what?

- After registration there's still some work to be done for you to get quantitative tissue information
- FSL does have the functions for you to achieve this
  - **BET** – Brain Extraction
  - **FAST** – Tissue Segmentation
  - **FIRST** – Subcortical Structure Segmentation
  - **FSLSTATS** – Tissue Volume Calculation

Section 1:
Brain Extraction

Removing the skull from tissue

- We just want to get measurements from brain tissue
  - We don’t want skull, along with the skin and fat
- BET (Brain Extraction Tool) completely removes everything BUT brain area

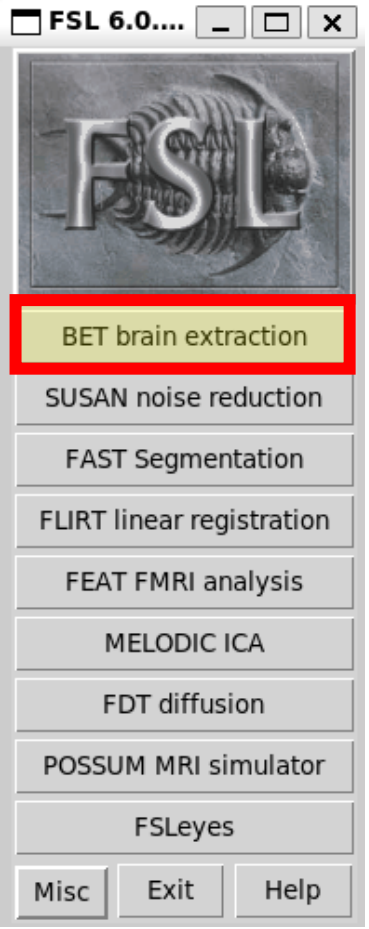


Demo: Running BET

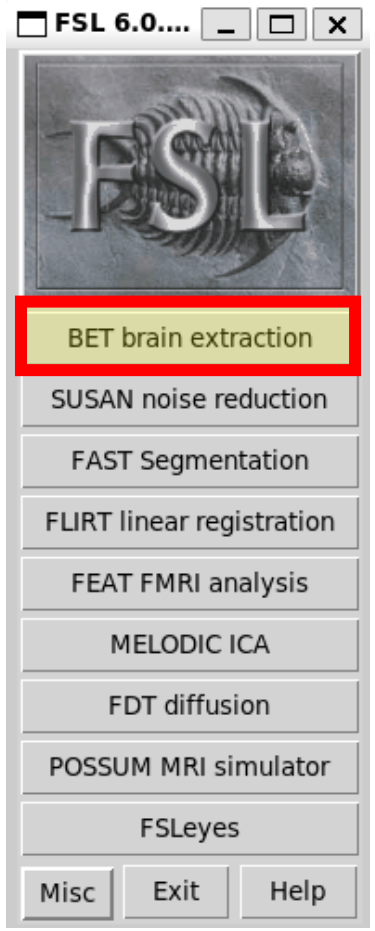
In GUI

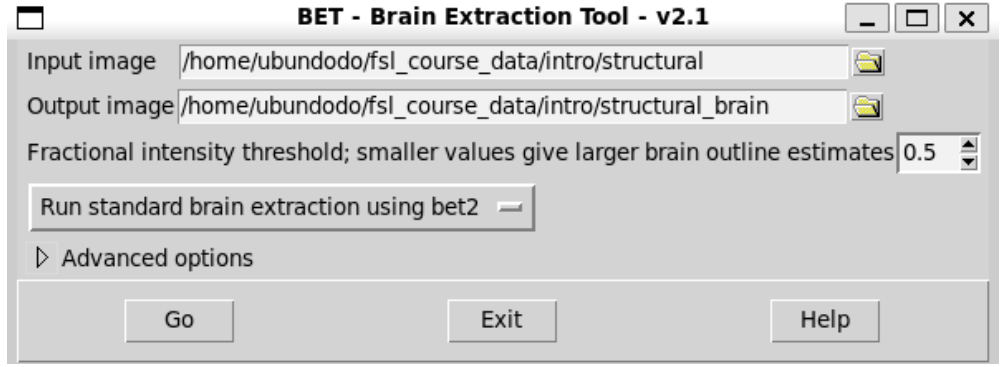
Exercise 1.1: Running BET (GUI)

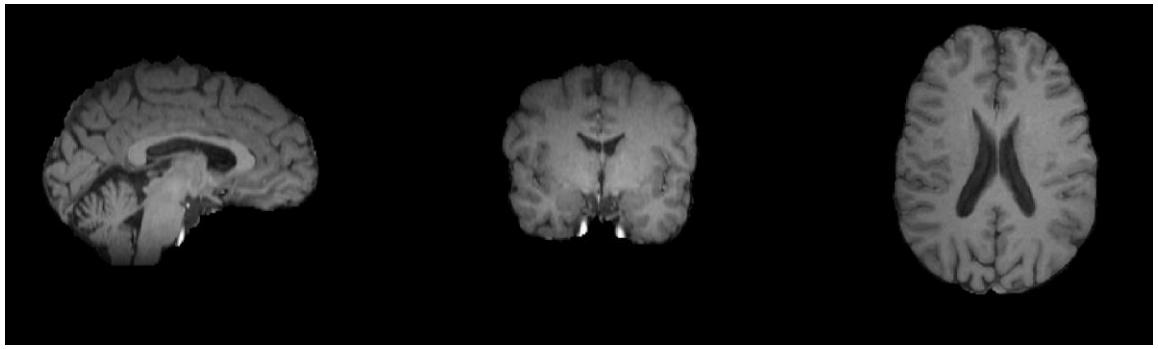
1. Run BET on **structural.nii.gz**
2. Examine the output
3. Overlay output on top of the input structural image and change the overlay colour



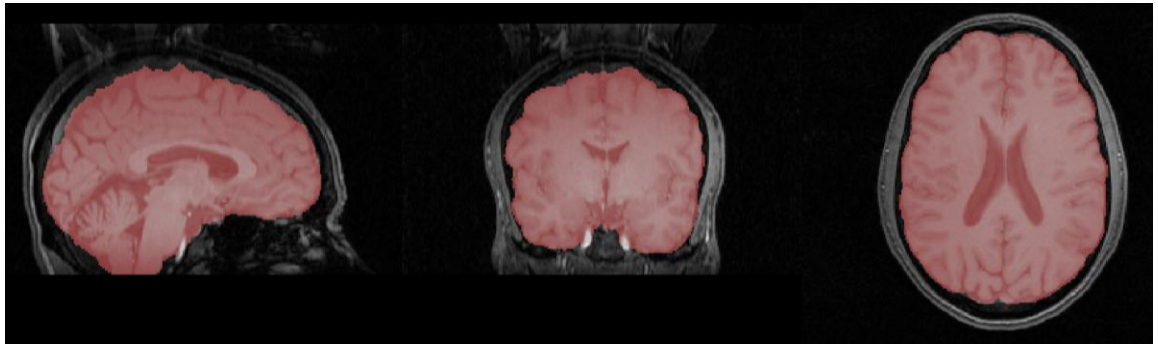
Exercise 1A): Running BET (GUI)





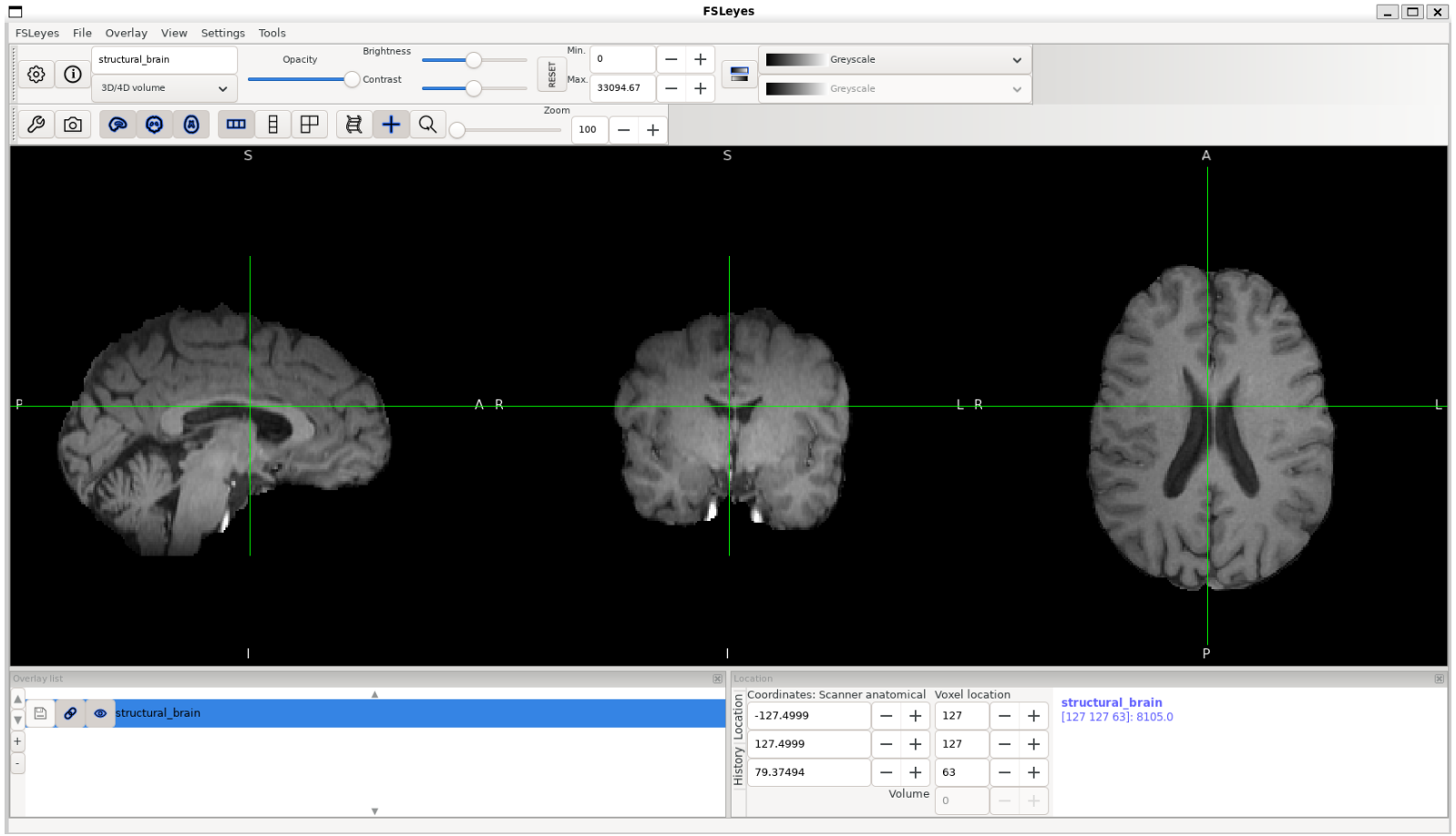


Output:
structural_brain

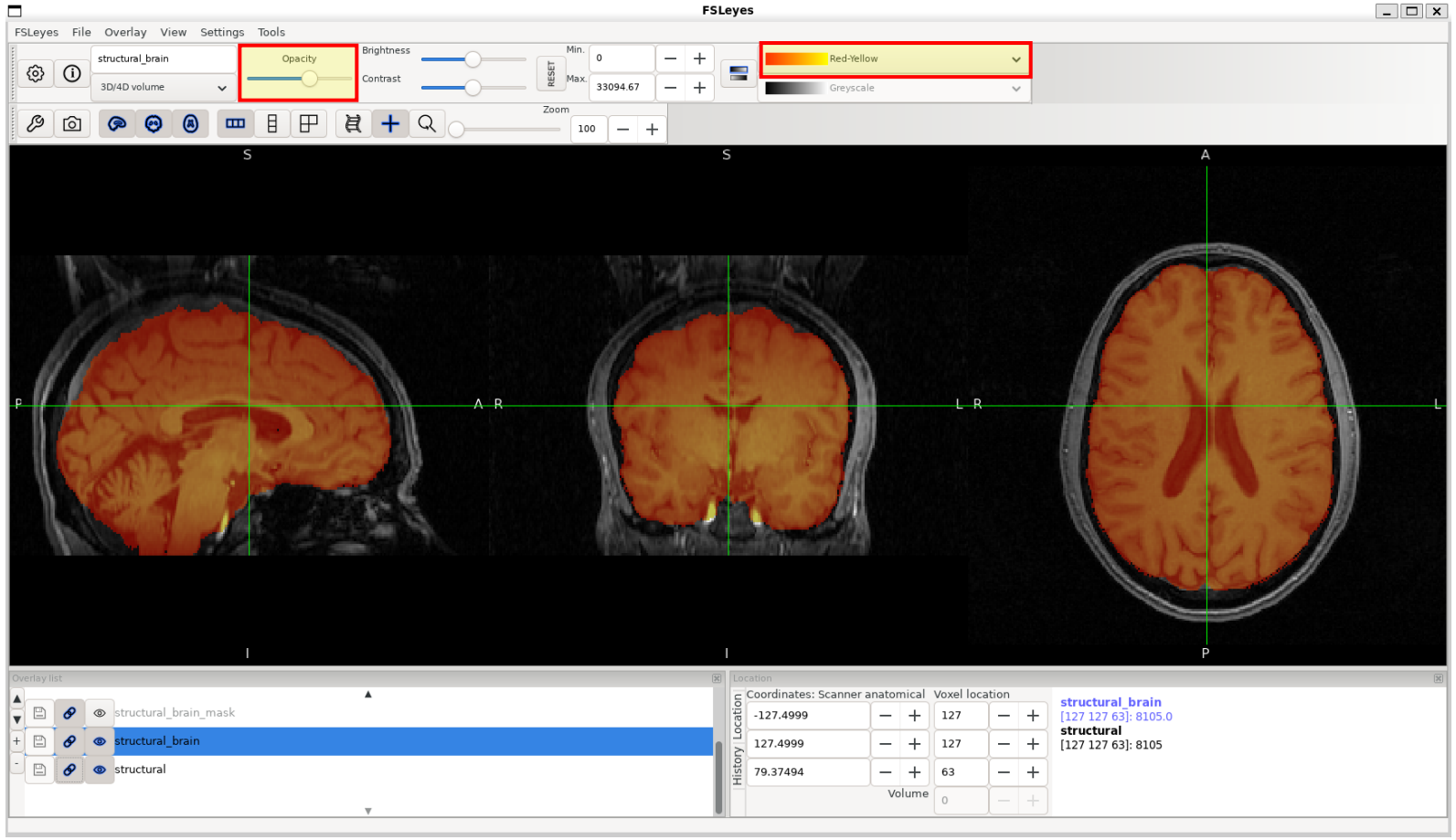


Overlay:
structural &
structural_brain

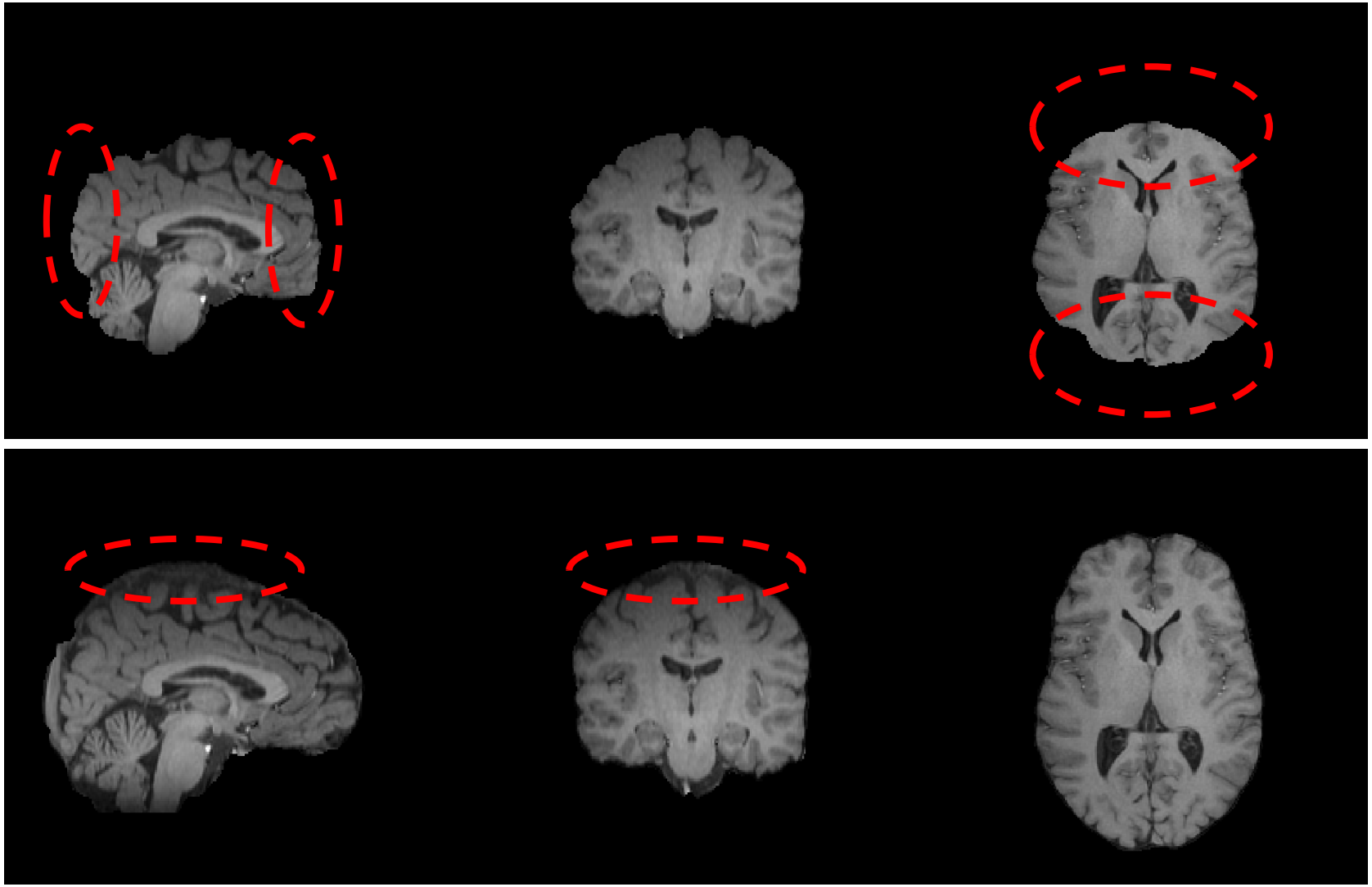
Results: BET


FSLeyes
FSLeyes File Overlay View Settings Tools
structural_brain
3D/4D volume
Opacity
Brightness
Contrast
RESET
Min.
0
Max.
33094.67
Greyscale
Greyscale
Zoom
100
S
S
A
P
A R
L R
L
I
I
P
Overlay list
structural_brain
Location
Coordinates: Scanner anatomical
Voxel location
-127.4999
127.4999
79.37494
127
127
63
Volume
0
History
Location
structural_brain
[127 127 63]: 8105.0

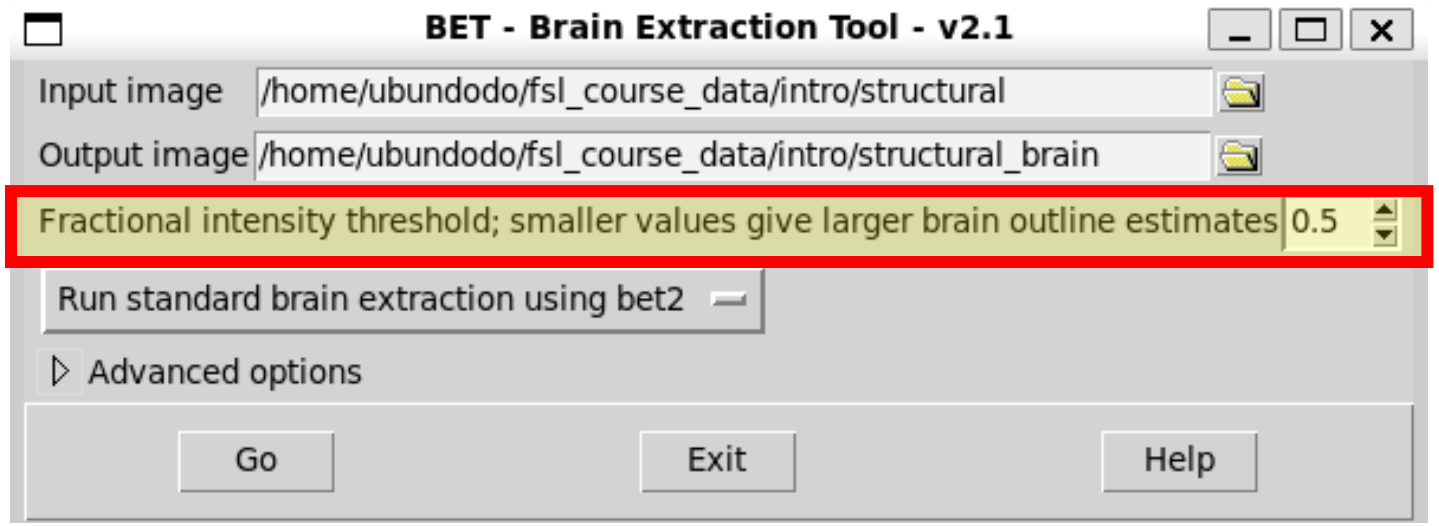
Results: BET (overlay)



What if BET Takes Off Too Much or Too Little?



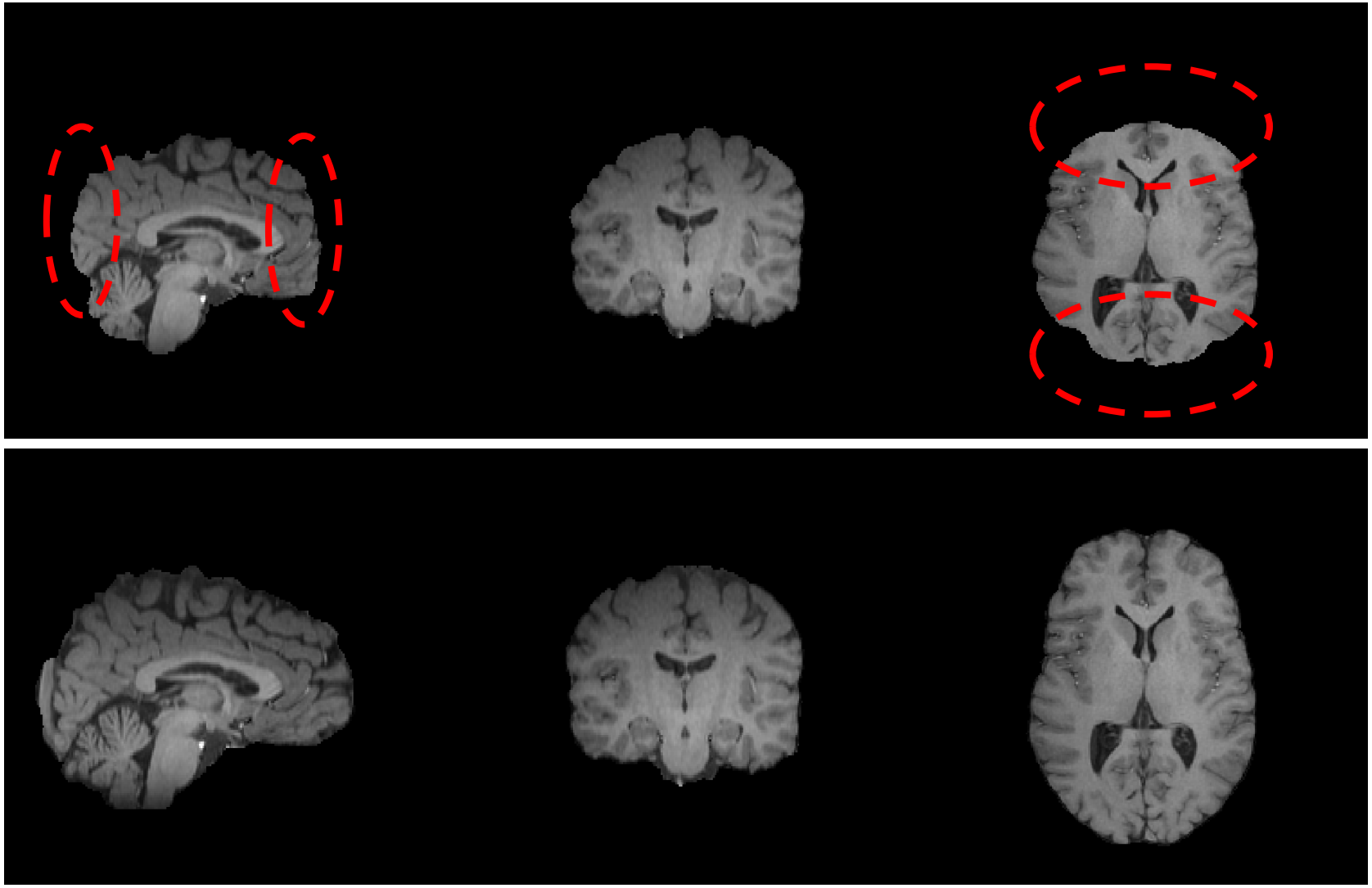
Adjusting the Fractional Intensity Threshold



Depending on your subject, you might have to adjust this value to properly extract the brain

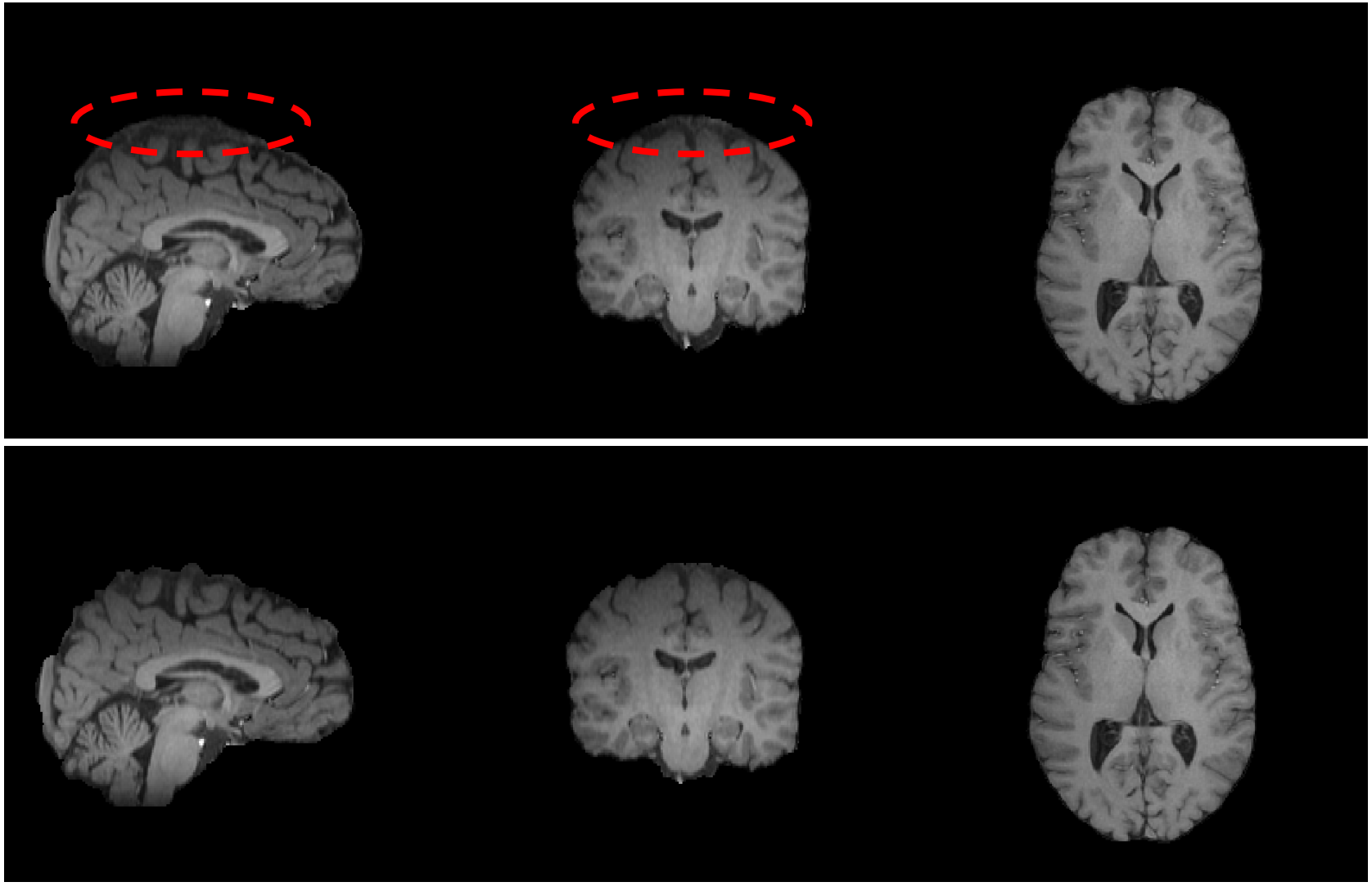
Too Much Brain is Cut Off!

Decrease the Threshold

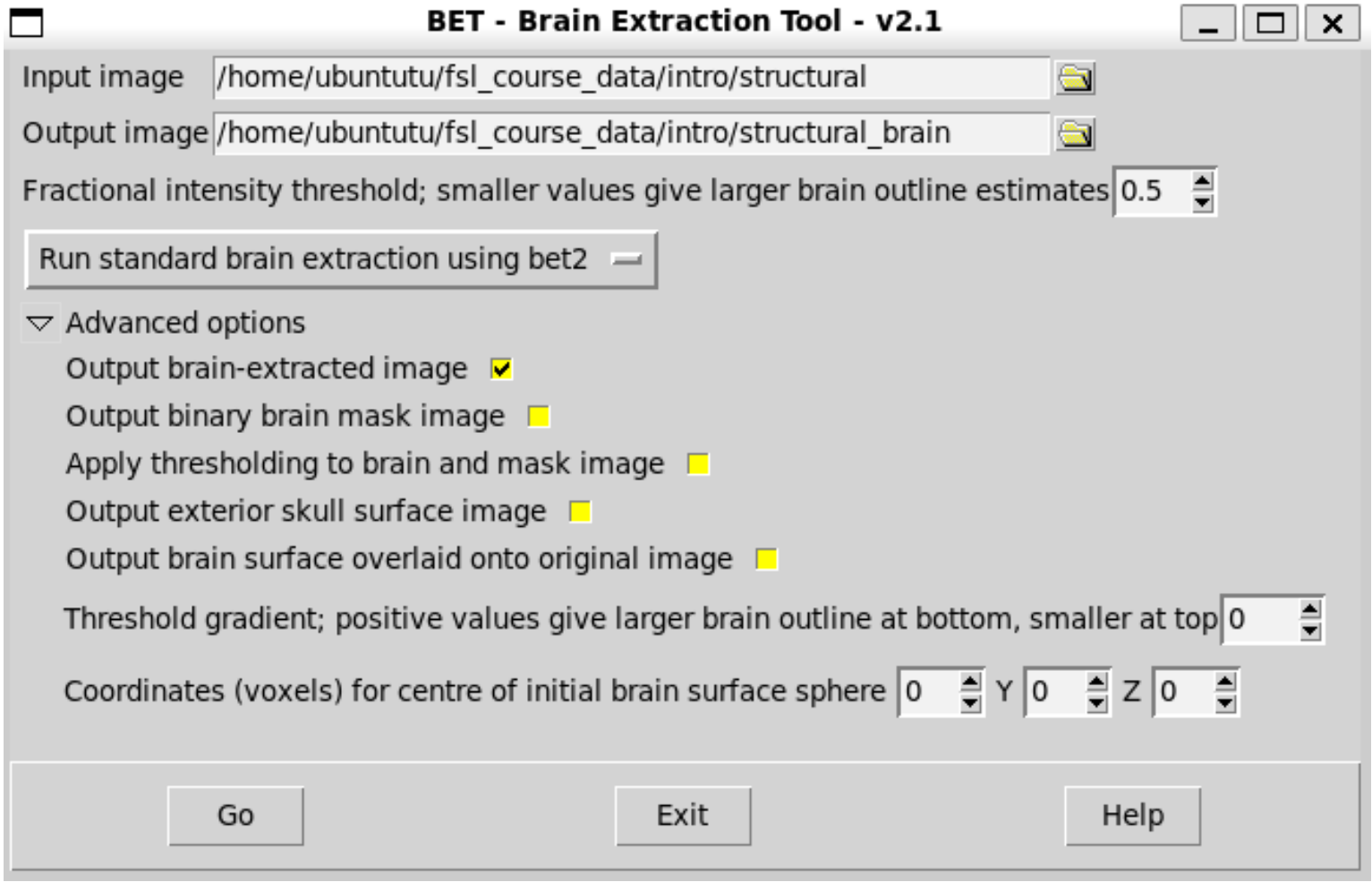


Too Much Non-Brain!

Increase the Threshold

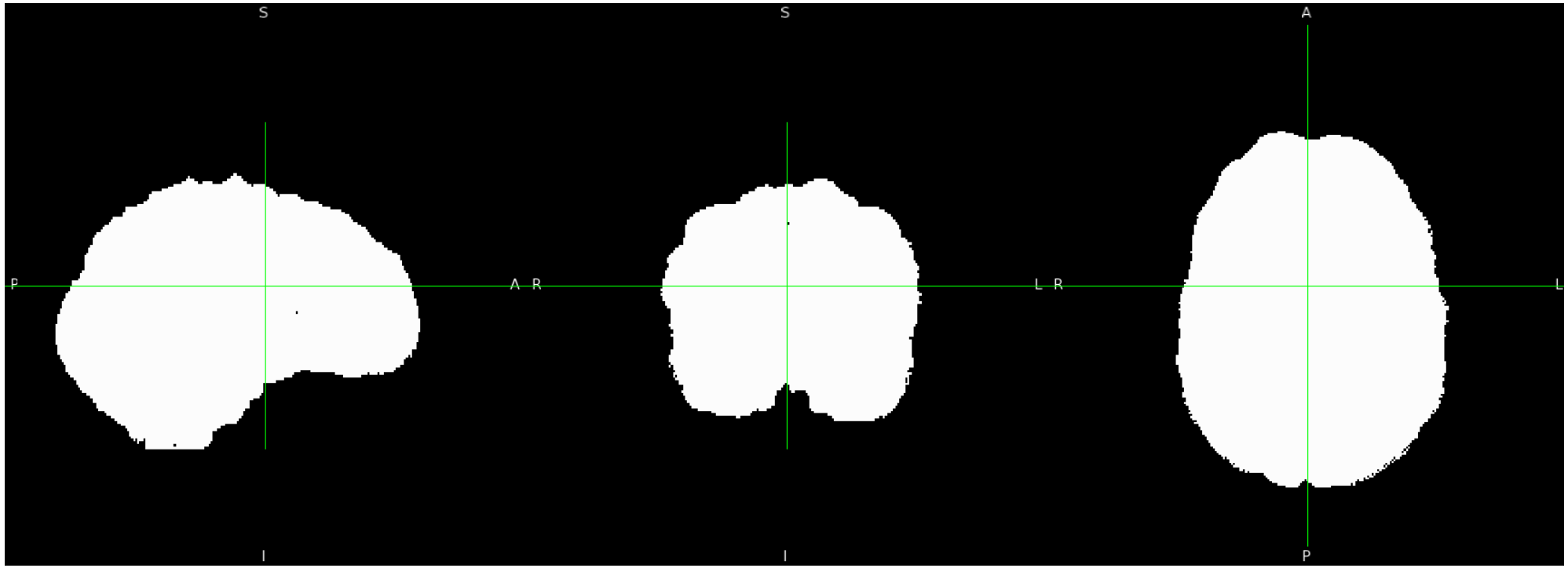


Other Outputs from BET



Other Outputs from BET

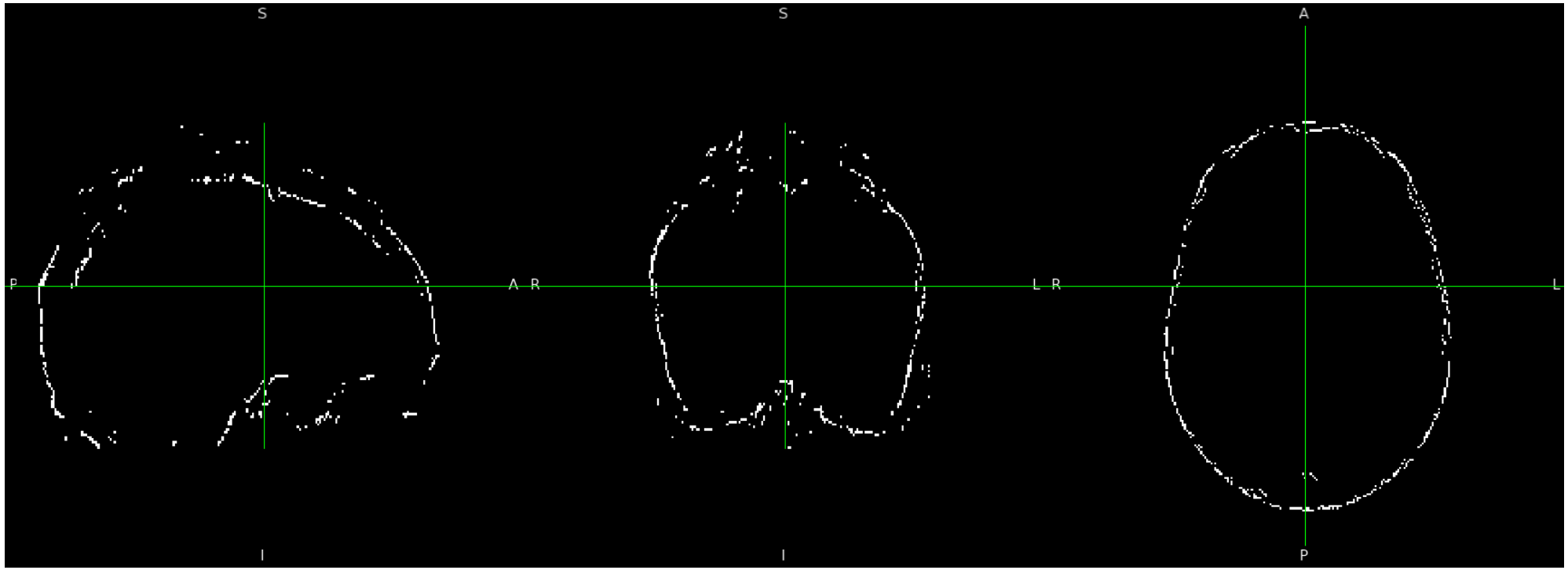
Binary Brain Mask: a binary image showing areas where there is brain (set as 1) and non-brain (set as 0)



Other Outputs from BET

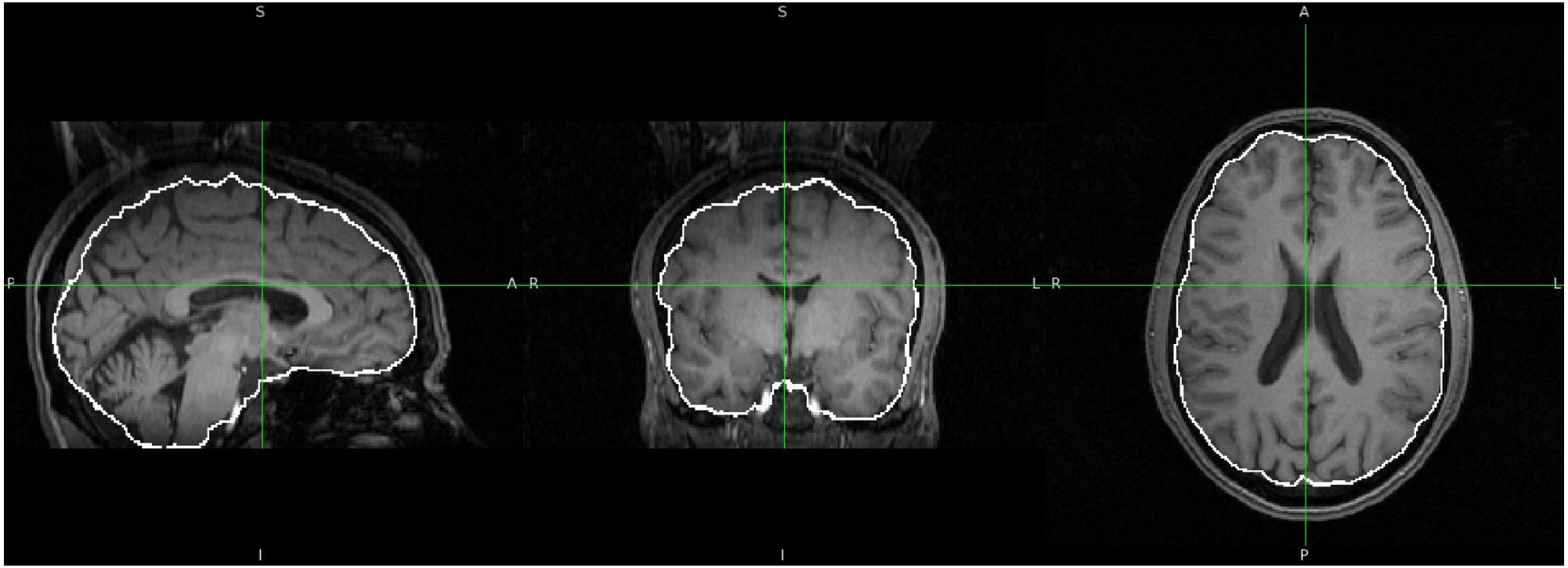
Exterior Skull Surface Image:

estimate of the exterior surface of the skull



Other Outputs from BET

Brain Surface Overlaid onto Original Image: output image will be a copy of the input image, but with the outline of the area where the brain has been extracted.



Demo: Running BET

In Command Line

Getting additional outputs from BET

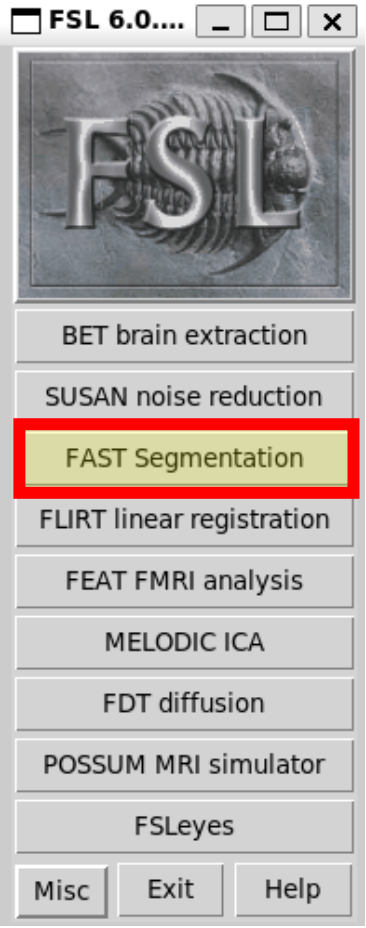
Exercise 1.2: Running BET (Command Line)

1. Run BET on **structural.nii.gz** this time add the following options:
   - Change the output name to have a basename of **structural_CL**
   - Output images for: **brain mask**, **skull image**, and **brain outline image**
2. Examine the outputs

Section 2:
Tissue Segmentation

Tissue Segmentation

- Separating the brain with respect to tissue type
  - White Matter (WM)
  - Grey Matter (GM)
  - Cerebral Spinal Fluid (CSF)
- In FSL, the **FAST Segmentation** tool will perform this task for you
- Segmentation only works with **Brain Extracted Images!!**



Demo: Running FAST

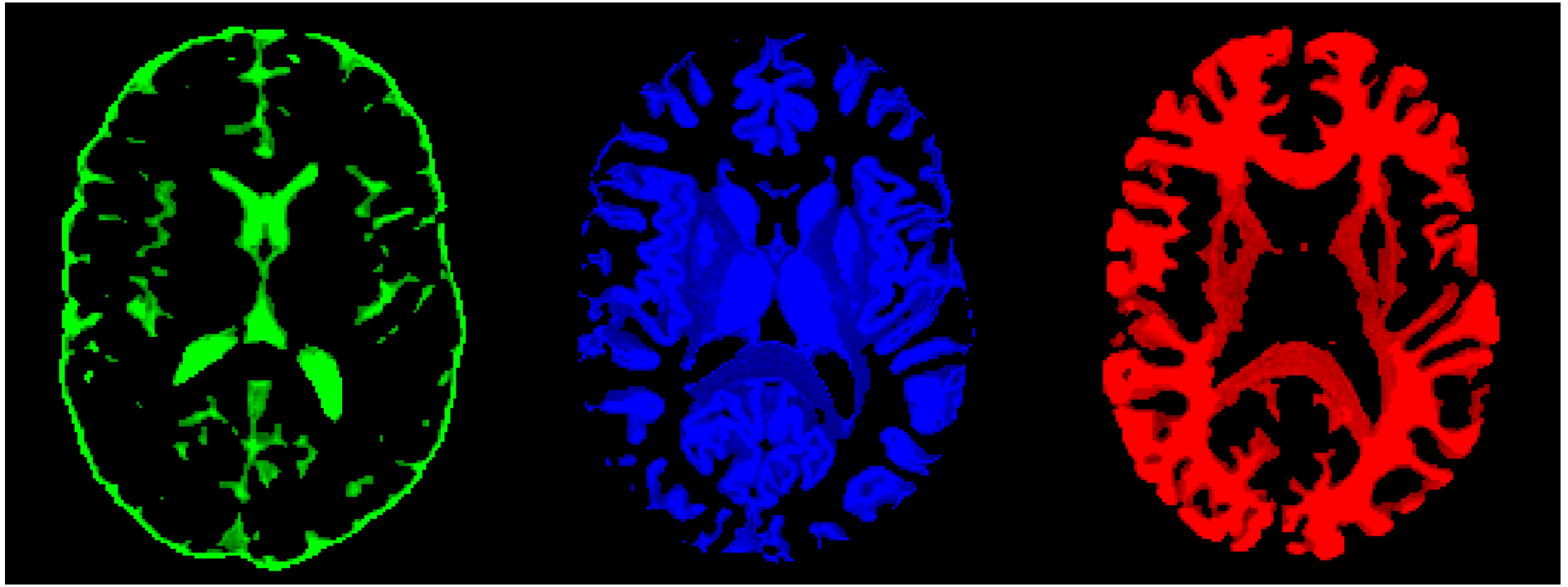
Determining the size of the image using **fslsize**

Trimming down the image size using **fslroi** command

Running **FAST** in GUI

FAST Outputs

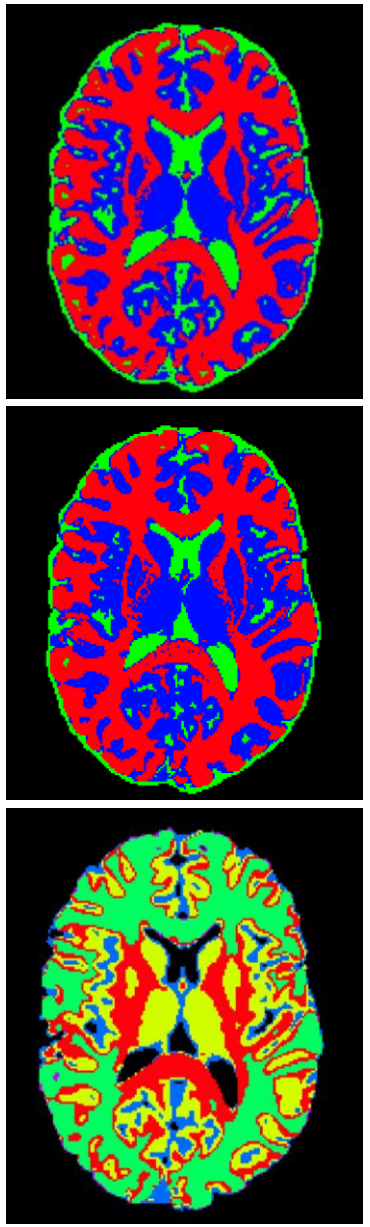
- **structural_brain_roi_pve_[0,1,2]**: partial volume estimate maps of CSF, GM, and WM, respectively



CSF **GM** **WM**

FAST Outputs

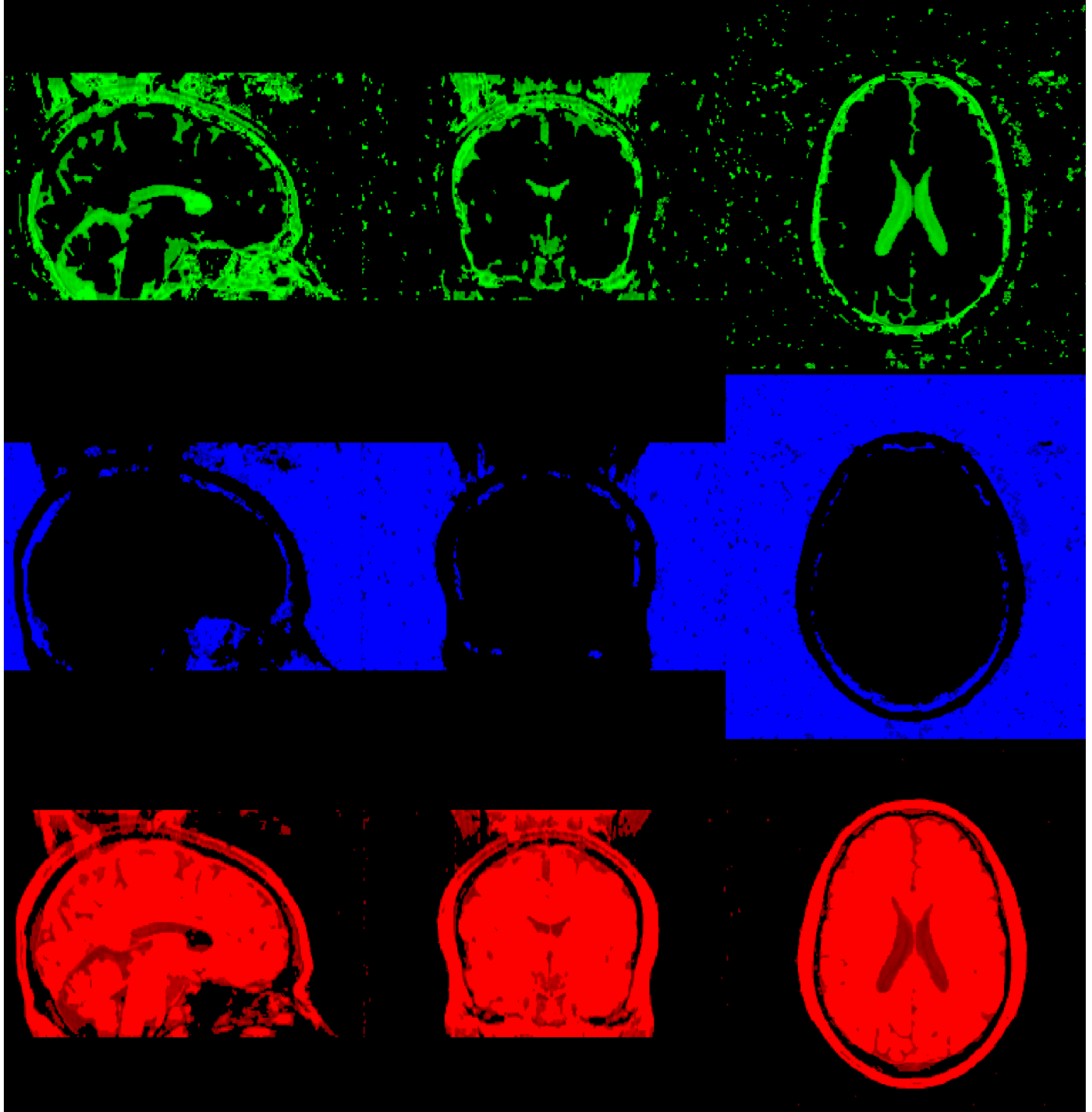
- **structural_brain_roi_seg**: image showing hard segmentation of GM, WM, and CSF
- **structural_brain_roi_pveseg**: image showing binary segmentation of GM, WM, and CSF with respect to PVE
- **structural_brain_roi_mixeltype**: image showing binary segmentation of GM, WM, and CSF, along with mixed tissue voxels



If You Forget to BET

- Since it characterises tissue based on voxel intensity, the input image must be brain-only
- Otherwise, FAST will include the skull and lipids, as well as skewing your tissue information

When You Don’t BET!



CSF

GM

WM

Exercise 2.1: Running FAST (GUI)

1. Use BET to extract the brain
2. Use **fslsize** determine the size of our image and **fslroi** to reduce the image size to 5 slices starting at position 100 in the **axial** plane
3. View the trimmed down image
   Run **FAST** for tissue segmentation
4. View the results from **FAST**

Partial Volume Estimates

- You will have voxels that are 100% GM, WM, or CSF

- However, there will be a lot that have a certain percentage of two (or more) tissue types

- One could simply have it so the most dominate tissue type be sole representative of the voxel, but that wouldn't be accurate
- With partial volume maps (*_pve*), each voxel contains a value (from 0.0 to 1.0) representing the proportion of tissue in that voxel

Tissue Volumes using **FSLSTATS**

- To get volume estimates use **fslstats** in the terminal window:
  - **fslstats structure_brain_roi_pve_1 -V** will give you number of non-zero GM voxels, both in <# of voxels> and in <mm^3>
  - **fslstats structure_brain_roi_pve_1 –M** will give you the mean intensity of the GM voxels (from 0->1)
  - By multiplying the two outputs together, you will get the GM volume estimate

Saving into Variables

- **Vol=` fslstats structure_brain_roi_pve_1 -V | awk ‘{print $2}’ `**
  - **Note:** The <` > character is the grave accent (or backquote), located next to “**1**” in the number row (in the US keyboard layout)
  - awk ‘{print $2}’: saves the second output from fslstats
- **Mean =` fslstats structure_brain_roi_pve_1 –M `**
- **Tissuevol=` echo “$mean * $vol” | bc –l `**

- You can output the variable (such as Tissuevol) in command line using:
  - **echo $Tissuevol**

- This can also be condensed into one line:
  - **Tissuevol=` fslstats structure_brain_roi_pve_1 -M –V | awk ‘{print $1 * $3}’ `**

Demo: Running FAST (Command Line) & Getting Tissue Volumes

Running **FAST** in Command Line

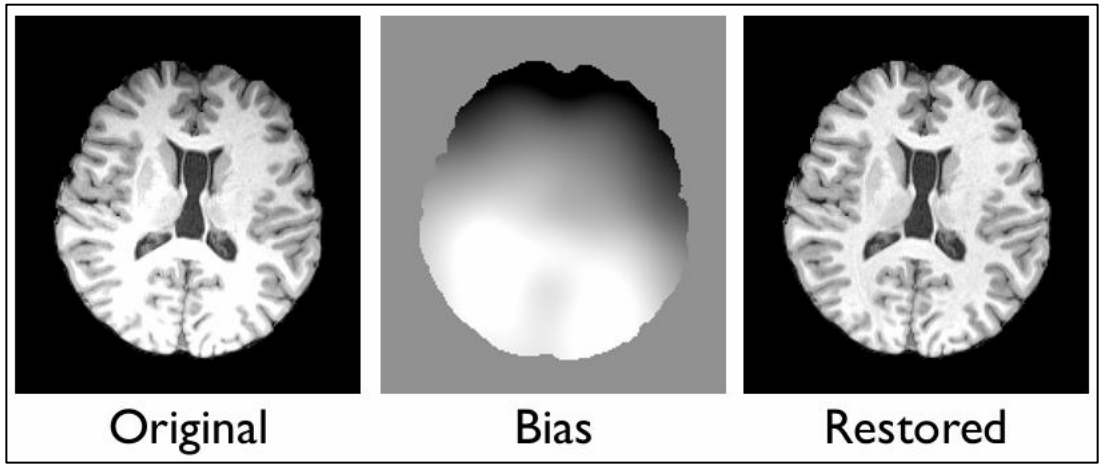
Getting Tissue Volumes via **FSLSTATS**

Exercise 2.2: Running FAST (Command) &Getting Tissue Metrices

1. Run **fast** in command line on the trimmed down "**structural_brain_roi**" image**.** This time, have the output name be: "**structural_brain_CL**".
2. View the outputs.
3. Use **fslstats** to determine the amount of GM, WM, and CSF volume in your image
4. Compare the tissue volumes from the PVE and the pure segmented outputs

Bias Field Correction

- During imaging, there will be MRI RF inhomogeneity
- Commonly seen in multi-channel RF coils and at ultra-high field strengths (B_0>3T)
- This will cause problems with segmentation as some areas will be unnaturally brighter



- Is corrected by default in **FAST segmentation**

Demo:
Bias Fields and FAST on 7T data

Viewing image with bias field

Effects of bias field on output volumes

Exercise 3: Bias Fields and FAST on 7T data

1. Run **fslroi** to trim down the image. Bring down the axial dimension to 5 slices, starting at position 100.
2. Run **FAST** in GUI, with "**Restored Output**" and "**Estimated Bias Field**" options enabled.
3. Observe the restored vs the original image. See the difference in image contrast and intensity. Look at the bias field image.
4. Run **FAST** in command line. Have the option to **not bias correct** on and name the output as "**structural_brain_7T_wbias**".
5. Get tissue volume estimates from the bias corrected, and bias non-corrected PVEs. Compare the outputs.

Section 3:
Subcortical Segmentation

Subcortical Segmentation

- The previous segmentation mainly looks for overall WM, GM, and CSF
- What if we are interested in subcortical structures?
  - Thalamus
  - Caudate Nucleus
  - Brainstem
  - Amygdala
  - Etc?

FSL Utility **FIRST**

- **run_first_all** <options> –i <input image> -o <output image>
- It is a script which sequentially runs: **first_flirt**, **run_first**, and **first**
- By default, the reference used is the **MNI152_1mm** standard
- Options:
  - **-b**: put this if the image is already brain extracted
  - **-s <substruct name>**: only output the specified subcortical structure
    - Putamen ([L/R]_Puta)
    - Caudate nucleus ([L/R]_Caud)
    - Nucleus accumbens ([L/R]_Accu)
    - Globus pallidus ([L/R]_Pall)
    - Hippocampus ([L/R]_Hipp)
    - Amygdala ([L/R]_Amyg)
    - Thalamus ([L/R]_Thal)
    - Brainstem (BrStem)
  - **-a <affine matrix>**: allows you to input an affine matrix, if you previously ran **first_flirt**
- This script will only work with **T1-weighted** images

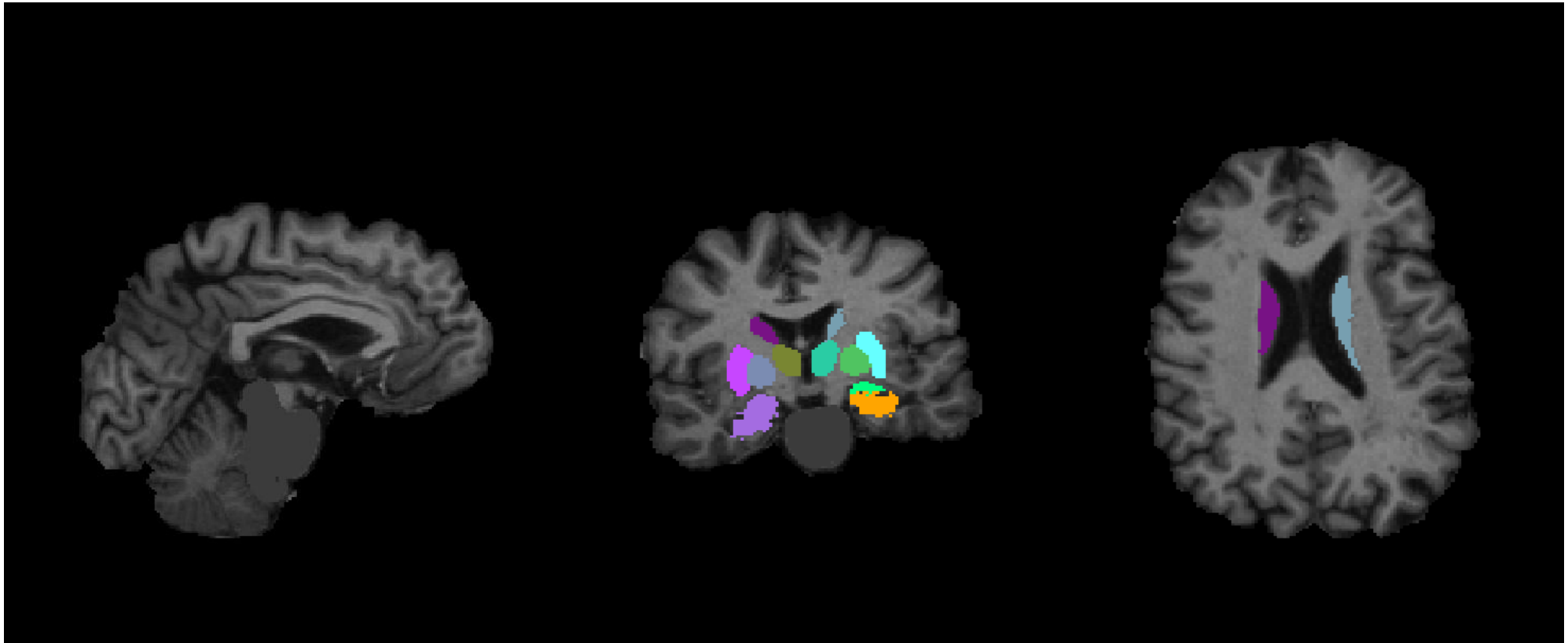
Demo: Subcortical segmentation with FIRST

Running **FIRST** to segment subcortical structures

Viewing structures

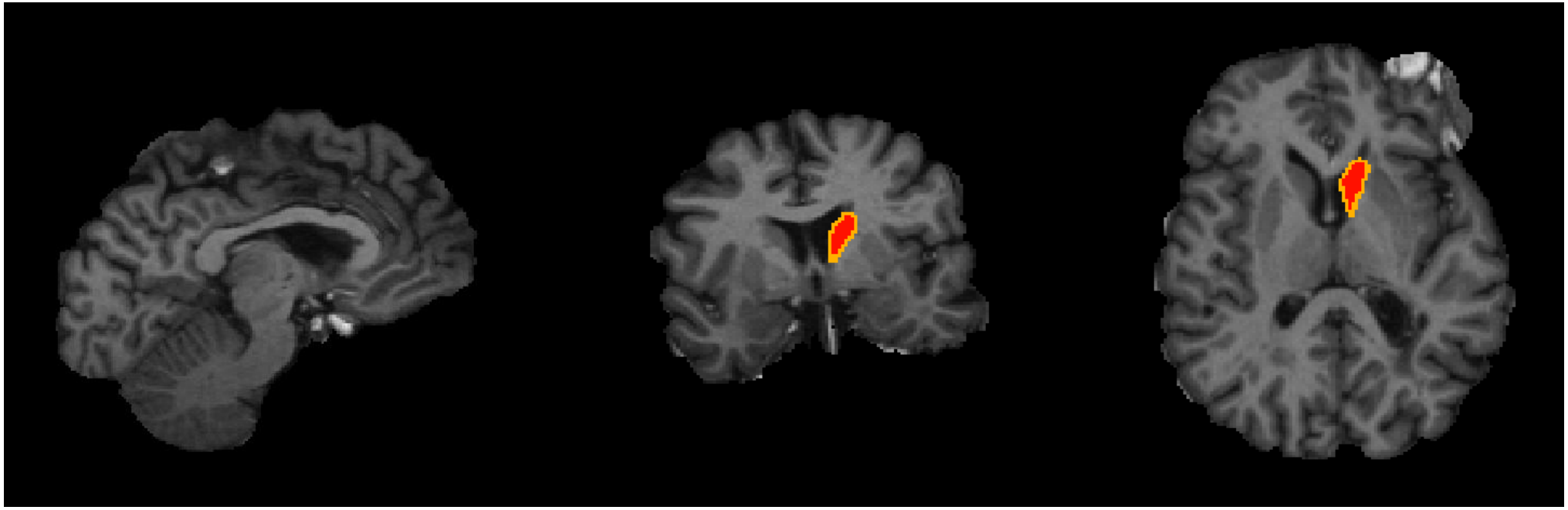
Outputs from **run_first_all**

- **con0047_all_fast_firstseg.nii.gz**:
  - Single imaging showing all segmented output of the structure(s). Structures are boundary corrected to ensure no overlap and labelled.



Outputs from **run_first_all**

- **con0047_all_fast_origsegs.nii.gz**:
  - 4D image containing the individual structure segmentations. Each structure will have its own 3D image, and you will be able to cycle through all the individual segmented structures. Structures are not corrected for boundary overlap.



Outputs from **run_first_all**

- **con0047_*_first.vtk**:
  - mesh representation of the final segmentation
- **con0047_*_first_bvars**:
  - Don’t delete this file. Contains the mode parameters and the model used. This along with the model files, can be used to reconstruct the other outputs. This can be used later to perform vertex analysis or as a shape prior to segment other shapes.
- These are the files used for further analysis, such as Vertex Analysis

Exercise 4.1: Subcortical segmentation with FIRST

1. Use **run_first_all** to segment the subcortical structures of the image
2. Overlay **con0047_brain** and **con0047_all_fast_firstseg**
3. Overlay **con0047_brain** and **con_0047_all_fast_origseg**. Cycle through the various structures to see them overlaid onto the original image.

Getting Subcortical Volume Estimates

- Remember that with our "**con0047_all_fast_firstseg.nii.gz**" output, each structure is labeled with a specific integer
- You can use **FSLSTATS** with an option to threshold the output to a particular number
- Example, for Brain Stem (Label = 16):
  fslstats con0047_all_fast_firstseg –l 15.5 –u 16.5 –v
  - This will limit the range **fslstats** looks for voxels in the integer range of [**15.5 to 16.5**]

Structure	Abbreviated name	Integer label	Modes
Left Thalamus	L_Thal	10	40
Left Caudate	L_Caud	11	30
Left Putamen	L_Puta	12	40
Left Pallidum	L_Pall	13	40
Brain Stem	BrStem	16	40
Left Hippocampus	L_Hipp	17	30
Left Amygdala	L_Amyg	18	50
Left Accumbens	L_Accu	26	50
Right Thalamus	R_Thal	49	40
Right Caudate	R_Caud	50	30
Right Putamen	R_Puta	51	40
Right Pallidum	R_Pall	52	40
Right Hippocampus	R_Hipp	53	30
Right Amygdala	R_Amyg	54	50
Right Accumbens	R_Accu	58	50

*This reference table is from:FIRST

Demo: Getting Subcortical Volume Estimates

Using **FSLSTATS** with thresholding on subcortical structures

Exercise 7: Subcortical Volume Metrics

1. Run **fsltats** with thresholding option to get the volumes of various subcortical volumes
   - Left Thalamus (Label = 10)
   - Left Hippocampus (Label = 17)
   - Right Caudate (Label = 50)
   - Right Amygdala (Label = 54)

Structure	Abbreviated name	Integer label	Modes
Left Thalamus	L_Thal	10	40
Left Caudate	L_Caud	11	30
Left Putamen	L_Puta	12	40
Left Pallidum	L_Pall	13	40
Brain Stem	BrStem	16	40
Left Hippocampus	L_Hipp	17	30
Left Amygdala	L_Amyg	18	50
Left Accumbens	L_Accu	26	50
Right Thalamus	R_Thal	49	40
Right Caudate	R_Caud	50	30
Right Putamen	R_Puta	51	40
Right Pallidum	R_Pall	52	40
Right Hippocampus	R_Hipp	53	30
Right Amygdala	R_Amyg	54	50
Right Accumbens	R_Accu	58	50

Other Advanced Metrics (and Programs)

- FSL
  - Vertex Analysis (change in subcortical structure shape between groups)
  - Voxel-based morphometry (GM density/concentration)
- SPM (MATLAB-based)
  - Longitudinal tissue changes
  - Cortical Thickness
- Freesurfer
  - Gold standard for cortical thickness/volume and surface-based morphometry

The End: Tutorial Complete!

Thank you for attending the SNSS Structural Module

Reference Information and Other Tools

- https://open.win.ox.ac.uk/pages/fslcourse/website/index.html
- https://fsl.fmrib.ox.ac.uk/fsl/docs/#/
- https://web.mit.edu/fsl_v5.0.10/fsl/doc/wiki/FSL.html

  Informative video from Dr. Sriranga Kashyap, giving overview of different programs for segmentation and registration:
  - MRITogether2024-S8T2: Open-source methods for MR image segmentation and registration
- Advanced toolkits from people at SRI:
  - GitHub - srikash/presurfer: Scripts to prepare MP2RAGE for Freesurfer
  - fahsuanlin/fhlin_toolbox: MATLAB routines for fMRI/EEG/MEG data processing and visualization with FreeSurfer