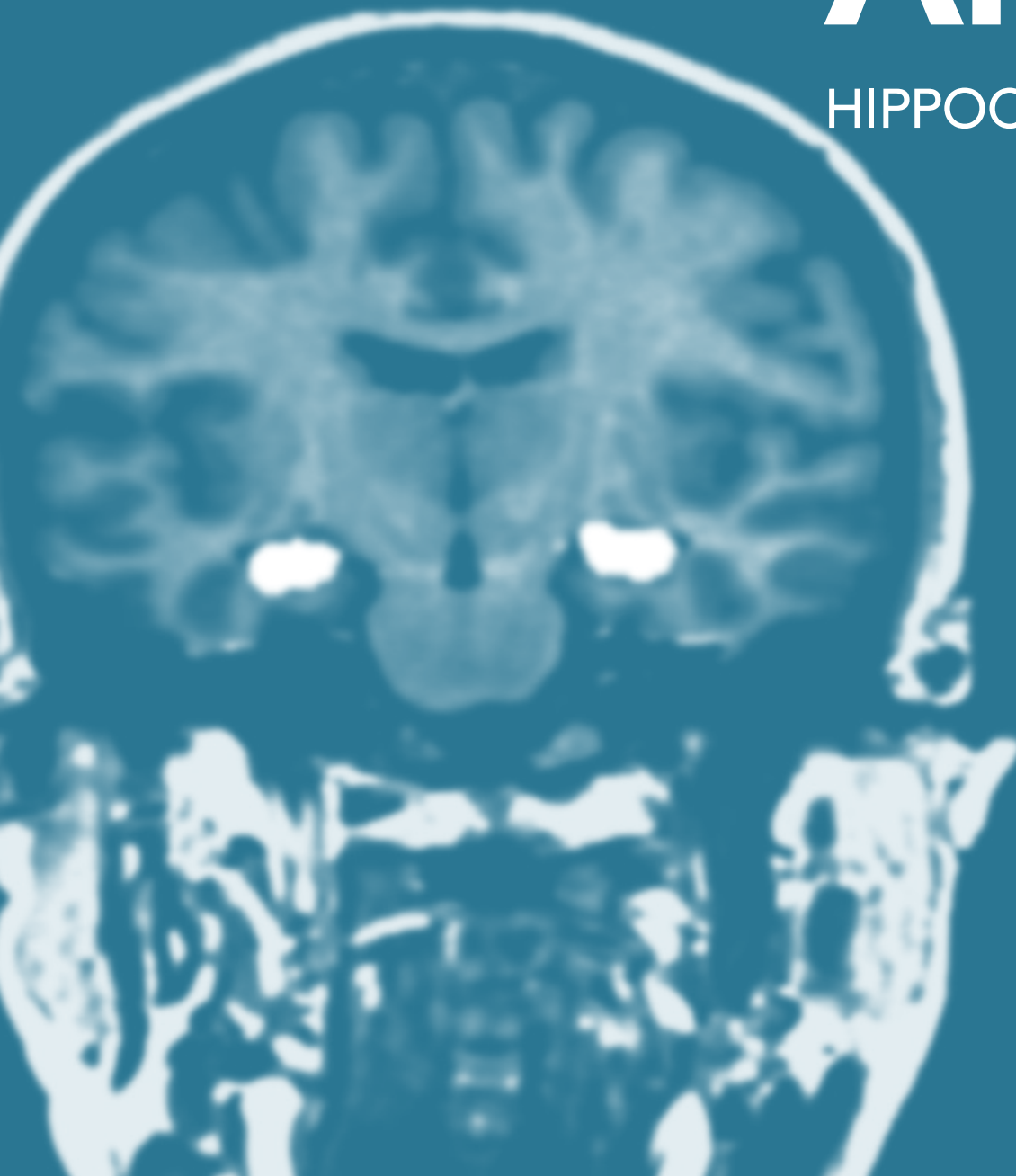


AnalyzePro

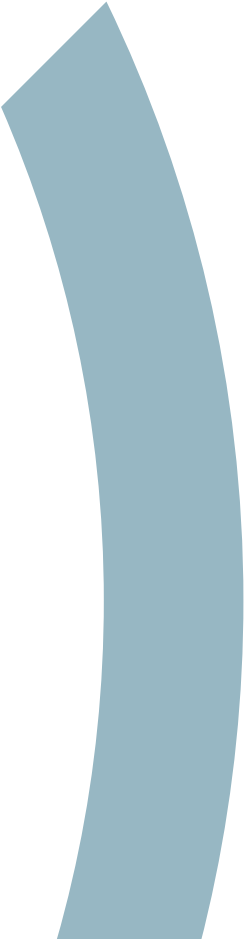

HIPPOCAMPAL VOLUME ASSESSMENT





HIPPOCAMPAL VOLUME ASSESSMENT GUIDE

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Introduction

The volume of the hippocampus is a commonly used structural MRI measurement in brain research. Many studies focus on a decrease in hippocampal volume associated with a wide variety of neurological and psychiatric conditions including depression¹, posttraumatic stress disorder (PTSD)^{2,3}, borderline personality disorder (BPD)⁴, schizophrenia^{5,6}, alcohol abuse⁷ and Alzheimer's⁸, Parkinson's and Huntington's diseases⁹.

It is as yet unclear what mechanisms underlie the decrease in hippocampal volume associated with major depressive disorder (MDD)⁹. Cross-sectional studies are incapable of determining definitively whether decreased hippocampal volume is caused by MDD or is a contributing factor to MDD, so it is recommended that longitudinal studies be conducted to determine the causal relationship between MDD and hippocampal volume¹⁰. Decreased hippocampal volume in MDD patients may lead to recollection memory impairment. It may also be related to an increase in glucocorticoids caused by stressful events and exacerbated by decreased inhibition of the hypothalamic-pituitary-adrenal (HPA) axis as a result of decreased hippocampal volume^{1,11}. It has been found that antidepressants used during episodes of depression may protect the hippocampus from decreasing in size¹² and that there is an association between duration of depression and reduced hippocampal volume^{13,14}.

Decreased hippocampal volume has been identified in Vietnam veterans with PTSD, and hippocampal volume was directly correlated with combat exposure. This correlation may be explained by combat stress causing PTSD, which in turn damages the hippocampus, or by an increased likelihood of individuals with smaller hippocampi either being assigned to combat units or developing PTSD when exposed to combat stress². Decreased hippocampal volume has not been found to be a necessary risk factor for developing PTSD and does not occur within six months of the onset of PTSD. The lower hippocampal volume observed in some PTSD patients may be related to chronic or complicated PTSD or related distress. The frontal lobe may be the area of the brain most significantly affected by PTSD, rather than the hippocampus³. In adults who sustained childhood trauma in the form of abuse or maltreatment, hippocampal volume is reduced¹⁵. This effect is not observed until adulthood, suggesting there is a delay between the childhood causal factors and the reduced hippocampal volume effect^{16,17}.



Hippocampal volume is frequently used in the study of Alzheimer's disease (AD) and as such may be developed into a standardized biomarker for dementia and AD^{18,19}. The volume of the hippocampus, adjusted for age and gender, has been found to best discriminate controls from early-onset AD cases in comparison to other medial temporal lobe structures²⁰. However, a stronger correlation was found between change in hemispheric volume measurements (whole brain and ventricle) and cognitive performance compared to the correlation between medial temporal lobe volume decrease and cognitive performance²¹. MRI-based hippocampal volume measurement may become a useful diagnostic tool in cases of memory impairment which may indicate early-onset AD²⁰, and measurement of whole brain atrophy rates may be preferable for some clinical trials, especially considering that it can more easily be automated²¹.

Findings from the Nun Study have shown that hippocampal volume as measured by postmortem MRI was able to identify differences between Braak stages of AD pathology as determined by histopathology. This suggests that hippocampal volume could be used to identify AD at early stages in patients long before they show clinical symptoms. This should be considered along with findings that 43-67% of individuals fulfill neuropathologic criteria for diagnosis of AD at autopsy yet never exhibited dementia²². Measurements of hippocampal volume from MRI have been used to show that patients with mild cognitive impairment (MCI) who have smaller hippocampal volume are more likely to convert to AD than MCI patients with larger hippocampi²³.

In order to be widely used as a biomarker in clinical practice and clinical trials, hippocampal segmentation will have to be automated, but the gold standard of hippocampal segmentation remains manual tracing¹⁸. The bottom line is that structural volume measurements as determined by MRI provide a more reliable assessment of AD progression than cognitive tests alone. Imaging should be used in conjunction with standard cognitive tests in AD therapeutic trials²¹.



The population in the developed world is increasingly aging, and researchers are trying to confirm ways of preventing or reversing cognitive atrophy. One group found that higher fitness levels were associated with increased left and right hippocampal volume in nondemented adults, which was also associated with increased performance on spatial memory tests²⁴. Spatial navigation training maintained hippocampal volume in both younger and older men, while controls experienced a decline in hippocampal volume over time²⁵.

Insulin resistance is a cause of cardiovascular disease, diabetes, and obesity, which are in turn risk factors for dementia and AD²⁶. Insulin receptors in the central nervous system are mostly located in or near the hippocampus, and a negative correlation has been demonstrated between insulin resistance and hippocampal volume²⁷.

Note that the sample data set shown is a 1.5 Tesla T1-weighted MR image. Because of the relatively low field strength, the boundaries of the hippocampus are not always clear due to partial volume effects. Data acquired at a higher field strength such as 3T or even 7T would have a higher resolution and signal-to-noise ratio, and an atlas of the hippocampus has even been created from 9.4T postmortem scans²⁸. Partial volume effects can also be reduced by acquiring the data orthogonal to the long axis of the hippocampus.

Using a higher field strength would also allow for the segmentation of the hippocampus into its subregions. In particular, reduced hippocampal tail volume has been observed in patients with MDD and schizophrenia^{29,30}. This guide focuses on the steps necessary to segment hippocampi and measure total hippocampal volume in AnalyzePro.

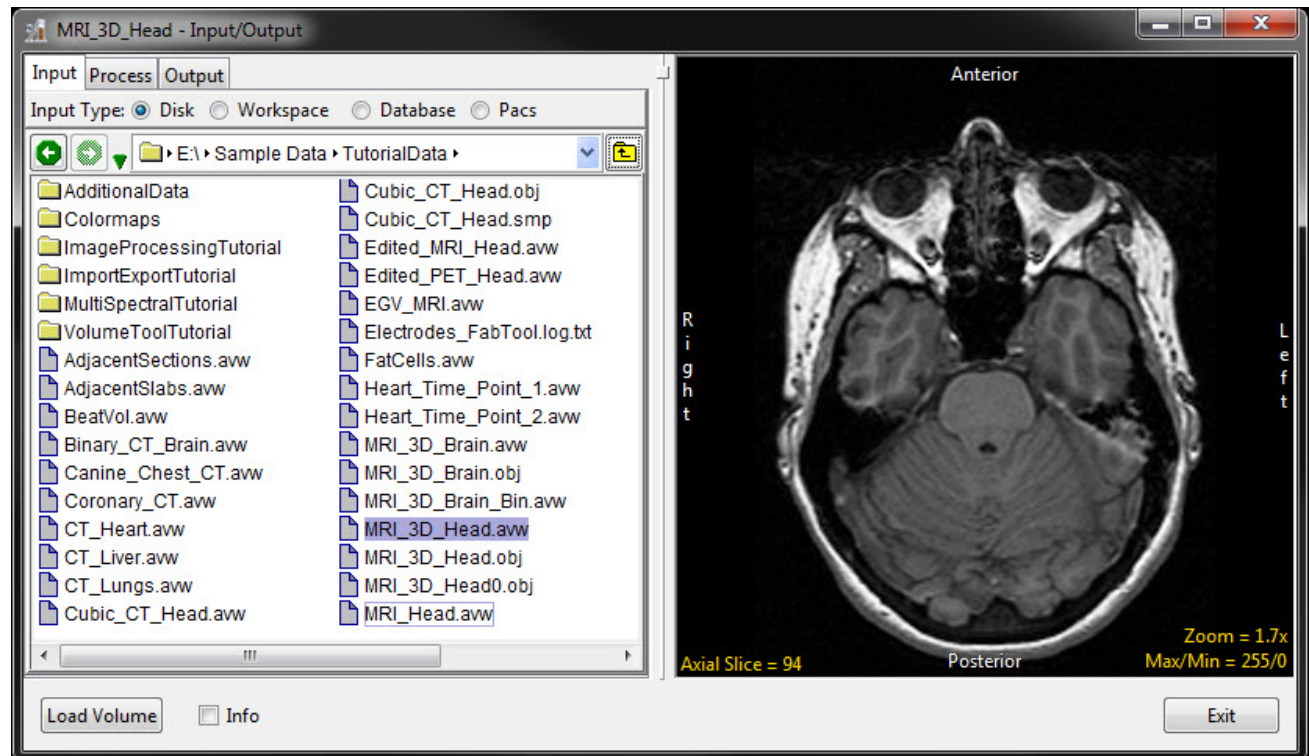


Loading Data

- Open Input/Output.
- Navigate to and select the MRI_3D_Head data set.
- Click Load Volume.
- Once the data is loaded into AnalyzePro, close Input/Output.



Download the MRI_3D_Head data set to follow along
<http://analyzedirect.com/data/>





Preprocessing Steps

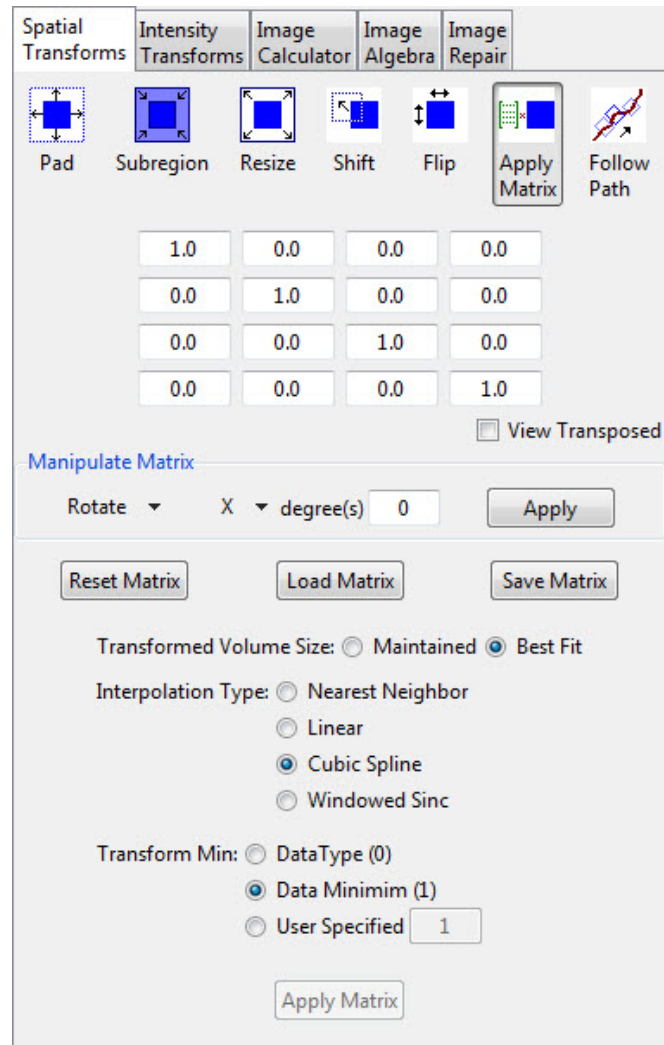
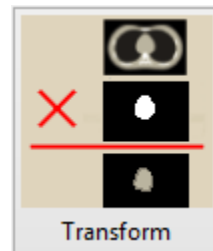
There are a number of data preprocessing steps that will aid in manual hippocampus segmentation. It is recommended to reslice the data orthogonal to the AC-PC line for consistency. In addition, upsampling the data through interpolation to create a smaller voxel size will allow you to define hippocampal regions more precisely. Finally, cropping or subregioning the data to zoom in on the region containing the hippocampus will reduce the size of the data set and speed up processing and rendering times, especially on slower systems.



Manual AC-PC Alignment of Brain Data

According to the current standards set by EADC (European Alzheimer's Disease Consortium) and ADNI (Alzheimer's Disease Neuroimaging Initiative)^{31,32}, the structural MRI brain data should be AC-PC aligned before segmentation. The brain data can be aligned along the AC-PC axis as described in the following section.

- Select the data set to align along the AC-PC axis and open Transform.
- Navigate to Spatial Transforms.
- Select the Apply Matrix tool.

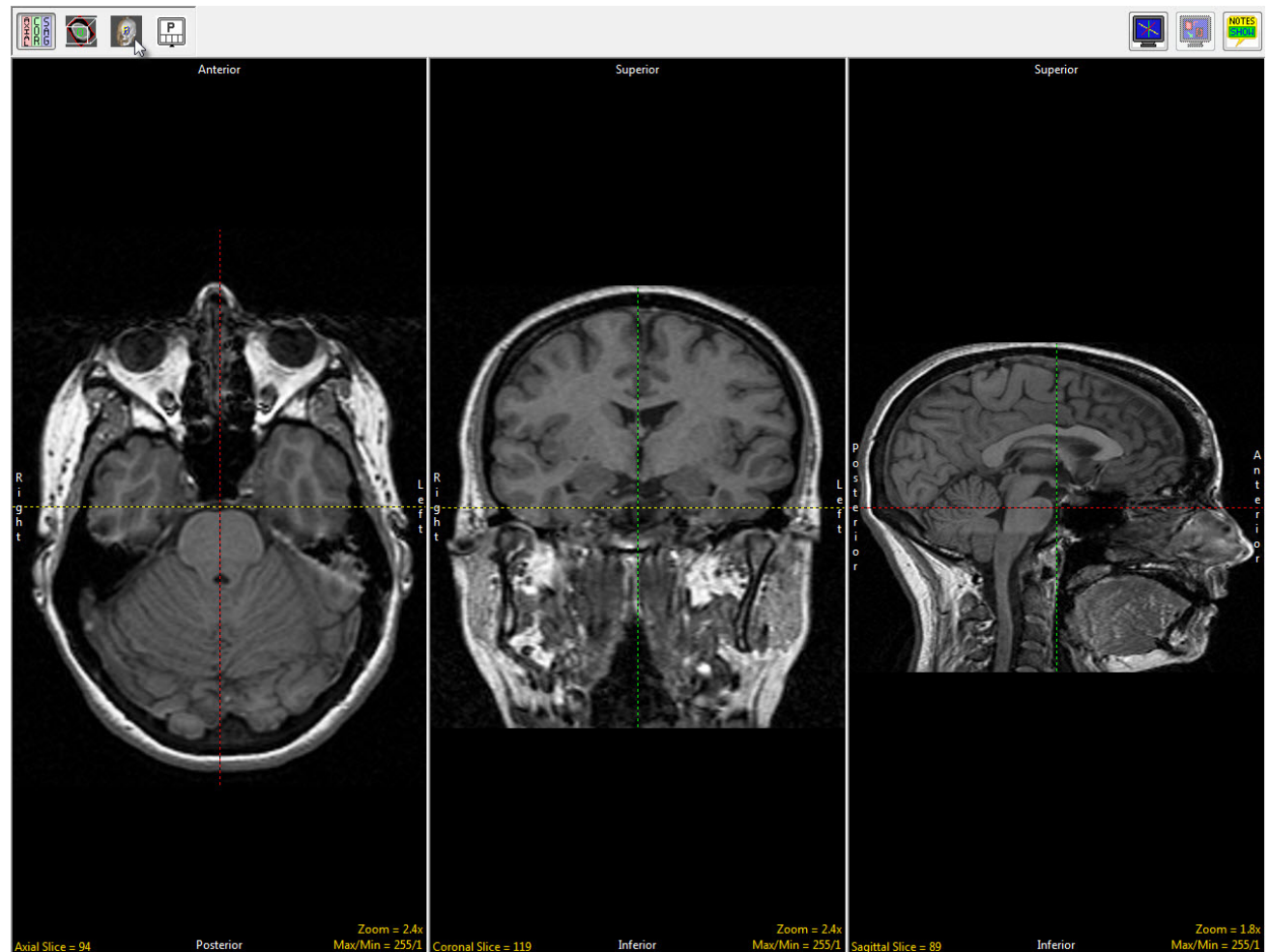


The optimal screen layout for AC-PC alignment can be set as follows:

- Switch off the rendering using the Toggles Rendering button.



- Now the three orthogonal views are shown.



- Switch on the oblique.



- Now the oblique and the three orthogonal views are shown.



- Toggle the orthogonal orientations to a single image using the Toggles All Orients button.



- Set this image to the sagittal view by clicking twice on the Orient parameter at the bottom left of the image.
- Now the sagittal orientation and the oblique image are shown.



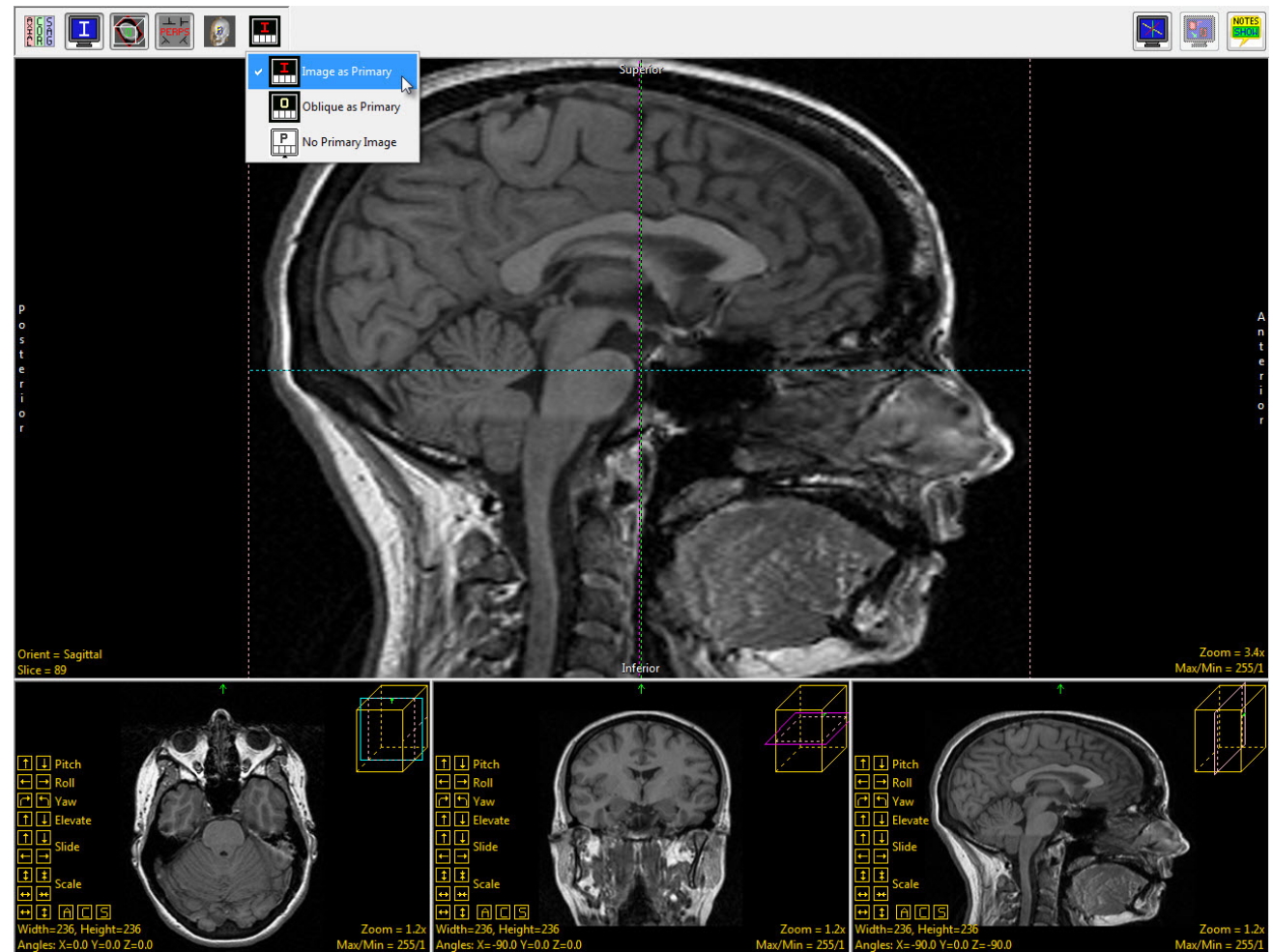
- Switch on the perpendicular images using the Toggles Perpendicular button.



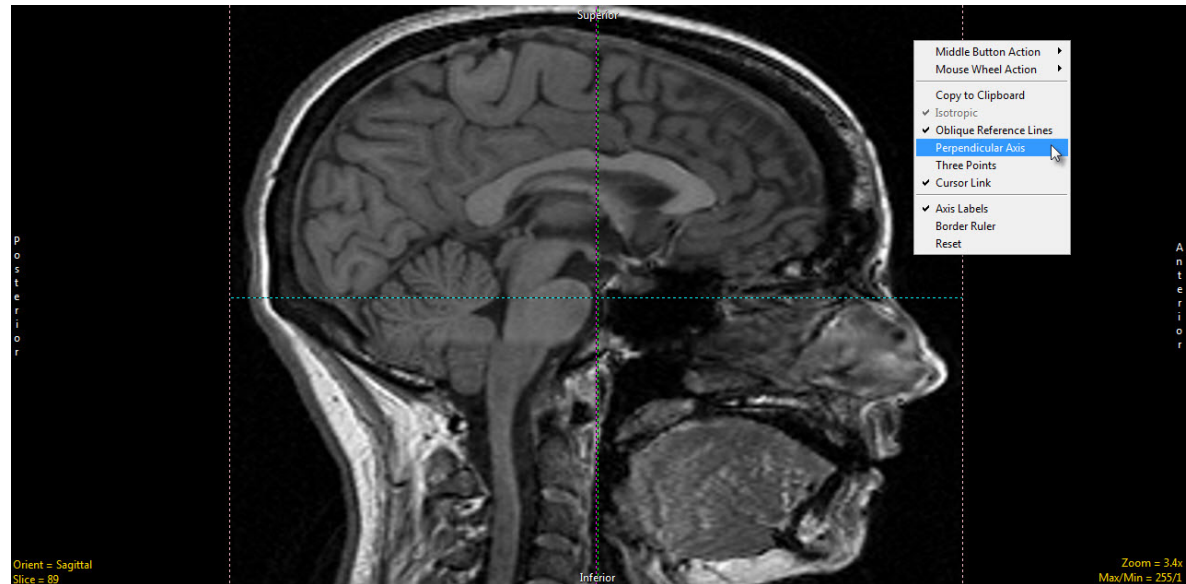
These are the planes perpendicular to the oblique plane as it is defined at any moment and can be used to verify the choice of oblique plane. Now the sagittal orientation, the oblique image and the two planes perpendicular to the oblique image are shown.



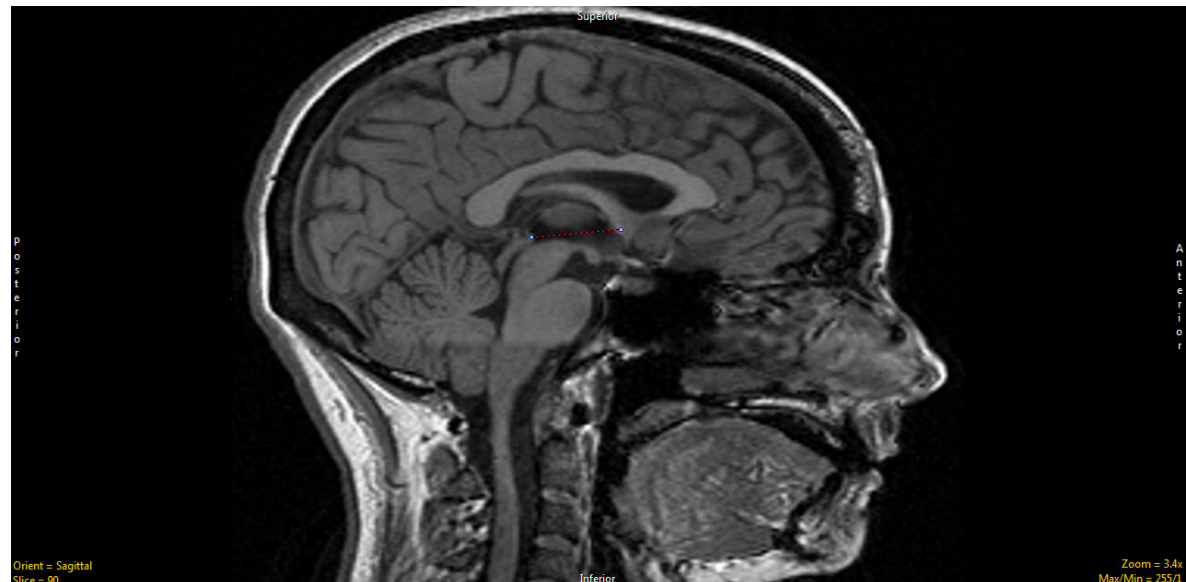
- The AC-PC line will be defined on the sagittal view, so double-click the sagittal image or select Image as Primary in the dropdown menu.



- Right-click on the sagittal image and select Perpendicular Axis to turn on the perpendicular axis tool.



- Navigate to the mid-sagittal slice, which for this data set is slice 90.
- Drag the left endpoint of the perpendicular axis to the posterior commissure.
- Drag the right endpoint of the perpendicular axis to the anterior commissure.

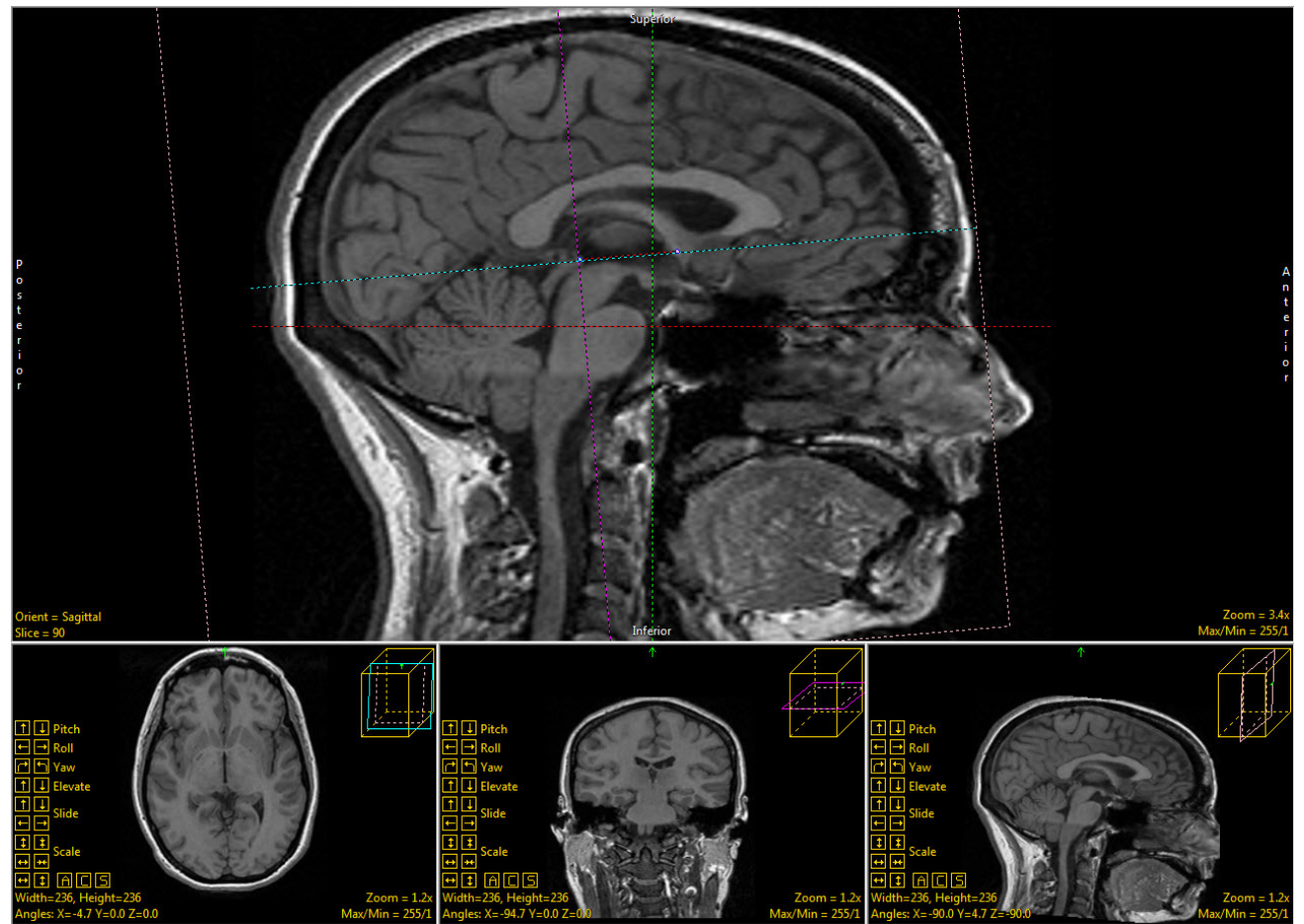




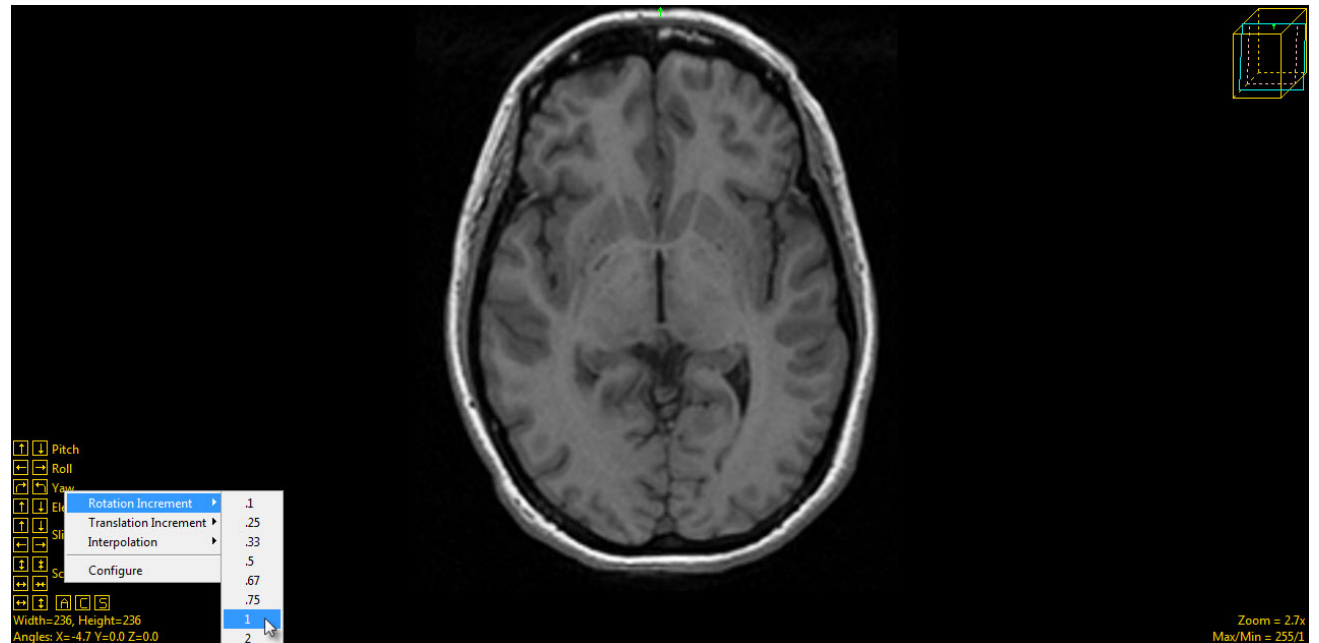
- To set the oblique plane to be parallel, rather than perpendicular, to the AC-PC plane, right-click on one of the perpendicular axis endpoints and set the Matrix to Create to Parallel.



- Now the main oblique image (the leftmost of the three) is an AC-PC aligned axial image.
- Double-click this image to make it the primary image.

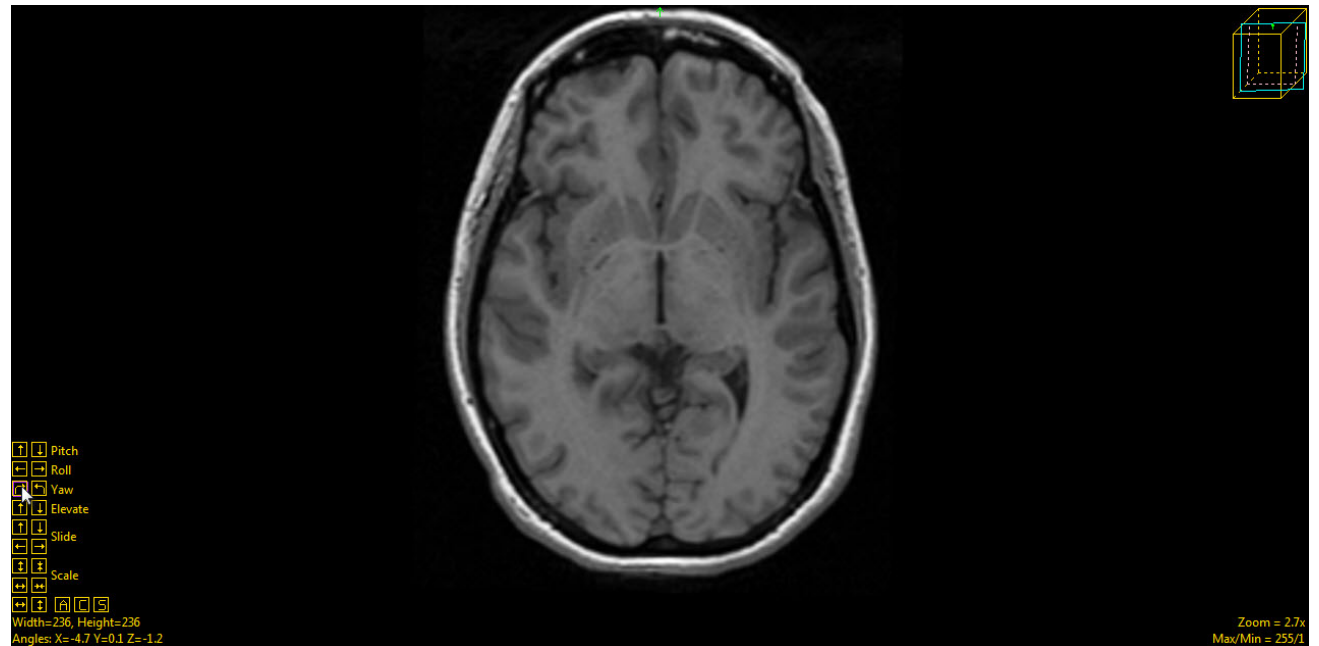


- The AC and PC can both be seen on the same oblique slice, indicating good AC-PC alignment.
- To adjust vertical alignment of the data set, right-click Yaw and set the Rotation Increment to 1.



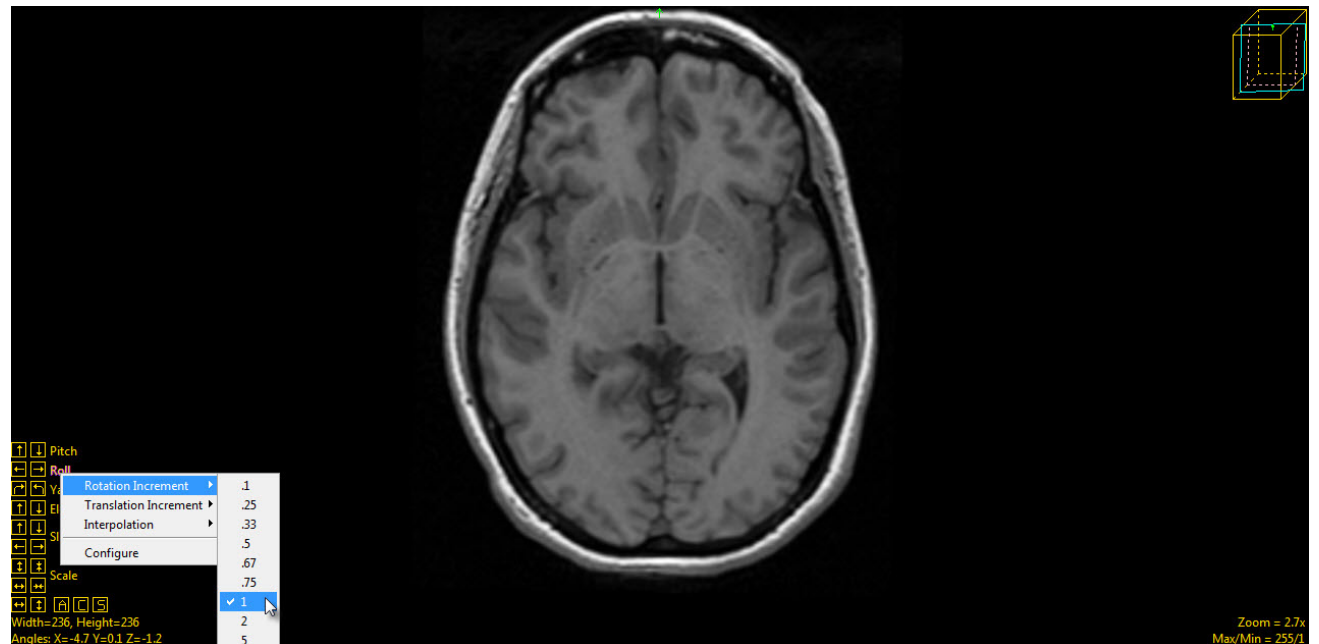


- Use the left and right Yaw buttons to align the data set vertically. The increment can be made even smaller to make smaller adjustments as needed.



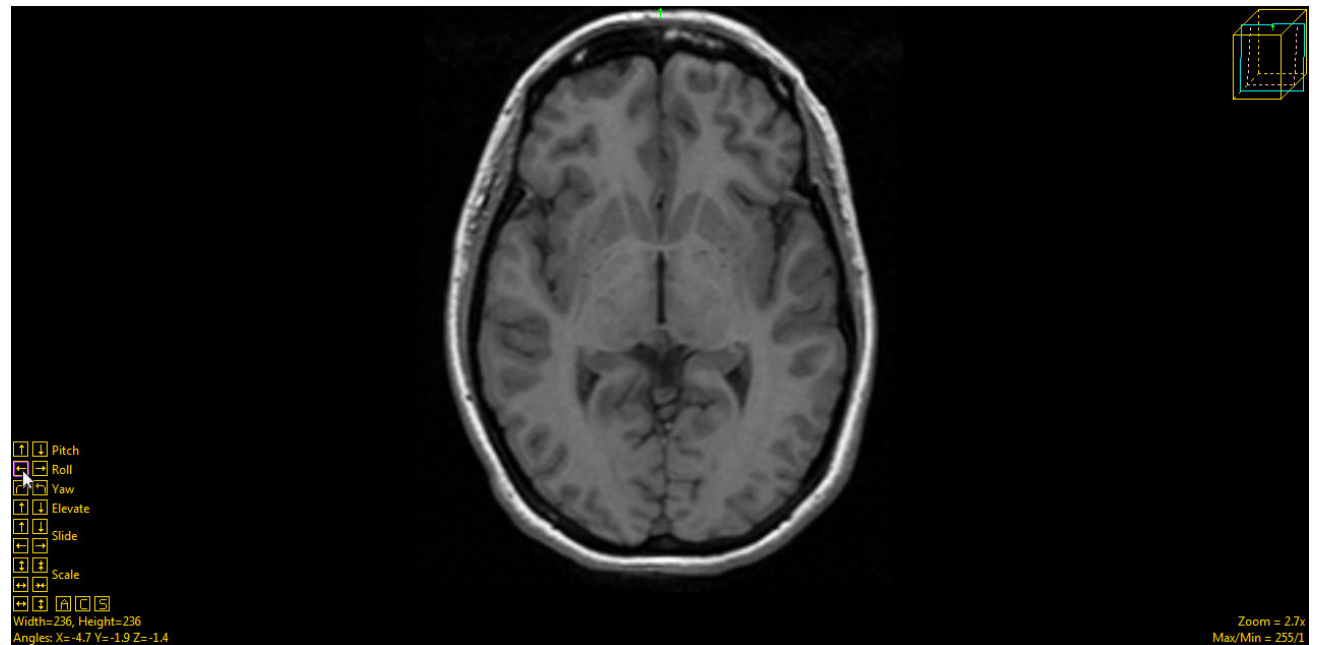


- The last adjustment to be made to the oblique plane will be done using the Roll function.
- Right-click on Roll and change the Rotation Increment to 1.





- Click on the left and right roll buttons to adjust the data set until the anatomical structures appear symmetrical.





- Click the Apply Matrix button to apply the transformation.

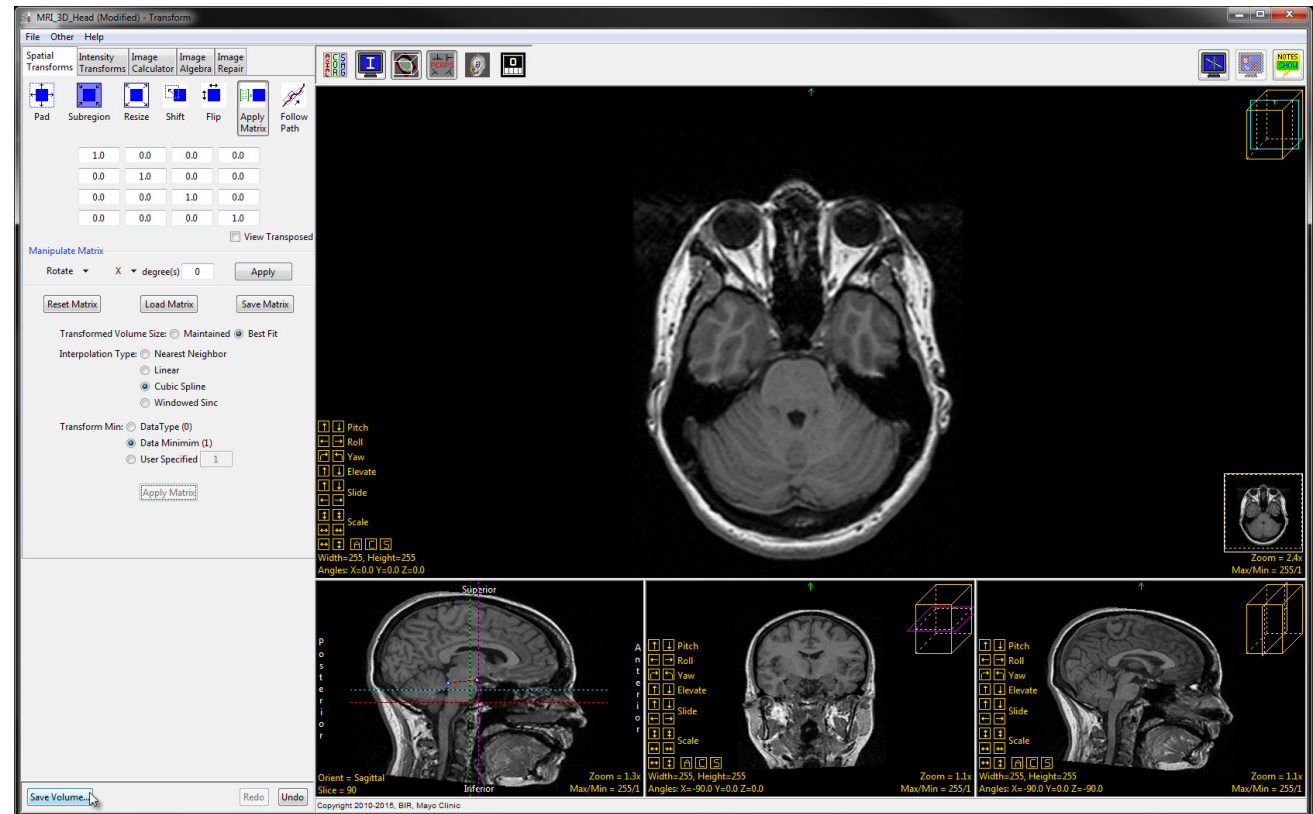
The screenshot shows the 'Apply Matrix' dialog box in the AnalyzePro software. At the top, there are icons for 'Pad', 'Subregion', 'Resize', 'Shift', 'Flip', 'Apply Matrix', and 'Follow Path'. Below these icons is a 4x4 matrix of input fields containing the following values:

0.999238	0.020560	-0.03315	0.106856
-0.01745	0.995657	0.091437	19.57691
0.034894	-0.09078	0.995258	-21.9265
0.0	0.0	0.0	1.0

Below the matrix is a checkbox labeled 'View Transposed' which is currently unchecked. Under the heading 'Manipulate Matrix', there is a 'Rotate' dropdown menu, a 'X' dropdown menu, a 'degree(s)' input field with the value '0', and an 'Apply' button. Below this section are three buttons: 'Reset Matrix', 'Load Matrix', and 'Save Matrix'. Further down, there are three radio button options for 'Transformed Volume Size': 'Maintained', 'Best Fit' (which is selected), and 'Linear'. Below these are three radio button options for 'Interpolation Type': 'Nearest Neighbor', 'Linear', 'Cubic Spline' (which is selected), and 'Windowed Sinc'. Below these are three radio button options for 'Transform Min': 'DataType (0)', 'Data Minimum (1)' (which is selected), and 'User Specified' with an input field containing the value '1'. At the bottom of the dialog is a large 'Apply Matrix' button with a mouse cursor pointing at it.

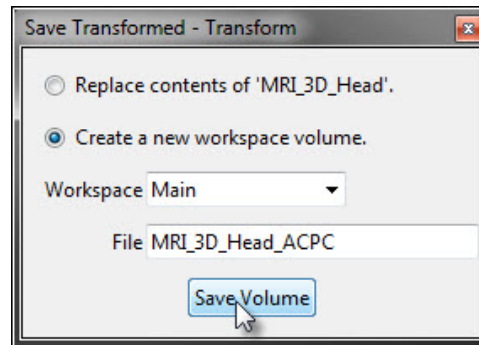


- Click Save Volume button at the bottom of left of the window to save the transformed data set.





- In the Save Transformed window, choose to create a new workspace volume.
- Rename the file and click Save Volume.
- Close Transform.



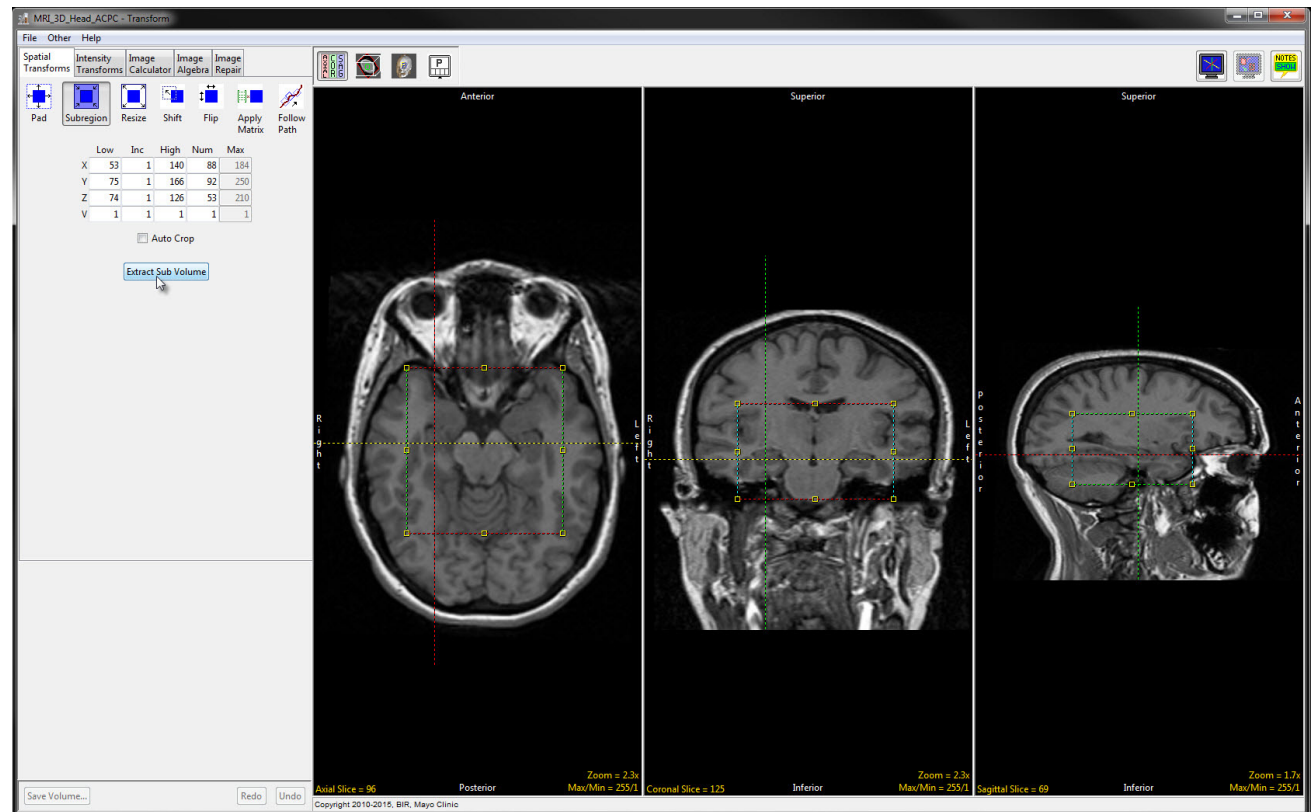
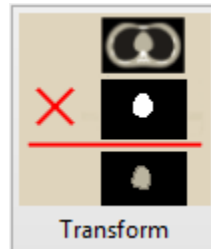


Cropping and Upsampling for Improved Segmentation

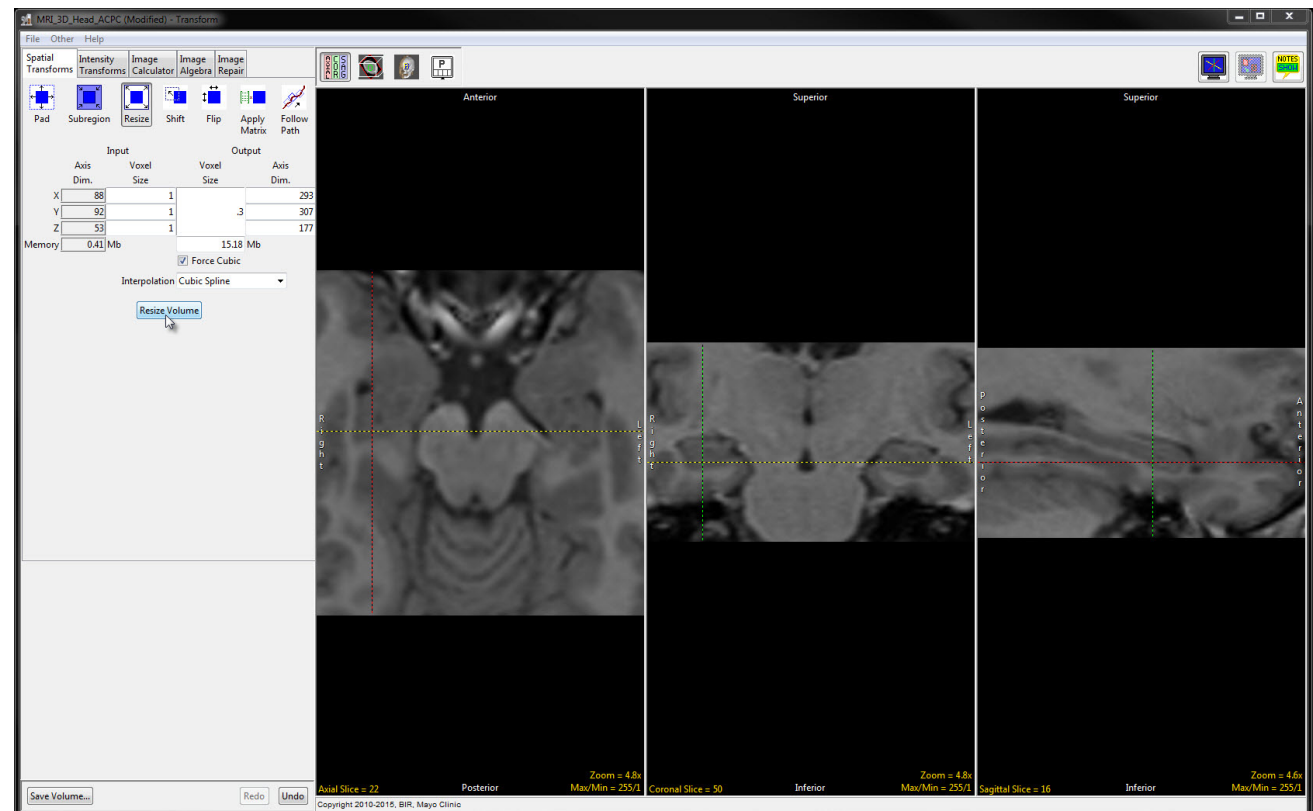
The hippocampus is a relatively small structure compared to the whole brain. For this reason, unless the data was taken at an extremely high resolution, the definition of the hippocampal region in the original data set will likely not be completely smooth due to large voxel size compared to the size of the hippocampus. To be able to more precisely define the hippocampus, the data can be upsampled (interpolated) to create a smaller voxel size.

However, upsampling the entire data set may result in too large a file size. To reduce the size of the data set, it can be cropped (subregioned). The cropping and upsampling steps are optional and can also be performed individually, depending on which are necessary for the data.

- Select the AC-PC aligned data and open Transform.
- Under the Spatial Transforms tab, select the Subregion tool.
- Click and drag the yellow control points around the image in all three orthogonal orientations to crop around the part of the brain containing the hippocampus.
- Click Extract Sub Volume to complete the crop.

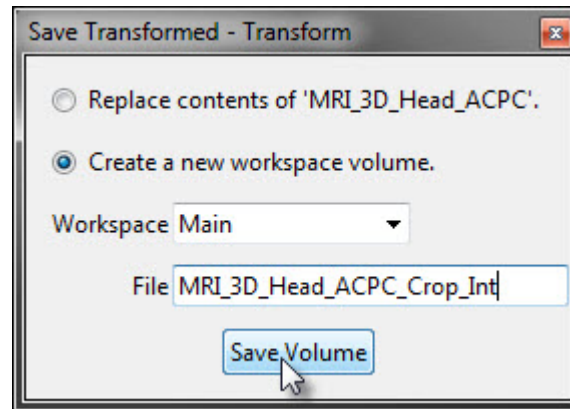


- To upsample the data, navigate to the Resize tool.
- Check the Force Cubic checkbox and change the voxel size to a smaller value.
- Use the drop-down menu to select an interpolation type. Cubic Spline and Windowed Sinc are recommended as the most likely to preserve the data as it is interpolated.
- Click Resize Volume to complete the interpolation.





- Click the Save Volume button in the bottom left of the window.
- In the Save Transformed window, choose to create a new workspace volume.
- Choose a workspace from the drop-down menu.
- Rename the file.
- Click Save Volume to save the transformed volume.
- Close Transform.



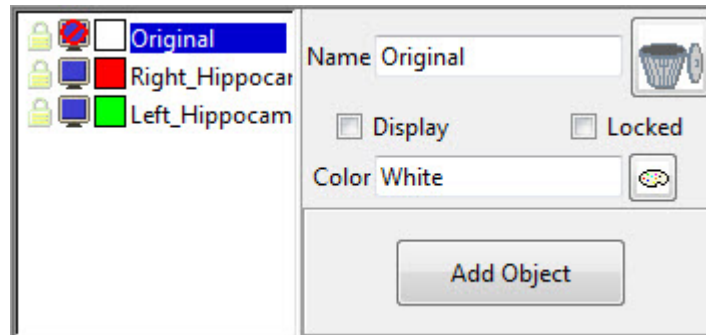
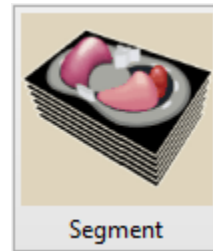


Manual Segmentation of the Hippocampus

The segmentation will be performed primarily in the coronal view, from rostral to caudal. The [EADC-ADNI Harmonized Protocol for Manual Hippocampal Segmentation](#) (HarP) requires 3D navigation for frequently checking anatomical structures against the sagittal and transverse views during the segmentation process. The linked cursor in Segment provides an optimal method for viewing structures in all orthogonal orientations.



- Open the AC-PC aligned, optionally preprocessed T1 MRI brain data set in Segment.
- Click the Add Object button in the bottom left of the window twice to add two new objects.
- Name the objects Right_Hippocampus and Left_Hippocampus.





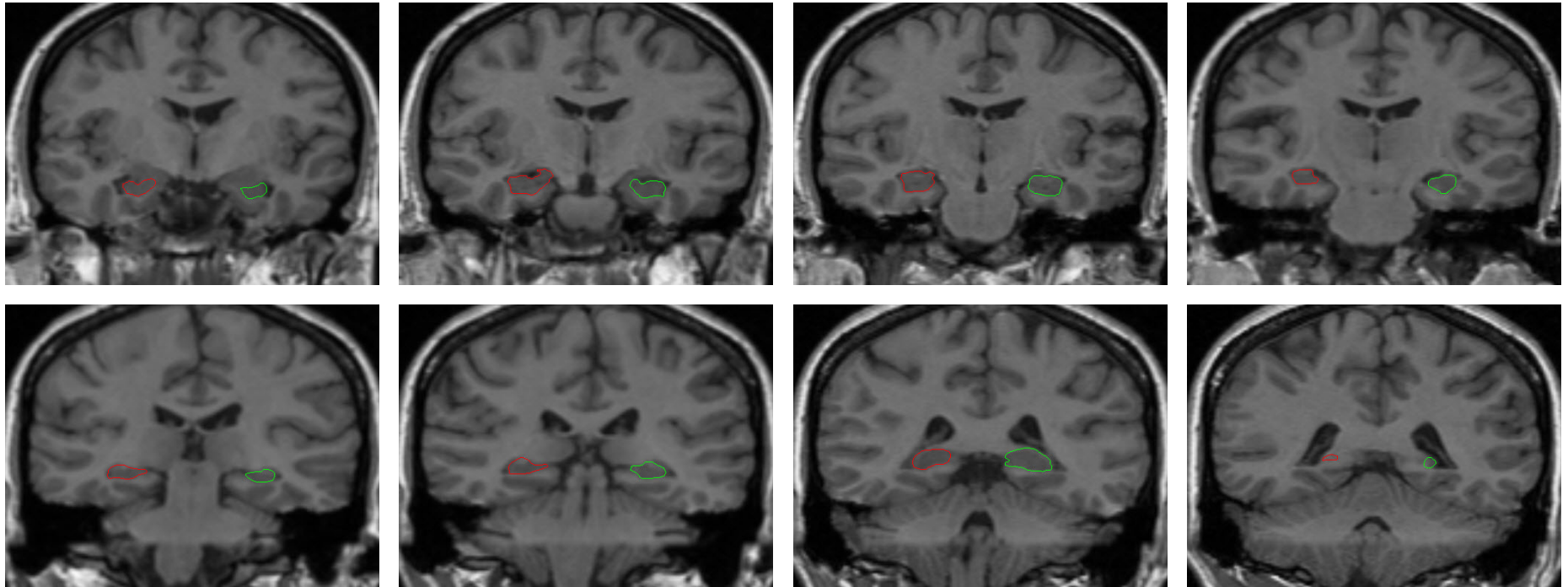
The segmentation method will not be described in anatomical detail here, as that is beyond the scope of this document. Please refer to the [EADC-ADNI protocol](#) for detailed instructions on manual hippocampus segmentation.

Note: AnalyzePro uses a left-handed coordinate system, so the sagittal images as seen in AnalyzePro are mirror images of the sagittal view as seen in the EADC-ADNI Protocol.


Use the manual trace tool to delineate hippocampal tissue. To do this, select the object to be defined, Right_Hippocampus or Left_Hippocampus, then click on the Manual Trace tool under the Manual tab, and trace around the hippocampus on the given coronal slice. Mistakes can be fixed by clicking the Undo button and retracing the contour. The Nudge Edit tool can also be used to expand the hippocampus contour (by clicking inside the hippocampus and dragging out along the inside of the contour) or to nudge the contour back from the outside (by clicking in the Original object and dragging along the outside of the contour). The size of the Nudge Edit tool can be adjusted by holding down the middle mouse button and moving the mouse up or down.

Move through the coronal slices, defining the Right_Hippocampus and Left_Hippocampus objects using the Trace tool and checking against anatomical landmarks as described in the EADC-ADNI protocol. Save the object map periodically during the segmentation process (File > Save Object Map).

A few representative coronal slices with the right and left hippocampal objects defined are shown below, from rostral to caudal.



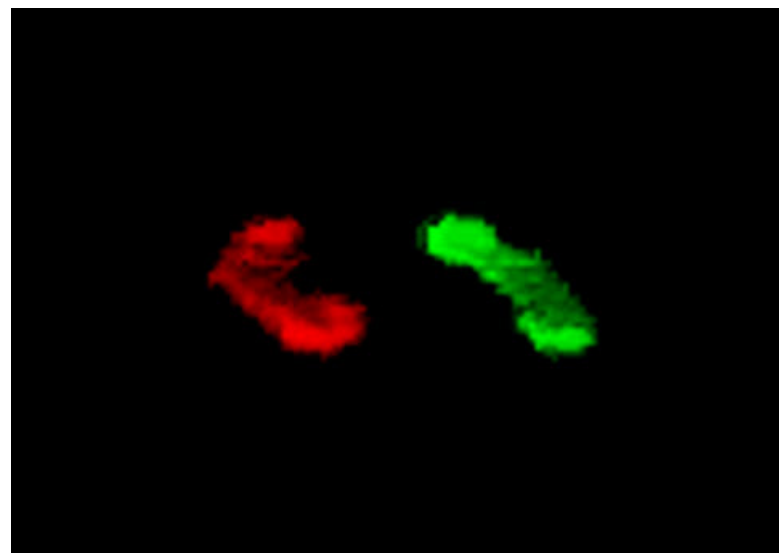
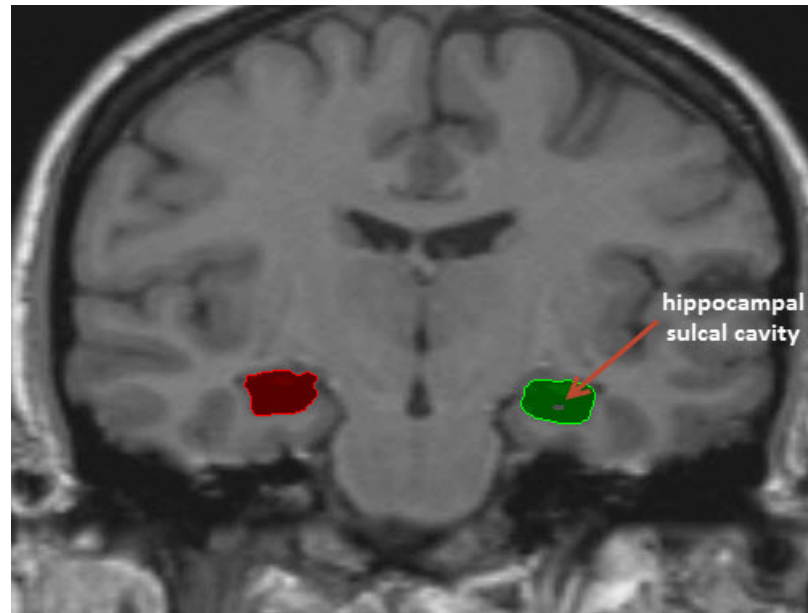


A hippocampal sulcal cavity³³⁻³⁵ appears on one of these slices, the segmentation of which can be more clearly seen by toggling the display using the Toggle Region Display button. 

To exclude a region from the hippocampus object, the Freehand Draw tool (under the Manual tab) can be used to assign such a cavity to the Original object.

An example rendering of the segmented hippocampi is shown below.

Note: Hippocampal subregions such as the tail can be manually segmented by adding further objects and assigning voxels to these objects using manual tools as described for the whole hippocampus above.

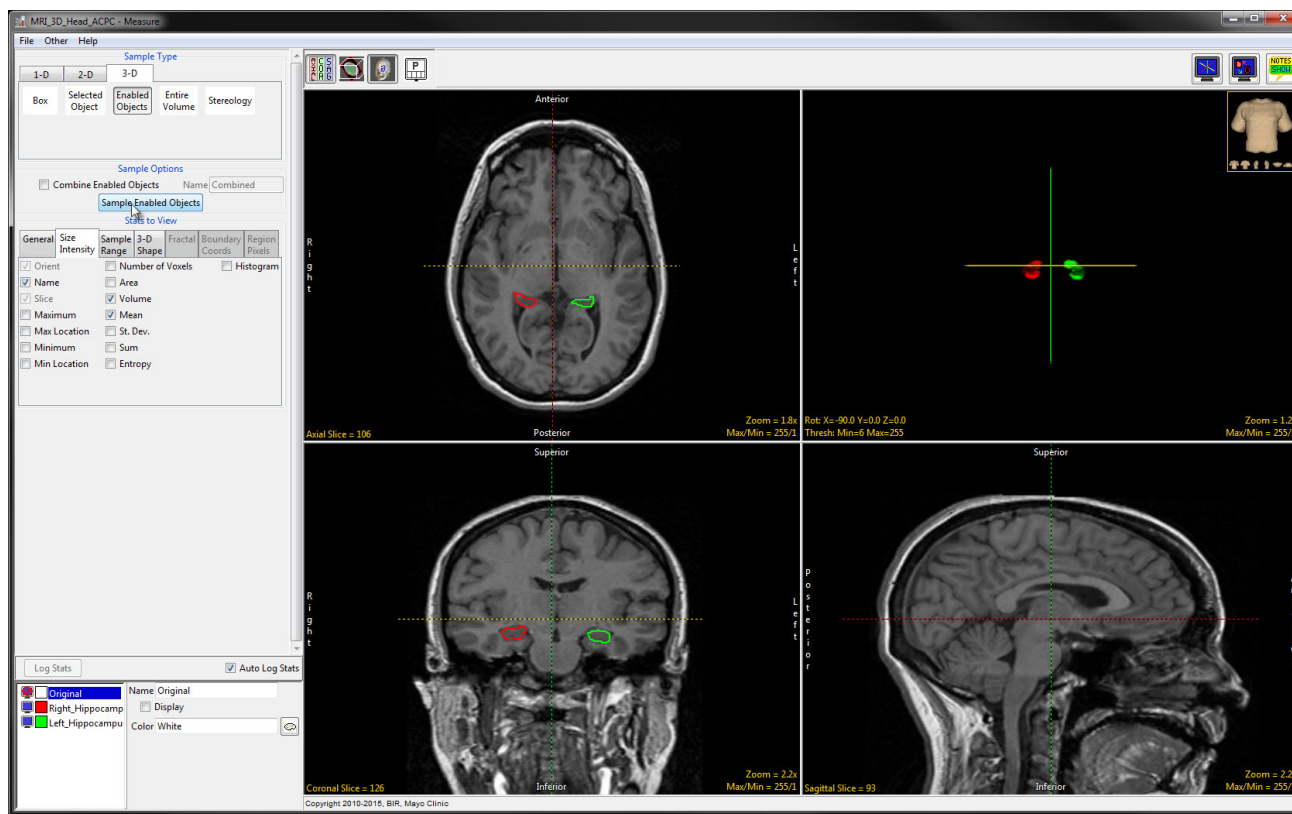
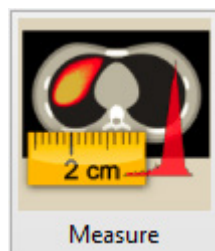




Measurement of Hippocampal Volume

As discussed in the introduction, the volume of the hippocampus is the measurement of interest.

- Select the data set and open Measure. Load the object map saved at the end of the segmentation (File > Load Object Map).
- Under the 3-D tab, select the Enabled Objects tool.
- Select the Size Intensity tab and make sure Volume is checked.
- Check the Auto Log Stats checkbox below the Stats to View area.
- Click Sample Enabled Objects to calculate the volume of each hippocampus.





Note: If subregions of the hippocampi were identified during the segmentation process, these objects can all be selected and their volumes separately measured. After the individual subregion volumes are measured and logged, all subregions for either the left or right hippocampus can be enabled and the Combine Enabled Objects checkbox checked. This measurement will generate the total hippocampal volume from the subregion volumes. Once this has been done for one side, the same can be done for the other side. A further option would be to add the subregion volumes in a separate analysis software such as Microsoft Excel. If this approach is used, a high enough number of decimal places should be chosen for the measurement to minimize rounding error in the sums.



- The volume of each hippocampus will be shown in the Stats box in the bottom left of the window, and recorded in the log below the images.
- The log can be saved by right-clicking.
- Click Save Log to output the measurements as a .stats file, which can then be opened in a spreadsheet and used for further analysis.



Acknowledgments

Thanks to Dr. Jerome Maller for his inputs regarding hippocampal segmentation and acquisition of hippocampal images. His knowledge of research in this area was extremely helpful in putting together this guide.

Dr. Maller can be found online at <http://www.maprc.org.au/dr-jerome-maller>.

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