









# **Table of Contents**

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## Introduction

Thalamic volume has been studied using MRI for a range of brain disorders. Reduced volume of the thalamus has been observed in schizophrenia, major depressive disorder, Alzheimer's disease, progressive supranuclear palsy, frontotemporal dementia and Huntington's disease<sup>1</sup>. Increased volume of the thalamus has been observed in untreated obsessive-compulsive disorder<sup>1</sup>.

A protocol for manual segmentation of the thalamus has been proposed by Power *et al.*<sup>1</sup>. This guide will provide instructions for using Analyze to segment the left and right thalami according to this protocol. This guide will also provide the steps to make volume measurements of the thalami using Analyze. For more specific guidelines as to the anatomical boundaries of the thalamus, please refer to the thalamus segmentation protocol.

# **Preprocessing Steps**

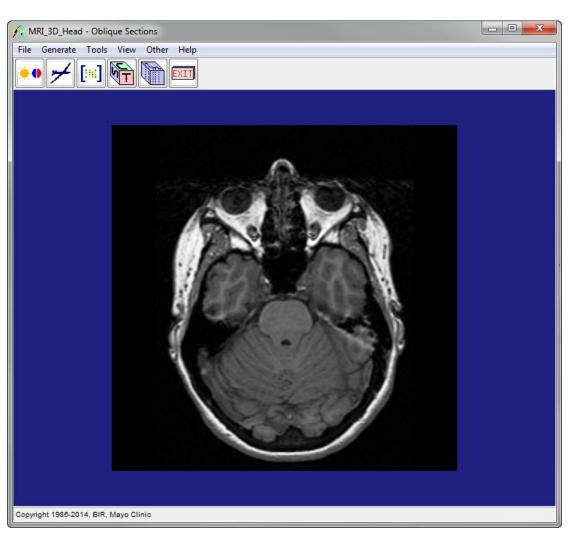
There are a number of data preprocessing steps that will aid in manual thalamic segmentation. It is recommended to reslice the data orthogonal to the AC-PC line for consistency in the segmentation process. In addition, upsampling the data through interpolation to create a smaller voxel size will allow you to define the thalamus more precisely.

#### Manual AC-PC Alignment

The thalamic segmentation protocol outlined by Power *et al.*<sup>1</sup> proceeds along the intercommissural plane. The brain data can be aligned along the AC-PC axis as described in the following section, or as described in the <u>Oblique Sections: Manual</u> <u>AC-PC Realignment</u> document. For users who have access to the Mayo 3D Brain Atlas add-on module, there is a tool specifically designed for easy AC-PC alignment of data, which is described in steps 3-8 of the <u>Mayo 3D Brain Atlas Training Guide</u> document.

Load the MRI\_3D\_Head.avw data set from the TutorialData folder or your MRI brain data set into

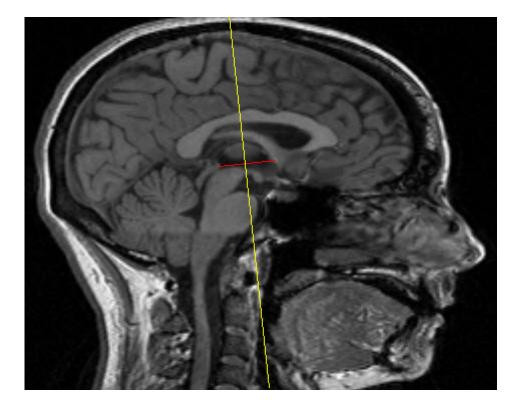




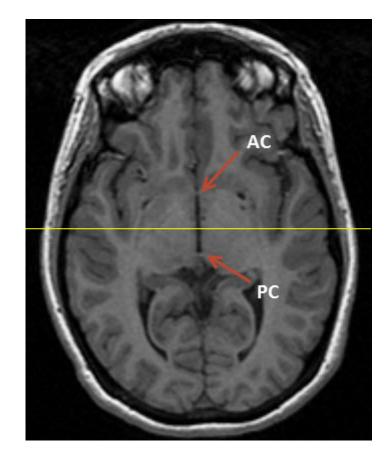
Open the Orthogonals tool from the Oblique Sections module window (**Tools > Orthogonals**). In order to better visualize the sagittal view in which the AC-PC axis will be defined, change the size of that view to double by right-clicking on the image, selecting Size, then selecting Double.



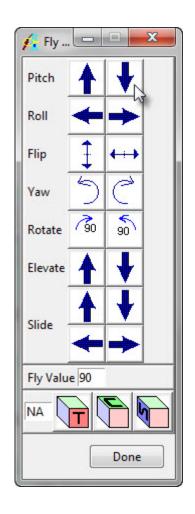
In the sagittal view, on the mid-sagittal slice, click on the PC, then drag the red line to the location of the AC and release the mouse button. The yellow line will be generated as the orthogonal plane. This step can be repeated if necessary to define the correct plane. If you wish to view the orthogonal plane interactively, check the Interactive Perp Axis option in the Orthogonals window.



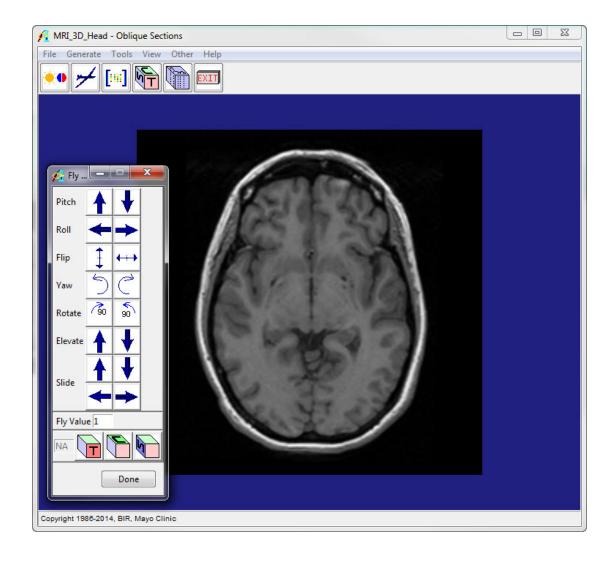
Correct AC-PC alignment can be checked for by viewing the transverse slice in the Orthogonals window. The AC and PC should both be clearly visible.



Open the Fly tool from the Oblique Sections window (**Generate > Fly**). Change the Fly Value to 90 degrees at the bottom of the Fly tool window, and click one of the Pitch buttons. The oblique image in the main Oblique Sections window should have the anterior part of the brain at the top. If it is upside-down, then click the same Pitch button twice to flip the oblique plane by 180 degrees.



Change the Fly value to a small value between 1-5 degrees. Use the Roll and Yaw buttons to make small adjustments to the oblique plane such that the brain structures appear symmetrical.



To output the data set resliced along the oblique plane, open the Output window (**File > Output**). Rename the data set and choose where to save the resliced data. Change the method to Reformat Entire Volume and select Change to best fit data. Click Generate Slices to output the reformatted data to the specified location.

| 🗘 Output - Oblique Sections 📃 💷 💌   |  |  |  |  |  |  |  |
|---|--|--|--|--|--|--|--|
| Destination () File () WorkSpace<br>Name MRI_3D_Head_ACPC                   |  |  |  |  |  |  |  |
| Method Reformat Entire Volum 💌  |  |  |  |  |  |  |  |
| Transformed volume size should be:<br>Maintained<br>Change to best fit data |  |  |  |  |  |  |  |
| ✓ Display Reformat ✓ Images ○ Object Map                                    |  |  |  |  |  |  |  |
| Generate Slices Done  |  |  |  |  |  |  |  |

#### Upsampling for Improved Segmentation

Interpolating between large voxels allows the voxel dimensions of the data set to be reduced, which allows for smoother curves to be defined during segmentation.

Open Load As (**File > Load As**) and drag the reformatted data set from the workspace into the File area of the window.

| 👫 Load As - Load As                 |                 |   |     |        | 23                                     |
|-------------------------------------|-----------------|---|-----|--------|--|
| File<br>Intensities Flip/Shift SubR | MRI_30_Head_ACP | /Orient Output                                |     |        | ************************************** |
| C Threshold                         | c               | <ul> <li>Intensity</li> <li>Output</li> </ul> |     | 2      |  |
| DataType<br>Maximum<br>Minimum      |                 |   | -   |        |  |
| Auto Exit After Load                | Invert          | [[  | Loi | ad Col | lormap                                 |
| Load Previ                          | ew              | Info  |     | Ca     | ancel                                  |

Navigate to the Resize tab and check the Force Cubic checkbox. Choose an interpolation type from the dropdown menu. Cubic Spline and Windowed Sinc are recommended as they will best preserve the data as it is interpolated.

You will need to choose a voxel size. To change the voxel size, click in the Voxel Size box and type in a new voxel size. The box showing the size of the data set will automatically update.

Click the Load button to load the data into the Analyze workspace. When prompted, click Create New to create a separate preprocessed data set. You may wish to rename the preprocessed data set once it is loaded, according to your own naming conventions.

| Intensities | Flip/Shift | SubRegion Resize | Pad/Orient Outpu | it 📲 |
|-------------|------------|------------------|------------------|------|
|             |            | Input            | Out              | tput |
|             | Axis       | Voxel            | Voxel            | Axis |
|             | Dim.       | Size             | Size             | Dim. |
| X           | 105        | 1                |                  | 350  |
| Y           | 79         | 1                | 0.3              | 263  |
| z           | 41         | 1                |                  | 137  |
| Memory      | 0.32       | Mb               | 12.03            | Mb   |
|             |            |                  | Force Cubic      |      |
|             |            | Interpolation    | Windowed Sinc    | -    |
|             |            |                  |                  |      |
|             |            |                  |                  |      |
|             |            |                  |                  |      |

## **Manual Segmentation of the Thalamus**

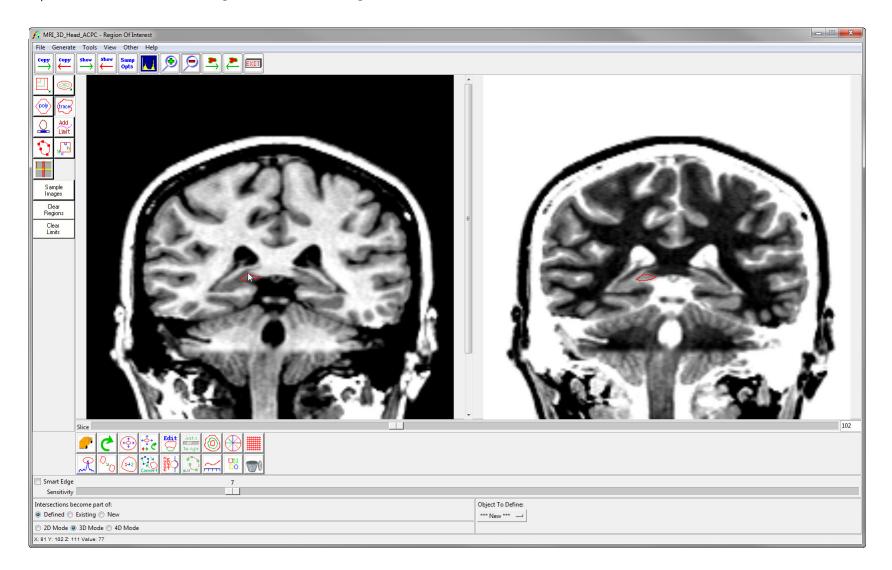
The segmentation will be performed in the coronal view, from caudal to rostral. In order to improve visualization of the thalamus, the data set and its inverse will be viewed side by side in the Region of Interest module.

Select the data set and open the Region of Interest module (Measure > Region of Interest). Open the Input / Output ports (File > Input/Output Ports). Drag the same data set into the Related Volume port. To invert the related volume, open the Intensity Related Volume window (View > Intensities > Related Volume) and check the Invert box. Adjust the intensity display range to improve the visibility of the thalamus. To adjust the intensity display range of the loaded volume (on the left), open the Intensity Loaded Volume window (View > Intensities > Loaded Volume).

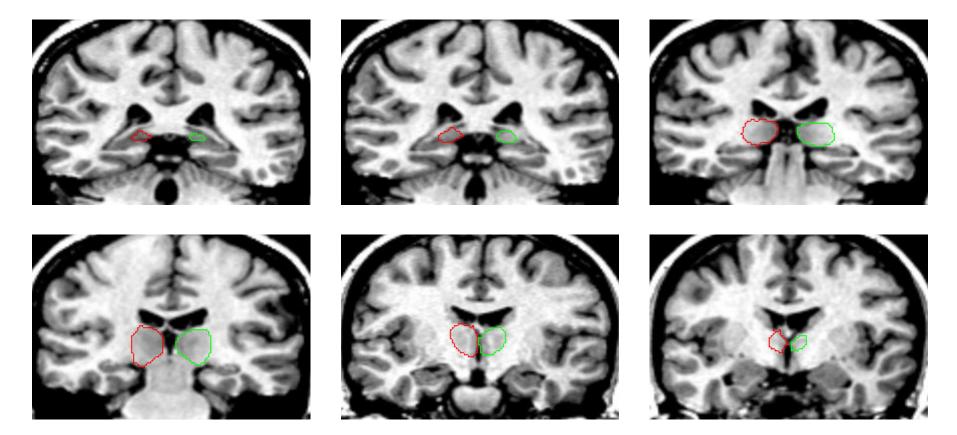
| 👍 Intensity Rela | ted Volume - Region Of Interest |            |
|------------------|---------------------------------|------------|
| Type: Window     | •                               |            |
| Max/Min          | Level/Width                     |            |
| Minimum          | 🔲 Histogram                     | Maximum    |
| 46               |                                 | 116        |
|                  | Range 🖲 Volume 🔘 DataType       | :          |
| Invert           |                                 | 🔽 Show Now |
| N2               |                                 | Done       |

Close the Input / Output ports (File > Input/Output Ports) and change the orientation to coronal (Generate > Orientation). Zoom in to the data using the Double Current Size button.

Choose the Manual Trace tool in the left toolbar, and trace around the right thalamus on the most caudal slice where it appears. The trace can be performed on either the original or inverted image.



After the first trace, change the Object to Define to 1.Object. Move through the slices using the + and – keys on the keyboard and continue to trace the right thalamus. A detailed protocol is outlined by Power *et al.*<sup>1</sup>. Once the right thalamus has been defined, change the Object to Define to \*\*\*New\*\*\* and trace the left thalamus on the most caudal slice where it appears. After the first trace of the left thalamus, change the Object to Define to 2.Object and continue to move through the slices until the left thalamus has been completely traced.



Review the object map in all orientations (**Generate > Orientation**) and make any adjustments necessary. If you wish, rename the objects to Right\_Thalamus and Left\_Thalamus in the Objects window. To do this, navigate to **View > Objects**, then choose Attribute, set Attribute to Name, and retype the object names. Save the object map (**File > Save Object Map**).

# **Measurement of Thalamic Volume**

To open the measurement tool, click **Generate > Sample Options**. In the Sample Options window:

- Set Sample Type to Object(s)
- Make sure the Right\_Thalamus and Left\_Thalamus objects are selected
- Set Summing to On
- Sample to All Slices
- Sequence Display to Off
- Choose the desired number of decimal places
- Set Log Stats to On
- Click Configure Log Stats

| 🖌 Sample Options - Region Of Interest 📃 💷 💌  |                                       |  |  |  |  |  |  |  |
|--|---------------------------------------|--|--|--|--|--|--|--|
| Sample Type       Image: Constraint of the second sec   |                                       |  |  |  |  |  |  |  |
|  | Select All Invert Selections          |  |  |  |  |  |  |  |
| Combine Objects:   No   Yes  |                                       |  |  |  |  |  |  |  |
| Minimum Sample Max/Min Maximum   |                                       |  |  |  |  |  |  |  |
| Range  Volume  Range  R |                                       |  |  |  |  |  |  |  |
| Summing  On  Off Auto Reset  On  Off   |                                       |  |  |  |  |  |  |  |
| Sample   |                                       |  |  |  |  |  |  |  |
| Stat Type       Intensity       2D Shape       3D Shape       Fractal         Boundary Coordinates       Region Pixels         Decimal Places       2  |                                       |  |  |  |  |  |  |  |
| Log Stats 💿 On 🔘   | Log Stats  On Off Configure Log Stats |  |  |  |  |  |  |  |
| Sample Images Done   |                                       |  |  |  |  |  |  |  |

The ROI Stats window will open, showing the measurements that can be generated. Uncheck the Area measurement, as this is not applicable.

| 🜈 ROI S  | itats - Region Of Interest   | - O X           |  |  |  |  |  |  |  |
|----------|------------------------------|-----------------|--|--|--|--|--|--|--|
| Plot Log | Plot Log                     |                 |  |  |  |  |  |  |  |
|          | File = MRI_3D_Head_ACPC      |                 |  |  |  |  |  |  |  |
|          | Object Map = MRI_3D_Head_ACP | C_Thalamus_Both |  |  |  |  |  |  |  |
|          | Vol. No. = 1                 |                 |  |  |  |  |  |  |  |
|          | Slice = 135                  |                 |  |  |  |  |  |  |  |
| <b>V</b> | Region Name = Original_sum   |                 |  |  |  |  |  |  |  |
|          | Maximum = 0.                 |                 |  |  |  |  |  |  |  |
|          | at (1, 1, 1)                 |                 |  |  |  |  |  |  |  |
|          | Minimum = 99999.             |                 |  |  |  |  |  |  |  |
|          | at (1, 1, 1)                 |                 |  |  |  |  |  |  |  |
|          | Mean = 0.                    |                 |  |  |  |  |  |  |  |
|          | St. Dev. = 0.                |                 |  |  |  |  |  |  |  |
|          | Sum = 0.                     |                 |  |  |  |  |  |  |  |
|          | Entropy = 0.                 |                 |  |  |  |  |  |  |  |
|          | Number Of Voxels = 0         |                 |  |  |  |  |  |  |  |
|          | Area = 0. mm2                |                 |  |  |  |  |  |  |  |
|          | Volume = 0. mm3              |                 |  |  |  |  |  |  |  |
|          | < 1 = 0                      |                 |  |  |  |  |  |  |  |
|          | > 255 = 0                    |                 |  |  |  |  |  |  |  |
|          | >= 1 & <= 255 = 0            |                 |  |  |  |  |  |  |  |
|          | Mean In Range = 0.           |                 |  |  |  |  |  |  |  |
|          | St. Dev. In Range = 0.       |                 |  |  |  |  |  |  |  |
|          | Sum In Range = 0.            |                 |  |  |  |  |  |  |  |
|          | Area In Range = 0. mm2       |                 |  |  |  |  |  |  |  |
|          | Volume In Range = 0. mm3     |                 |  |  |  |  |  |  |  |
|          | BAP = 0.                     |                 |  |  |  |  |  |  |  |
| Log      | Stats                        | Done            |  |  |  |  |  |  |  |

Click the Sample Images button at the bottom of the Sample Options window. This will initiate the measurement routine. The ROI Stat Log window will open, showing the two objects, the mean intensity value for each object and the standard deviation of intensity, as well as the number of voxels belonging to each object and the corresponding volume in cubic millimeters.

| f.     | ROI          | Stat         | Log - Re        | gion Of Interest   |          |          |        |         | x |
|--------|--------------|--------------|-----------------|--|----------|----------|--------|---------|---|
| #<br># | Vol:<br>Samj | umel<br>plel | File=<br>Max= 2 | 1:27:09 CDT 2015<br>MRI_3D_Head_ACPC<br>255 SampleMin= 1<br>1 VoxelHeight= 1 Vox | elDepth= | 1        |        |         | ~ |
| # #    | Vol          | _# :         | Slice           | Name   | Mean     | Std.Dev. | Voxels | Vol_mm3 |   |
| T      |              | 1            | 250             | Right Thalamus sum   | 93.55    | 9.78     | 6872   | 6872.00 |   |
|        |              | 1            | 250             | Left_Thalamus_sum  | 95.90    | 7.96     | 6504   | 6504.00 |   |
| •      | Si           | ave          |                 |  |          |          |        | Dor     | Þ |

Click Save to output the measurements as a .stats file, which can then be opened in a spreadsheet and used for further analysis.

### References

 Power, B. D. et al. Validation of a protocol for manual segmentation of the thalamus on magnetic resonance imaging scans. Psychiatry Res. Neuroimaging 1–8 (2015). doi:10.1016/j.pscychresns.2015.02.001

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