

CSP Segmentation Protocol Using 3D Slicer

Objective:

This protocol details the procedure for manually segmenting the **Cavum Septum Pellucidum (CSP)** in MRI scans using the program **3D Slicer**.

Step 1: Create New Segmentation

1.1 Open 3D Slicer and Add Data

- Launch **3D Slicer** on your workstation. At the top of the screen, click **File** and **Add data**.
- Choose the designated T1 image for upload (“.....nii.gz file”) and ensure **Volume** is selected under the description tab.

1.2 Select the Segment Editor Module

- From the **Module Finder**, search for and select the **Segment Editor** module. This is where all segmentation-related tasks will be performed.

1.3 Create a New Segmentation

- In the **Segment Editor**, click **Create new segmentation** to initialize the segmentation process.
- Select the current file name as the **Source volume**.
- Click **Add**, and rename your segment per the following naming convention:
 - “Segment_#####_XX”
 - ##### is the file case number, XX will be your first and last initial

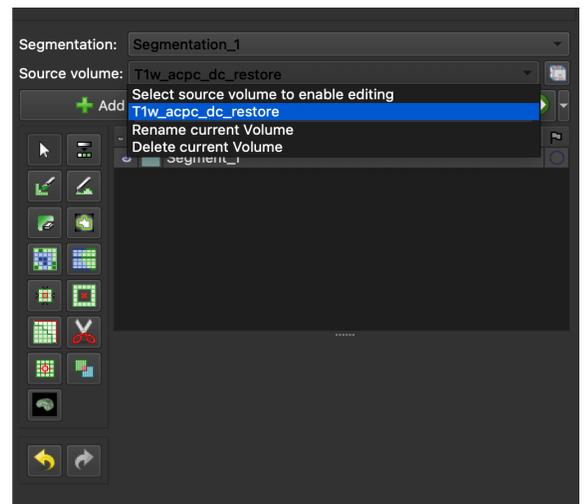


Figure 1

Step 2: Locate the CSP on coronal views

2.1 Find the Slice with the Largest CSP

- Focusing on the **coronal view** (*green*), navigate through the slices.
- Locate the slice where the CSP appears largest and has the highest contrast compared to the surrounding regions.
- This slice will serve as your reference for initial thresholding; take note of the alternate views and position within the brain.



Step 3: Apply Thresholding for Initial Segmentation

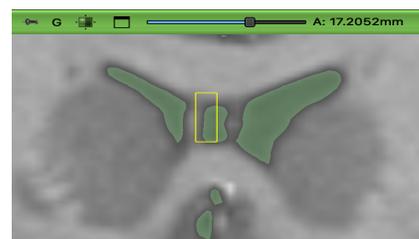
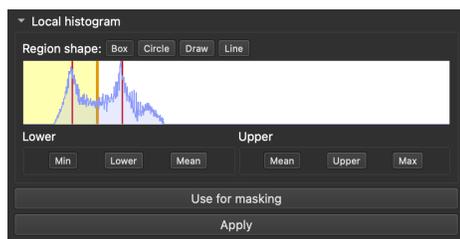
3.1 Open the Threshold Tool

- In the **Segment Editor**, click the **Threshold** tool to define an intensity range for the CSP.



3.2 Applying Proper Threshold and Masking Region

- Navigate to the lower portion of the menu, and expand the **Local Histogram** section.
- Select **Box** and use the cursor to select a portion of the CSP.
 - The portion selected should create a histogram with two distinct peaks on the low and high intensity ends of the histogram, and reflect an accurate voxel intensity composition per the visually confirmed CSP. The placement of the box should optimally include equal parts of the CSP and the adjacent structure. Finding the optimal position to place the histogram box will require trial and error. Note that separate histograms should be used for CSF-white borders and CSF-gray borders.

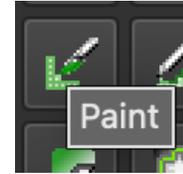


- Using the cursor, click on the vertical axis of the right peak within the histogram and drag until the cursor reaches the vertical axis of the left peak of the histogram.
- Select **Min** as the lower bound, and **Mean** as the upper bound.

Step 4: Create the Segment

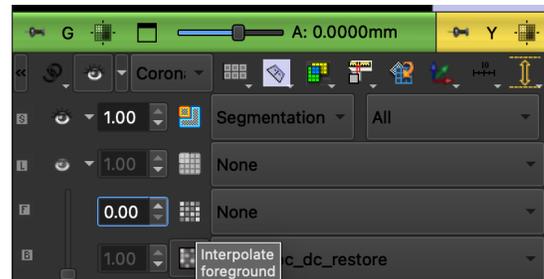
4.1 Select Segmentation Tool

- Choose the **Paint Tool** in the Segment Editor and highlight the CSP on your initial reference slide for thresholding.
- Navigate to slices both anterior and posterior to the initial reference slide and complete the same process until the CSP has been fully segmented.



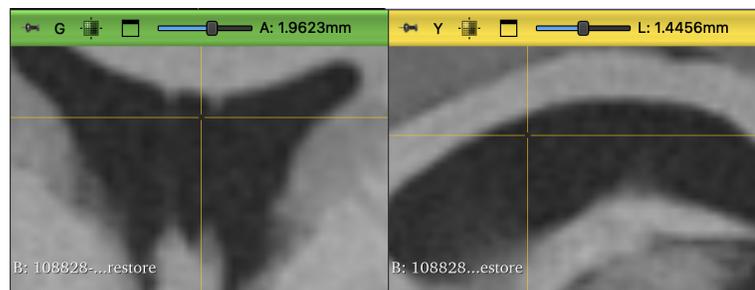
4.2 Quality Check the Segmentation

- The threshold will guide the segmentation, but manual adjustments may be necessary:
 - In the threshold editor, **turn off the Editable Intensity Range** to avoid unintentionally altering the threshold during manual editing.
 - **Enable/Disable Interpolated View** by clicking the pin icon followed by the double arrows, and lastly the interpolate background icon to enhance voxel visibility.
 - **Analyze the voxel intensities:** Look for outliers or unmarked regions that could be part of the CSP.
 - Use **Zoom and Slice Views** to examine voxel intensities from different planes.



4.3 Check for Intensity Outliers and Questionable Voxels

- Large CSPs that do not appear to have a posterior border are considered as a separate category – the cavum vergae. Note this, and exclude such cases from segmentation of the CSP.
- If you identify voxels that are not continuous with the thresholded segment but are likely to be CSP, continue as follows.
 - First, repeat the thresholding process detailed in step 3.2 and attempt to isolate the questionable region using the threshold tool. If able to do so, select the voxel as



part of the segment. If unable to confirm the aberrant voxel through thresholding, do not include it in the segment, however take note for future discussion and peer review. To aid in this analysis, use the **crosshair tool** (top right of toolbar) to locate the voxel and compare its intensity relative to surrounding tissue in different views.

Step 5: Refinement of the CSP

5.1 Examine Anterior-Posterior Regions

- Repeat the quality control process and focus on the **anterior** and **posterior** ends of the CSP. For both the anterior and posterior borders, segmentation based on thresholding in the sagittal view may be useful; there are more voxels in this view that may allow for a better threshold estimate.



Step 6: Review and Finalize Segmentation

6.1 Inspect Segmentation and Validate Shape

- Review the segmented region in **zoomed-out coronal, sagittal, and axial views**, as well as the **3D model**.
- Inspect the overall shape of the segmented CSP in a non-interpolated view to ensure the segmentation creates natural contours without jagged or irregular boundaries of the CSP.
- Check for any inconsistencies, gaps, or regions where the segmentation does not match the expected anatomy.
- For questionable voxels attempt to confirm inclusion through the above criteria. If any questions persist, take note for further discussion and agreement with study staff.

6.2 Use the Segment Statistics module to obtain a volume.

6.3 Save Segmentation

- Save the file using the following naming convention:
 - “#####_CSP_XX”
 - ##### is the file case number, XX will be your first and last initials.