

Structural MRI of Amygdala

Tutorial: Observation, Segmentation, Quantification

The **FMRIB Software Library (FSL)** is a powerful tool that allows users to observe the human brain in various planes and dimensions, segment structures, and quantify these segments.

FSL is capable of analyzing gray matter, white matter, and the following eight structures.

Accumbens (area): left and right

Amygdala: left and right

Caudate: left and right

Hippocampus: left and right

Pallidum: left and right

Putamen: left and right

Thalamus (*proper*): left and right

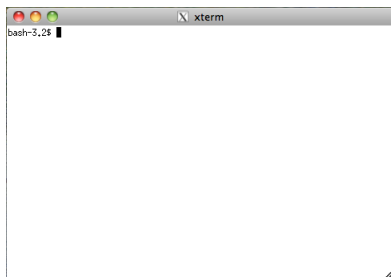
Brain Stem/ 4th Ventricle: whole

This tutorial will guide you in using **FSL** to analyze the left and right amygdalae in brains captured by **Structural MRI**.

(01) Launch X11

Locate the **X11** icon in the **Dock** on your **iMac**. Click it to launch the application.

An **xterm** window will appear.

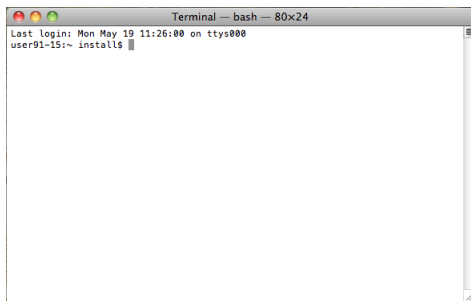


Note that you will need this program open, but you will not be typing any commands into this window. Instead, you will be entering commands into **Terminal**.

(02) Launch Terminal

Locate the **Terminal** icon in the **Dock** on your **iMac**. Click it to launch the application.

A **Terminal** window will appear.



Note that the last line you see in the window ends with a “\$” followed by a grey box. This is the command line, and the grey box indicates where your typed commands will begin.

(03) Launch FSLView (via Terminal) to view subject’s MRI scan

In the **Terminal** window, type the following **change directory** command, then hit **Return** on the keyboard.

cd

Note that this command (without any directory path specified) will change directory to the computer’s default directory. This is sort of a “clear history” action, so that you can move into other directories without any error.

In the **Terminal** window, type the following **list** command, then hit **Return** on the keyboard.

ls

Note that this command will list the contents (files and folders) contained in the current directory (the directory that the Terminal has been asked to “look” at).

In the **Terminal** window, type the following command to change directory to your subject’s **files** folder, then hit **Return** on the keyboard.

cd ~/Desktop/MRI_ImageDB_Partial/<subj>/<subj>_MR/scans/anat-anat/resources/NIfTI/files/

Note that this command will change directory to the path specified, allowing **Terminal** to view and access the image files in this folder. Right now, this includes your subject’s original (un-extracted) brain file (anat.nii.gz).

In the **Terminal** window, type the following **list** command, then hit **Return** on the keyboard.

ls

Check to make sure your subject’s original (un-extracted) brain file (anat.nii.gz) is listed.

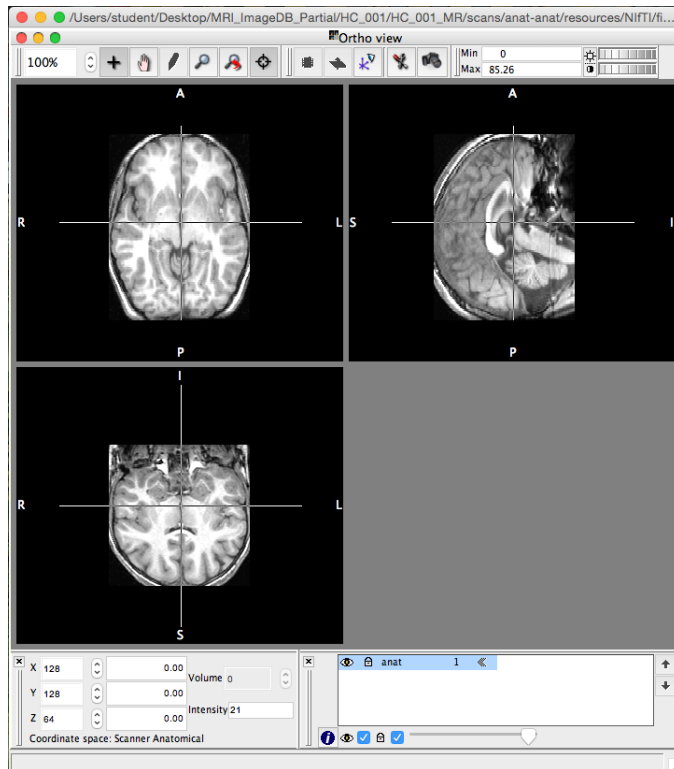
In the **Terminal** window, type the following command, then hit **Return** on the keyboard.

fslview anat.nii.gz

Note that if this command fails try the following instead.

fslview anat.nii

Your subject's MRI scan will appear in a **FSLView** window.



Scroll through the images to become familiar with each view, and locate the amygdala.

When you are finished viewing your subject's MRI scan, quit **FSLView**.

(04) Launch FSL (via Terminal)

In the **Terminal** window, type the following command, then hit **Return** on the keyboard.

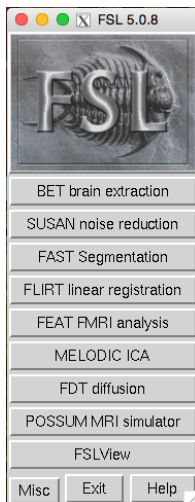
```
fsl
```

Note that if you receive a DISPLAY error message, enter the following commands (hitting **Return** after each) instead.

```
export DISPLAY=:0
```

```
fsl
```

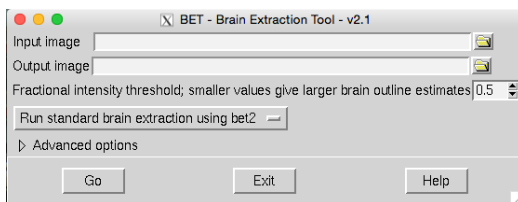
An **FSL** window will appear.



(05) Launch Brain Extraction Tool (BET) to extract subject's brain

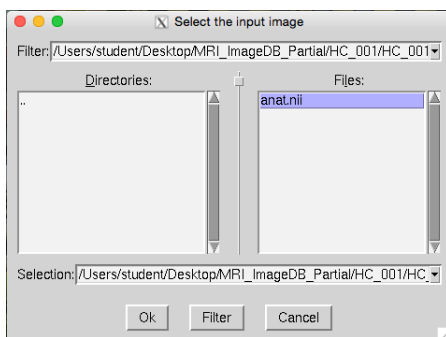
Click the **BET brain extraction** button in the **FSL** window.

A **BET – Brain Extraction Tool** window will appear.



Click the folder button to the right of the **Input image** field.

A **Select the input image** window will appear.



In the **Files:** section on the right, select the following file.

anat.nii.gz

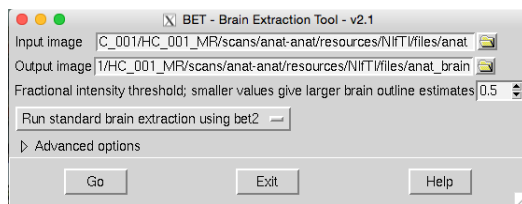
Note that if the file (anat.nii.gz) does not appear in the **Files:** section, move to the correct directory manually. In the **Directories:** section on the left, double-click (to open) the following directories.

Desktop
MRI_ImageDB_Partial
<subject>
<subject>_MR
scans
anat-anat
resources
NiftI
files
anat.nii.gz

Also note that the **Output image** field will automatically be assigned the same folder path, with the following new file name.

anat_brain.nii.gz

Make sure the correct file paths appear in the **Input image** and **Output image** fields.



In the **Fractional intensity threshold** section, leave the default amount as 0.5. Also leave the default drop menu option to **Run standard brain extraction using bet2**. Then click the **Go** button to extract your subject's brain.

Check to make sure the assigned output file (anat_brain.nii.gz) has been created in your subject's **files** folder. Again, it is in the same folder location as the input file.

Click the **Exit** button to exit out of the **BET Brain Extraction Tool**.

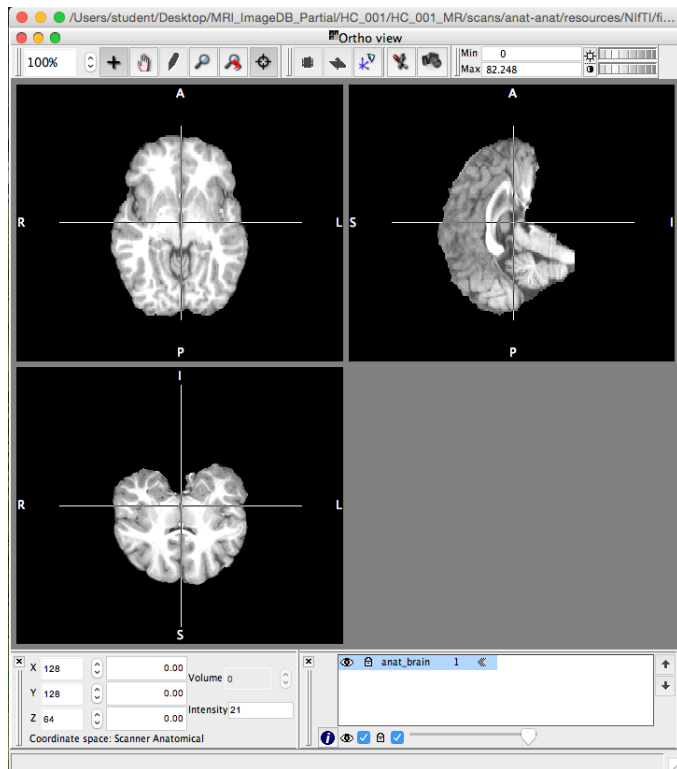
Close the **Exit** button on the **FSL** window to exit out of the program.

(06) Launch FSLView (via Terminal) to view subject's extracted brain

In the **Terminal** window, type the following command, then hit **Return** on the keyboard.

fslview anat_brain.nii.gz

Your subject's extracted brain will appear in the **FSLView** window.



Scroll through the images to become familiar with each view, and locate the amygdala.

When you are finished viewing your subject's extracted brain, quit **FSLView**.

(07) Flip subject's extracted brain (via Terminal)

In the **Terminal** window, type the following **ls** command, then hit **Return** on the keyboard.

ls

Check to make sure your subject's un-extracted brain file (anat.nii.gz) and extracted brain file (anat_brain.nii.gz) are listed.

In the **Terminal** window, type the following command to flip your subject's extracted brain, then hit **Return** on the keyboard.

fslswapdim anat_brain.nii.gz x -y z anat_brain_flipped

Note that this command will flip the extracted brain and save the flipped image as the following name (which is specified at the end of the command).

anat_brain_flipped.nii.gz

Check to make sure this output file has been created in your subject's **files** folder.

(08) Segment left amygdala in subject's flipped extracted brain (via Terminal)

In the **Terminal** window, type the following command to segment your subject's left amygdala, then hit **Return** on the keyboard.

```
run_first_all -i anat_brain_flipped.nii.gz -b -s L_Amyg -o anat_brain_flipped
```

Note that this task may take a few minutes to complete. The **Terminal** window will indicate when the task is finished (by bringing back the command line).

Also note that this command will segment out the left amygdala from the flipped extracted brain and save some new files. The following file is the only one you need to work with regarding the left amygdala.

```
anat_brain_flipped-L_Amyg_first.nii.gz
```

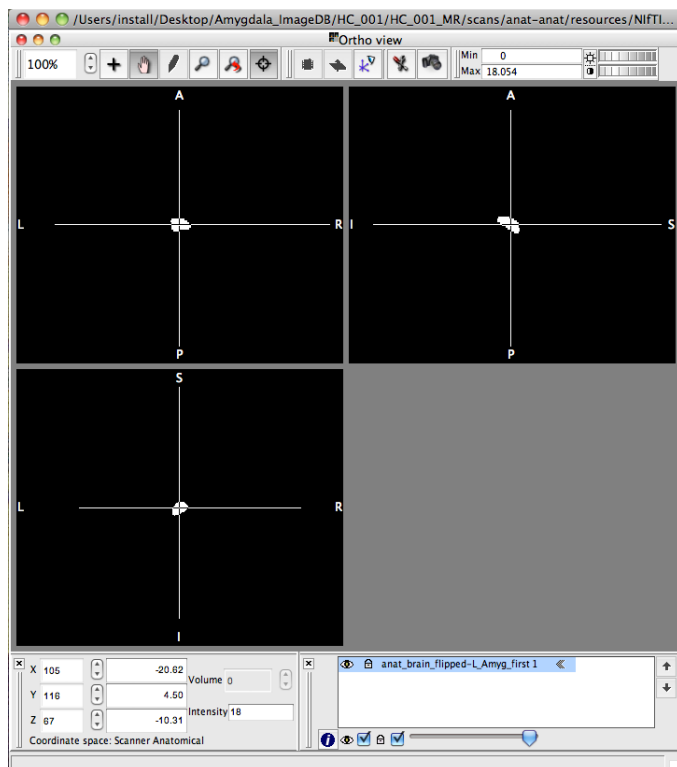
Check to make sure this output file has been created in your subject's **files** folder.

(09) Launch FSLView (via Terminal) to view subject's left amygdala and flipped extracted brain

In the **Terminal** window, type the following command, then hit **Return** on the keyboard.

```
fslview anat_brain_flipped-L_Amyg_first.nii.gz
```

Your subject's segmented left amygdala will appear in the **FSLView** window.



Scroll through the images to become familiar with each view, and center the amygdala (similar to above).

Note that once the amygdala is centered, you may want to switch from the **Crosshair Tool** (plus icon) to the **Pan Tool** (hand icon) in the **Main modes tool** toolbox at the top of the **FSLView** window. This will prevent you from accidentally moving the crosshairs and losing the view of the amygdala.

In **FSLView**, select **File** from the menu bar, then select **Add...**, then select the following file from the list, then click the **Open** button.

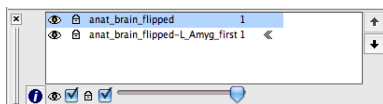
anat_brain_flipped.nii.gz

Note that if the file is not shown, navigate through the following folders to open your subject's flipped extracted brain, then click the **Open** button.

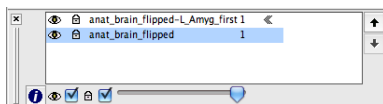
Desktop
MRI_ImageDB_Partial
 <subject>
 <subject>_MR
 scans
 anat-anat
 resources
 NiftI
 files
 anat_brain_flipped.nii.gz

Your subject's flipped extracted brain will appear in the **FSLView** window.

In the **Overlay settings** toolbox on the bottom right of the **FSLView** window, click on your subject's flipped extracted brain file (anat_brain_flipped.nii.gz) to select it (it will be highlighted blue if selected).

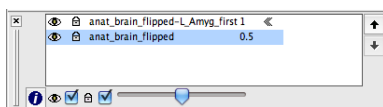


Use the **Move overlay up/ down** arrow buttons to move this file down below your subject's segmented left amygdala file (anat_brain_flipped-L_Amyg_first.nii.gz).

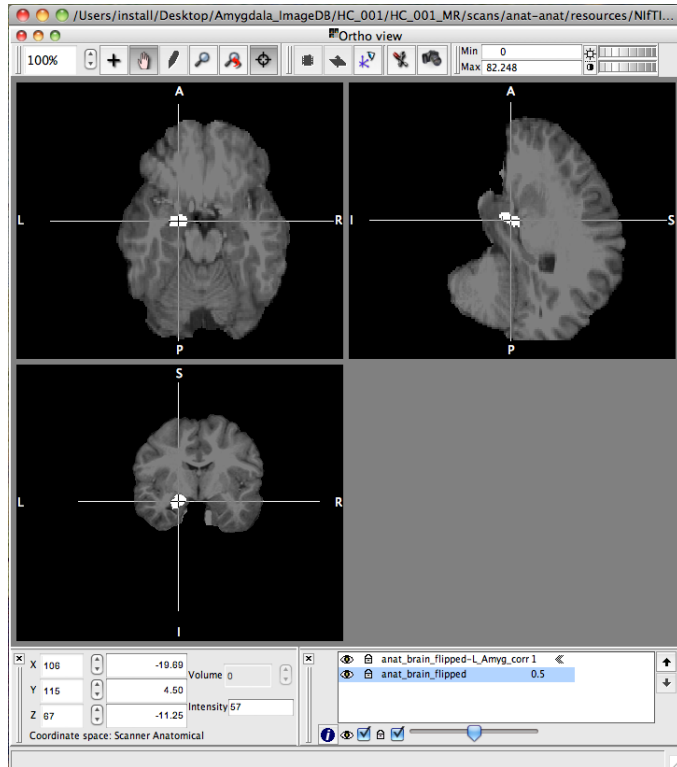


Note that the segmented left amygdala image is superimposed onto the flipped extracted brain image.

While your subject's flipped extracted brain file is still selected in the **Overlay settings** toolbox, use the **Transparency slider** to reduce the opacity to around 50%.



Observe the superimposed images in **FSLView**.



When you are finished viewing your subject's brain, quit **FSLView**.

(10) Measure volume of subject's left amygdala (via Terminal)

In the **Terminal** window, type the following command to measure the volume of your subject's left amygdala, then hit **Return** on the keyboard.

```
fsmaths anat_brain_flipped-L_Amyg_first.nii.gz -thr 17.5 -uthr 18.5 Amyg_Left
```

Note that this command will calculate the volume of your subject's left amygdala and save the information as the following name (which is specified at the end of the command).

Amyg_Left.nii.gz

Check to make sure this output file has been created in your subject's **files** folder.

In the **Terminal** window, type the following command to view the volume of your subject's left amygdala, then hit **Return** on the keyboard.

```
fsstats Amyg_Left.nii.gz -V
```

*****Make sure to use UPPER-CASE V, lower-case will give bad data!*****

Note that two numbers appear in the **Terminal** window immediately following the command. The first number gives the structure's volume in voxels. The second number gives the structure's volume in cubed millimeters (mm^3).

Record these values with a descriptive label (like your name, subject, and segment).

*****Include these measurements in your lab report!!!*****

(11) Segment right amygdala in subject's flipped extracted brain (via Terminal)

In the **Terminal** window, type the following command to segment your subject's right amygdala, then hit **Return** on the keyboard.

```
run_first_all -i anat_brain_flipped.nii.gz -b -s R_Amyg -o anat_brain_flipped
```

Note that this task may take a few minutes to complete. The **Terminal** window will indicate when the task is finished (by bringing back the command line).

Also note that this command will segment out the right amygdala from the flipped extracted brain and save some new files. The following file is the only one you need to work with regarding the right amygdala.

```
anat_brain_flipped-R_Amyg_first.nii.gz
```

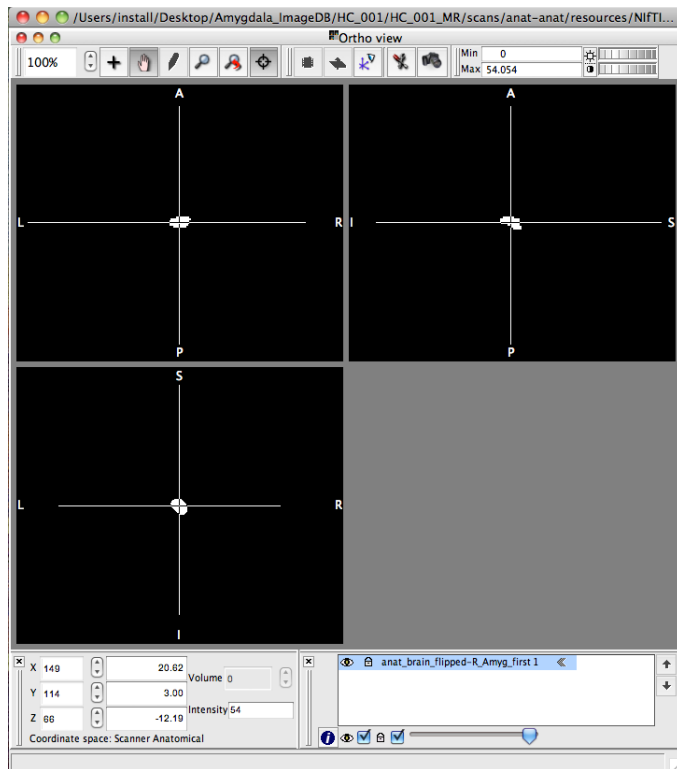
Check to make sure this output file has been created in your subject's **files** folder.

(12) Launch FSLView (via Terminal) to view subject's right amygdala and flipped extracted brain

In the **Terminal** window, type the following command, then hit **Return** on the keyboard.

```
fslview anat_brain_flipped-R_Amyg_first.nii.gz
```

Your subject's segmented right amygdala will appear in the **FSLView** window.



Scroll through the images to become familiar with each view, and center the amygdala (similar to above).

Note that once the amygdala is centered, you may want to switch from the **Crosshair Tool** (plus icon) to the **Pan Tool** (hand icon) in the **Main modes tool** toolbox at the top of the **FSLView** window. This will prevent you from accidentally moving the crosshairs and losing the view of the amygdala.

In **FSLView**, select **File** from the menu bar, then select **Add...**, then select the following file from the list, then click the **Open** button.

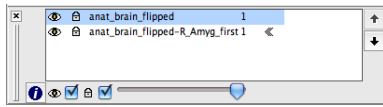
anat_brain_flipped.nii.gz

Note that if the file is not shown, navigate through the following folders to open your subject's flipped extracted brain, then click the **Open** button.

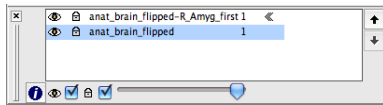
```
Desktop
MRI_ImageDB_Partial
<subject>
<subject>_MR
scans
anat-anat
resources
NIFTI
files
anat_brain_flipped.nii.gz
```

Your subject's flipped extracted brain will appear in the **FSLView** window.

In the **Overlay settings** toolbox on the bottom right of the **FSLView** window, click on your subject's flipped extracted brain file (anat_brain_flipped.nii.gz) to select it (it will be highlighted blue if selected).

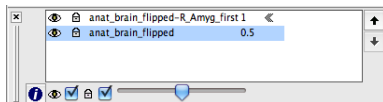


Use the **Move overlay up/ down** arrow buttons to move this file down below your subject's segmented right amygdala file (anat_brain_flipped-R_Amyg_first.nii.gz).

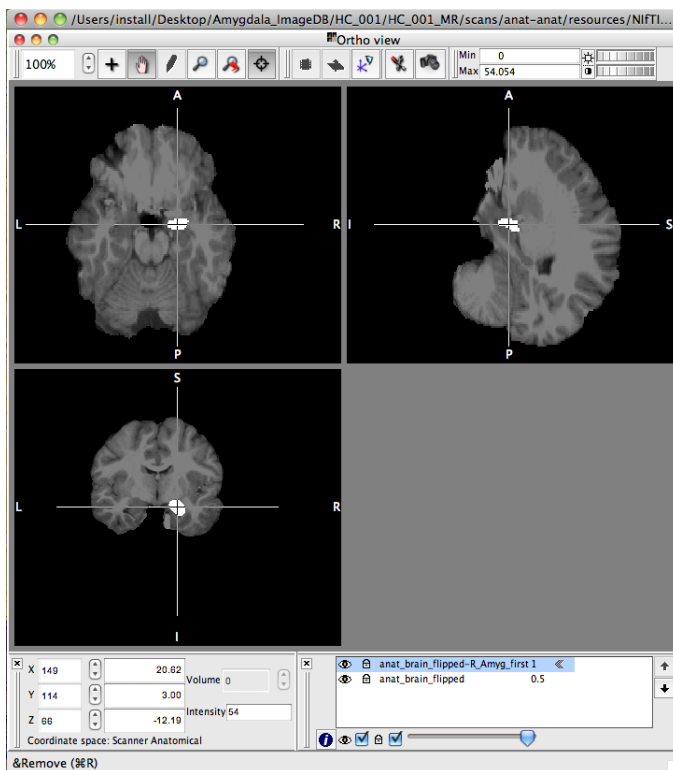


Note that the segmented right amygdala image is superimposed onto the flipped extracted brain image.

While your subject's flipped extracted brain file is still selected in the **Overlay settings** toolbox, use the **Transparency slider** to reduce the opacity to around 50%.



Observe the superimposed images in **FSLView**.



When you are finished viewing your subject's brain, quit **FSLView**.

(13) Measure volume of subject's right amygdala (via Terminal)

In the **Terminal** window, type the following command to measure the volume of your subject's right amygdala, then hit **Return** on the keyboard.

```
fslmaths anat_brain_flipped-R_Amyg_first.nii.gz -thr 53.5 -uthr 54.5 Amyg_Right
```

Note that this command will calculate the volume of your subject's right amygdala and save the information as the following name (which is specified at the end of the command).

Amyg_Right.nii.gz

Check to make sure this output file has been created in your subject's **files** folder.

In the **Terminal** window, type the following command to view the volume of your subject's right amygdala, then hit **Return** on the keyboard.

```
fsfstats Amyg_Right.nii.gz -V
```

*****Make sure to use UPPER-CASE V, lower-case will give bad data!*****

Note that two numbers appear in the **Terminal** window immediately following the command. The first number gives the structure's volume in voxels. The second number gives the structure's volume in cubed millimeters (mm³).

Record these values with a descriptive label (like your name, subject, and segment).

*****Include these measurements in your lab report!!!*****

(14) Launch FSLView (via Terminal) to view subject's superimposed images of both amygdalae and flipped extracted brain

In the **Terminal** window, type the following command, then hit **Return** on the keyboard.

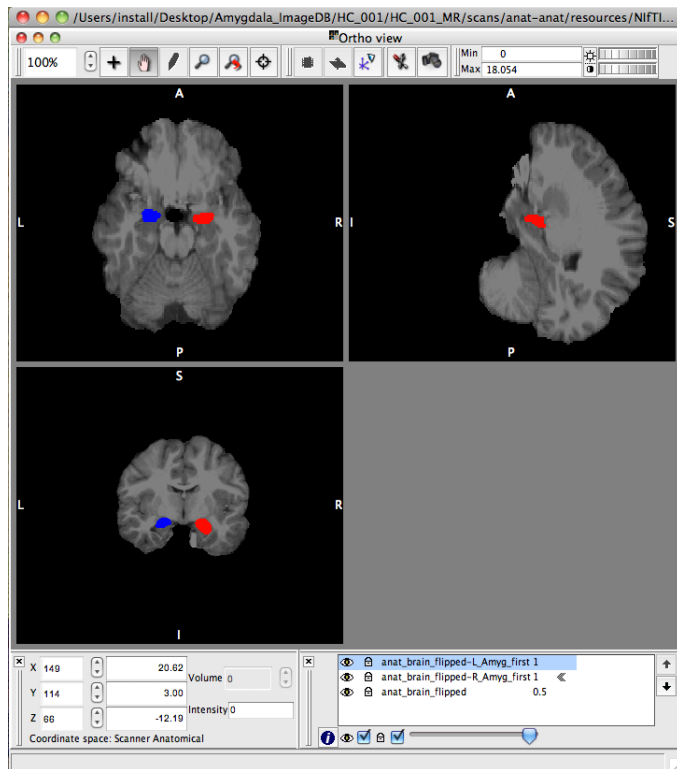
```
fslview anat_brain_flipped.nii.gz Amyg_Right.nii.gz -l "Red" Amyg_Left.nii.gz -l "Blue"
```

Observe the superimposed images in **FSLView**.

Select your subject's flipped extracted brain file in the **Overlay settings** toolbox, and use the **Transparency slider** to reduce the opacity to around 50%.

Scroll through the images to become familiar with each view. Select one of the amygdalae in the **Overlay settings** toolbox, and use the cross-hairs to center it.

Click the **Toggle cross-hairs on/off** (bullseye icon) in the **Main modes tool** toolbox at the top of the **FSLView** window to toggle off the cross-hairs from being seen over the images.



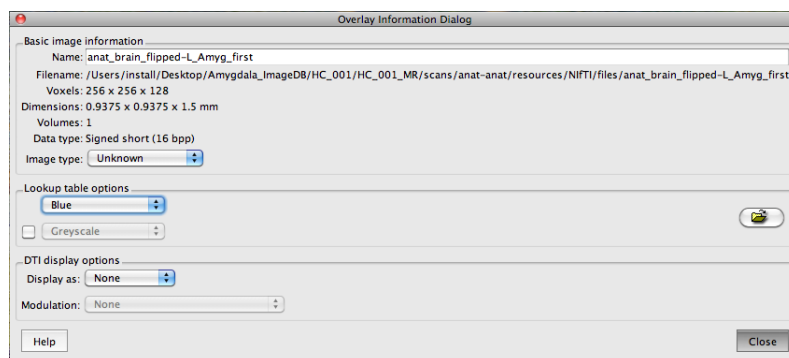
Note that if you don't see blue and red amygdalae, then do the following steps.

Select your subject's flipped extracted brain file in the **Overlay settings** toolbox, then use the **Transparency slider** to reduce the opacity to around 50%.

Click on your subject's segmented left amygdala file (Amyg_Left.nii.gz) to select it (it will be highlighted blue if selected), then click on the **Open information dialog** (letter "i" icon) button at the bottom left of the **Overlay settings** toolbox.

An **Open Information Dialog** window will appear.

In the **Lookup table options** box, use the drop menu to change the segmented left amygdala image color from **Greyscale** to **Blue**, then click the **Close** button.

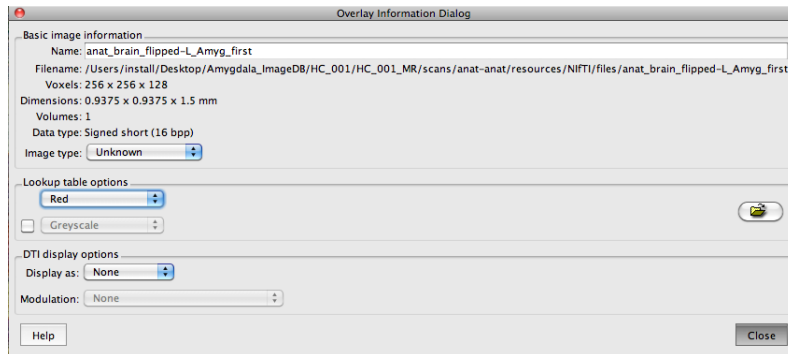


Note that the segmented left amygdala image is now blue.

Click on your subject's segmented right amygdala file (Amyg_Right.nii.gz) to select it (it will be highlighted blue if selected), then click on the **Open information dialog** (letter "i" icon) button at the bottom left of the **Overlay settings** toolbox.

An **Open Information Dialog** window will appear.

In the **Lookup table options** box, use the drop menu to change the segmented right amygdala image color from **Greyscale** to **Red**, then click the **Close** button.



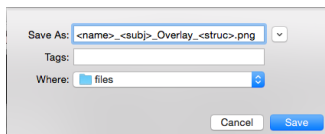
Note that the segmented right amygdala image is now red.

In the **Main mode tools** toolbox at the top of the **FSLView** window, click on the **Save snapshot to file** (camera icon) button.



Note that this tool will capture a screenshot of the images visible in the **FSLView** window.

Save the screenshot file to the **Desktop** with something descriptive (like your name, subject, "Overlay" and structure).



Note that you may also grab a screenshot by simultaneously typing **Cmd-Shift-4**, then using a crossing window (marquee) with your mouse to select the area of the screen you wish to capture. When you release the mouse, a **PNG** file of your selection will be saved to the **Desktop**.

*****Include this screenshot in your lab report!!!*****

When you are finished viewing your subject's brain, quit **FSLView**.

(15) Segment gray matter and white matter in subject's flipped extracted brain (via Terminal)

In the **Terminal** window, type the following command to segment your subject's gray matter and white matter, then hit **Return** on the keyboard.

```
fast -t 1 -n 3 -H 0.1 -l 4 -l 20.0 -g -o anat_brain_flipped_fast anat_brain_flipped.nii.gz
```

Note that this task may take a few minutes to complete. The **Terminal** window will indicate when the task is finished (by bringing back the command line).

Also note that this command will segment out the gray matter (indicated by “1” in the file name) and white matter (indicated by “2” in the file name) from the flipped extracted brain and save some new files. The following files are the only ones you need to work with regarding the right amygdala.

```
anat_brain_flipped_fast_seg_1.nii.gz  
anat_brain_flipped_fast_seg_2.nii.gz
```

Check to make sure this output file has been created in your subject's **files** folder.

(16) **Measure volume of subject's gray matter and white matter (via Terminal)**

Note that during segmentation of gray matter and white matter (in the previous step), respective volumes were already calculated, and this information was saved with the output files.

In the **Terminal** window, type the following command to view the volume of your subject's gray matter, then hit **Return** on the keyboard.

```
fsstats anat_brain_flipped_fast_seg_1.nii.gz -V
```

*****Make sure to use UPPER-CASE V, lower-case will give bad data!*****

Note that two numbers appear in the **Terminal** window immediately following the command. The first number gives the structure's volume in voxels. The second number gives the structure's volume in cubed millimeters (mm^3).

Record these values with a descriptive label (like your name, subject, and segment).

*****Include these measurements in your lab report!!!*****

In the **Terminal** window, type the following command to view the volume of your subject's white matter, then hit **Return** on the keyboard.

```
fsstats anat_brain_flipped_fast_seg_2.nii.gz -V
```

*****Make sure to use UPPER-CASE V, lower-case will give bad data!*****

Note that two numbers appear in the **Terminal** window immediately following the command. The first number gives the structure's volume in voxels. The second number gives the structure's volume in cubed millimeters (mm^3).

Record these values with a descriptive label (like your name, subject, and segment).

*****Include these measurements in your lab report!!!*****

When you are finished recording your subject's measurements, quit **Terminal**, and quit **X11**.

(17) Repeat these steps for remaining subjects

Make sure that you have completely quit **Terminal** and **X11** before moving on to your remaining subjects.

Go to the very beginning of this tutorial and repeat each of these steps to observe, segment, and quantify the left amygdala, right amygdala, gray matter, and white matter in the remainder of your subjects' MRI scans.

Note that you should carefully check to make sure you are using the correct subject's folders and files.

When you are finished, submit your data to your instructor, and email your screenshots and data to yourself.

END