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 any and all of these structures in brains captured by **Structural MRI**.

Note that the images contained in this tutorial were taken during analysis of just one subject. Your images may look slightly different.

---

(01) Launch X11

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

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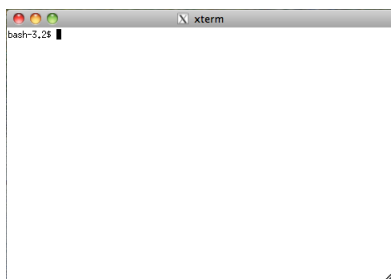
Note that you will need this program open, but you will not be typing any commands into this window. Instead, you will be entering commands into **Terminal**.

---

(02) Launch Terminal

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

A **Terminal** window will appear.

Terminal — bash — 80×24

Last login: Mon May 19 11:26:00 on ttys000
user91-15:~ install$
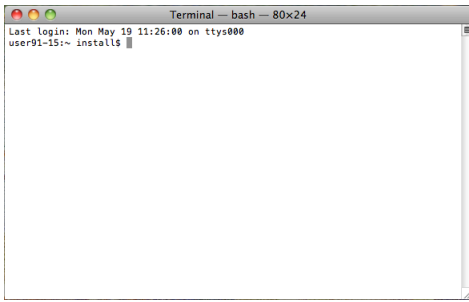
Note that the last line you see in the window ends with a "$" followed by a grey box. This is the command line, and the grey box indicates where your typed commands will begin.

---

(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 **print working directory** command, then hit **Return** on the keyboard.

pwd

Note that this command will show the path to the current directory (the directory that the Terminal has been asked to "look" at).

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.

In the **Terminal** window, type the following command to change directory to your subject's MRI folder, manually replace the "###" with your subject's number *(001-103)*, then hit **Return** on the keyboard.

cd ~/Desktop/MRI_ImageDB_Partial/###

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) and your subject's segmented cerebrum file (seg.nii).

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

pwd

Check to make sure your subject's path is correct.

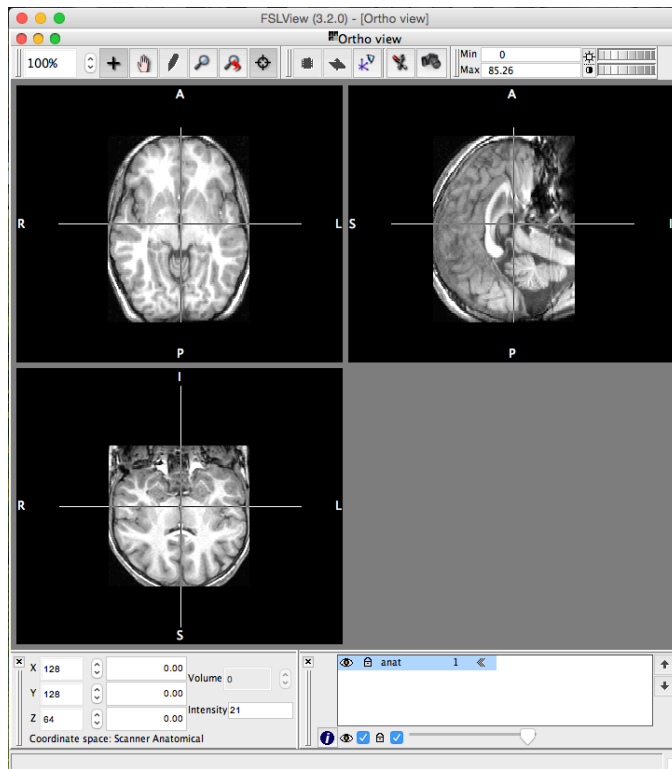In the **Terminal** window, type the following **list** command, then hit **Return** on the keyboard.

ls

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

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

fslview anat.nii

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

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

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

---

(04) Launch FSL (via Terminal)

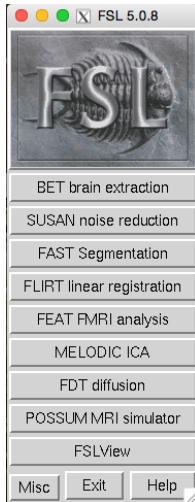In the **Terminal** window, type the following command, then hit **Return** on the keyboard.

fsl

Note that if you receive a DISPLAY error message, enter the following commands (hitting **Return** after each) instead.
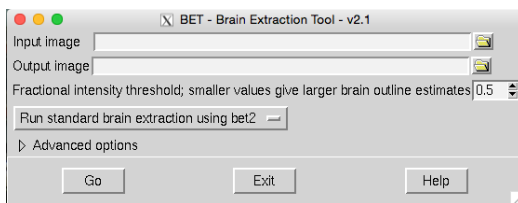
`export DISPLAY=:0`

`fsl`

An **FSL** window will appear.

---

(05) **Launch Brain Extraction Tool (BET) to extract subject's brain**
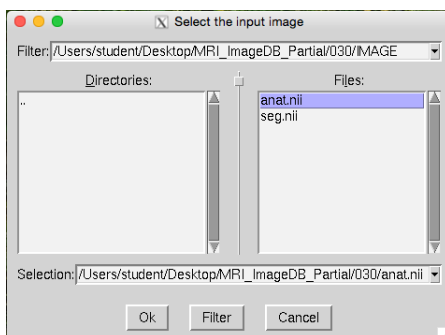
Click the **BET brain extraction** button in the **FSL** window.

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 so that it highlights blue/purple.

anat.nii

Note that if the file (anat.nii) 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.
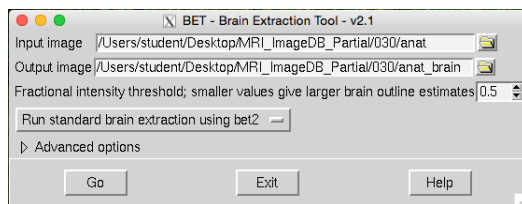
Desktop
  MRI_ImageDB_Partial
    ###
      anat.nii

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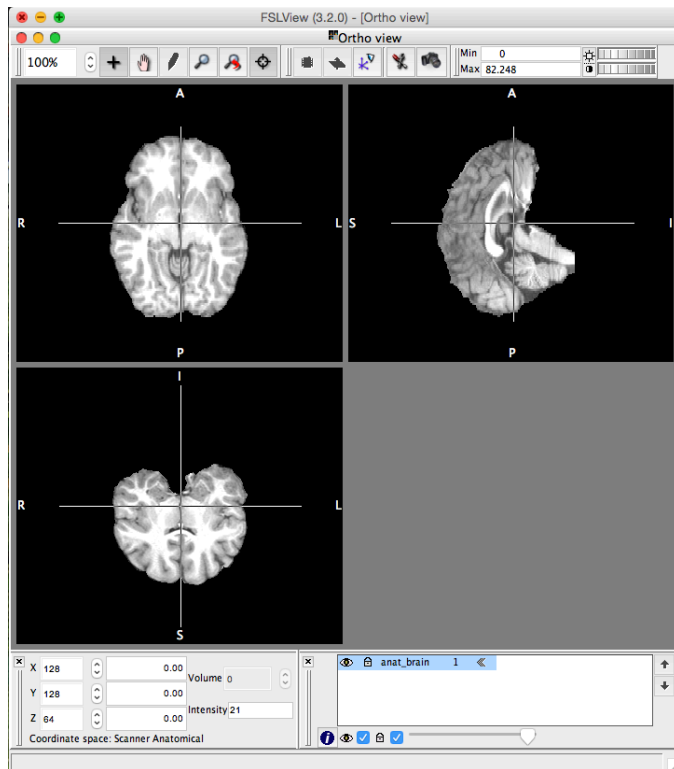
Scroll through the images to become familiar with each view, and locate the structure.

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

---

(07) Reorient to standard subject's extracted brain (via Terminal)

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

> fslreorient2std anat_brain.nii.gz anat_brain_std

Note that this command will reorient the extracted brain to the standard visual presentation of the MRI image and save this image as the following name (which is specified at the end of the command).

> anat_brain_std.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 standard 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.

> Left Amygdala:
> run_first_all -i anat_brain_std.nii.gz -b -s L_Amyg -o anat_brain_std

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 standard extracted brain and save some new files. The following file is the only one you need to work with regarding the left amygdala.

    Left Amygdala:
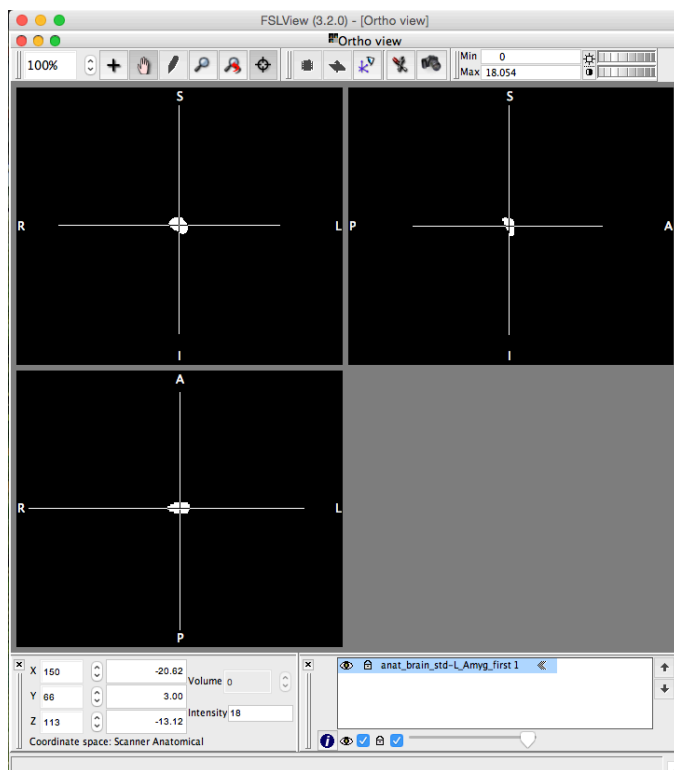   anat_brain_std-L_Amyg_first.nii.gz

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 standard extracted brain

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

    Left Amygdala:
        fslview anat_brain_std-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 use the crosshairs to center the amygdala in each view (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.

<mark>anat_brain_std.nii.gz</mark>

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

<mark>Desktop</mark>
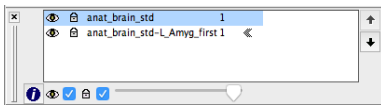 <mark>MRI_ImageDB_Partial</mark>
   ###
      <mark>anat_brain_std.nii.gz</mark>

Your subject's standard 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 standard extracted brain file (anat_brain_std.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_std-L_Amyg_first.nii.gz).
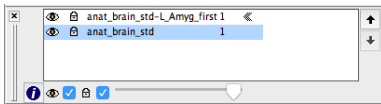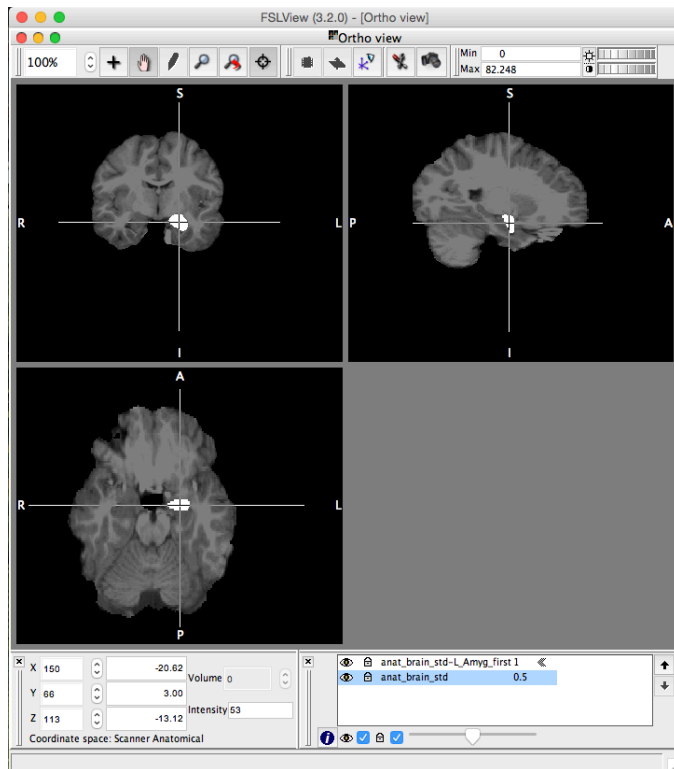

Note that the segmented left amygdala image is superimposed onto the standard extracted brain image.

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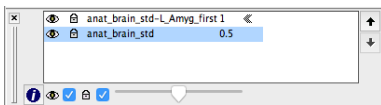
Observe the superimposed images in **FSLView**.

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 your subject's left amygdala, then hit **Return** on the keyboard.

Left Amygdala:
    fslmaths anat_brain_std-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).

Left Amygdala:
    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.

Left Amygdala:
    fslstats 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 standard 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.

Right Amygdala:
<mark>run_first_all -i anat_brain_std.nii.gz -b -s R_Amyg -o anat_brain_std</mark>

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 standard extracted brain and save some new files. The following file is the only one you need to work with regarding the right amygdala.

Right Amygdala:
anat_brain_std-R_Amyg_first.nii.gz

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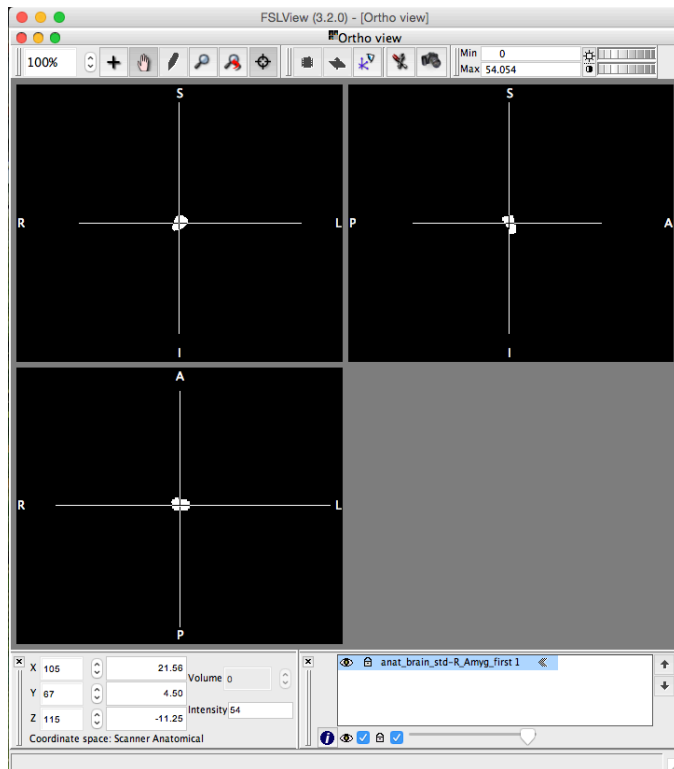
---

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

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

Right Amygdala:
<mark>fslview anat_brain_std-R_Amyg_first.nii.gz</mark>

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

Scroll through the images to become familiar with each view, and use the crosshairs to center the amygdala in each view (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_std.nii.gz

Note that if the file is not shown, navigate through the following folders to open your subject's standard extracted brain, then click the **Open** button.
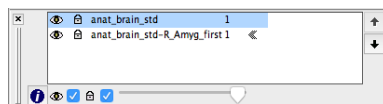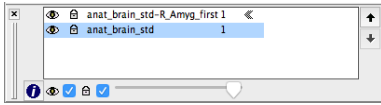
Desktop
 MRI_ImageDB_Partial
  ###
   anat_brain_std.nii.gz

Your subject's standard 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 standard extracted brain file (anat_brain_std.nii.gz) to select it (it will be highlighted blue if selected).
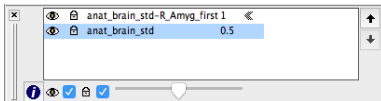
Use the **Move overlay up/ down** arrow buttons to move this file down below your subject's segmented right amygdala file (anat_brain_std-R_Amyg_first.nii.gz).
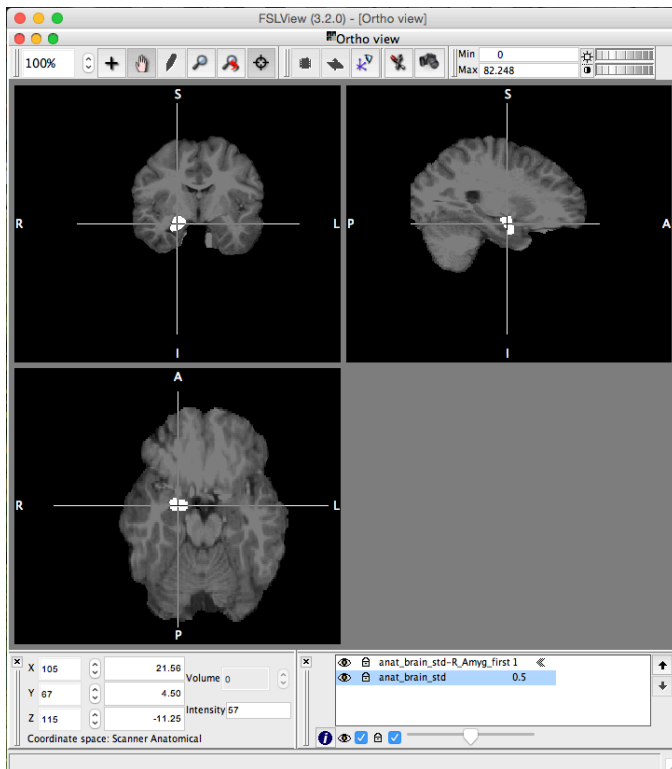

Note that the segmented right amygdala image is superimposed onto the standard extracted brain image.

While your subject's standard 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 your subject's right amygdala, then hit **Return** on the keyboard.

Right Amygdala:
fslmaths anat_brain_std-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).

Right Amygdala:
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.

Right Amygdala:
fslstats Amyg_Right.nii.gz -V

Make sure to use <u>UPPER-CASE</u> 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!!!

---

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

In the **Terminal** window, type the following command to view your subject's segmented amygdalae, then hit **Return** on the keyboard.

Amygdala:
fslview anat_brain_std.nii.gz Amyg_Left.nii.gz -l "Blue" Amyg_Right.nii.gz -l "Red"

Observe the superimposed images in **FSLView**.

Select your subject's standard extracted brain file in the **Overlay settings** toolbox, then 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 segmented amygdalae in the **Overlay settings** toolbox, and use the crosshairs to center it.

Click the **Toggle crosshairs on/off** (bullseye icon) in the **Main modes tool** toolbox at the top of the **FSLView** window to toggle off the crosshairs from being seen over the images.
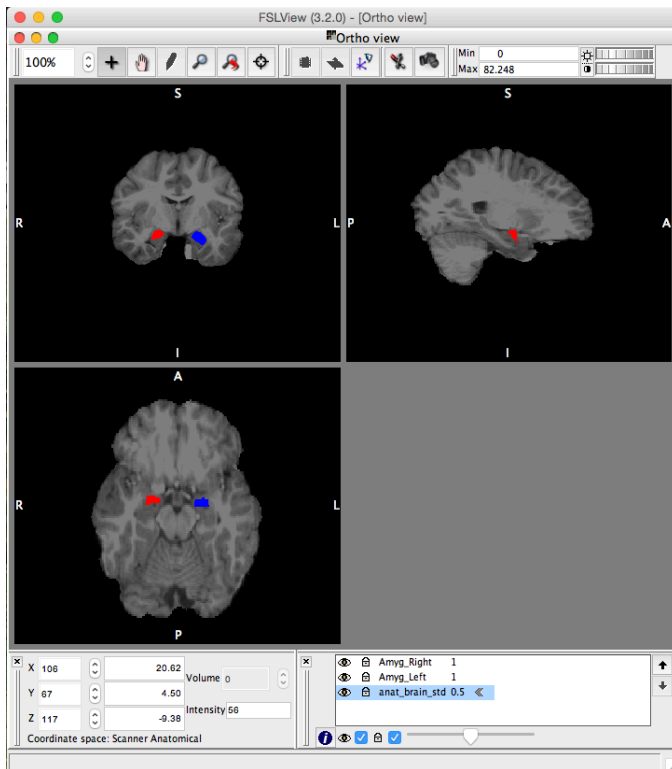
Note that if you don't see blue and red (or green) structures, then do the following steps.

Select your subject's standard 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 (Amyg_Left.nii.gz) file 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.
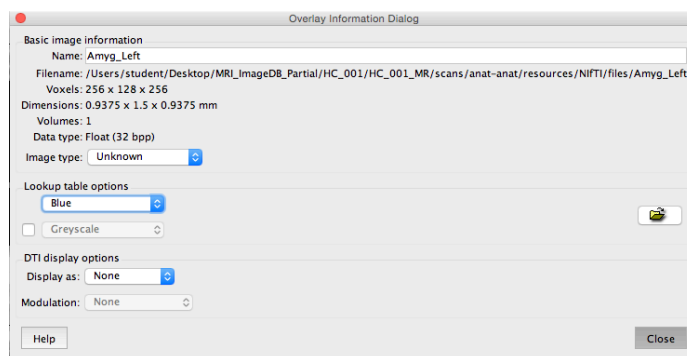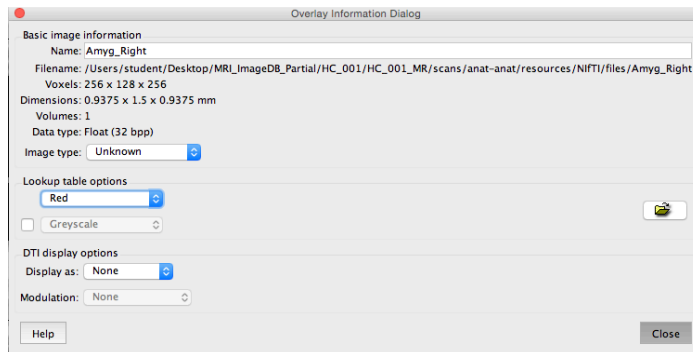

Note that the segmented left amygdala image is now blue.

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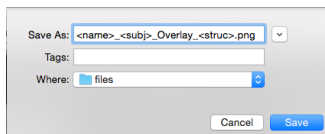
Note that you may also grab a screenshot by simultaneously typing **Cmd-Shift-4**, then using a crossing window (marquee) with your mouse to select the area of the screen you wish to capture. When you release the mouse, a **PNG** file of your selection will be saved to the **Desktop**.

Include this screenshot in your lab report!!!

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

---

(15) Reorient to standard subject's segmented cerebrum (via Terminal)

Verify that you are still in your same subject's MRI folder. In the **Terminal** window, type the following **print working directory** command, then hit **Return** on the keyboard.

pwd

Check to make sure your subject's path is correct.

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

<mark>ls</mark>

Check to make sure your subject's segmented cerebrum file (seg.nii) is listed.

In the **Terminal** window, type the following command to reorient your subject's segmented cerebrum to standard MRI presentation, then hit **Return** on the keyboard.

<mark>fslreorient2std seg.nii seg_std</mark>

Note that this command will reorient the segmented cerebrum to the standard visual presentation of the MRI image and save this image as the following name (which is specified at the end of the command).

seg_std.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)

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

<mark>fslmaths seg_std.nii.gz -thr 3 -uthr 3 -bin L_Gray</mark>

Note that this command will calculate the volume of your subject's left gray matter and save the information as the following name.

L_Gray.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 gray matter, then hit **Return** on the keyboard.

<mark>fslstats L_Gray.nii.gz -V</mark>

Make sure to use <u>UPPER-CASE</u> 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 measure your subject's right gray matter, then hit **Return** on the keyboard.

<mark>fslmaths seg_std.nii.gz -thr 42 -uthr 42 -bin R_Gray</mark>

Note that this command will calculate the volume of your subject's right gray matter and save the information as the following name.

R_Gray.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 gray matter, then hit **Return** on the keyboard.

fslstats R_Gray.nii.gz -V

Make sure to use <u>UPPER-CASE</u> 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 measure your subject's left white matter, then hit **Return** on the keyboard.

fslmaths seg_std.nii.gz -thr 2 -uthr 2 -bin L_White

Note that this command will calculate the volume of your subject's left white matter and save the information as the following name.

L_White.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 white matter, then hit **Return** on the keyboard.

fslstats L_White.nii.gz -V

Make sure to use <u>UPPER-CASE</u> 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 measure your subject's right white matter, then hit **Return** on the keyboard.

fslmaths seg_std.nii.gz -thr 41 -uthr 41 -bin R_White

Note that this command will calculate the volume of your subject's right white matter and save the information as the following name.

R_White.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 white matter, then hit **Return** on the keyboard.

<mark>fslstats R_White.nii.gz -V</mark>

Make sure to use <u>UPPER-CASE</u> 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!!!

---

(16) Launch FSLView (via Terminal) to view subject's left and right gray matter and left and right white matter

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

<mark>fslview L_Gray.nii.gz -l "Blue" R_Gray.nii.gz -l "Red" L_White.nii.gz -l "Blue-Lightblue" R_White.nii.gz -l "Red-Yellow"</mark>

Your subject's standard segmented cerebrum scans will appear, in an **FSLView** window.
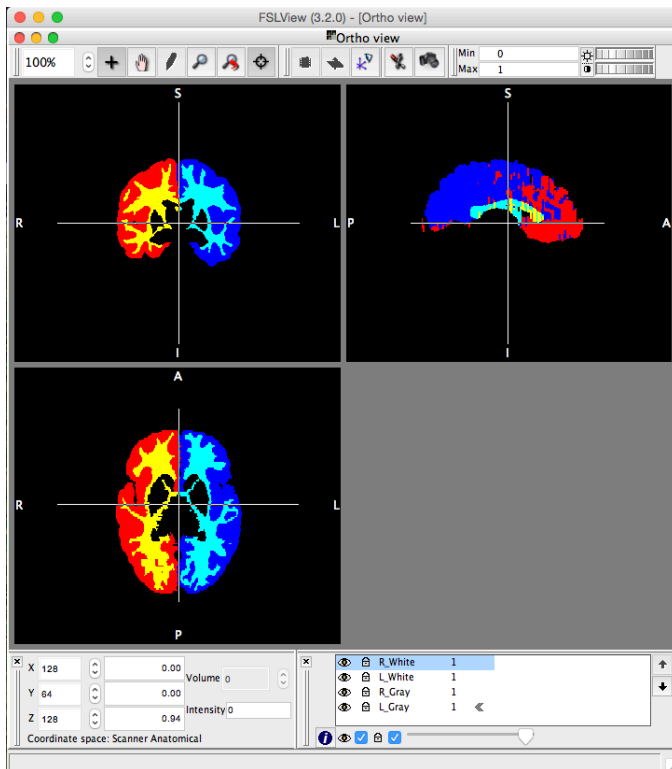
Note that the left gray matter, right gray matter, left white matter, and right white matter each have it's own color.

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

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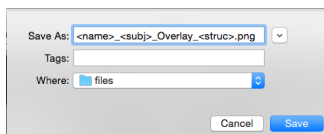
Note that you may also grab a screenshot by simultaneously typing **Cmd-Shift-4**, then using a crossing window (marquee) with your mouse to select the area of the screen you wish to capture. When you release the mouse, a **PNG** file of your selection will be saved to the **Desktop**.

Include this screenshot in your lab report!!!

When you are finished viewing your subject's segmented cerebrum scans, quit **FSLView**.

(17) <u>Repeat these steps for remaining subjects</u>

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 both the left and right structures (or whole brain stem/4th ventricle), left and right gray matter, and left and right 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