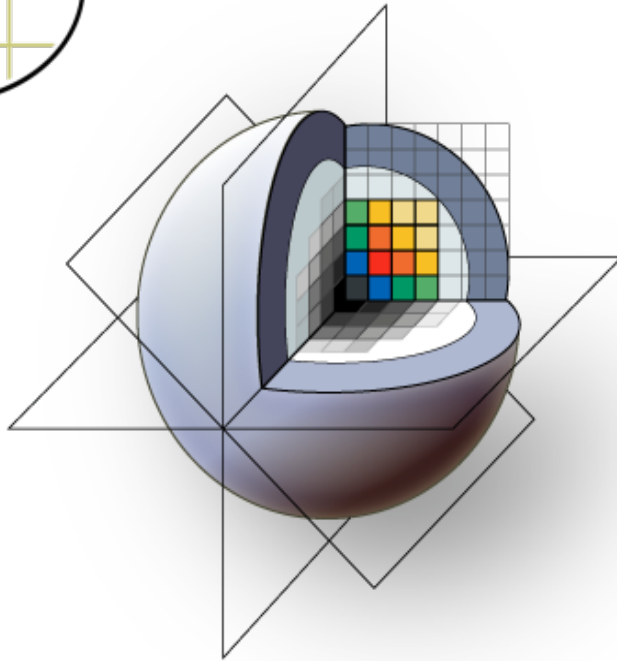




Slicer3 Training Compendium

Slicer3 Training Tutorial Using EM Segmenter with Non- Human Primate Images



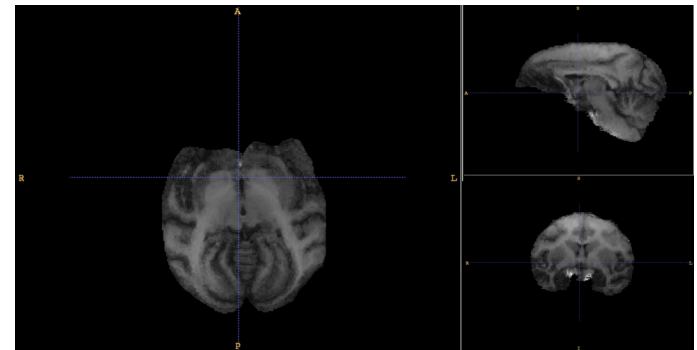
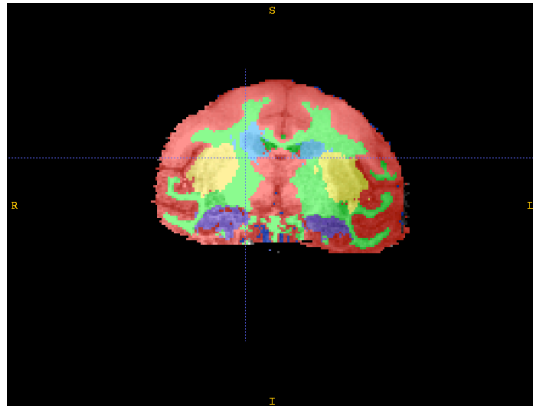
Vidya Rajagopalan
Christopher Wyatt
BioImaging Systems Lab
Dept. of Electrical Engineering
Virginia Tech

Learning Objective

The objective of this tutorial is to demonstrate how to use EM Segmenter to segment non-human primate images.

Non-human primates are widely used as models for human neuro-anatomical studies. Segmentation of primate MR images are critical to many of these studies.

We have used examples of vervet T1 images in this tutorial but the procedure has been used successfully for other species as well.





Prerequisites

This tutorial assumes that you have already completed the tutorial **Data Loading and Visualization**. Tutorials for **Slicer3** are available at the following location:

- **Slicer3** tutorials

<http://www.na-mic.org/Wiki/index.php/Slicer3.2:Training>



Prerequisites

We have developed two command-line tools for this procedure:

- i. MaskImage - Uses a binary image to mask required input image
- ii. RescaleIntensity - Rescale the intensity range of an image between user-specified lower and upper limits

These are available for download (using subversion) from:

<https://bsl-1.ece.vt.edu/svn/BSL-Slicer3-Modules/>

These tools can be installed by following the tutorials at:

http://wiki.na-mic.org/Wiki/images/4/46/Slicer3CourseForDevelopers_SPujol.ppt



Prerequisites

This procedure requires the use of a non-registration method. We recommend the use of Diffeomorphic Demons method which is available in Slicer3:

It can be obtained in two ways:

- i. CLI module in the latest developmental version of Slicer3 (Slicer3.3 Alpha).
- ii. As a part of Slicer3 NITRC modules, downloadable from: <http://www.nitrc.org/projects/brainsdemonwarp/>

In this tutorial we use the CLI module available in Slicer3.3 Alpha

Diffeomorphic Demons is also available from: <http://hdl.handle.net/1926/510>



Materials

This tutorial requires the installation of the **Slicer3** software and the tutorial dataset. They are available at the following locations:

- **Slicer3** download page (***Slicer 3.2***)

<http://www.slicer.org/pages/Downloads/>

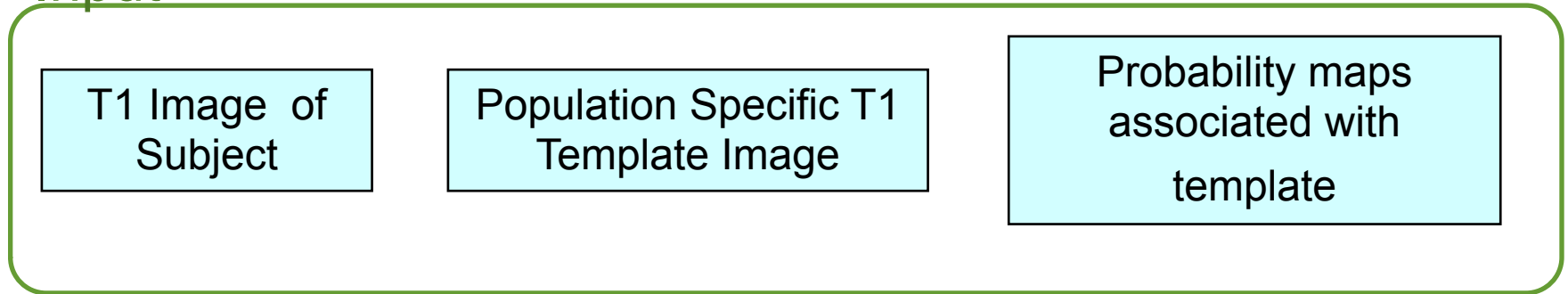
- Tutorial dataset (***Vervet Slicer Tutorial***)

http://www.bsl.ece.vt.edu/data/vervet_atlas/vervet.php

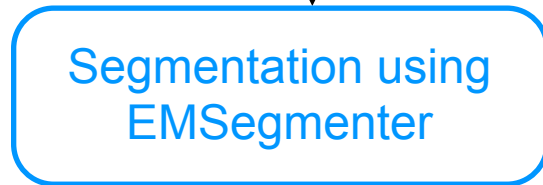
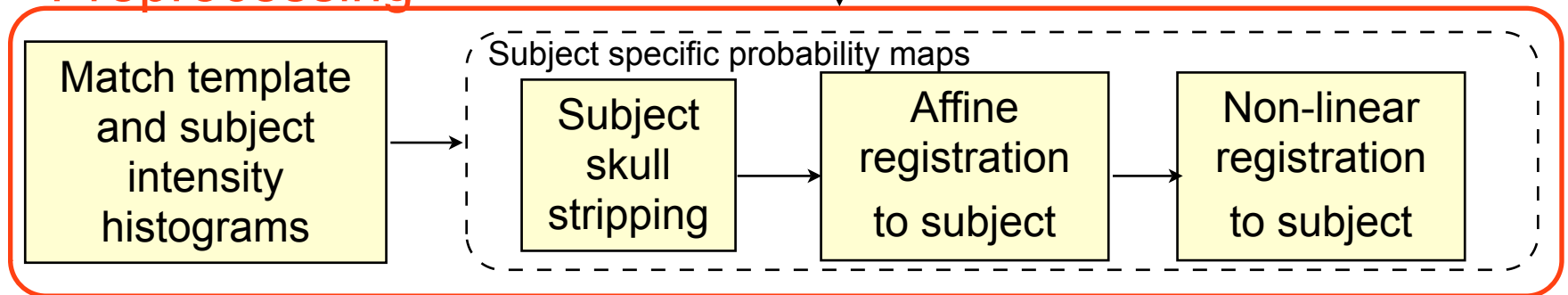
Disclaimer: *It is the responsibility of the user of Slicer to comply with both the terms of the license and with the applicable laws, regulations, and rules.*

Segmentation Procedure

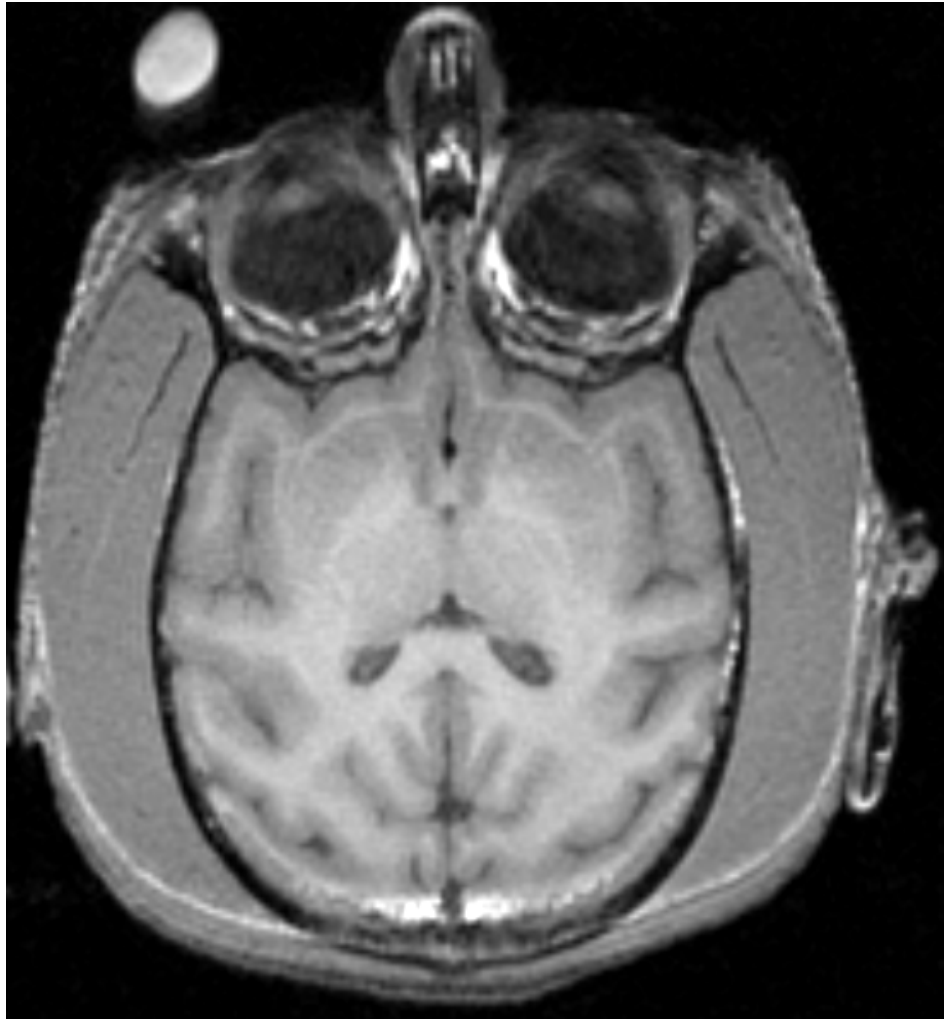
Input



Preprocessing



Input for Segmentation



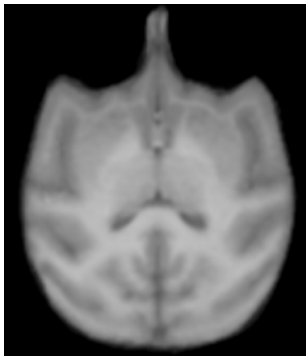
- The image to be segmented is the primary input.
- In this tutorial we deal with segmenting the T1 image of a vervet subject.
- This can be extended to multi-channel segmentation using the example in: http://wiki.na-mic.org/Wiki/images/2/2f/AutomaticSegmentation_SoniaPujol_Munich2008.ppt
- The subject T1 volume is loaded into Slicer.



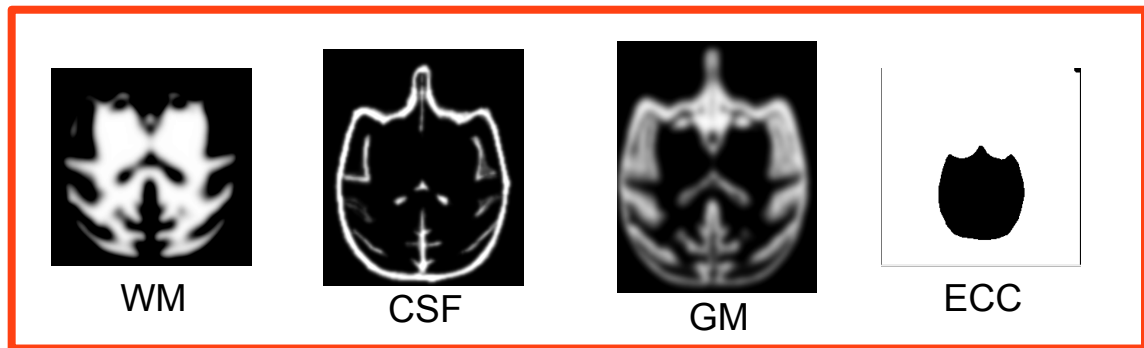
Input - Template Image and Probability Maps

- We now load the vervet template image and tissue probability maps
- In this tutorial we have used the template and probability maps available for download from:

http://www.bsl.ece.vt.edu/data/vervet_atlas/vervet.php



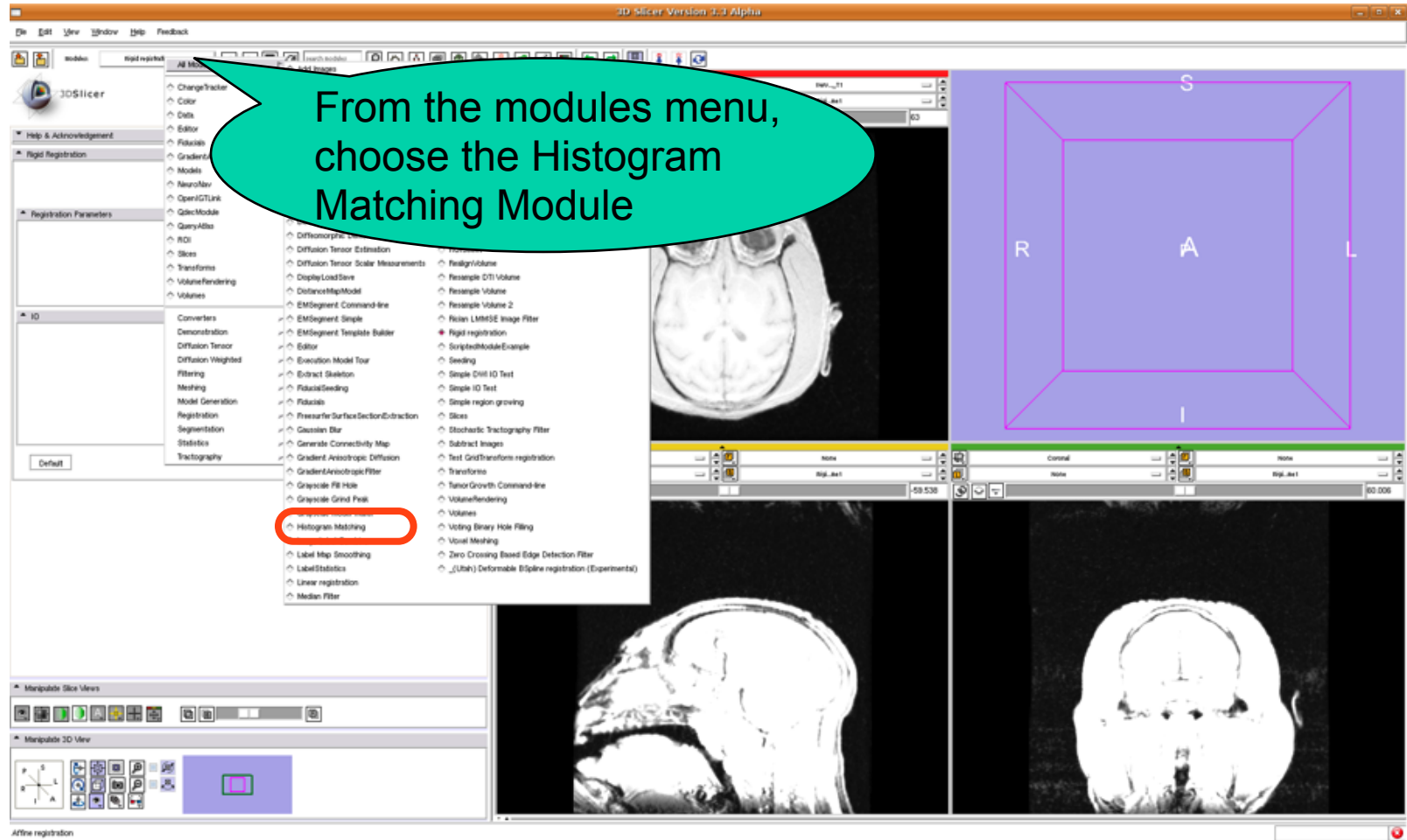
Template Image



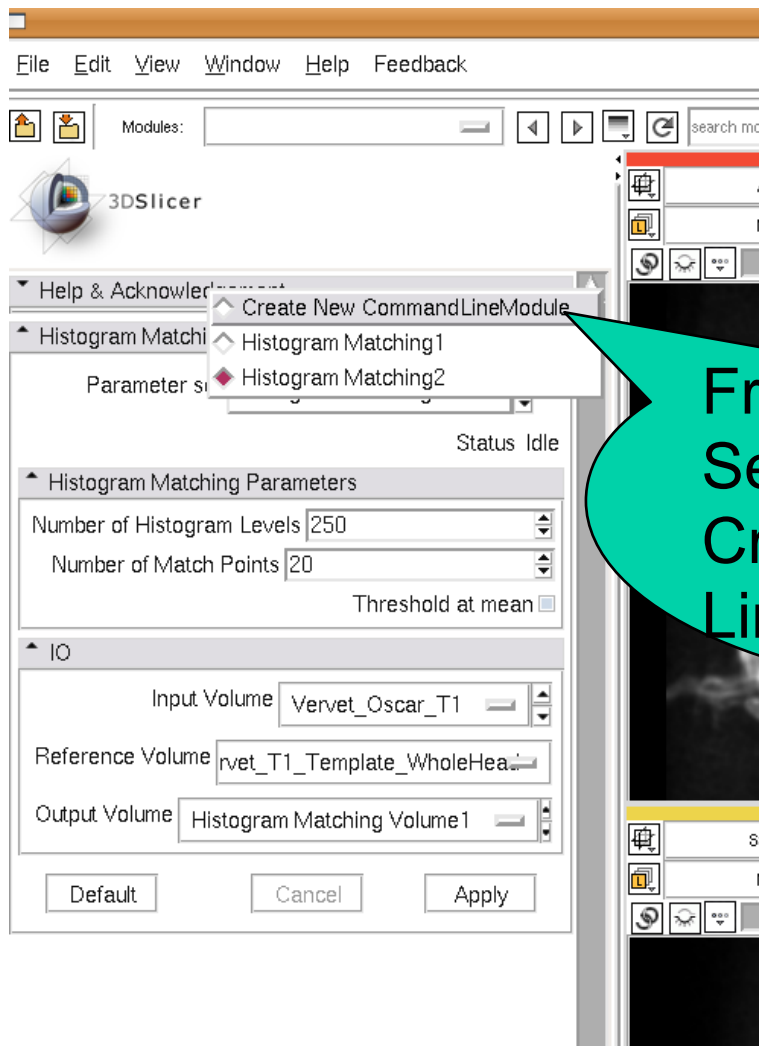
Probability Atlas

Preprocessing

Histogram Matching

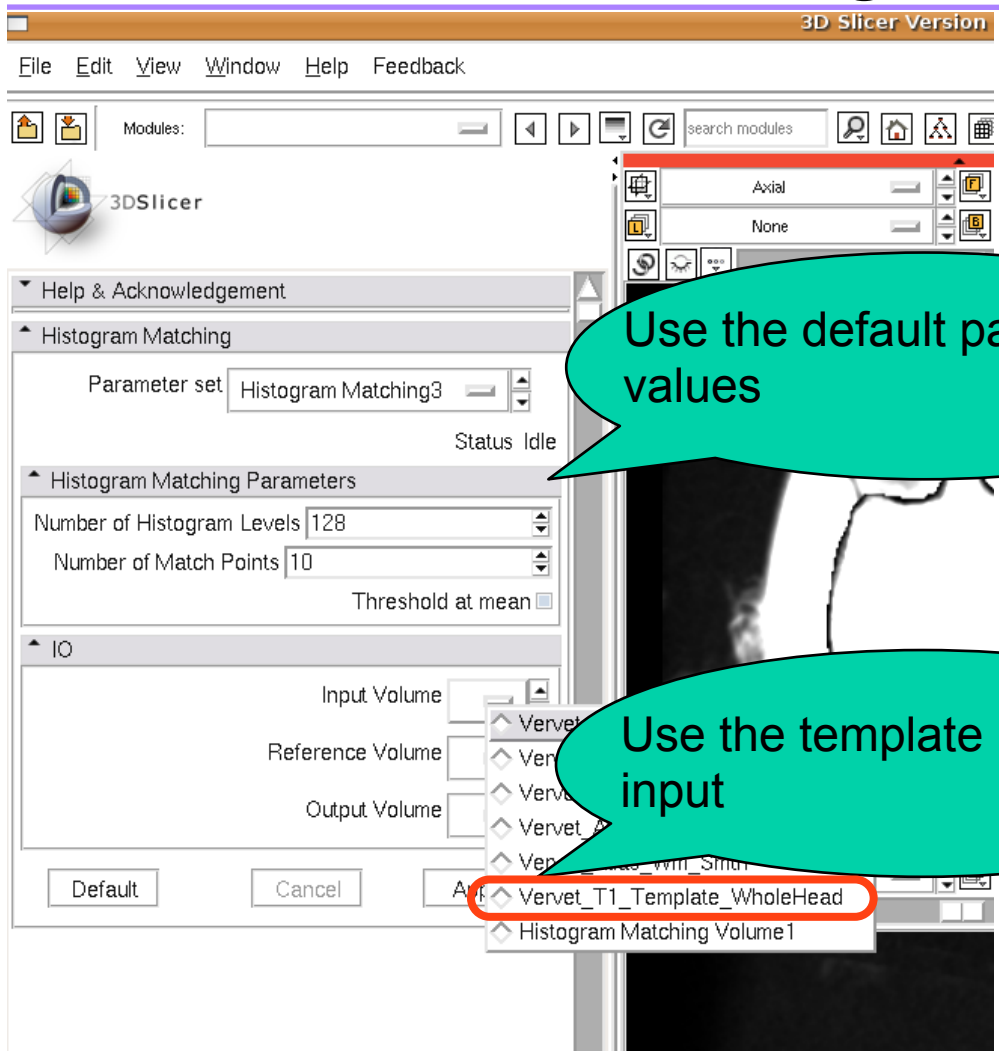


Histogram Matching



From the Parameter Set menu choose Create New Command Line Module

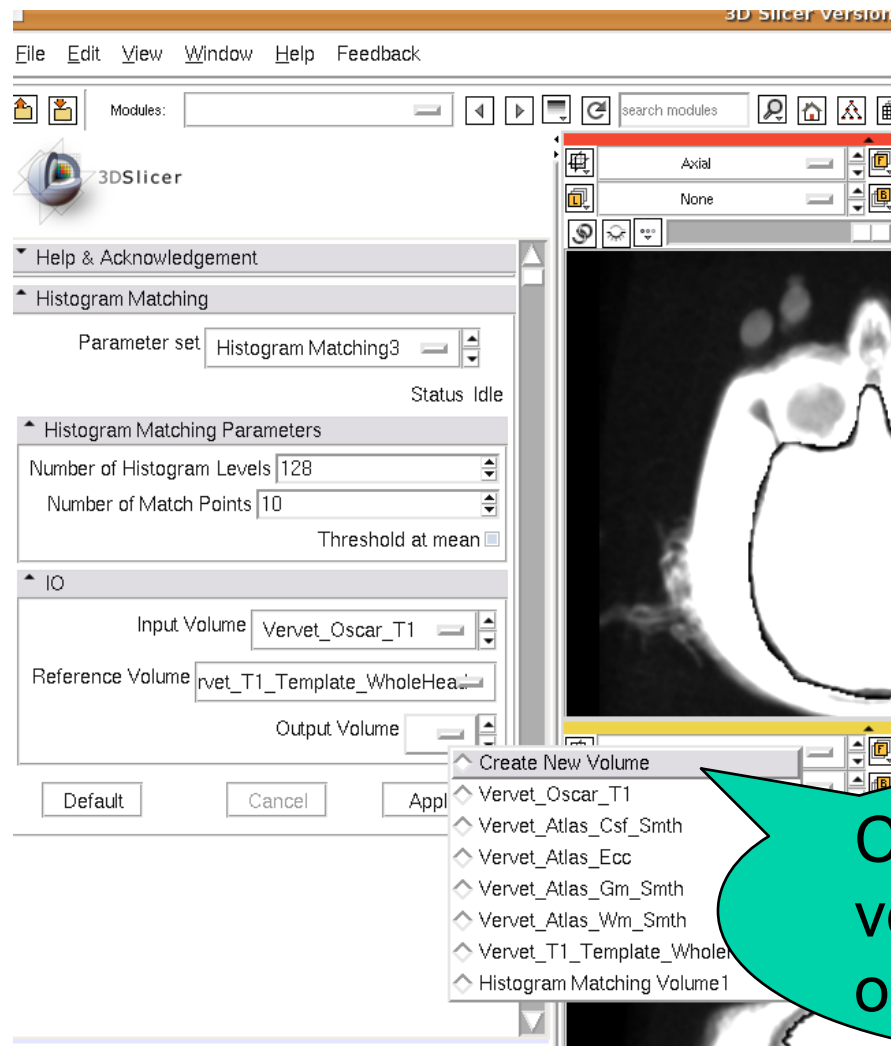
Histogram Matching



Use the default parameter values

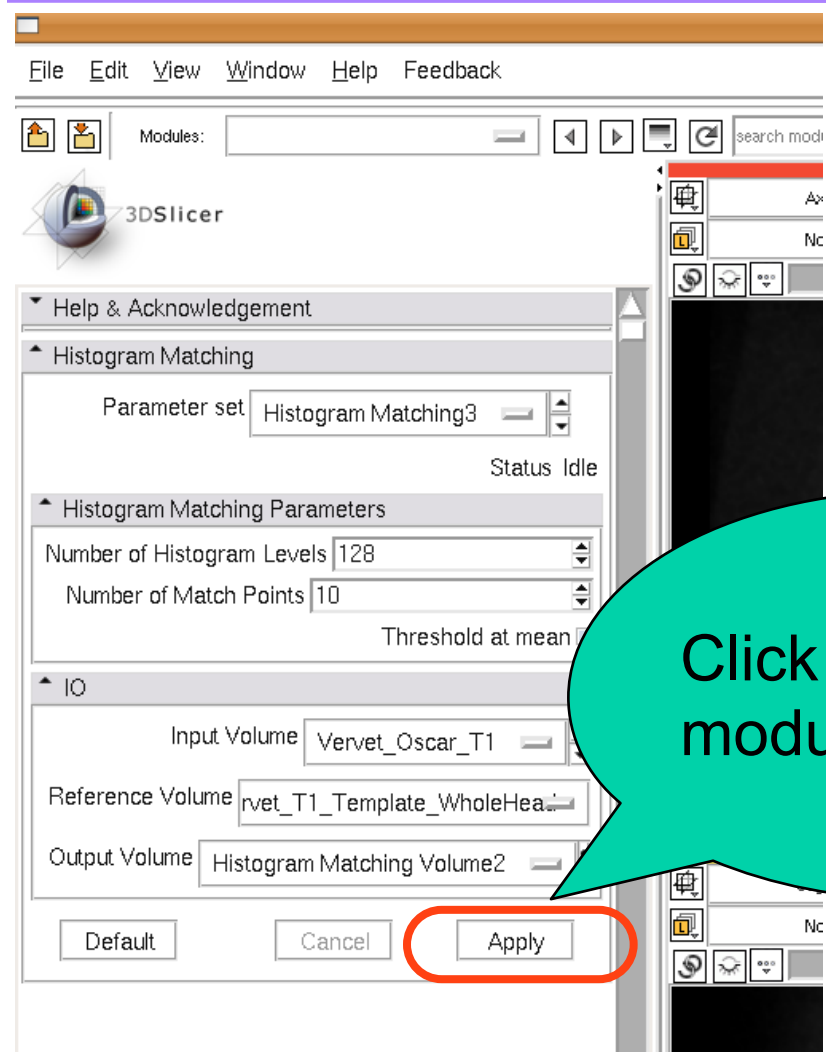
Use the template image as input

Histogram Matching



Create a new volume for the output image

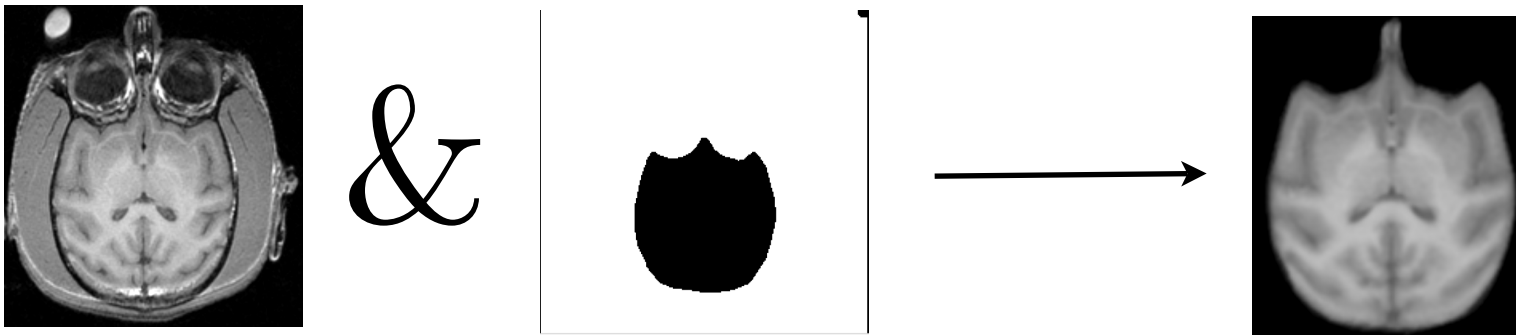
Histogram Matching



Click Apply to run module

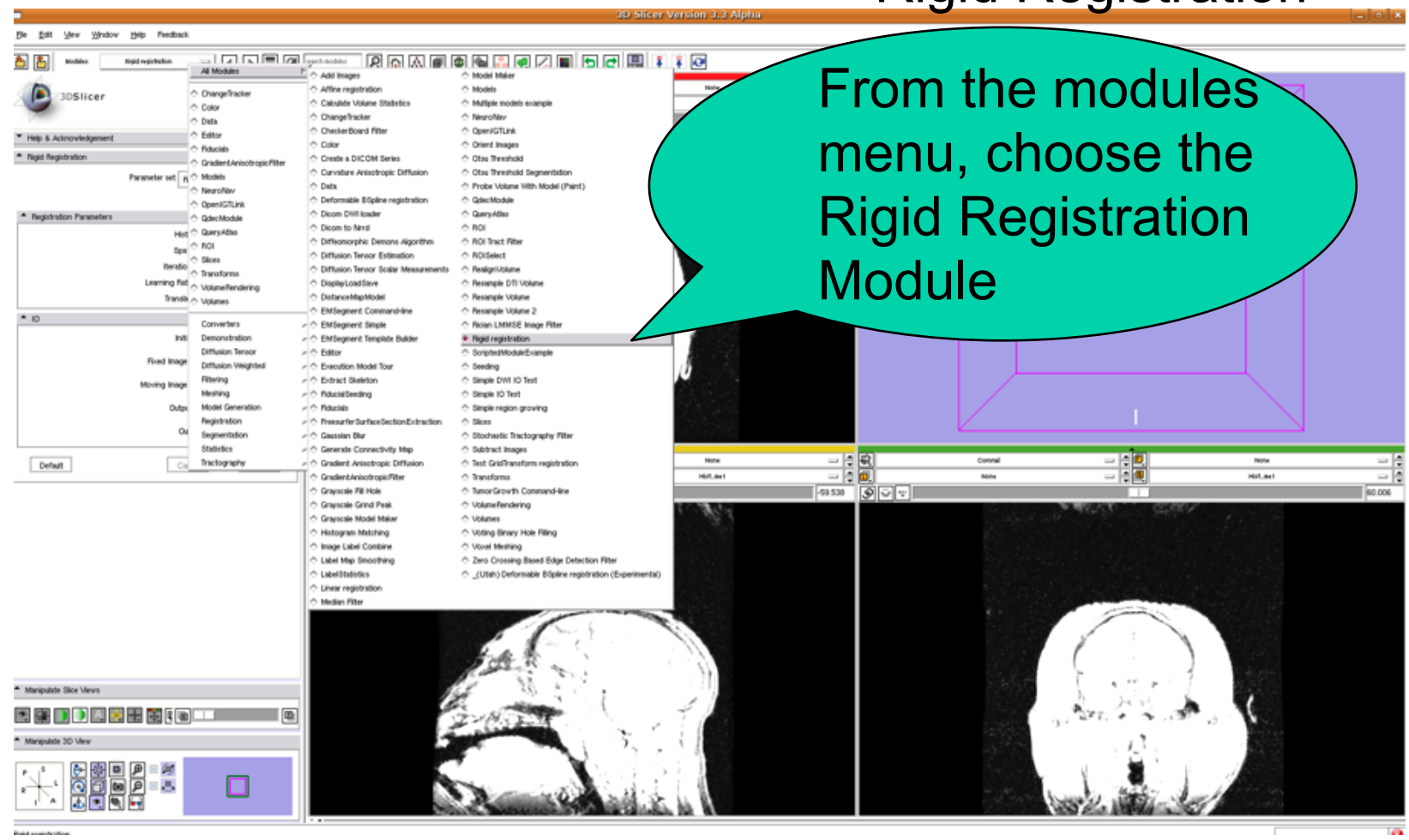
Subject Image Skull Stripping

- The Intra Cranial Content (ICC) of the subject is extracted.
- Improves probability map registration accuracy
- Creates more accurate patient specific atlas
- Two step procedure:
 - affine registration of ECC mask to subject
 - masking of subject by ECC mask



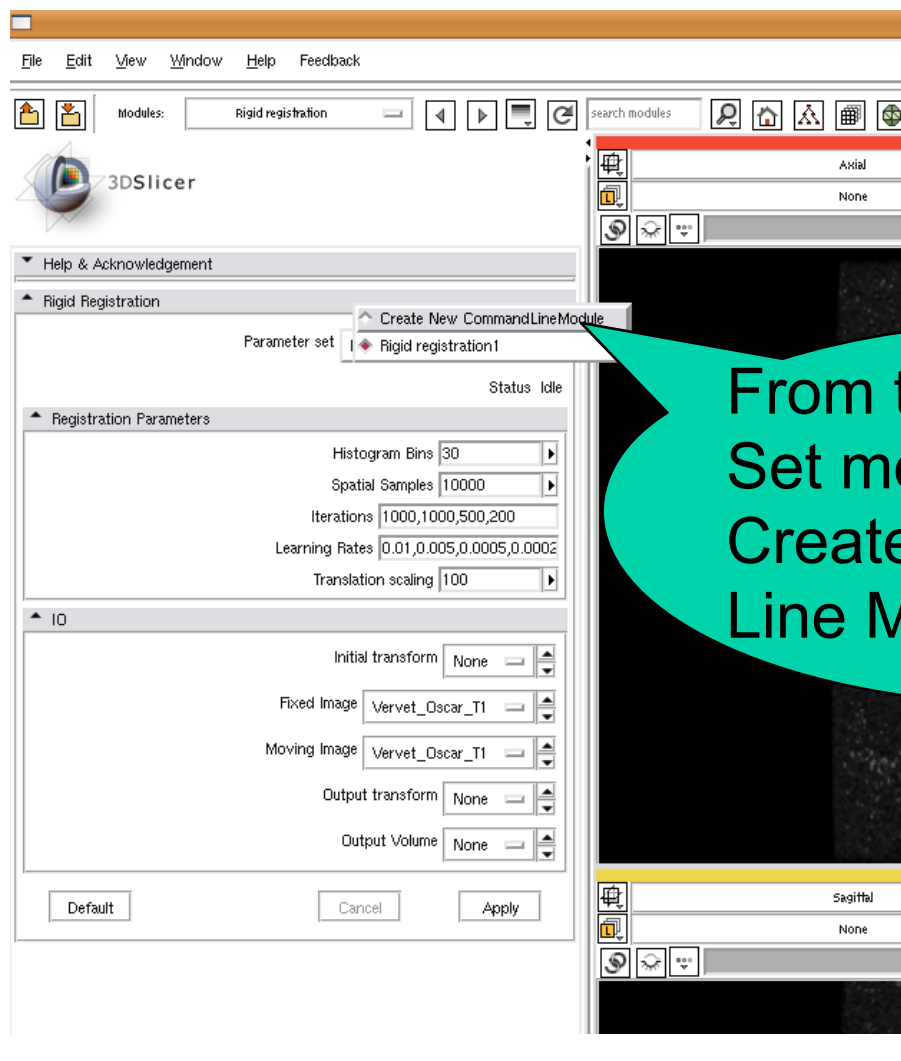
Subject Image Skull Stripping

Rigid Registration



Subject Image Skull Stripping

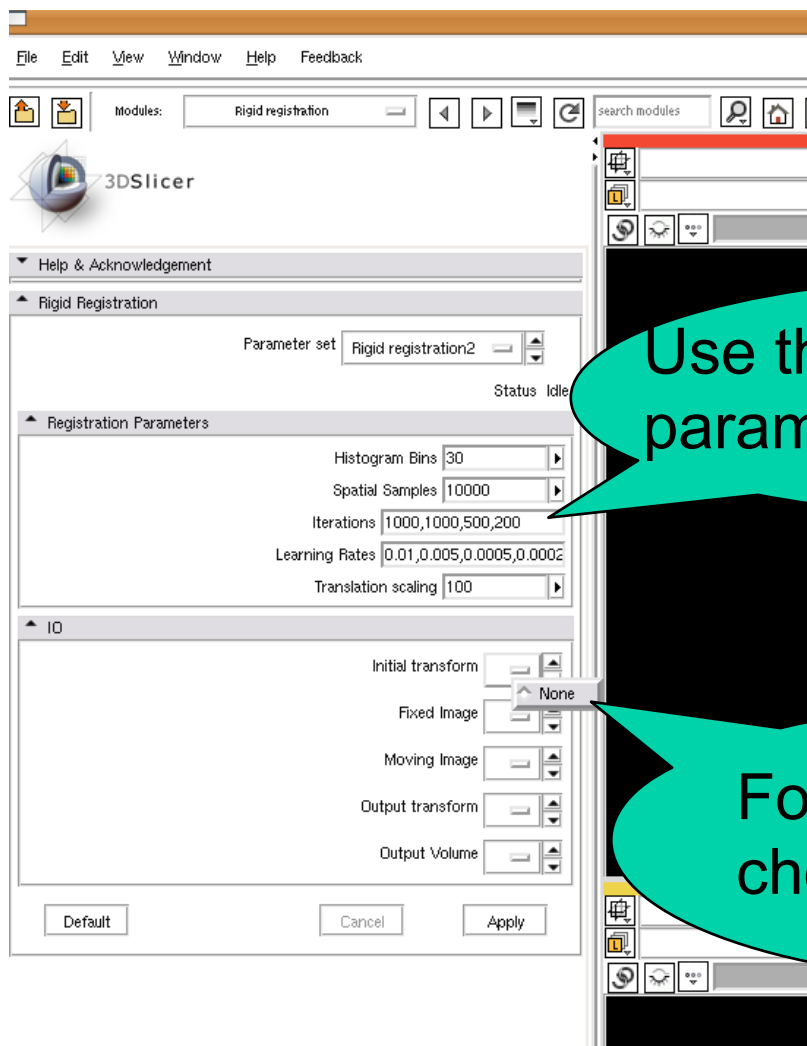
Rigid Registration



From the Parameter Set menu choose Create New Command Line Module

Subject Image Skull Stripping

Rigid Registration

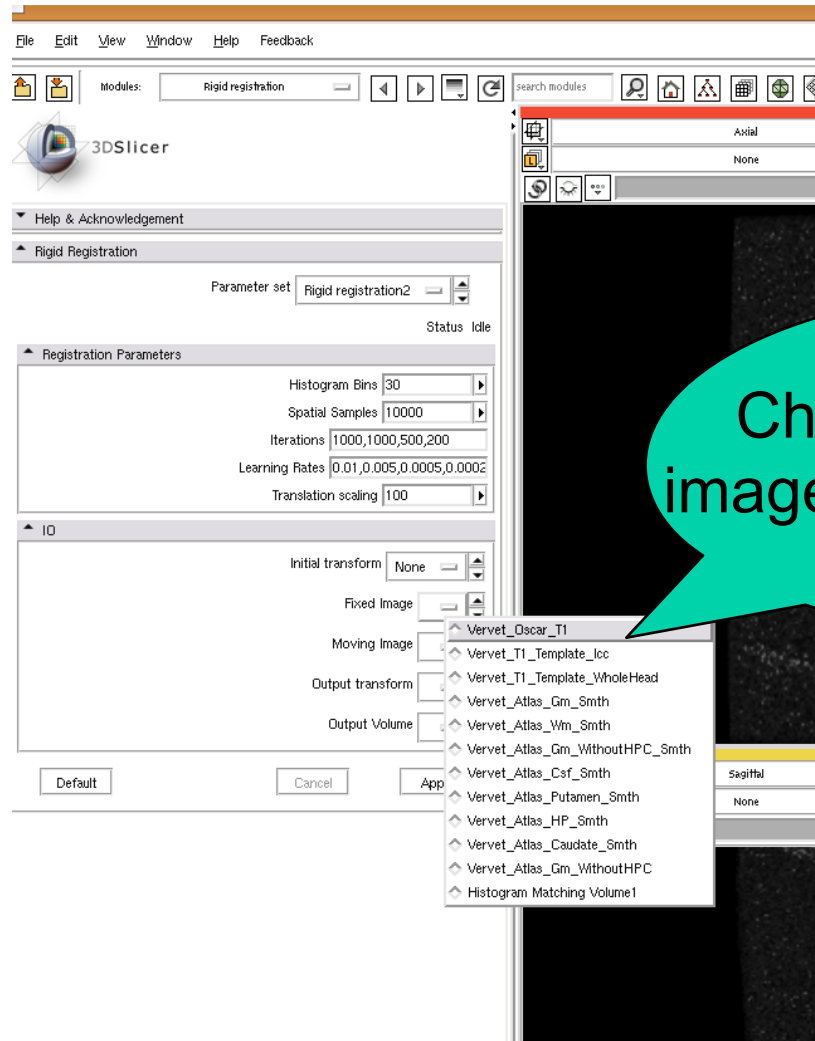


Use the default registration parameters

For initial transforms, choose None

Subject Image Skull Stripping

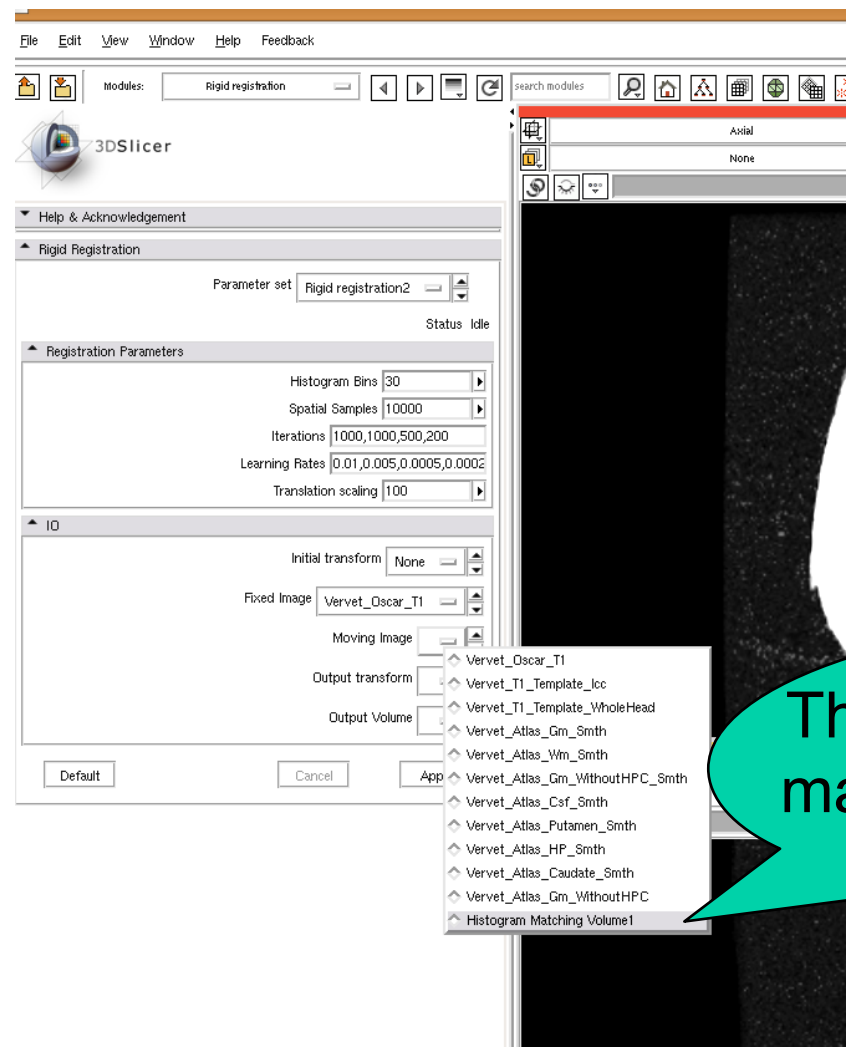
Rigid Registration



Choose the subject T1 image as the fixed image

Subject Image Skull Stripping

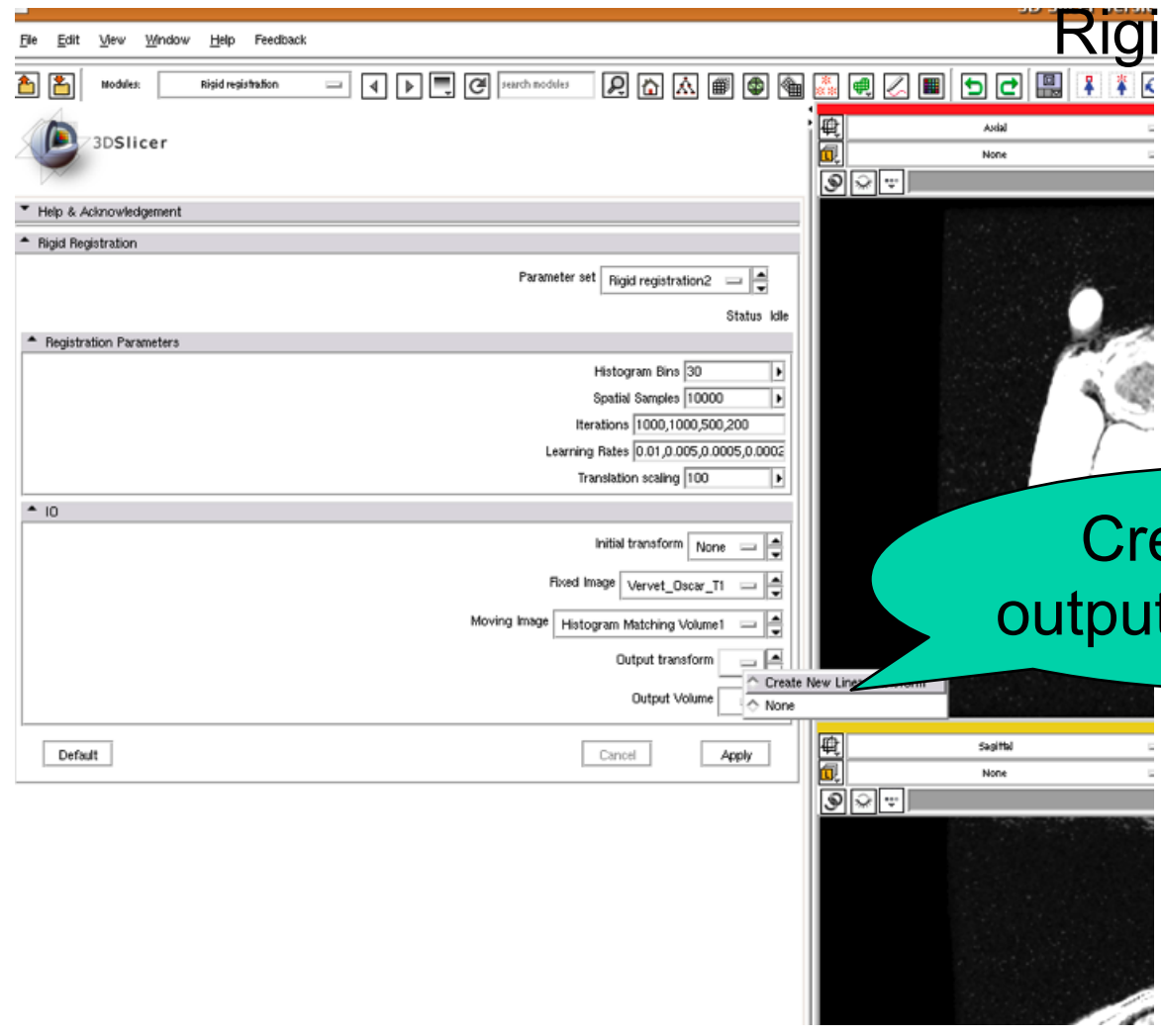
Rigid Registration



The output of the histogram matching filter is the moving image

Subject Image Skull Stripping

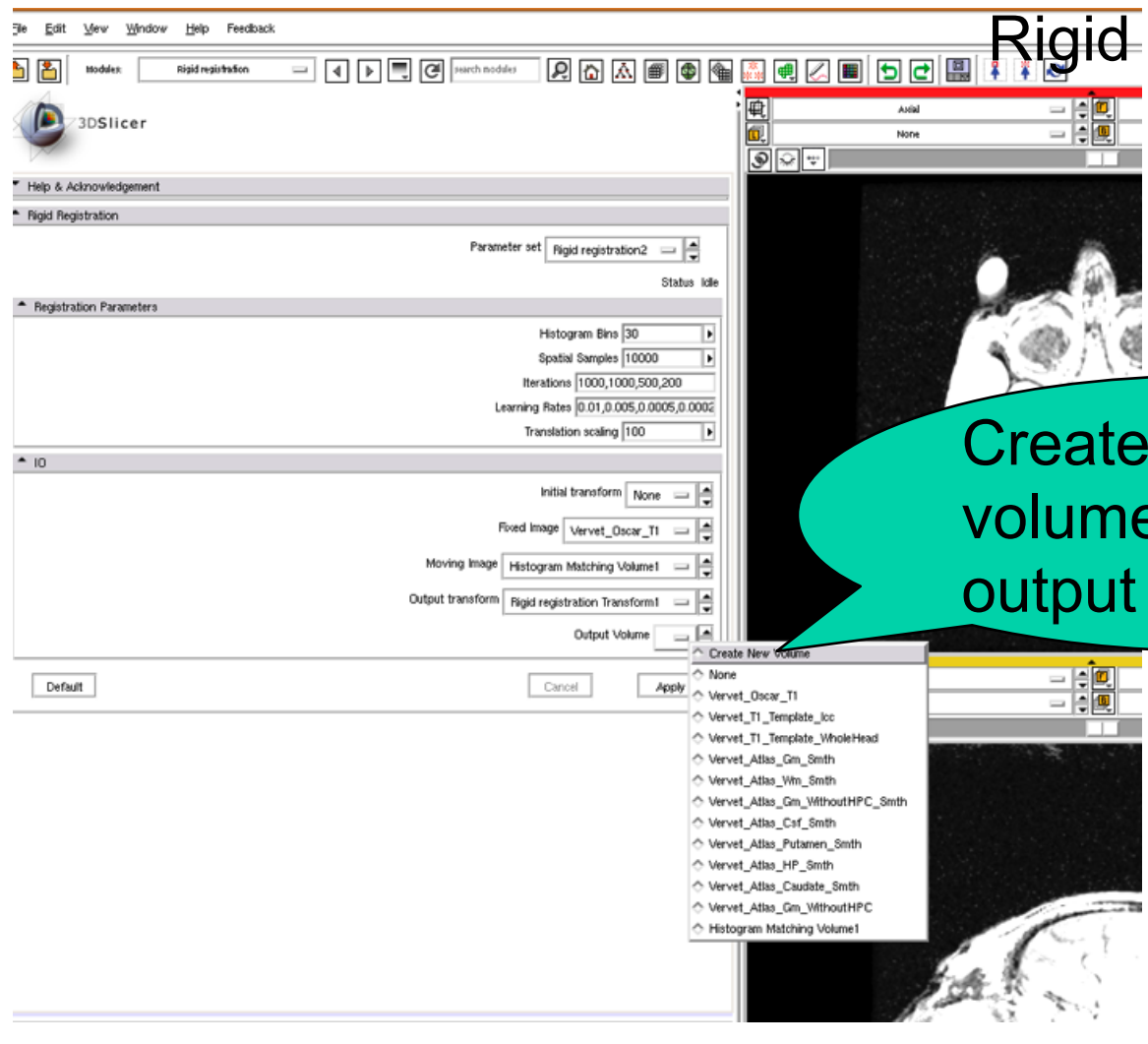
Rigid Registration



Create a new output transform

Subject Image Skull Stripping

Rigid Registration



Create a new volume for the output image



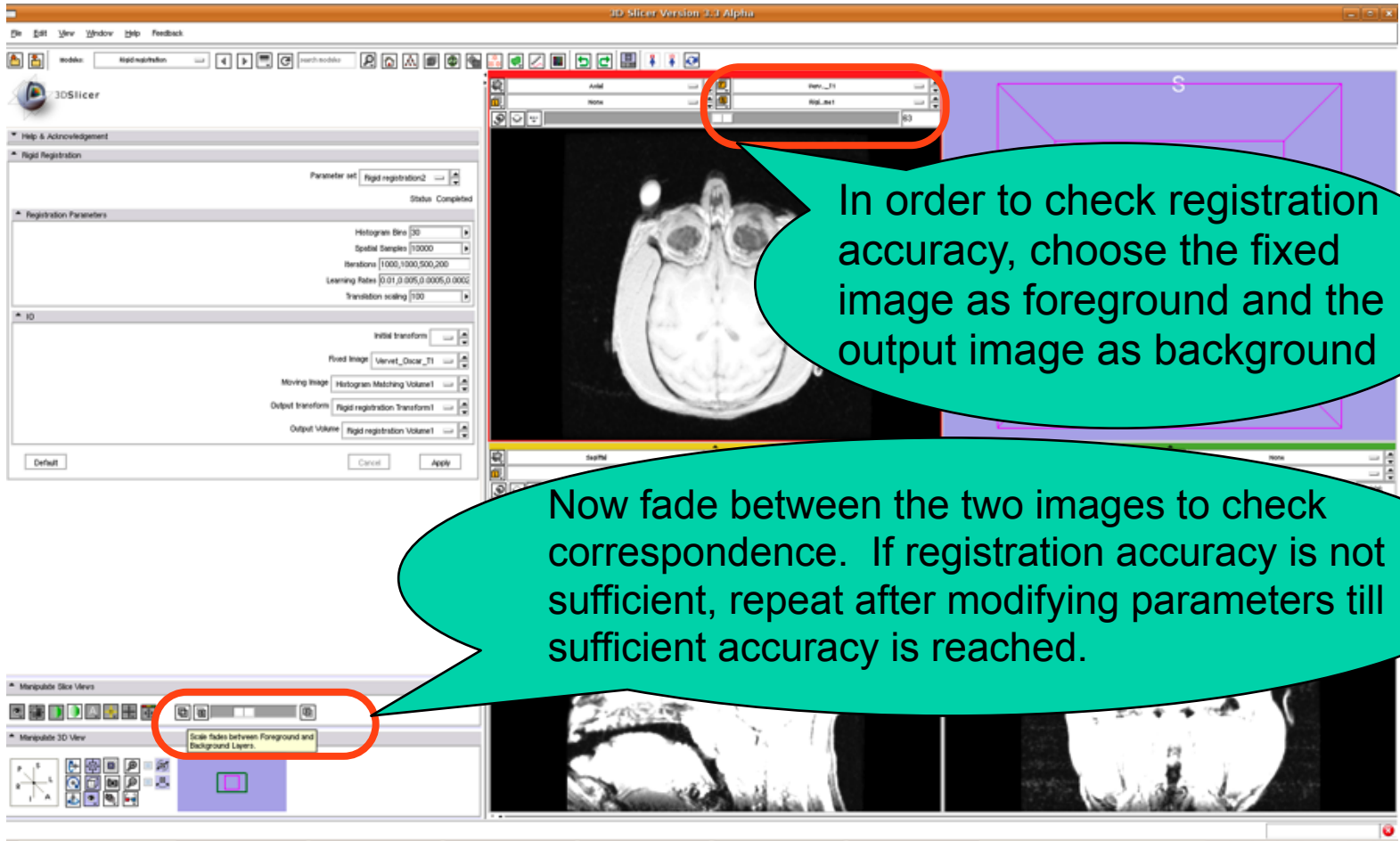
Subject Image Skull Stripping

Rigid Registration

The screenshot shows the 3DSlicer software interface with the Rigid Registration module active. The 'Registration Parameters' section is expanded, showing settings for Histogram Bins (30), Spatial Samples (10000), Iterations (1000, 1000, 500, 200), and Registration Rates (0.01, 0.005, 0.0005, 0.0002). The 'Apply' button at the bottom right is circled in red. A green speech bubble with the text 'Click Apply to perform registration' points to this button. The interface also shows a 'Parameter set' dropdown set to 'Rigid registration2' and a 'Status' field set to 'Idle'.

Subject Image Skull Stripping

Rigid Registration





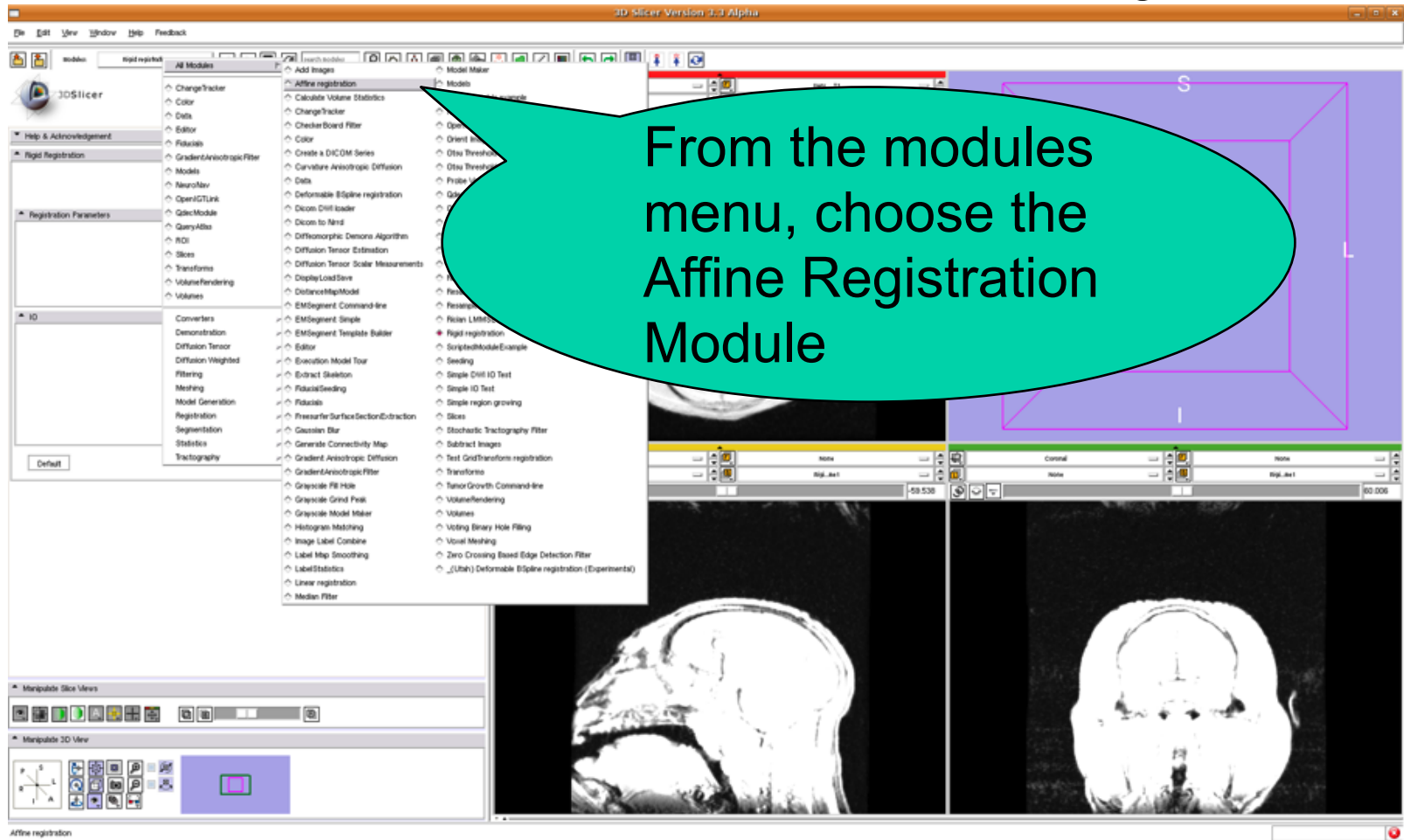
Subject Image Skull Stripping

Affine Registration

- The next step is to perform affine registration.
- The rigid transform is used as the starting point

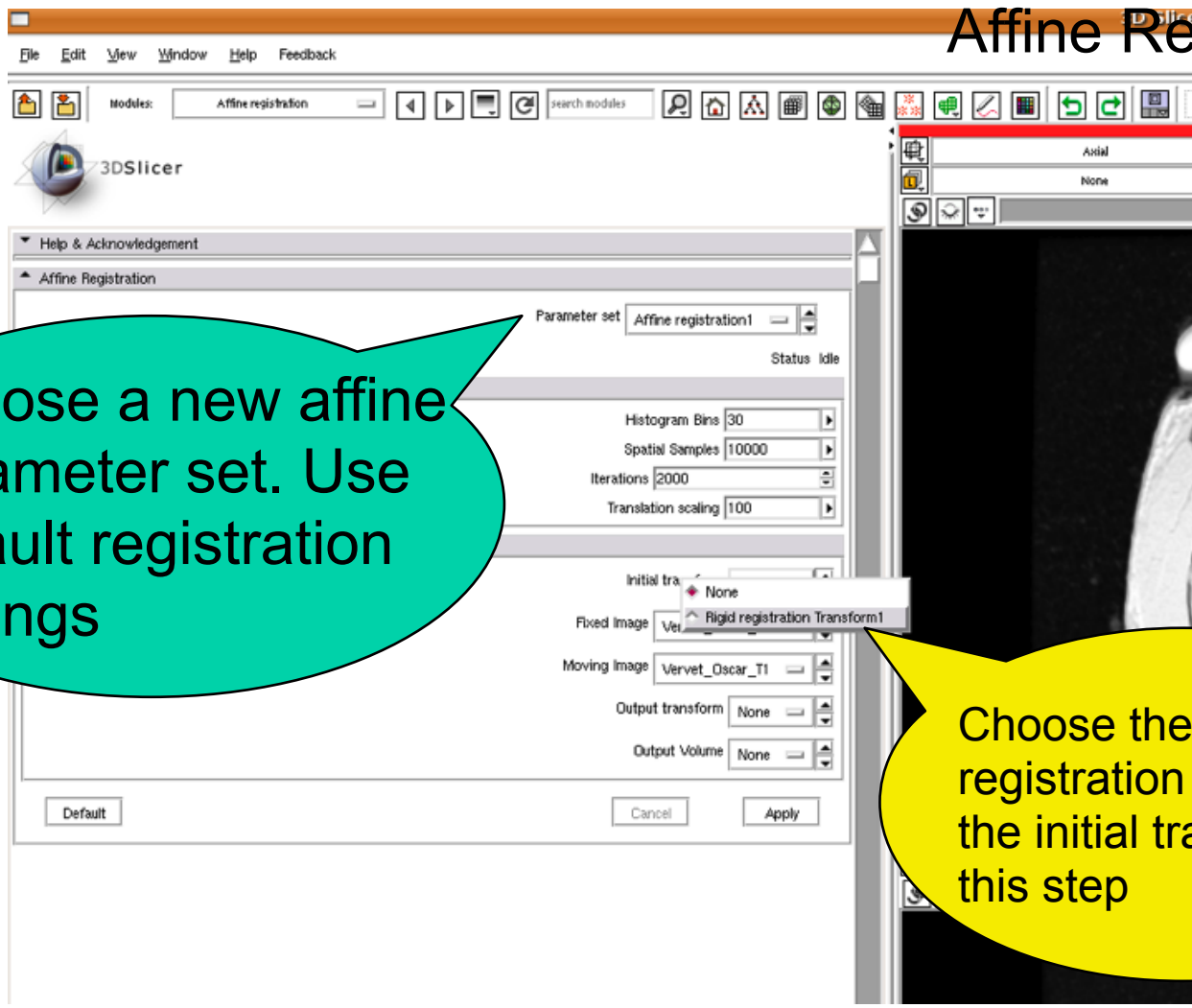
Subject Image Skull Stripping

Affine Registration



Subject Image Skull Stripping

Affine Registration

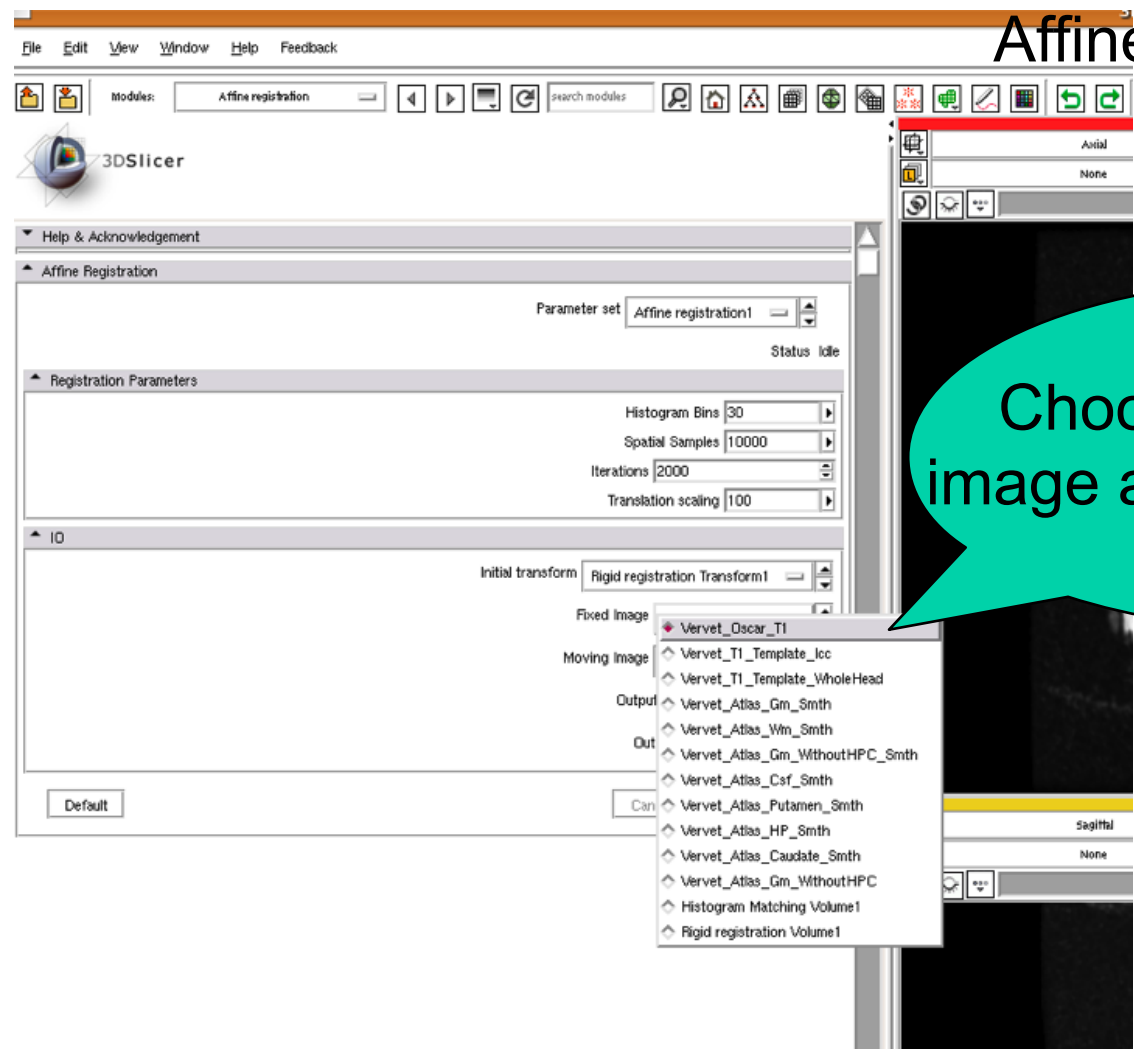


Choose a new affine parameter set. Use default registration settings

Choose the rigid registration transform as the initial transform for this step

Subject Image Skull Stripping

Affine Registration

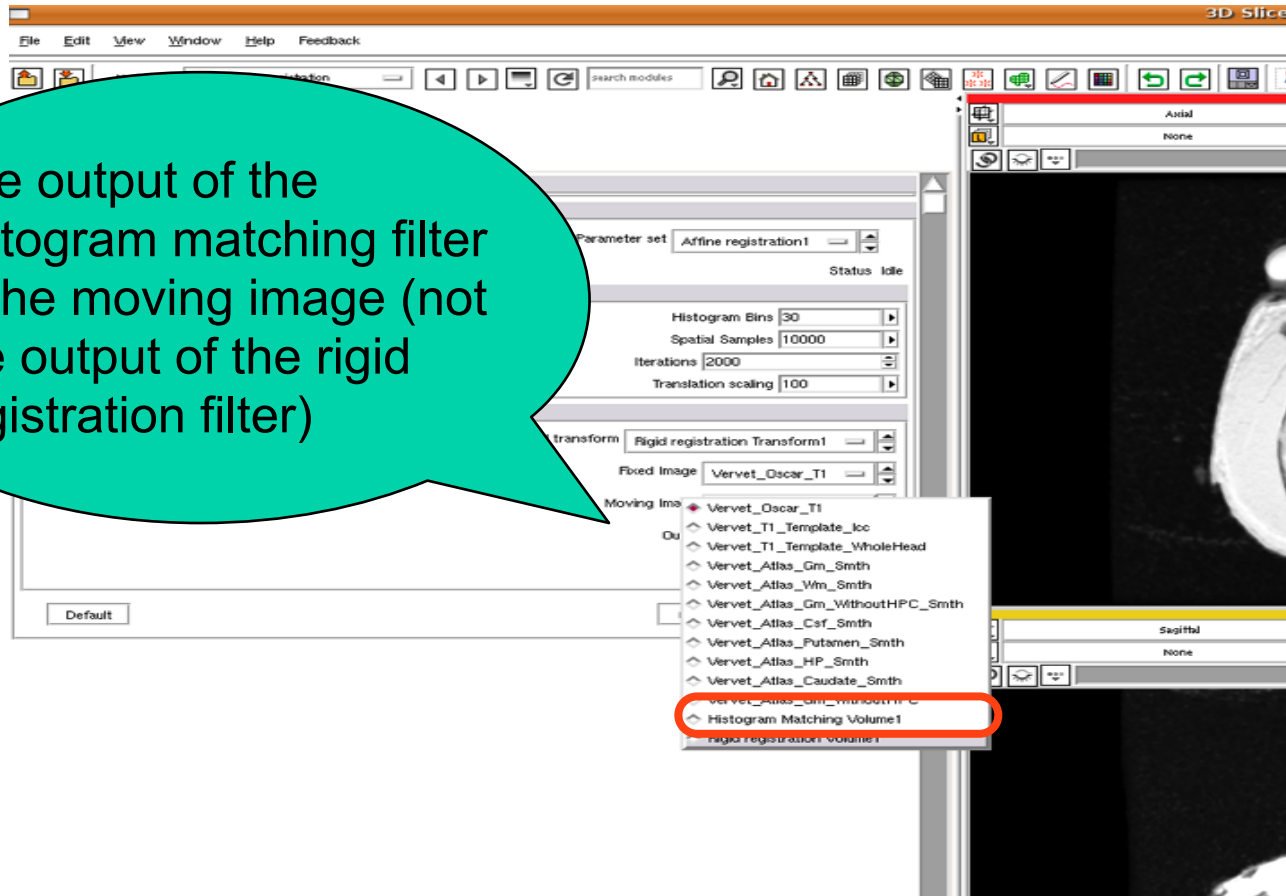


Choose the subject T1 image as the fixed image

Subject Image Skull Stripping

Affine Registration

The output of the histogram matching filter is the moving image (not the output of the rigid registration filter)





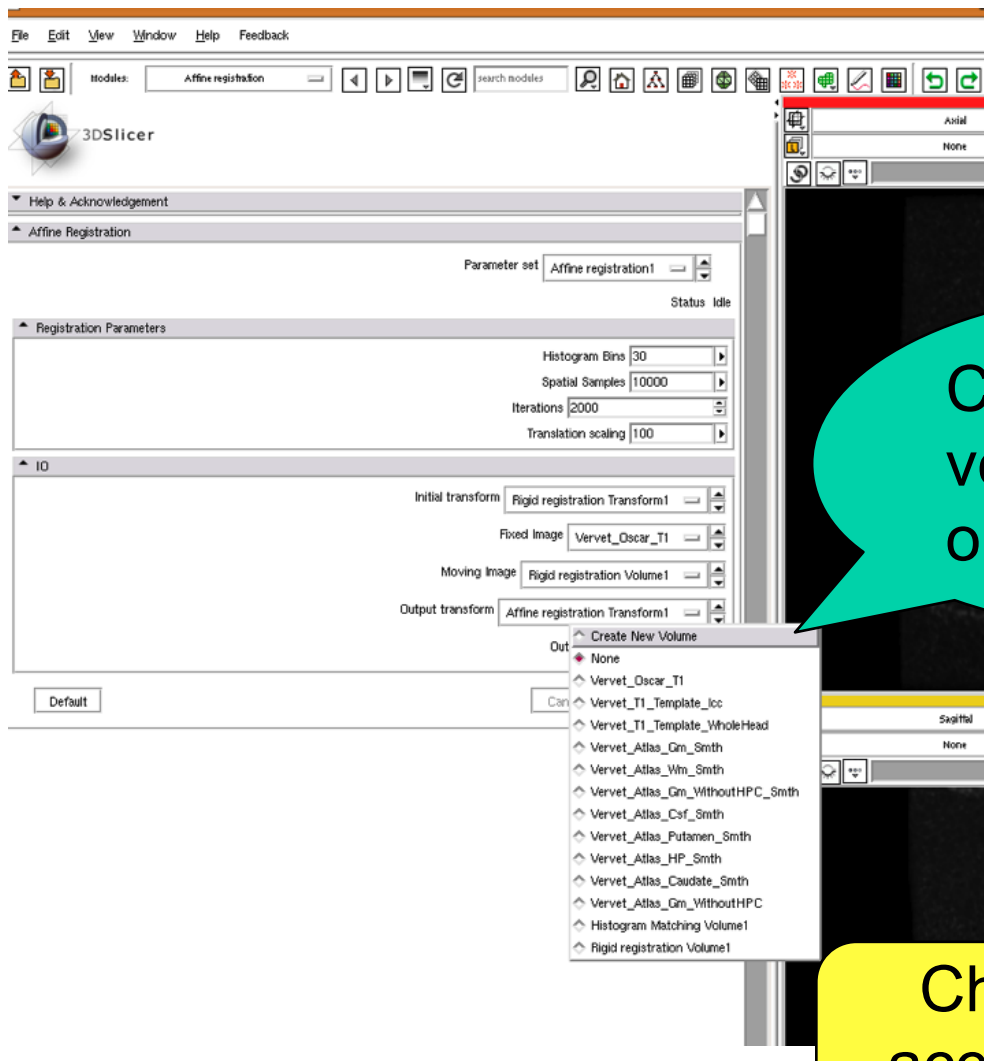
Subject Image Skull Stripping

Affine Registration

The screenshot shows the 3DSlicer software interface with the Affine Registration module active. The 'Affine Registration' panel is open, showing various parameters and a dropdown menu for the 'Output' field. A red callout bubble with the text 'Create a new output transform' points to the 'Output' dropdown menu, which is currently set to 'None'. The dropdown menu also shows options for 'Rigid registration Transform1' and 'Output Volume' set to 'None'. The 'Registration Parameters' section includes fields for 'Histogram Bins' (30), 'Spatial Samples' (10000), 'Iterations' (2000), and 'Relation scaling' (100). The 'IO' section shows 'Registration Transform1' and 'Input Image' (Vervet_Oscar_T1). Buttons for 'Default', 'Cancel', and 'Apply' are visible at the bottom of the panel.

Subject Image Skull Stripping

Affine Registration



Create a new volume for the output image

Click Apply to perform registration

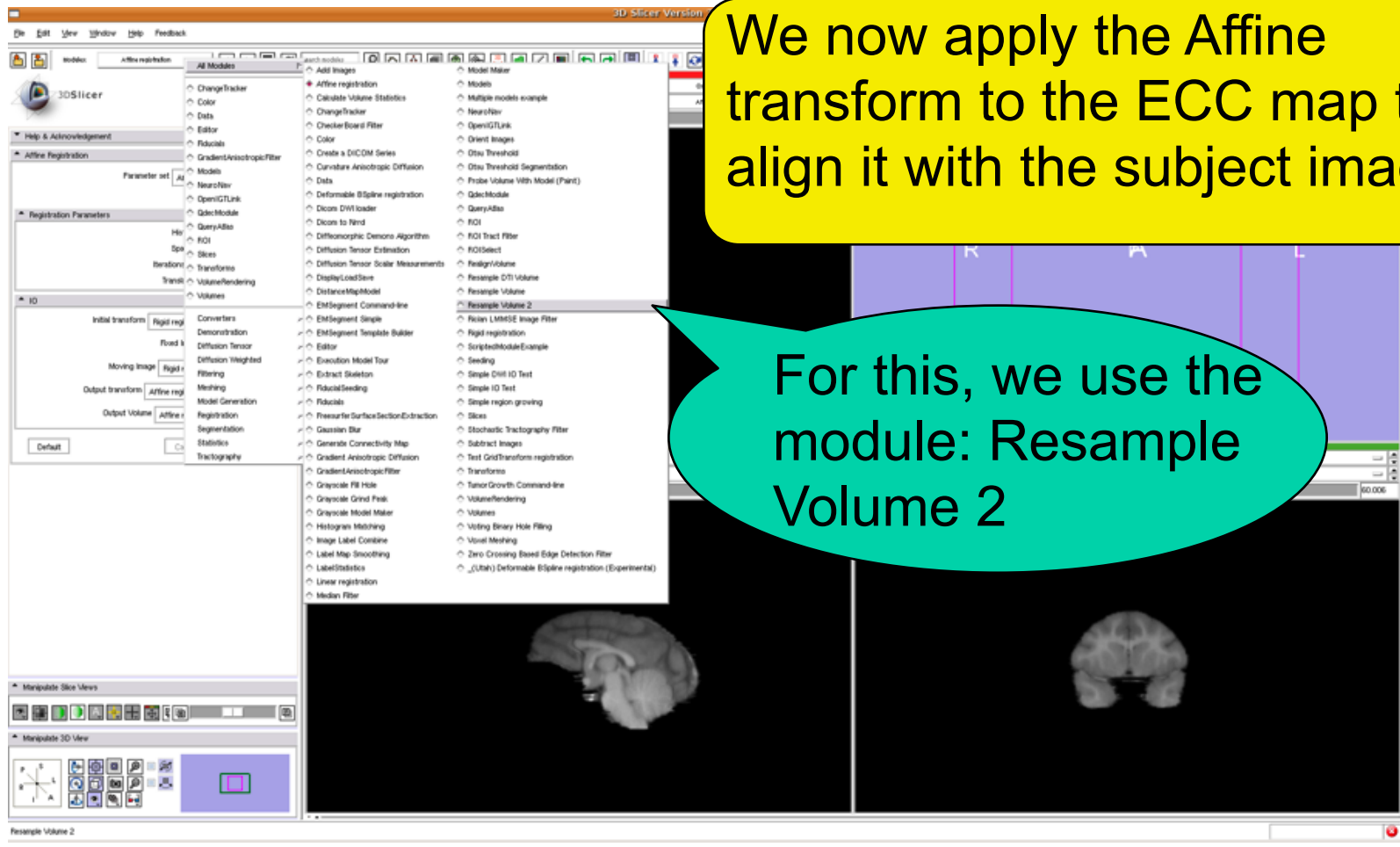
Check the registration accuracy as in the rigid registration step.

Subject Image Skull Stripping

Apply Transform to ECC Map

We now apply the Affine transform to the ECC map to align it with the subject image

For this, we use the module: Resample Volume 2



Subject Image Skull Stripping

Apply Transform to ECC Map

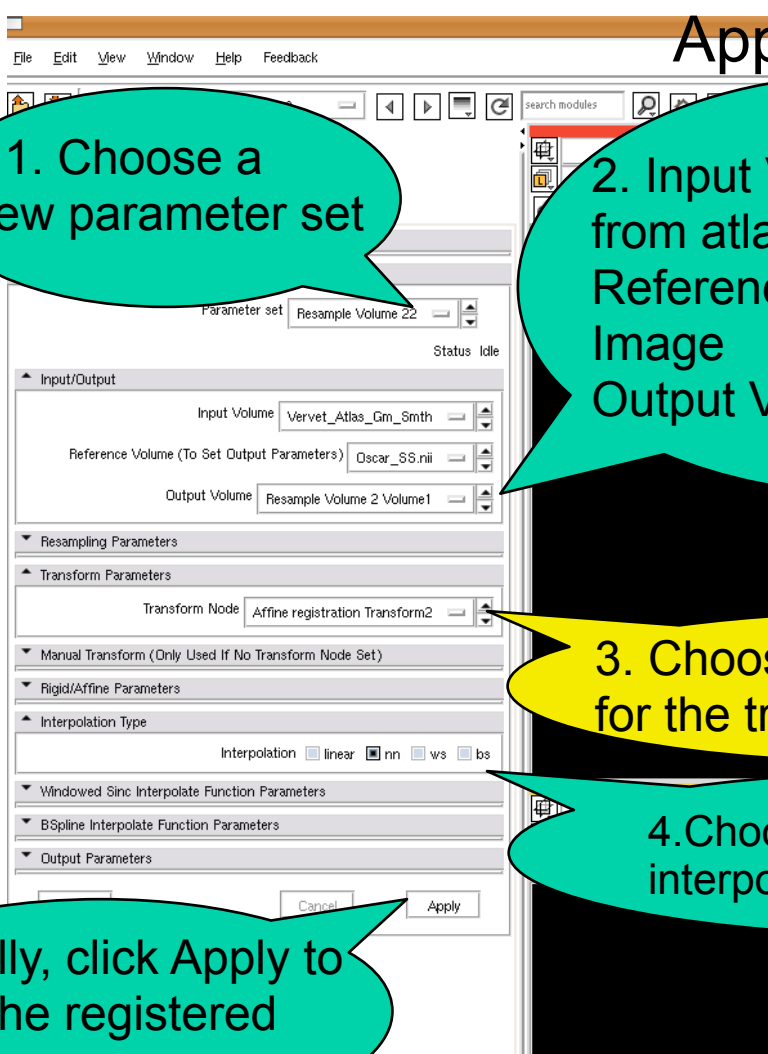
1. Choose a new parameter set

2. Input Volume: ECC map from atlas
Reference Volume: Subject Image
Output Volume: New

3. Choose the affine transform for the transformation node

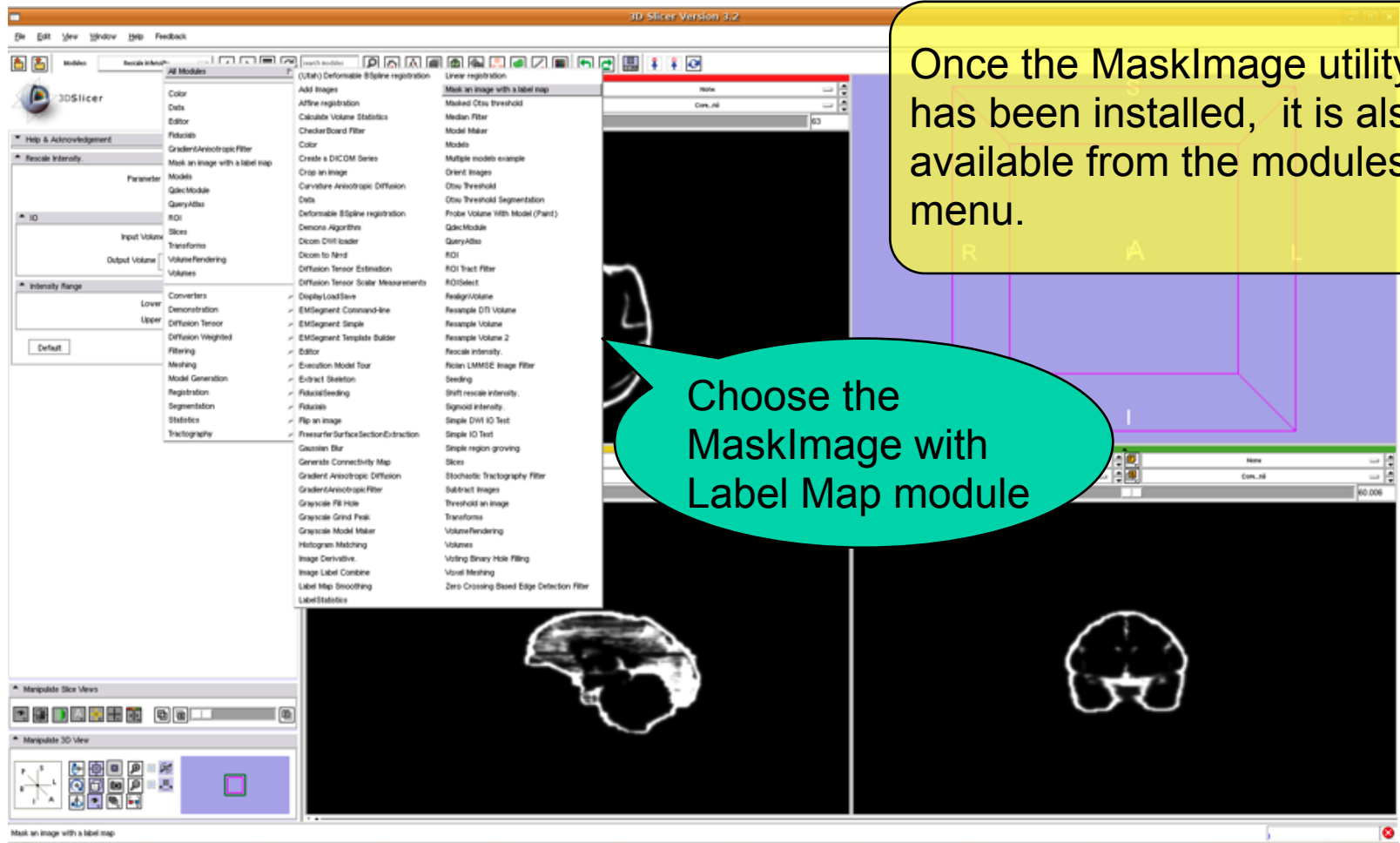
4. Choose nearest neighbour interpolation

Finally, click Apply to get the registered ECC map



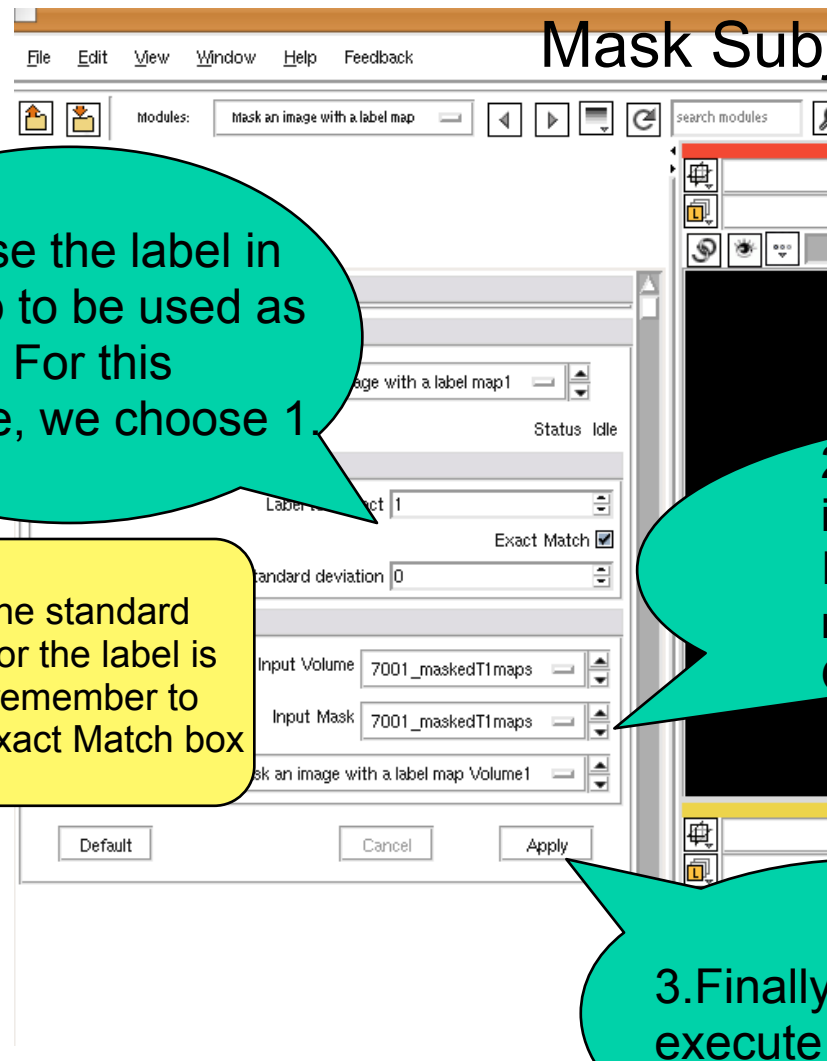
Subject Image Skull Stripping

Mask Subject Image with ECC Mask



Subject Image Skull Stripping

Mask Subject Image with ECC Mask



1. Choose the label in the map to be used as a mask. For this example, we choose 1.

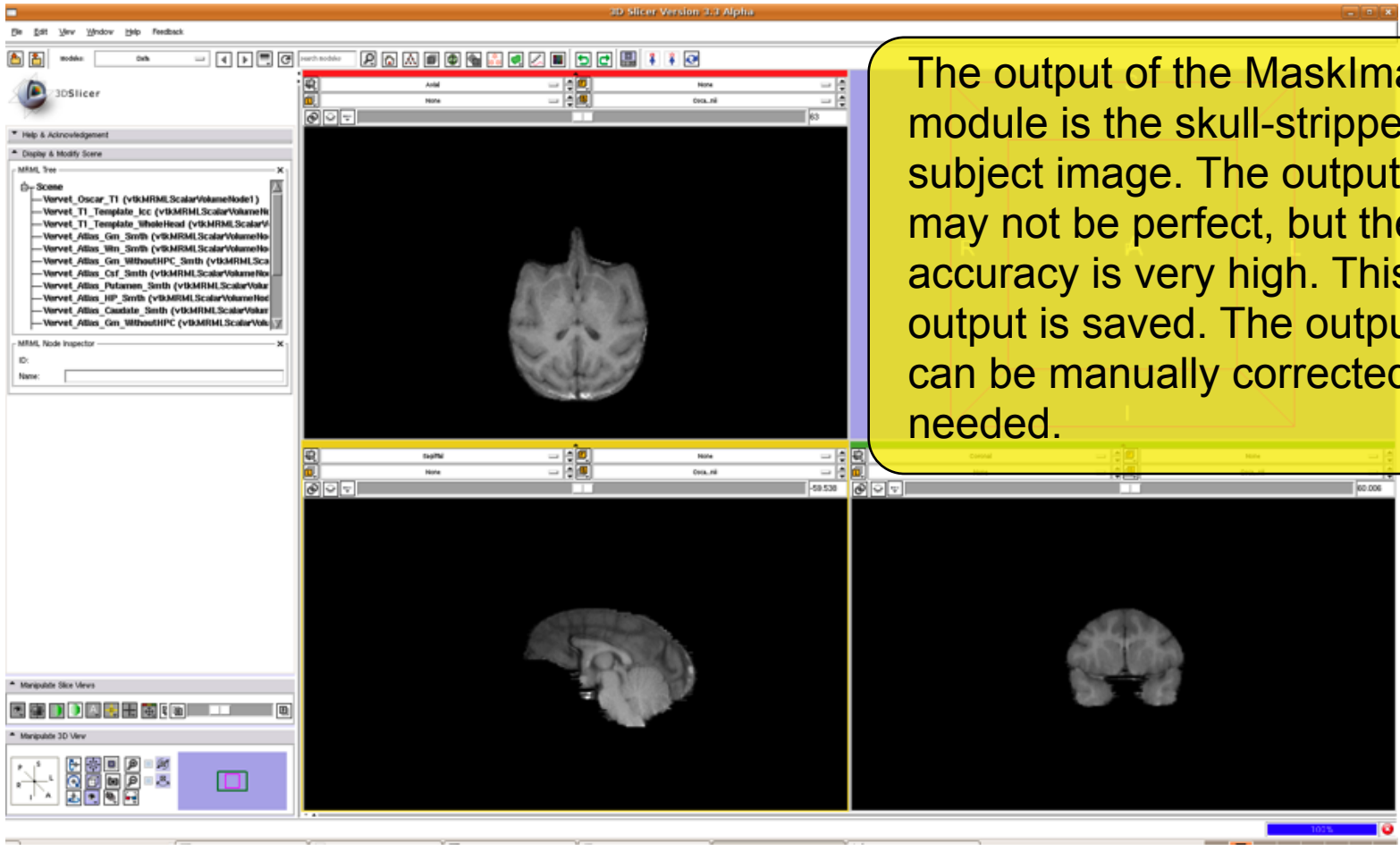
Note: If the standard deviation for the label is set to 0, remember to check the Exact Match box

2. Input Volume: Subject image
Input Mask: Registered ECC map
Output Volume: New

3. Finally, click Apply to execute module

Subject Image Skull Stripping

Mask Subject Image with ECC Mask



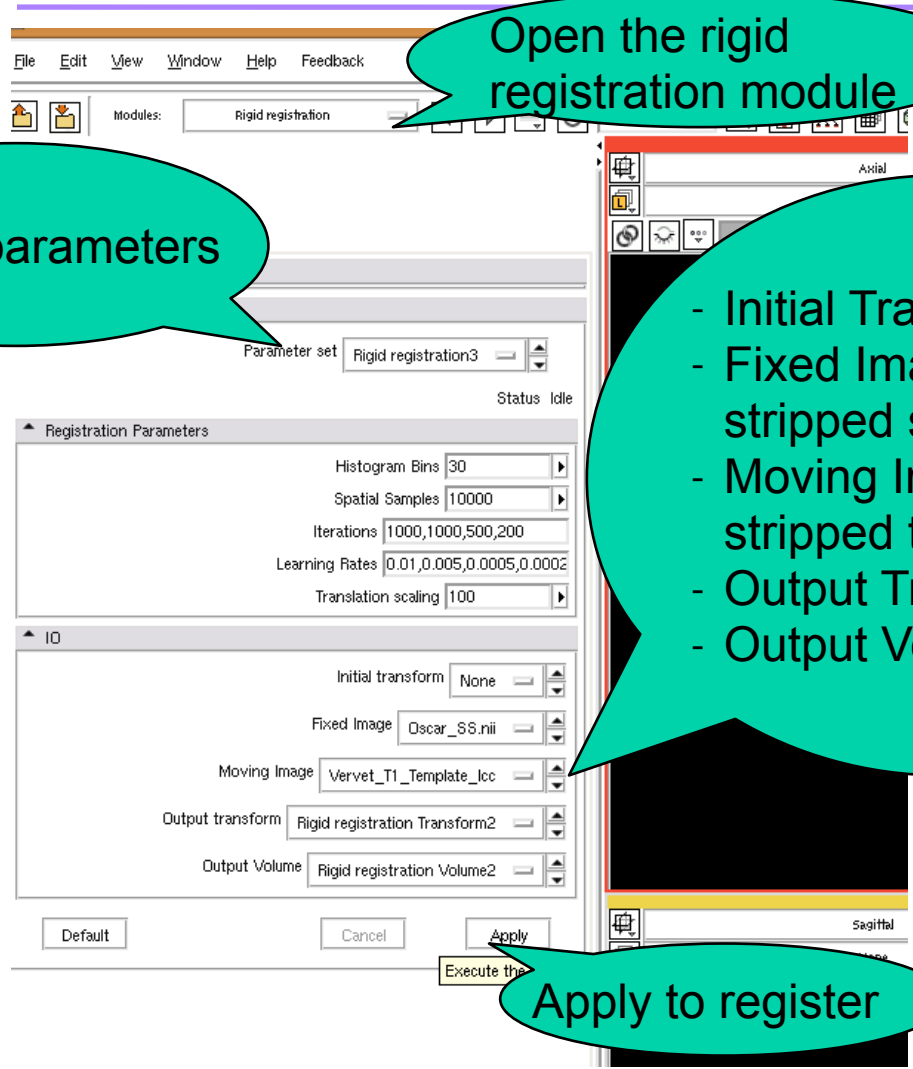
The output of the MaskImage module is the skull-stripped subject image. The output may not be perfect, but the accuracy is very high. This output is saved. The output can be manually corrected if needed.



Creating patient specific atlas

- Register skull-stripped subject image to skull-stripped template image
- Use affine registration followed by deformable registration
- Apply transformation to probability maps to get patient specific atlas
- Registered maps are rescaled to values between 0-255 to be used with EMSegmenter

Creating patient specific atlas - Rigid Registration



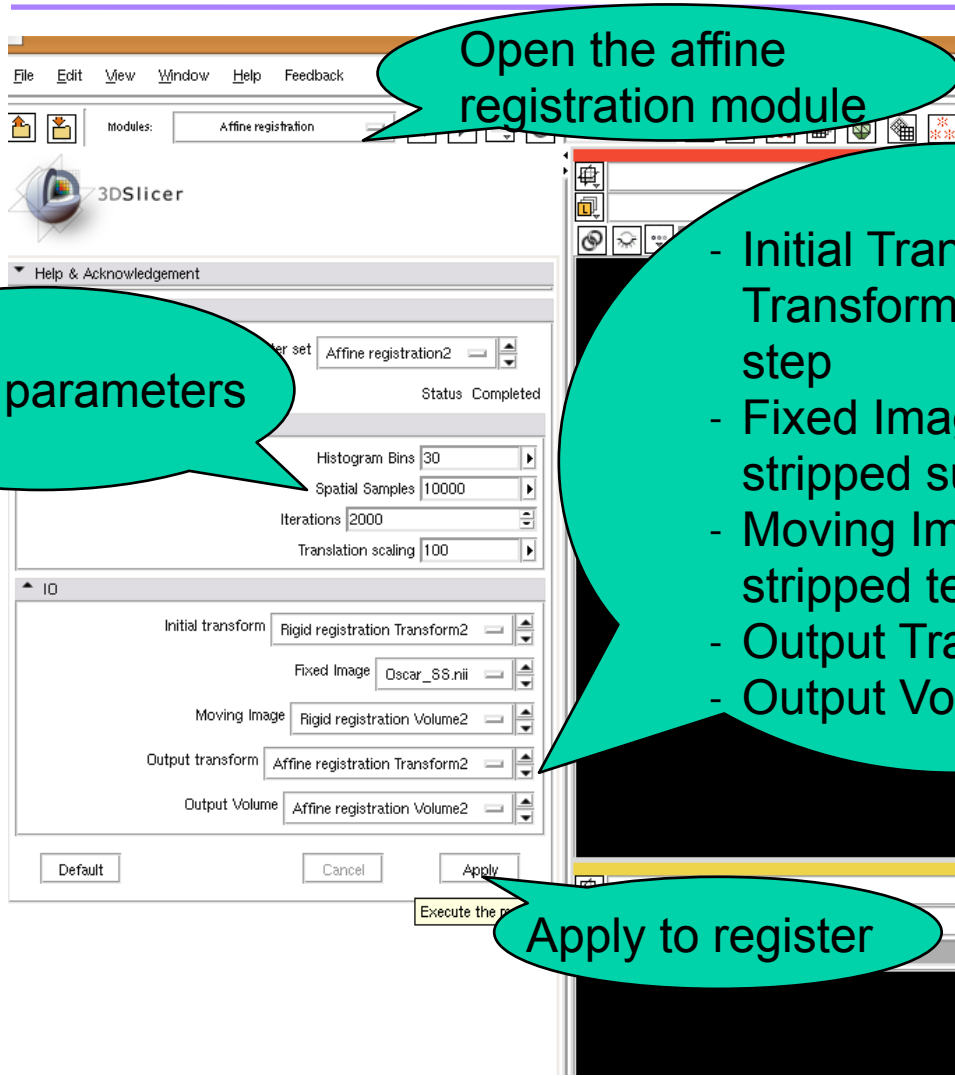
Open the rigid registration module

Default parameters

- Initial Transform: None
- Fixed Image: skull stripped subject image
- Moving Image: skull stripped template image
- Output Transform: New
- Output Volume: New

Apply to register

Creating patient specific atlas - Affine Registration



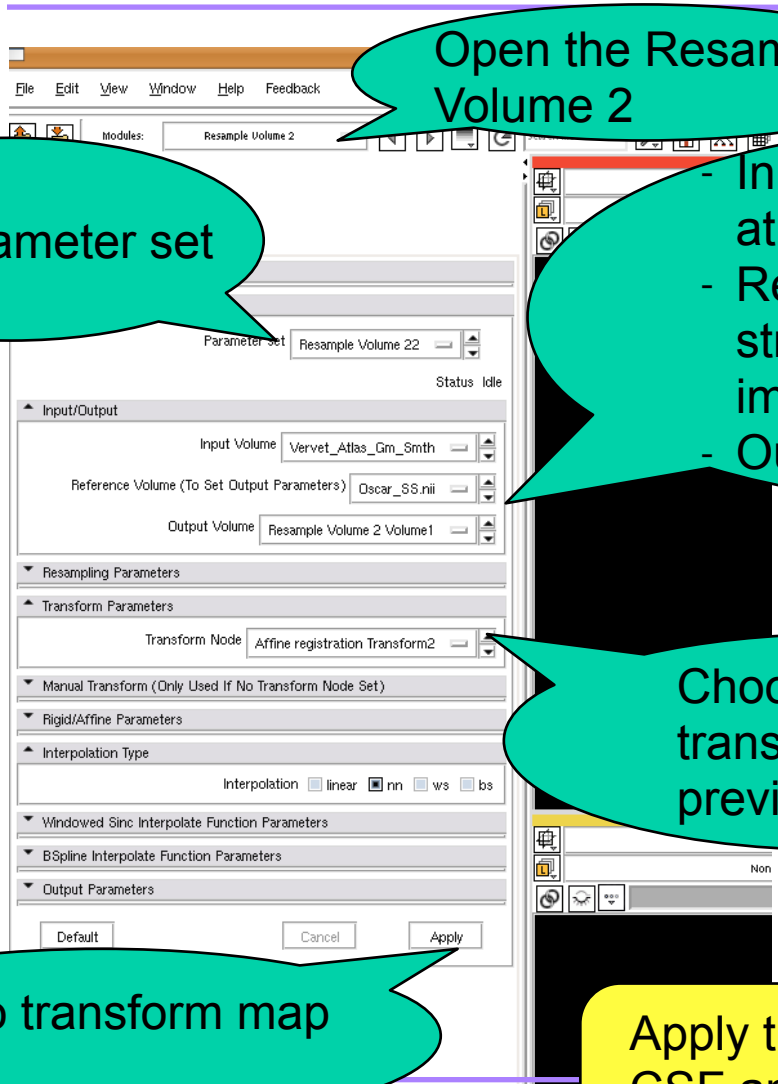
Open the affine registration module

Default parameters

- Initial Transform: Rigid Transform from previous step
- Fixed Image: skull stripped subject image
- Moving Image: skull stripped template image
- Output Transform: New
- Output Volume: New

Apply to register

Creating patient specific atlas - Apply Transform



Open the Resample Volume 2

New parameter set

- Input : GM map from atlas
- Reference: skull stripped subject image
- Output: New volume

Choose affine transform from previous step

Apply to transform map

Apply transform to all other maps (WM, CSF and ECC) by changing only the input each time. Save all the transformed maps as separate volumes.



Creating patient specific atlas - Non-Linear Registration

- To use the Diffeomorphic Demons CLI , open a new terminal to the directory containing: Slicer3-Build/lib/Slicer3/Plugins/
 - use the command: `./DemonsRegistration`
 - The skull stripped subject is the fixed image,
 - the affinely registered, skull-stripped template is the moving image and,
 - choose symmetrized gradient option.
 - For our application, we set the number of levels to 4 with the following iterations [90, 70,45,25]. The deformation field should be saved as a MHA file.
-



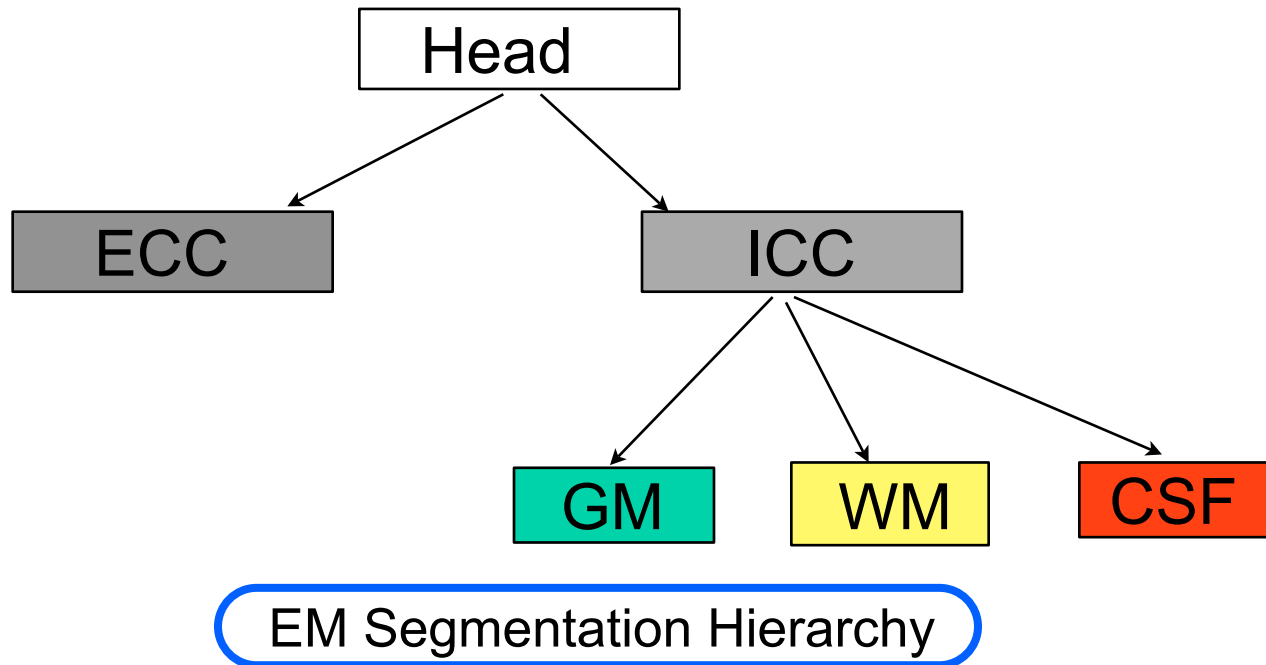
Creating patient specific atlas - Applying deformation field to probability maps

- ◉ Once registration has been completed, we use another tool in the same folder to apply the deformation field to the probability maps one at a time
 - ◉ use the command: `./applydeformationITK`
 - The GM probability map after affine registration is the moving image,
 - the diffeomorphic demons deformation field is the field to be apply and,
 - choose apply transformation option.
 - For our application, we set the interpolation to nearest neighbor
 - Repeat this for all other affinely registered probability maps by changing the moving image
-

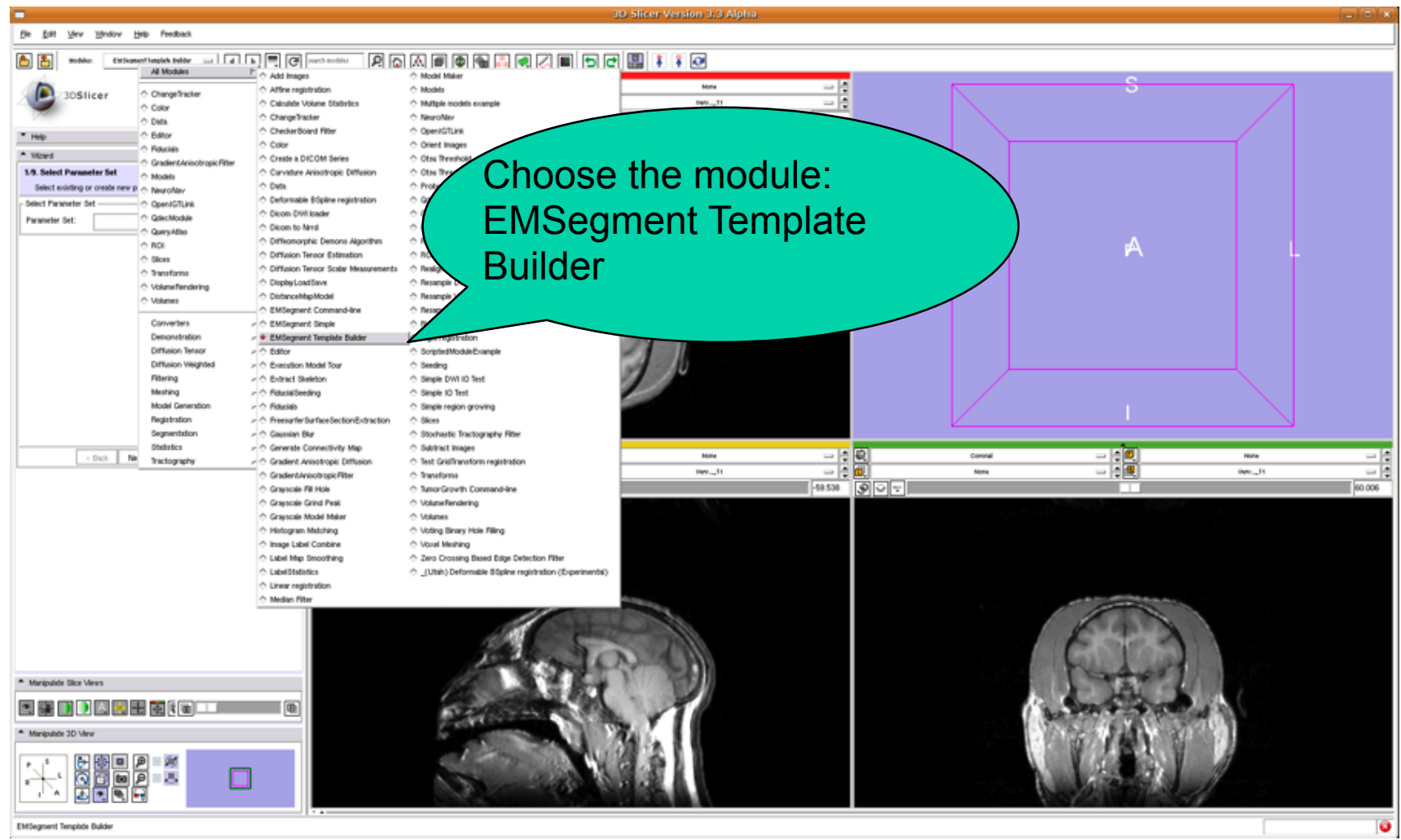
Segmentation using EMSegmenter

Segmentation

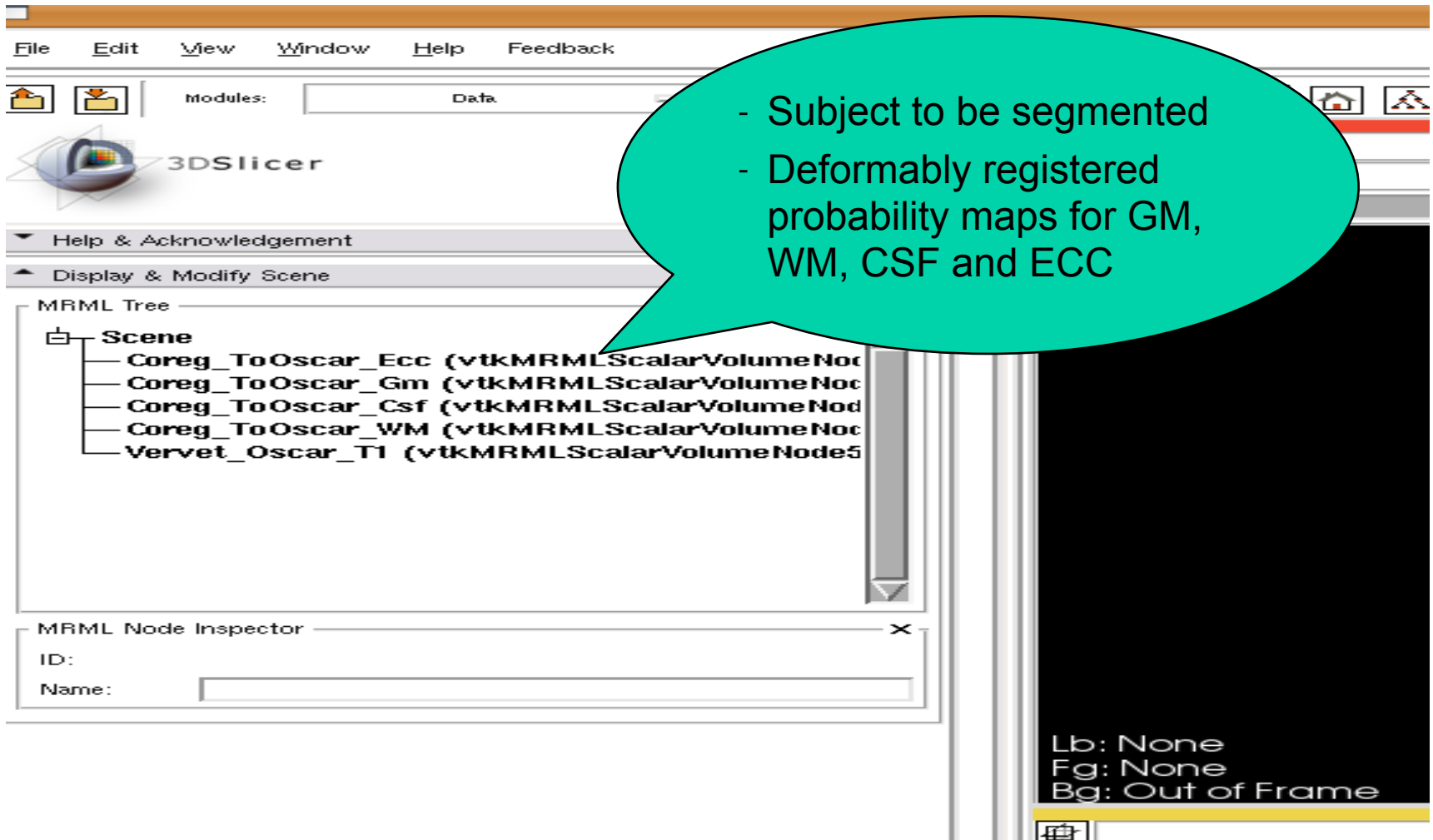
- Once the patient specific atlas has been created, we use that along with the subject image in EMSegmenter



Segmentation



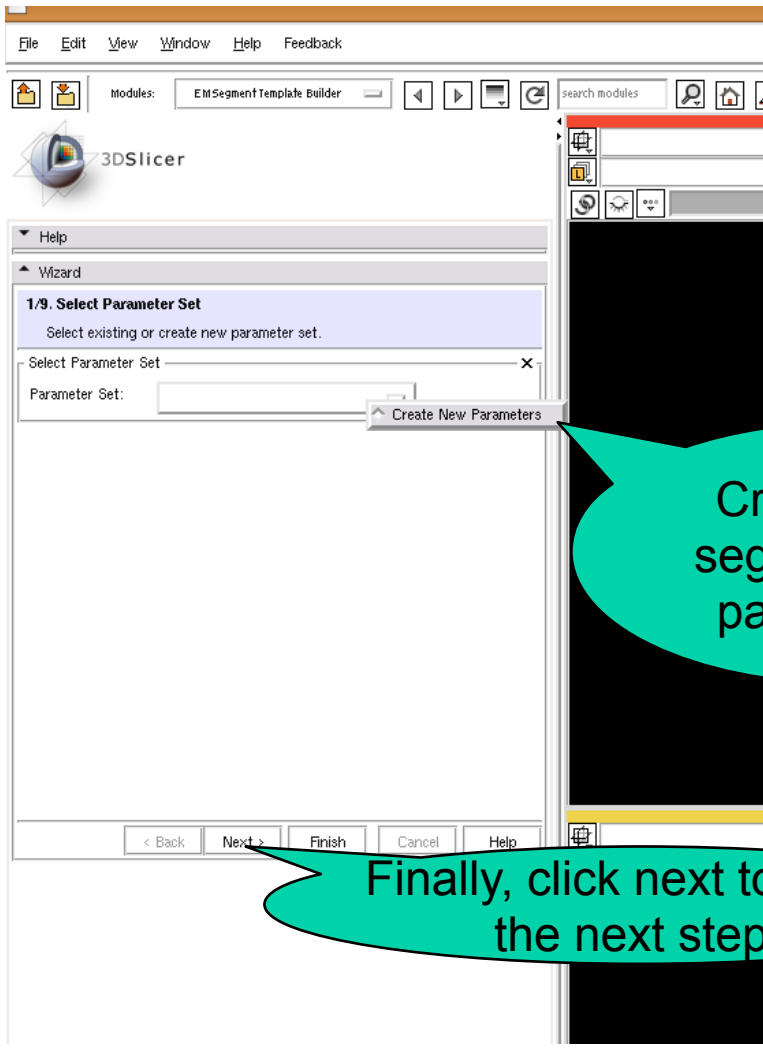
Segmentation - Input Data



The screenshot shows the 3DSlicer software interface. The top menu bar includes File, Edit, View, Window, Help, and Feedback. Below the menu bar, there are icons for home and refresh, and a 'Modules:' dropdown menu set to 'Data'. The 3DSlicer logo is visible on the left. The main interface is divided into several panels:

- Help & Acknowledgement**: A panel with a downward arrow.
- Display & Modify Scene**: A panel with an upward arrow.
- MRML Tree**: A tree view showing the scene structure:
 - Scene
 - Coreg_ToOscar_Ecc (vtkMRMLScalarVolumeNode)
 - Coreg_ToOscar_Gm (vtkMRMLScalarVolumeNode)
 - Coreg_ToOscar_Csf (vtkMRMLScalarVolumeNode)
 - Coreg_ToOscar_WM (vtkMRMLScalarVolumeNode)
 - Vervet_Oscar_T1 (vtkMRMLScalarVolumeNode)
- MRML Node Inspector**: A panel with fields for ID and Name.
- Property Panel**: A panel on the right showing properties: Lb: None, Fg: None, Bg: Out of Frame.

- Subject to be segmented
- Deformably registered probability maps for GM, WM, CSF and ECC

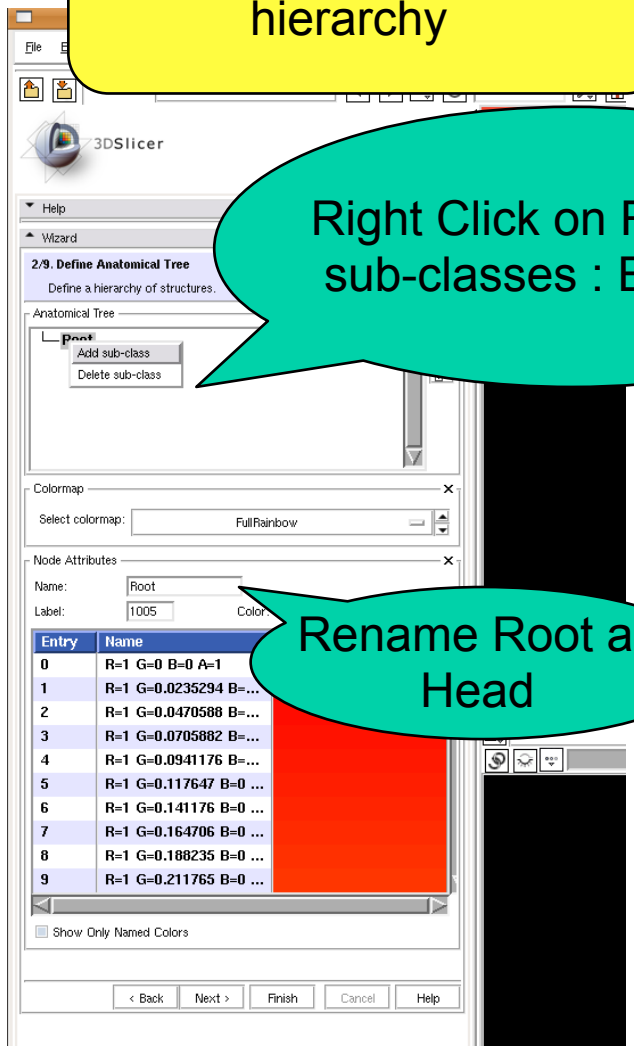


Create new segmentation parameters

Finally, click next to go to the next step

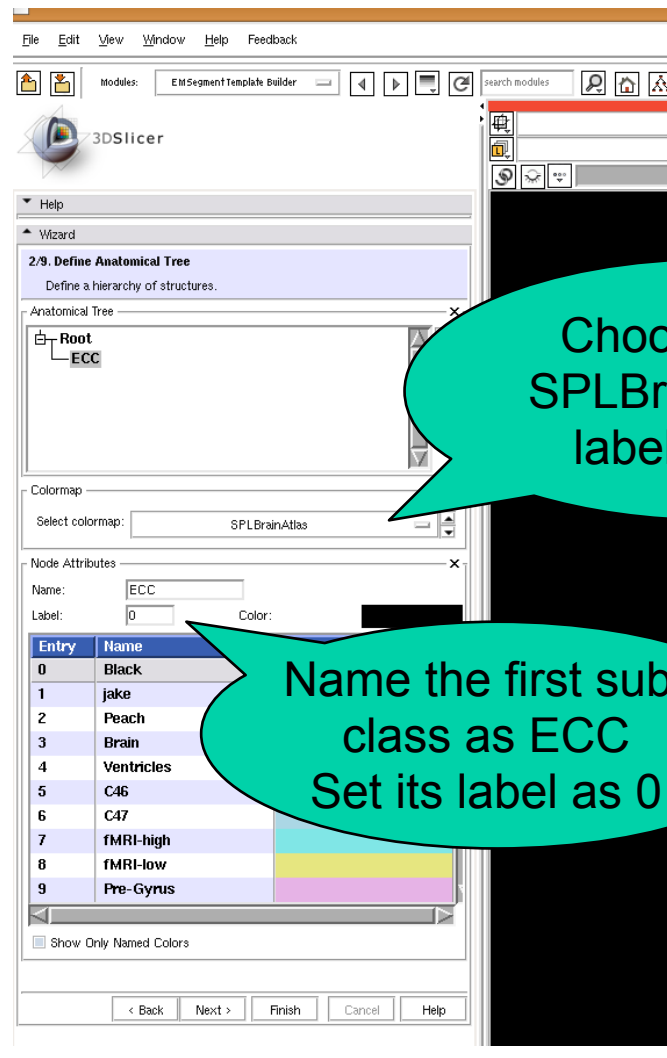
Segmentation

The second step is to specify the segmentation hierarchy



Right Click on Root to add 2 sub-classes : ECC and ICC

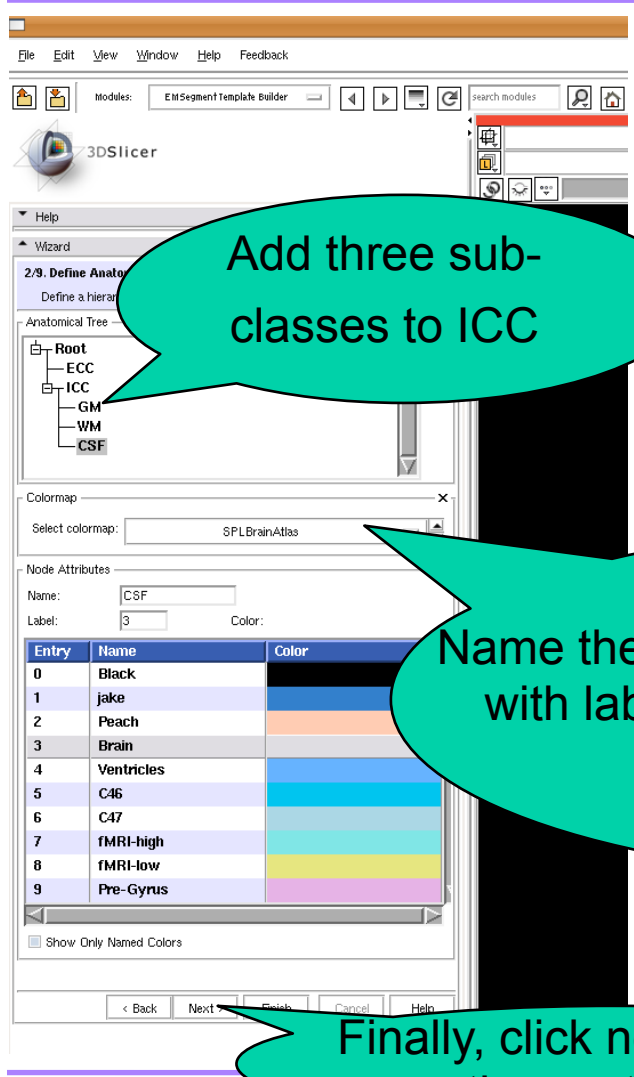
Rename Root as Head



Choose the SPLBrainAtlas labelmaps

Name the first sub-class as ECC
Set its label as 0

Segmentation

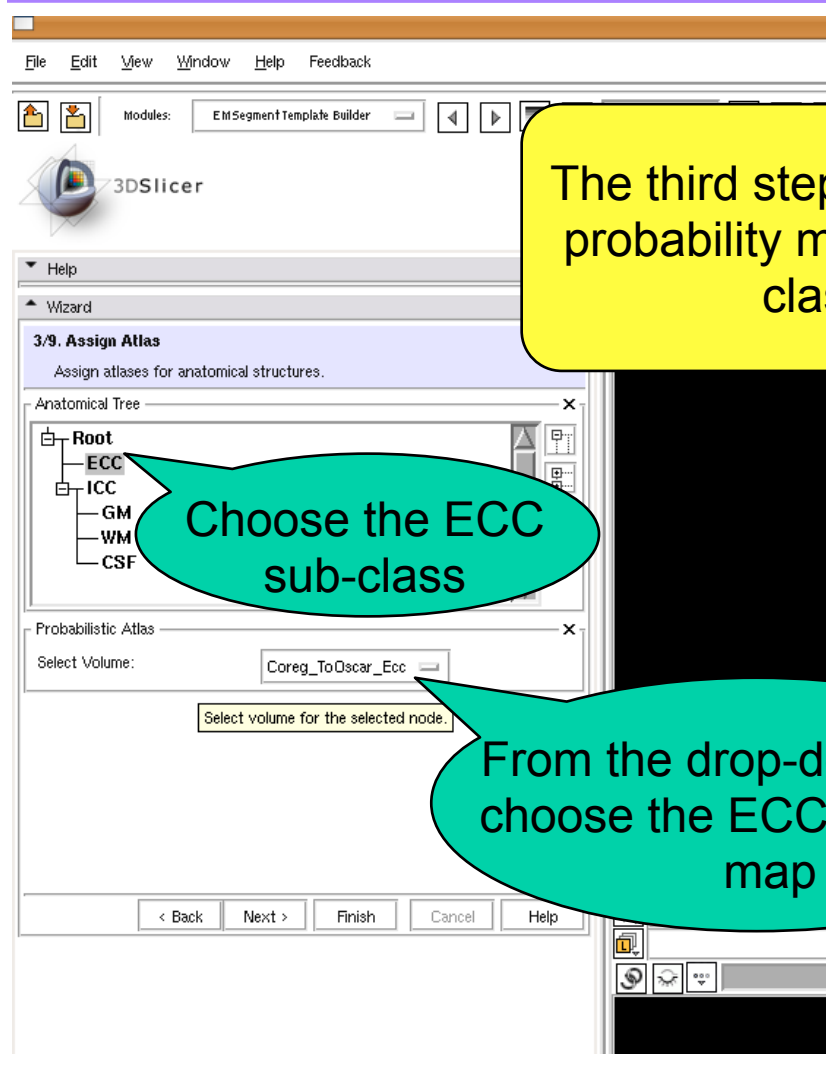


Add three sub-classes to ICC

Name the sub-classes as GM, WM and CSF with labels 1, 2 and 3 respectively in the SPLBrainAtlas labelmap

Finally, click next to go to the next step

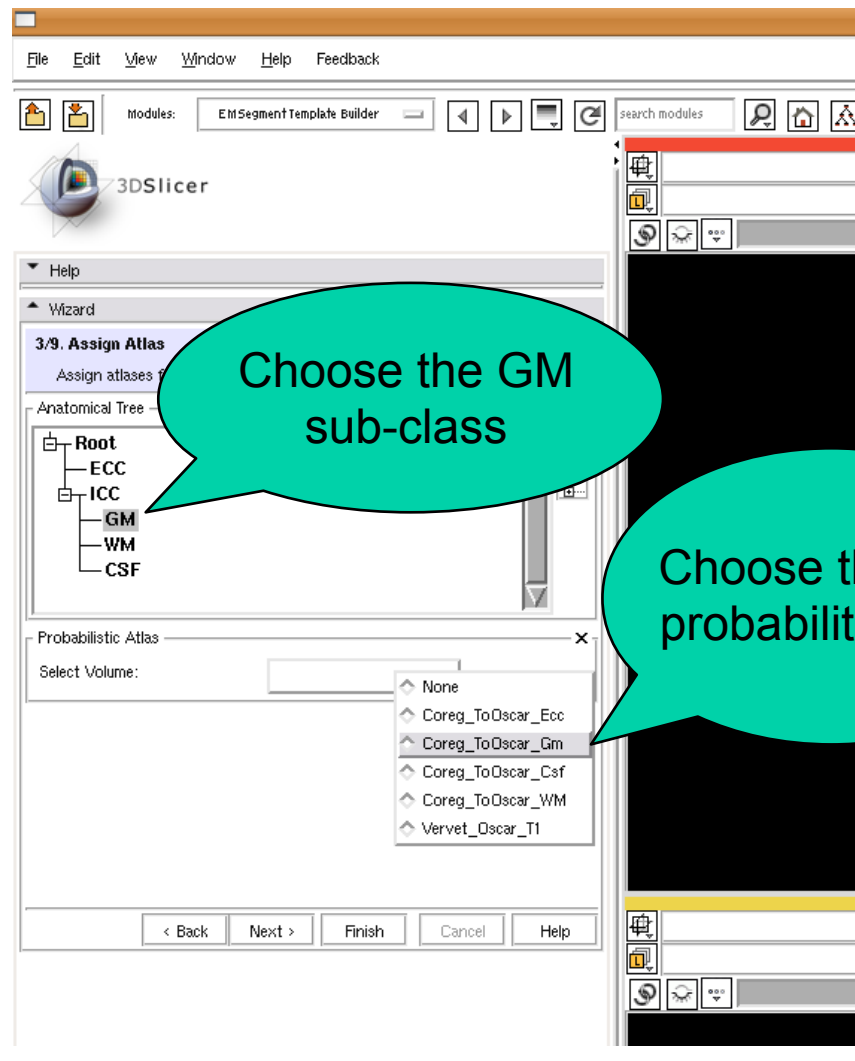
Segmentation



The third step is to assign probability maps to each class.

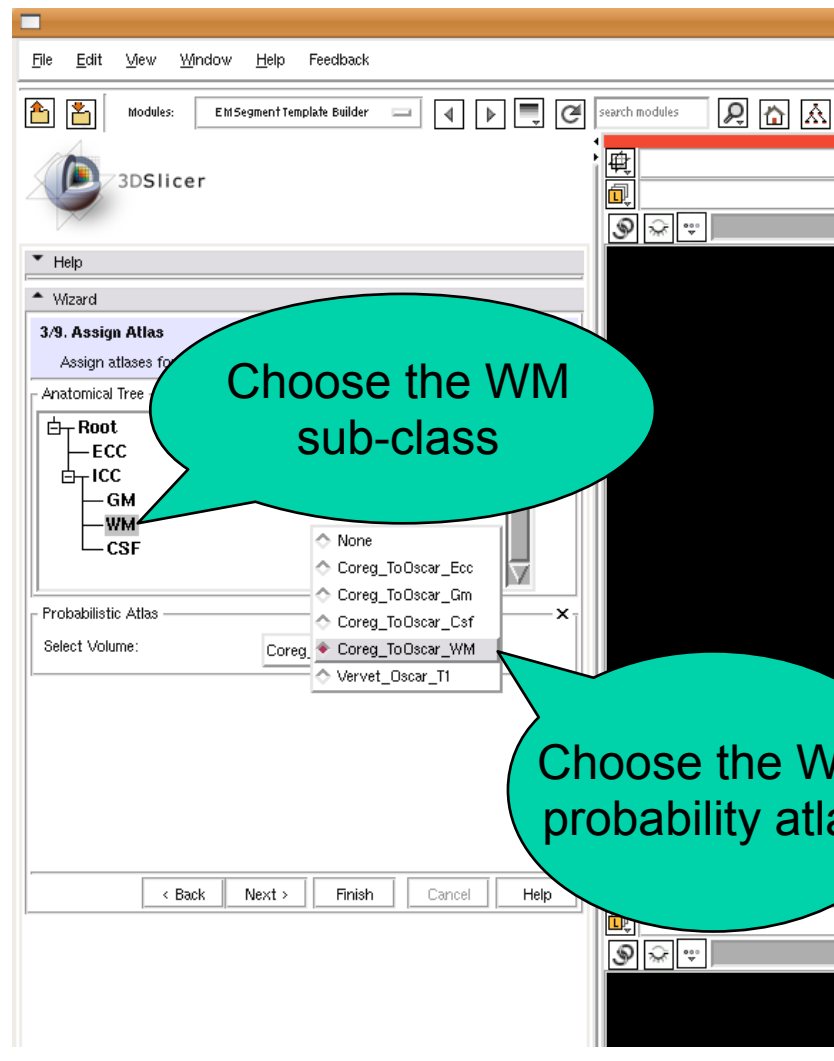
Choose the ECC sub-class

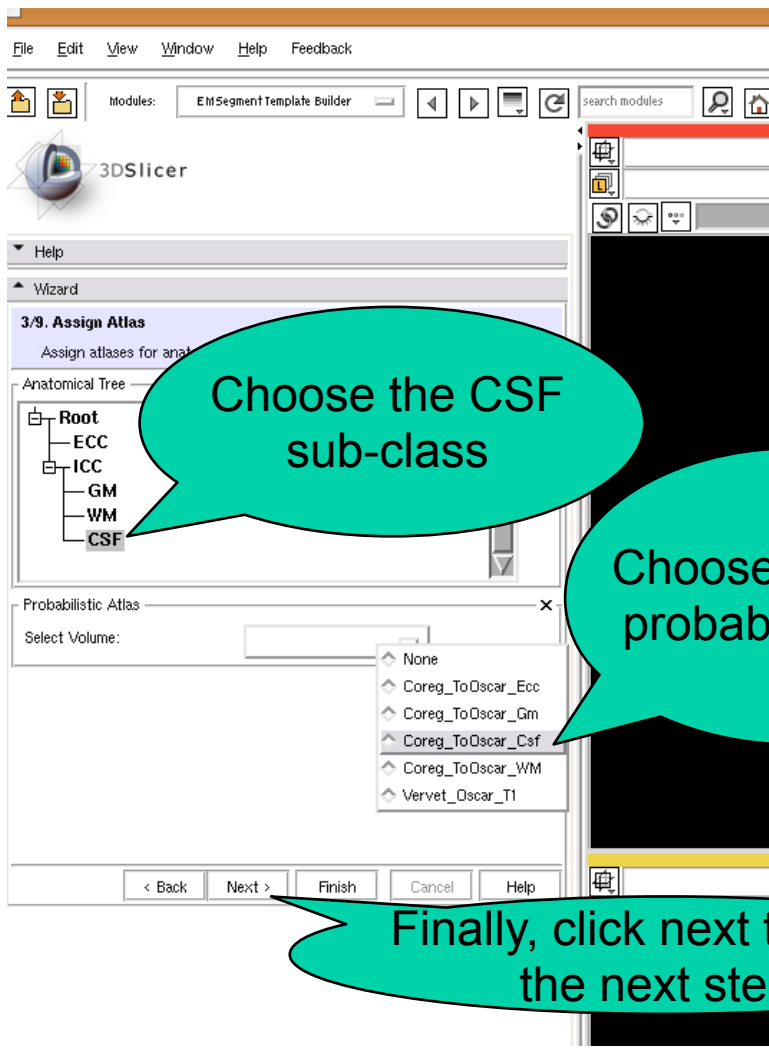
From the drop-down menu, choose the ECC probability map



Choose the GM sub-class

Choose the GM probability atlas





File Edit View Window Help Feedback

Modules: EMSegment Template Builder search modules

3DSlicer

Help

Wizard

3/9. Assign Atlas
Assign atlases for anat

Anatomical Tree

- Root
 - ECC
 - ICC
 - GM
 - WM
 - CSF**

Probabilistic Atlas

Select Volume:

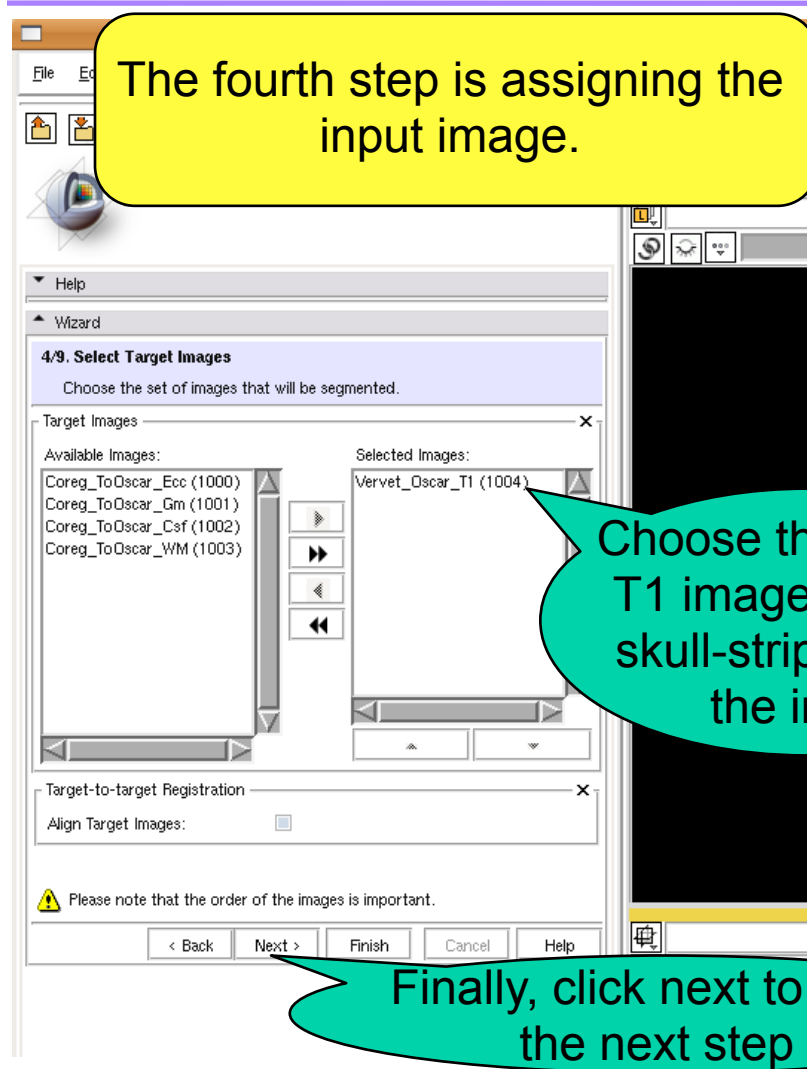
- None
- Coreg_ToOscar_Ecc
- Coreg_ToOscar_Gm
- Coreg_ToOscar_Csf**
- Coreg_ToOscar_WM
- Vervet_Oscar_T1

< Back Next > Finish Cancel Help

Choose the CSF sub-class

Choose the CSF probability atlas

Finally, click next to go to the next step



The fourth step is assigning the input image.

4/9. Select Target Images
Choose the set of images that will be segmented.

Target Images

Available Images:

- Coreg_ToOscar_Ecc (1000)
- Coreg_ToOscar_Gm (1001)
- Coreg_ToOscar_Csf (1002)
- Coreg_ToOscar_WM (1003)

Selected Images:

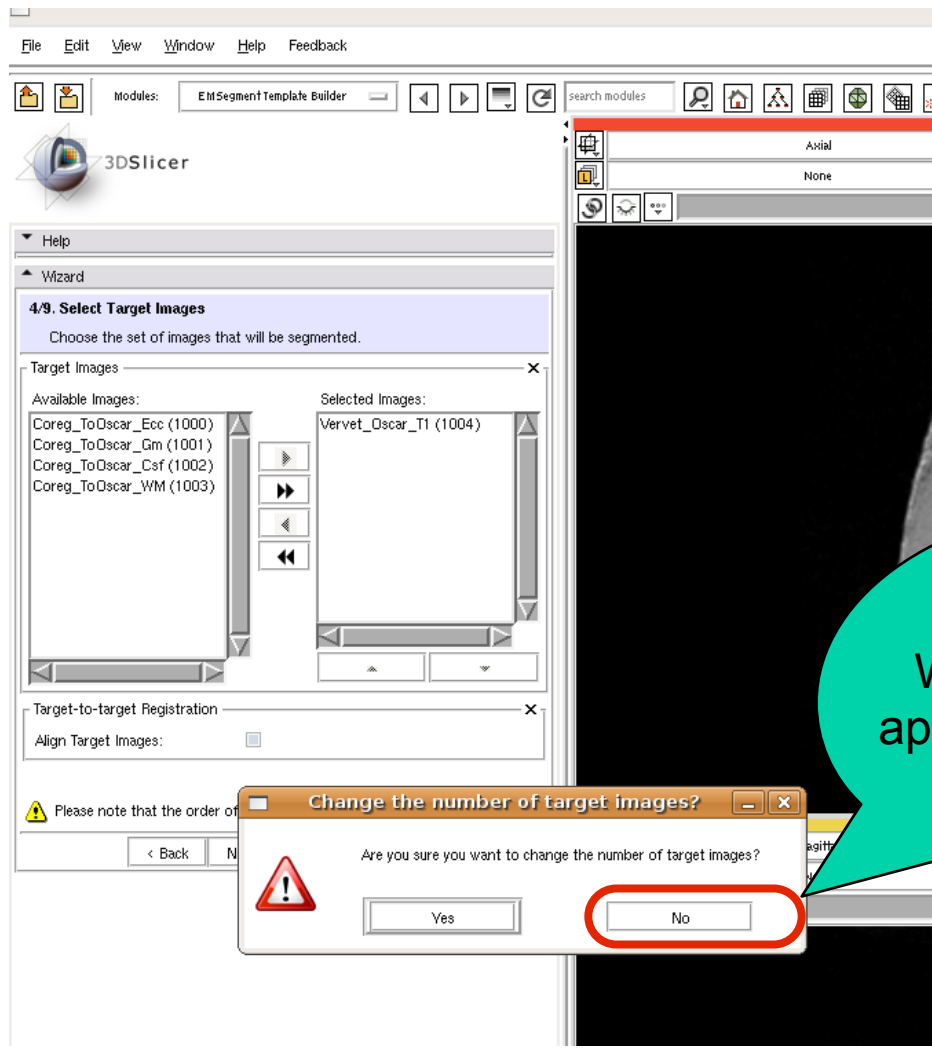
- Vervet_Oscar_T1 (1004)

Target-to-target Registration

Align Target Images:

Please note that the order of the images is important.

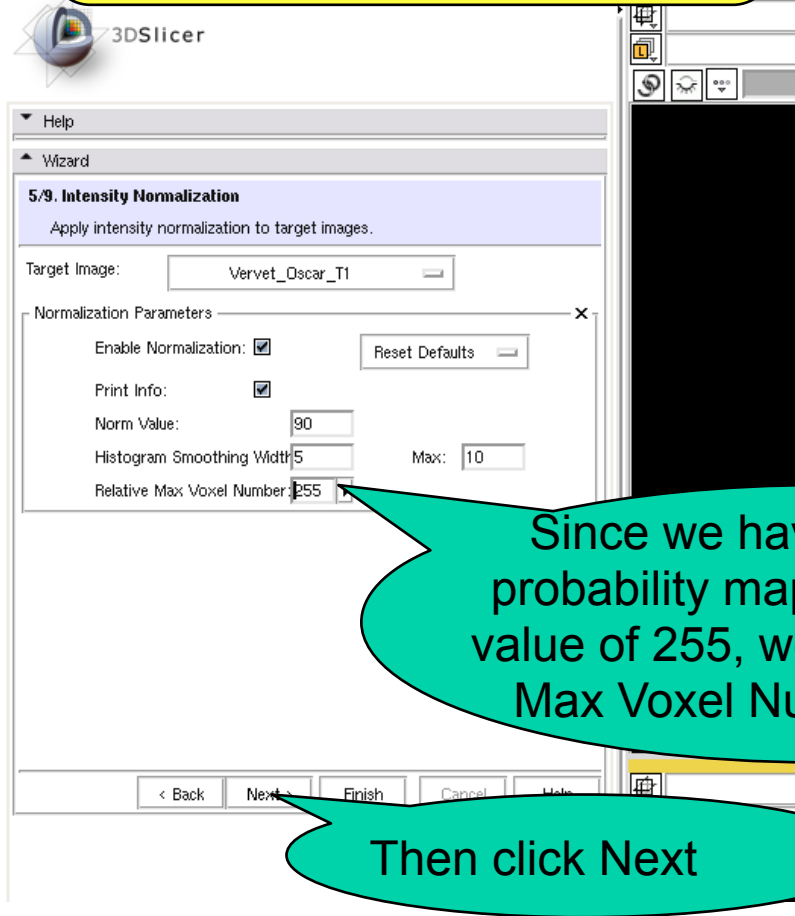
< Back Next > Finish Cancel Help



When this window appears , choose Yes

Segmentation

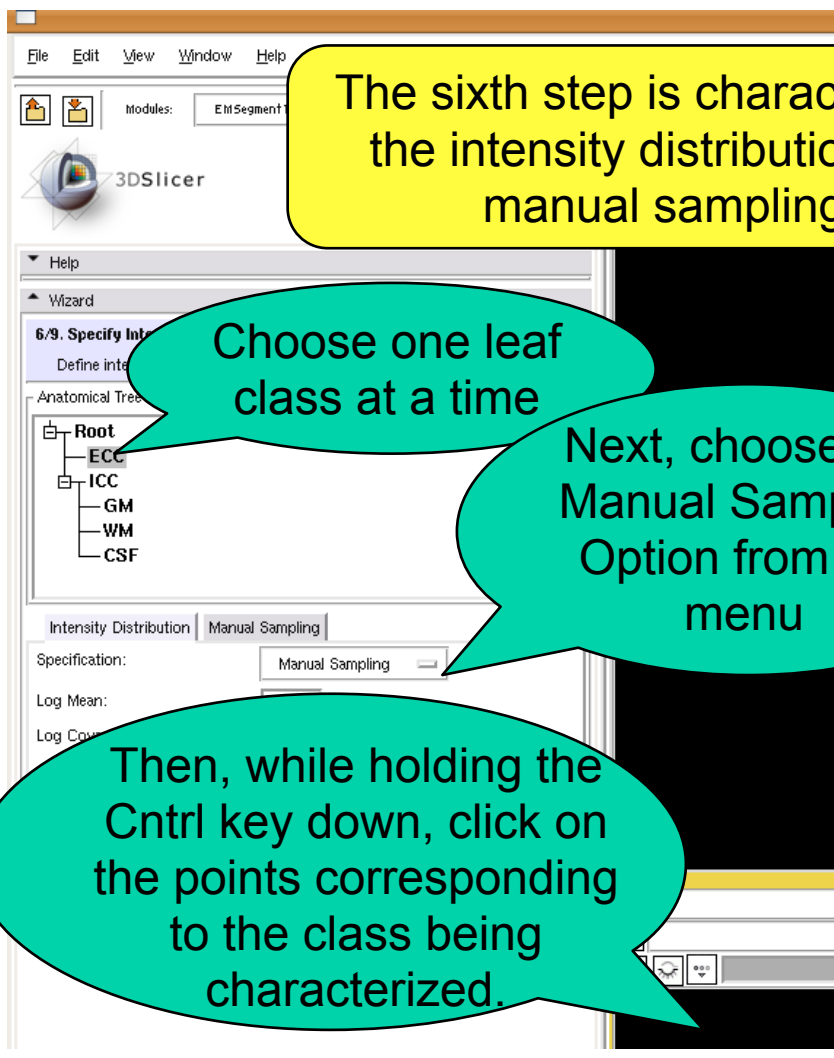
The fifth step is choosing normalization parameters.



Since we have rescaled the probability maps to a maximum value of 255, we choose Relative Max Voxel Number to be 255

Then click Next

Segmentation

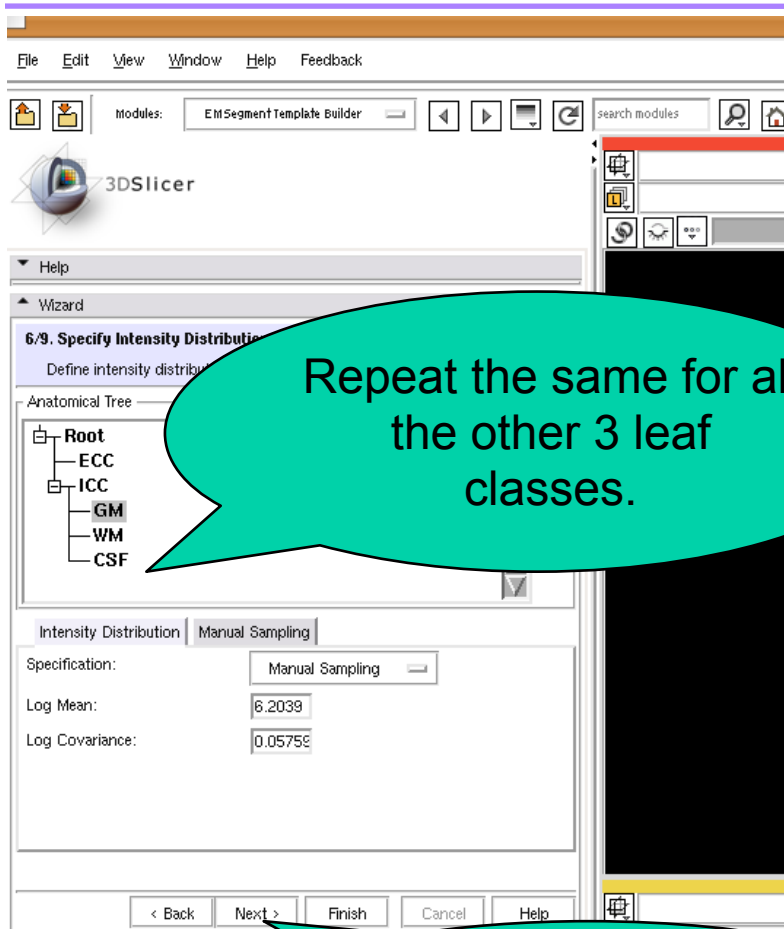


The sixth step is characterizing the intensity distributions by manual sampling

Choose one leaf class at a time

Next, choose the Manual Sampling Option from the menu

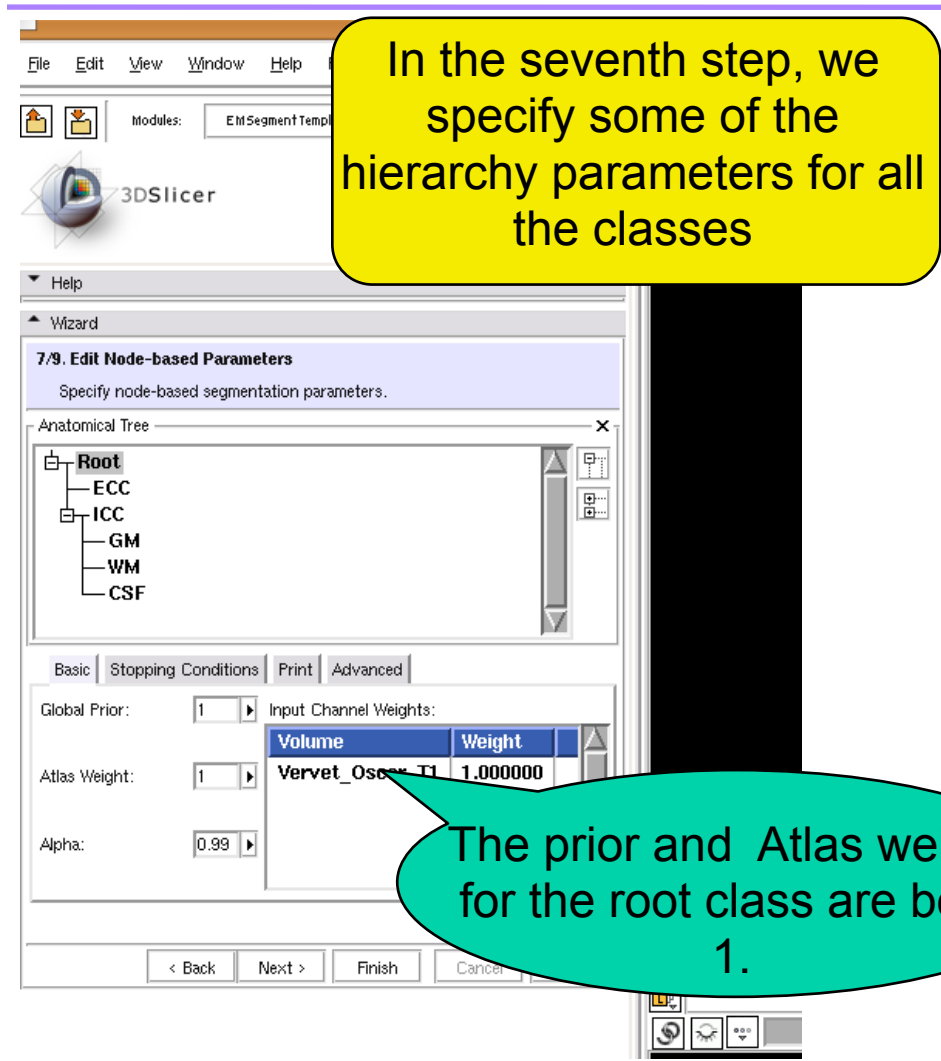
Then, while holding the Cntrl key down, click on the points corresponding to the class being characterized.



Repeat the same for all the other 3 leaf classes.

Finally, click next to move to the 7th step.

Segmentation

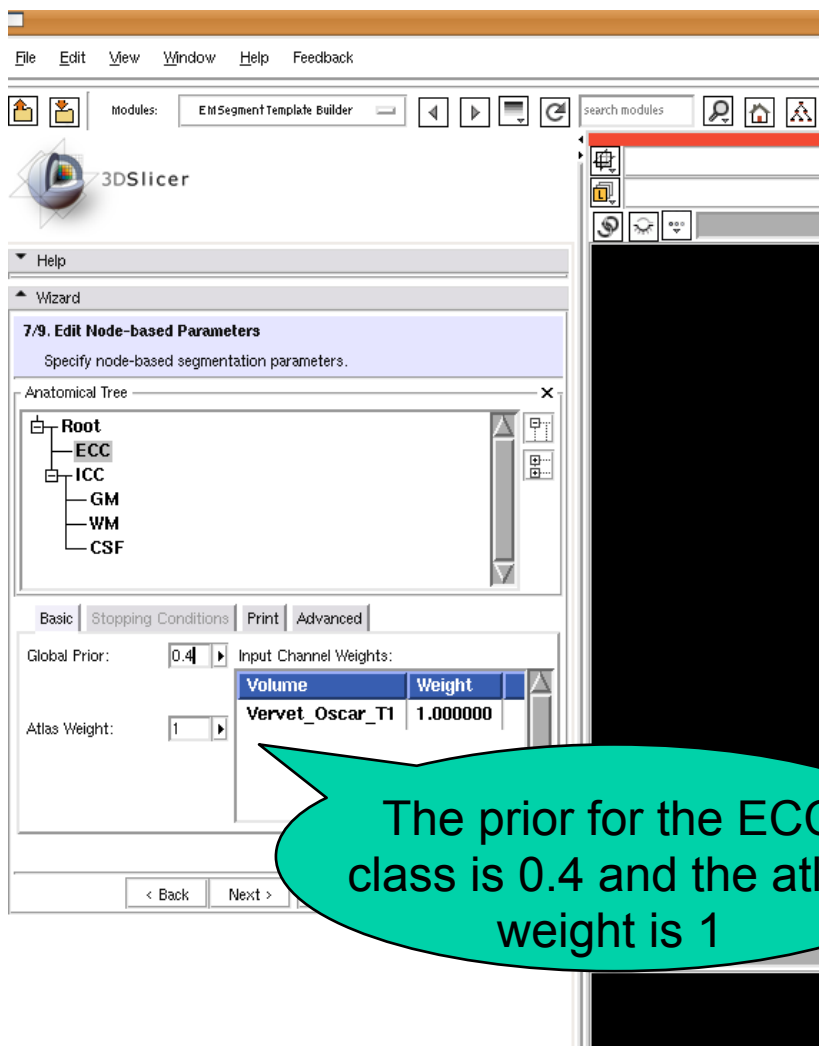


The screenshot shows the 3DSlicer interface during a segmentation wizard. The '7/9. Edit Node-based Parameters' step is active, with the instruction 'Specify node-based segmentation parameters.' The 'Anatomical Tree' on the left shows a hierarchy: Root, ECC, ICC, GM, WM, and CSF. The 'Basic' tab is selected, showing 'Global Prior' and 'Atlas Weight' both set to 1. The 'Input Channel Weights' table is visible, with the 'Volume' column and 'Vervet_Ossex_T1' row highlighted. A yellow callout box points to the anatomical tree, and a green callout box points to the Global Prior and Atlas Weight settings.

In the seventh step, we specify some of the hierarchy parameters for all the classes

Volume	Weight
Vervet_Ossex_T1	1.000000

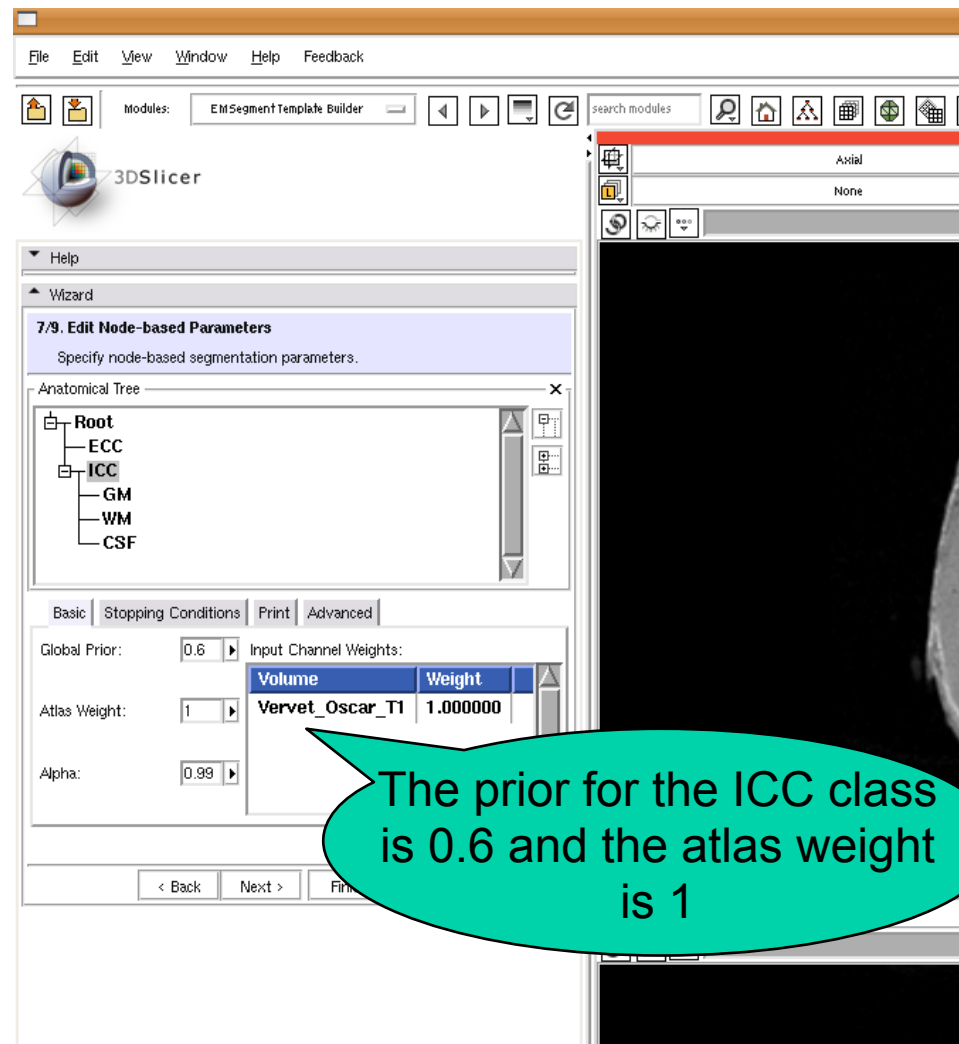
The prior and Atlas weight for the root class are both 1.



The screenshot shows the 3DSlicer interface with the EMSegmentTemplate Builder module active. The Wizard is at step 7/9, 'Edit Node-based Parameters'. The Anatomical Tree shows a hierarchy: Root -> ECC -> ICC -> GM, WM, CSF. The 'Basic' tab is selected, showing 'Global Prior' set to 0.4 and 'Atlas Weight' set to 1. The 'Input Channel Weights' table is visible below.

Volume	Weight
Vervet_Oscar_T1	1.000000

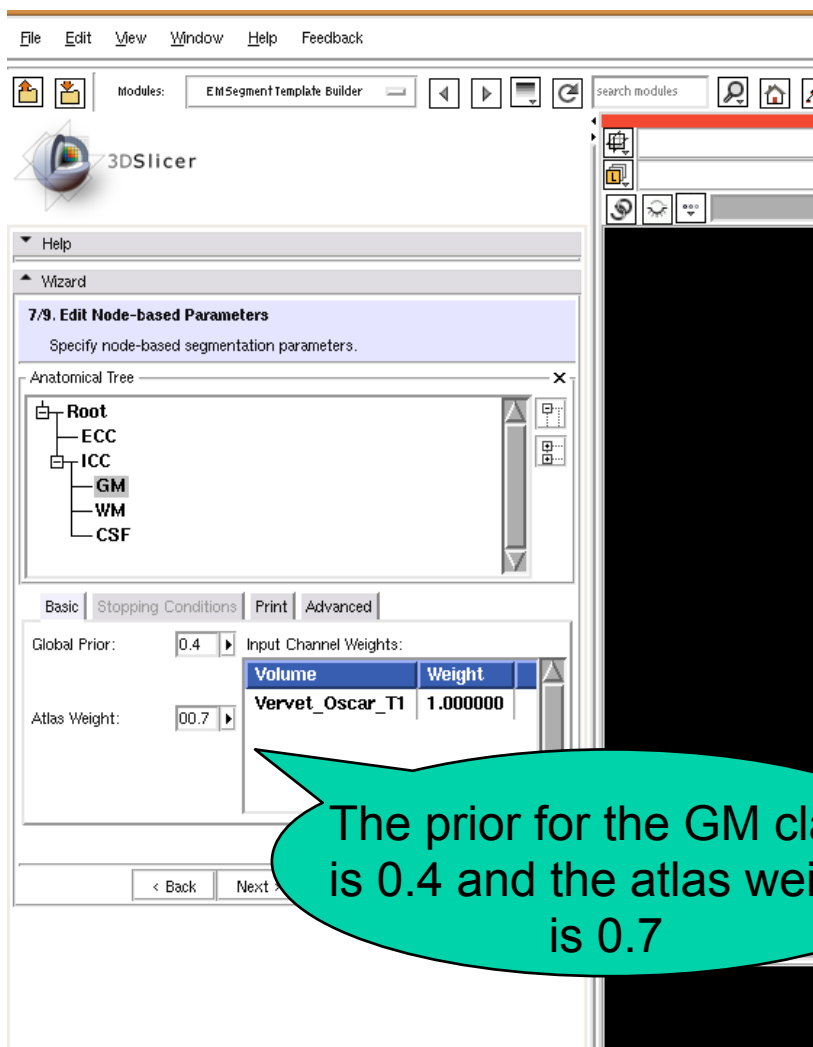
The prior for the ECC class is 0.4 and the atlas weight is 1



The screenshot shows the 3DSlicer interface with the 'EMSegment Template Builder' module active. The 'Anatomical Tree' on the left shows a hierarchy where 'ICC' is selected. The 'Basic' tab of the parameter panel is open, showing 'Global Prior' set to 0.6 and 'Atlas Weight' set to 1. The 'Input Channel Weights' table is as follows:

Volume	Weight
Vervet_Oscar_T1	1.000000

A callout bubble points to these values with the text: "The prior for the ICC class is 0.6 and the atlas weight is 1".



File Edit View Window Help Feedback

Modules: EMSegment Template Builder search modules

3DSlicer

Help

Wizard

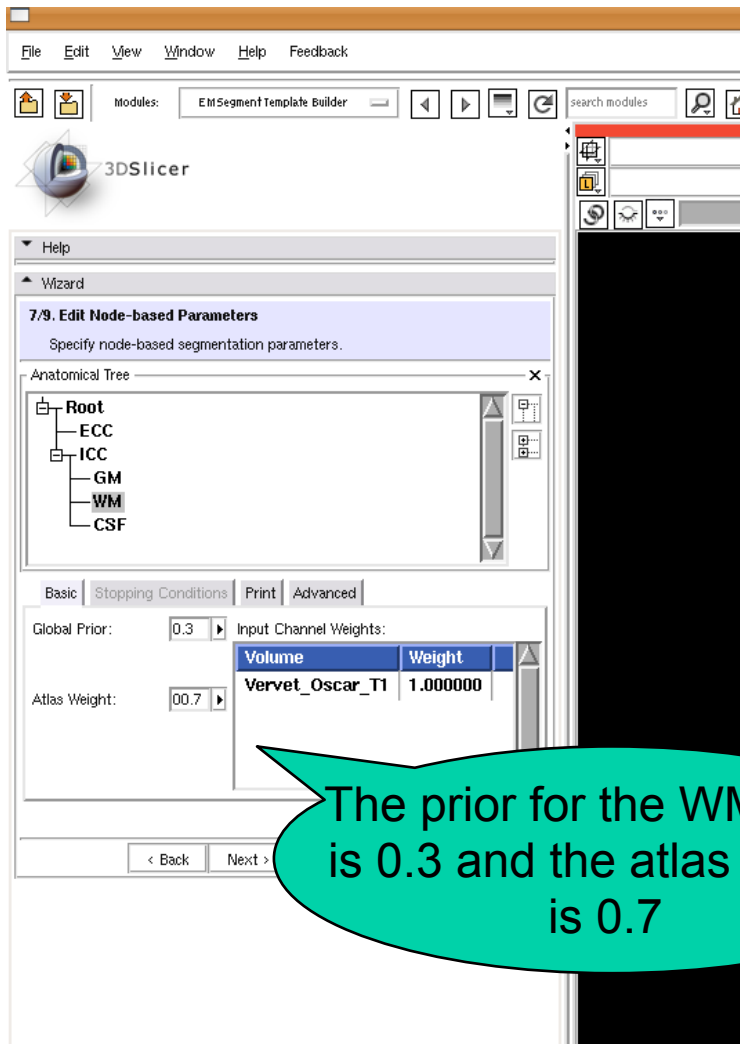
7/9. Edit Node-based Parameters
Specify node-based segmentation parameters.

Anatomical Tree

- Root
 - ECC
 - ICC
 - GM**
 - WM
 - CSF

The prior for the GM class is 0.4 and the atlas weight is 0.7

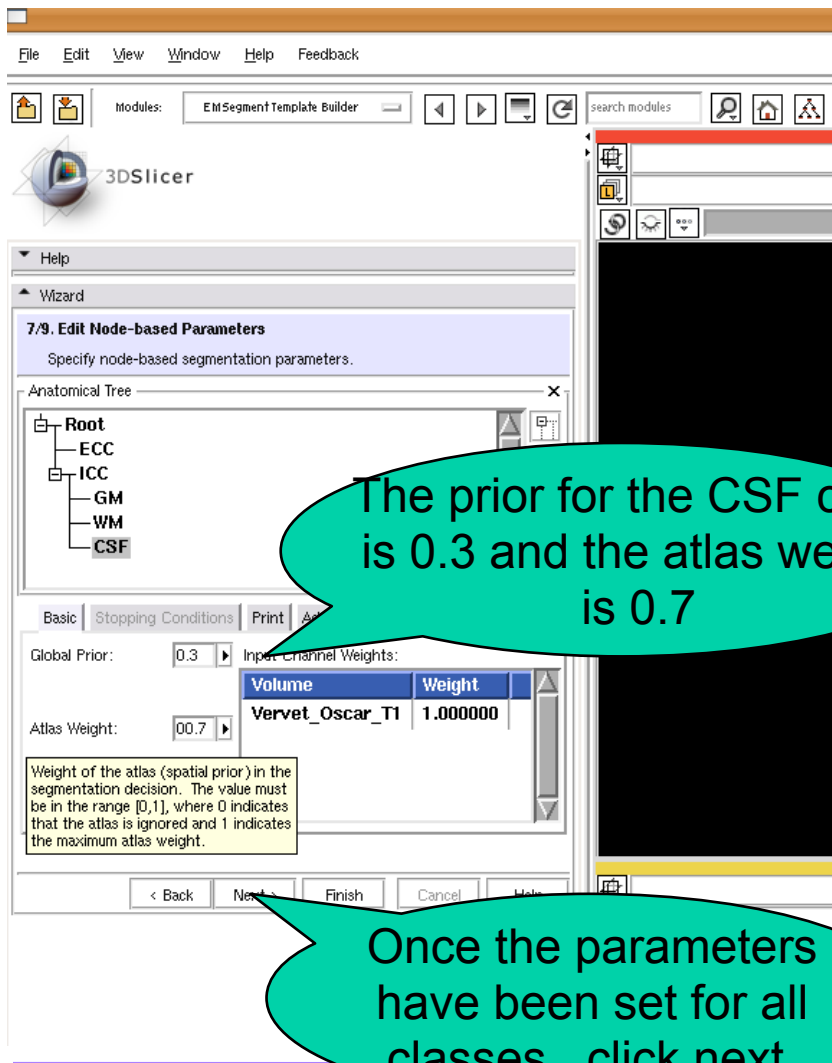
Segmentation



The screenshot shows the 'EM Segment Template Builder' window in 3DSlicer. The 'Wizard' section is active, specifically '7/9. Edit Node-based Parameters'. The 'Anatomical Tree' shows a hierarchy: Root -> ECC -> ICC -> GM -> WM -> CSF. The 'Basic' tab is selected, showing 'Global Prior' set to 0.3 and 'Atlas Weight' set to 0.7. The 'Input Channel Weights' table is as follows:

Volume	Weight
Vervet_Oscar_T1	1.000000

The prior for the WM class is 0.3 and the atlas weight is 0.7



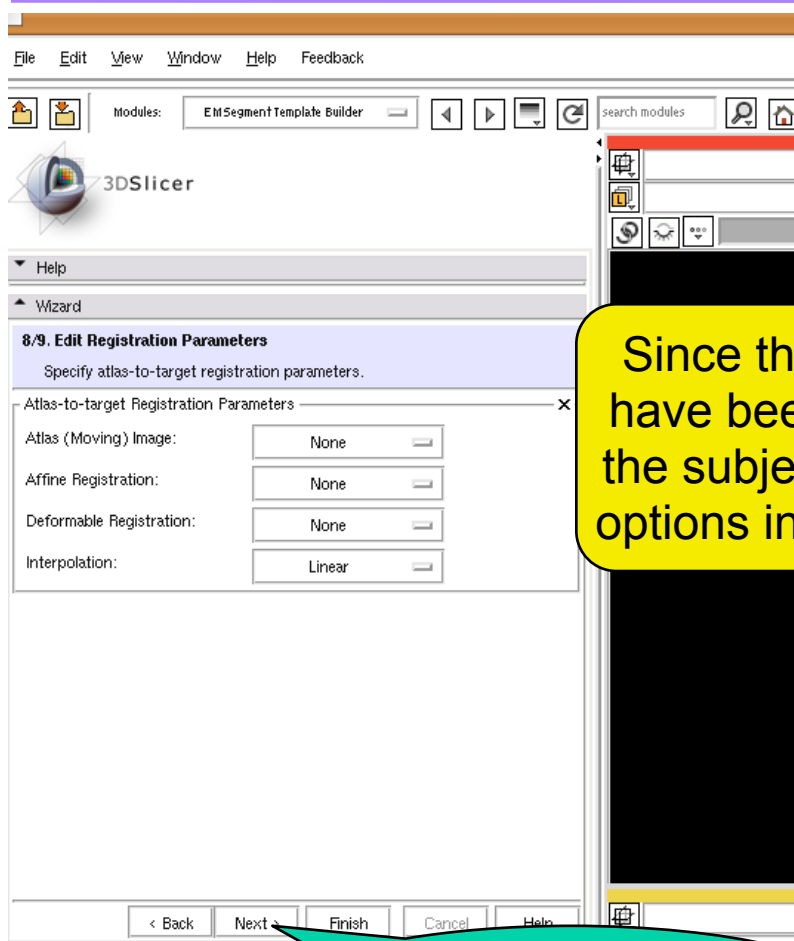
The screenshot shows the 3DSlicer interface with the EMSegmentTemplate Builder module active. The wizard is at step 7/9, 'Edit Node-based Parameters'. The Anatomical Tree shows a hierarchy: Root -> ECC -> ICC -> GM, WM, CSF. The 'Basic' tab is selected, showing a 'Global Prior' of 0.3 and an 'Atlas Weight' of 0.7. A table for 'Input Channel Weights' is visible, with one entry: Vervet_Oscar_T1 with a weight of 1.000000. A tooltip explains the Atlas Weight parameter.

Volume	Weight
Vervet_Oscar_T1	1.000000

Weight of the atlas (spatial prior) in the segmentation decision. The value must be in the range [0,1], where 0 indicates that the atlas is ignored and 1 indicates the maximum atlas weight.

The prior for the CSF class is 0.3 and the atlas weight is 0.7

Once the parameters have been set for all classes, click next.



Since the probability maps have been pre-registered to the subject image, we set all options in this step to: NONE

and then click Next

Segmentation

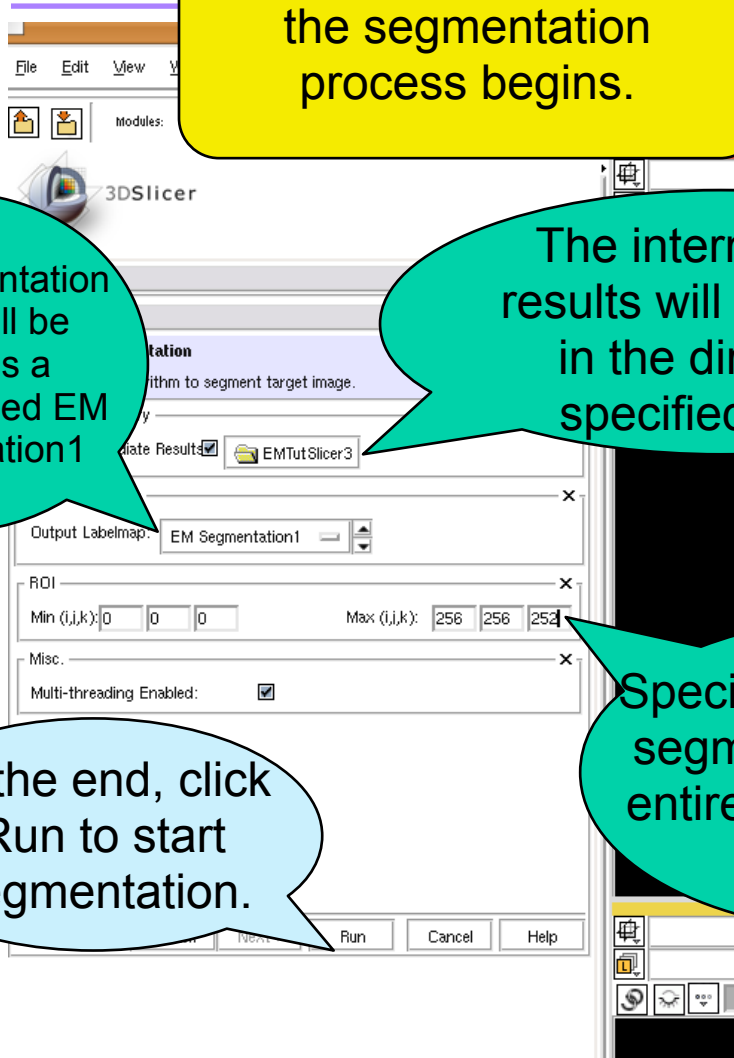
This is final step before the segmentation process begins.

The segmentation output will be saved as a volume called EM Segmentation1

The intermediate results will be saved in the directory specified here.

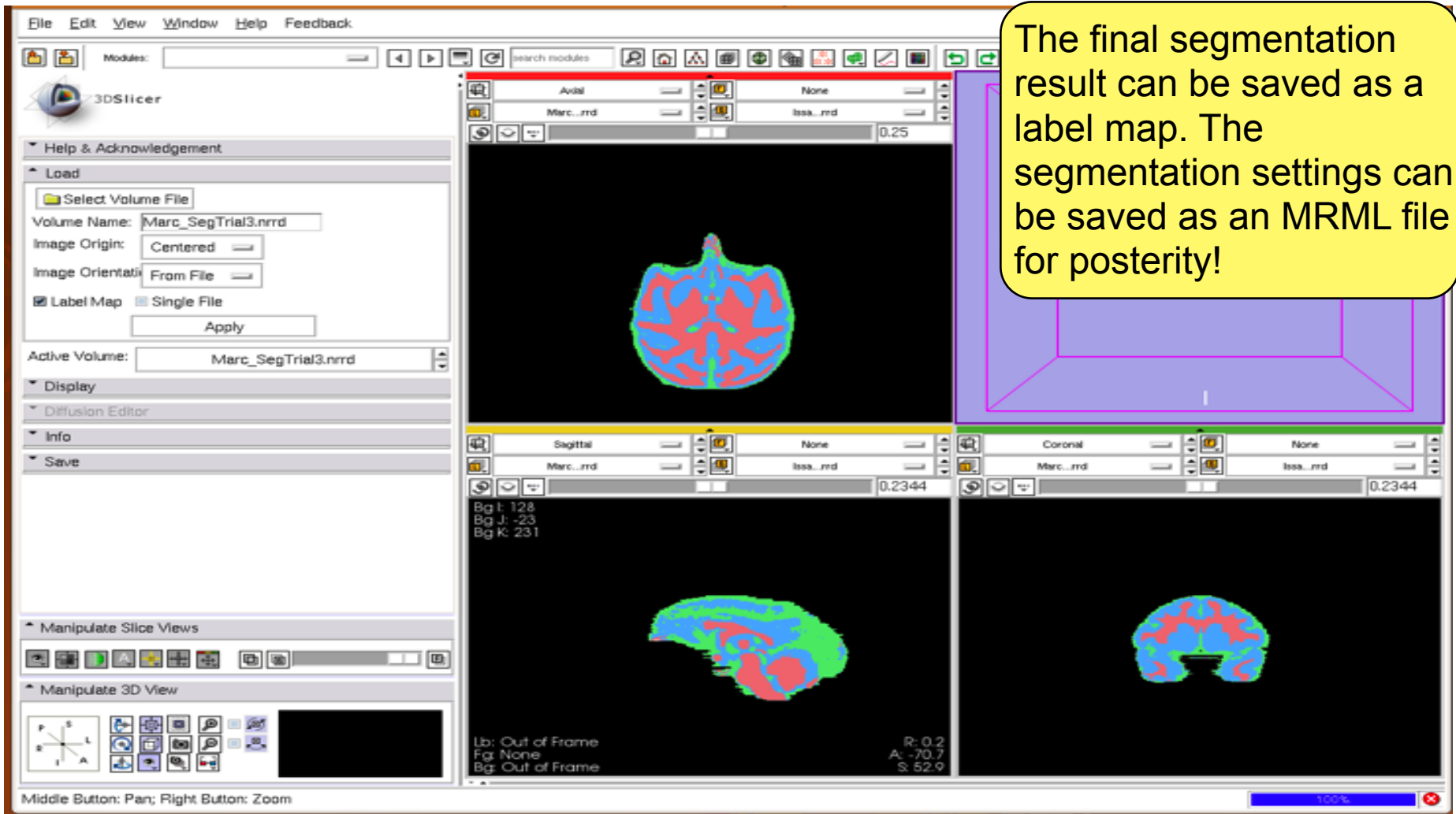
At the end, click Run to start segmentation.

Specify the slices to be segmented. Here the entire image has to be segmented.



Result - Segmentation Label Map

The final segmentation result can be saved as a label map. The segmentation settings can be saved as an MRML file for posterity!



- The segmentation result can be saved as a labelmap
- The segmentation hierarchy can be modified to include sub-cortical structures.
- Probability maps for sub-cortical structures are also available for download along with the other maps.



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