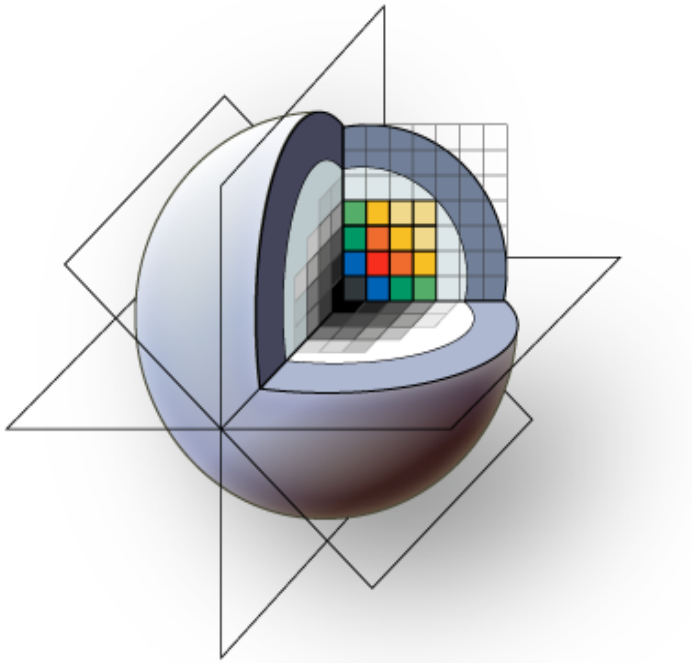
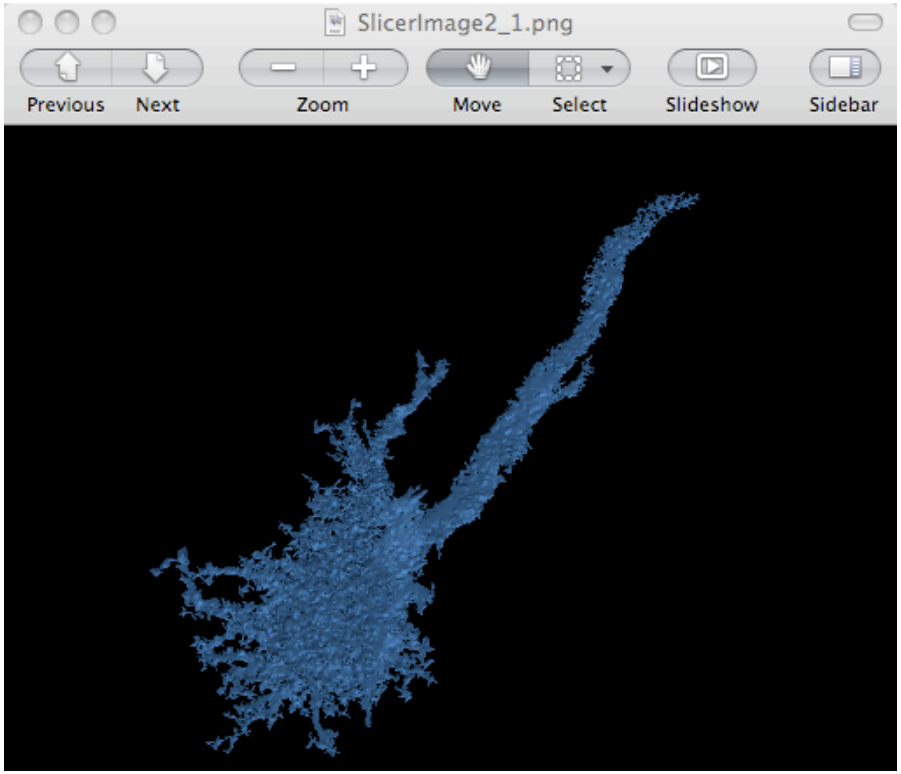
Introduction to the Use of Slicer in Confocal Microscopy



Learning Objective



Guiding you step by step through the process of loading confocal microscopy data, working with that data, and creating a 3D model for visualization.

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

- At least 300 MB free disk space

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 3.4***)

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

- Tutorial dataset (***Data from CCDB***)

http://ccdb.ucsd.edu

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.*

Overview

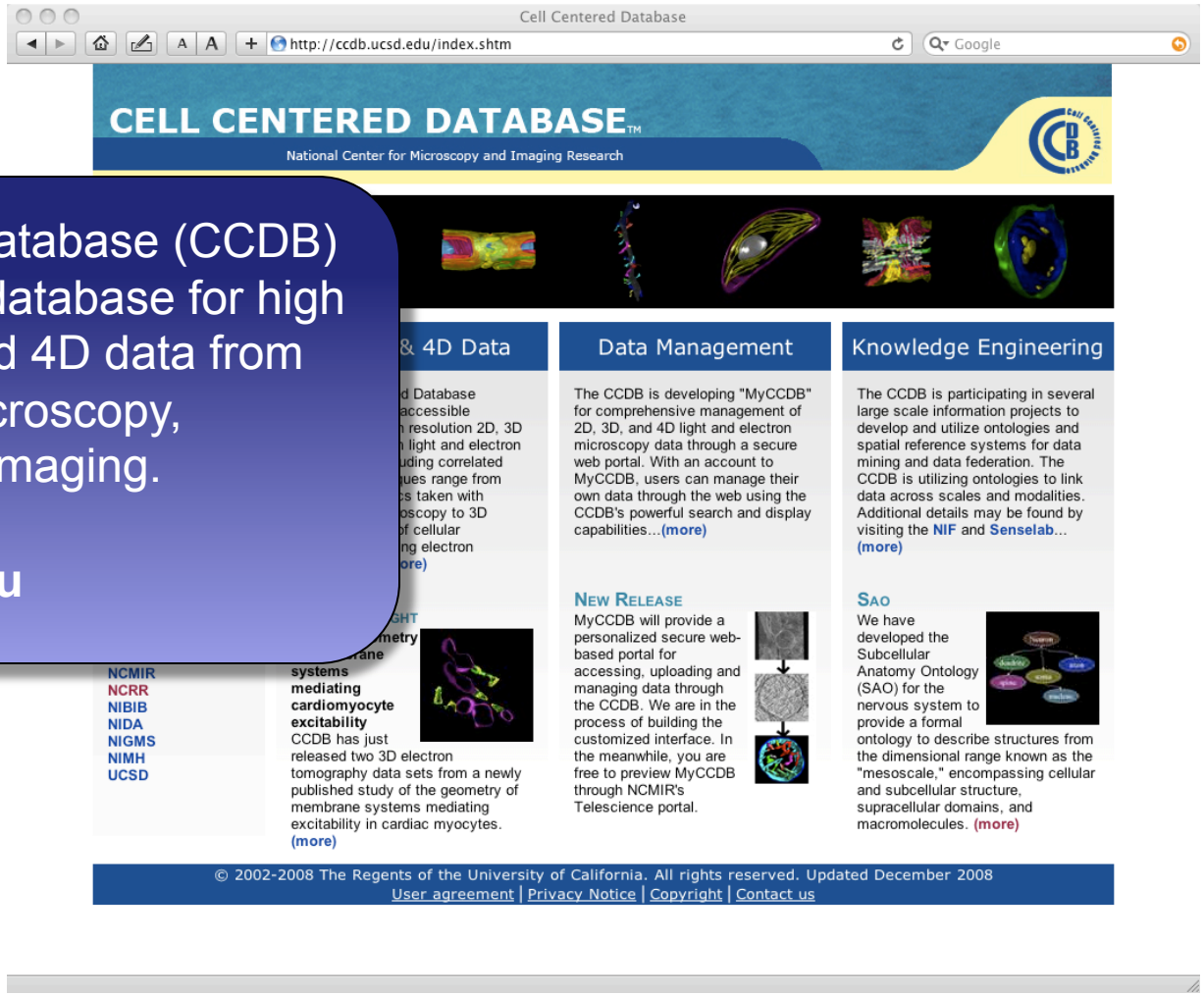
Basic Data Handling and Visualization

Note: this section uses a larger dataset

Downloading Sample Data

The Cell Centered Database (CCDB) is a web accessible database for high resolution 2D, 3D and 4D data from light and electron microscopy, including correlated imaging.

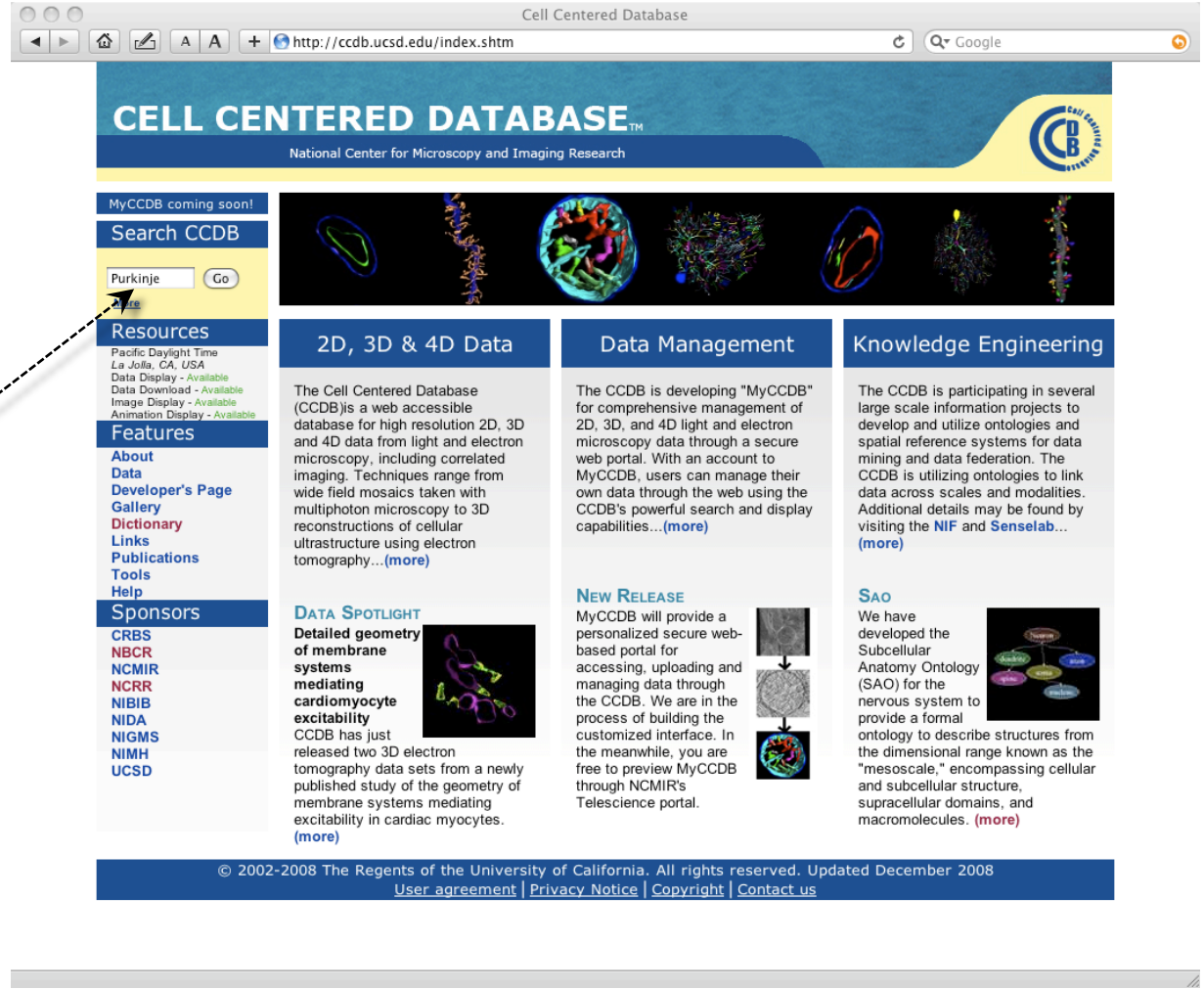
http://ccdb.ucsd.edu



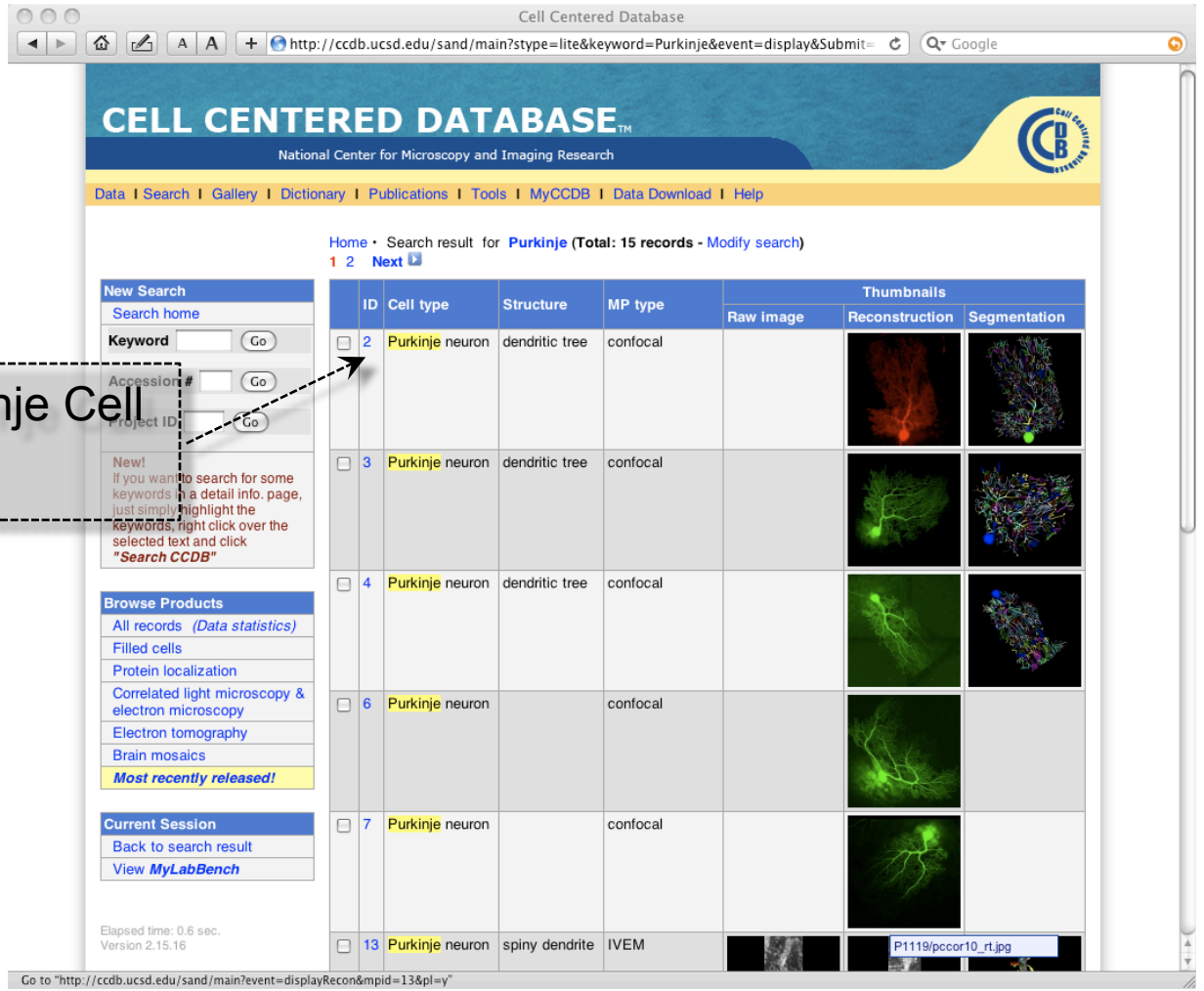
Downloading Sample Data

1) Enter Search Term:
   Purkinje

2) Select 'Go'



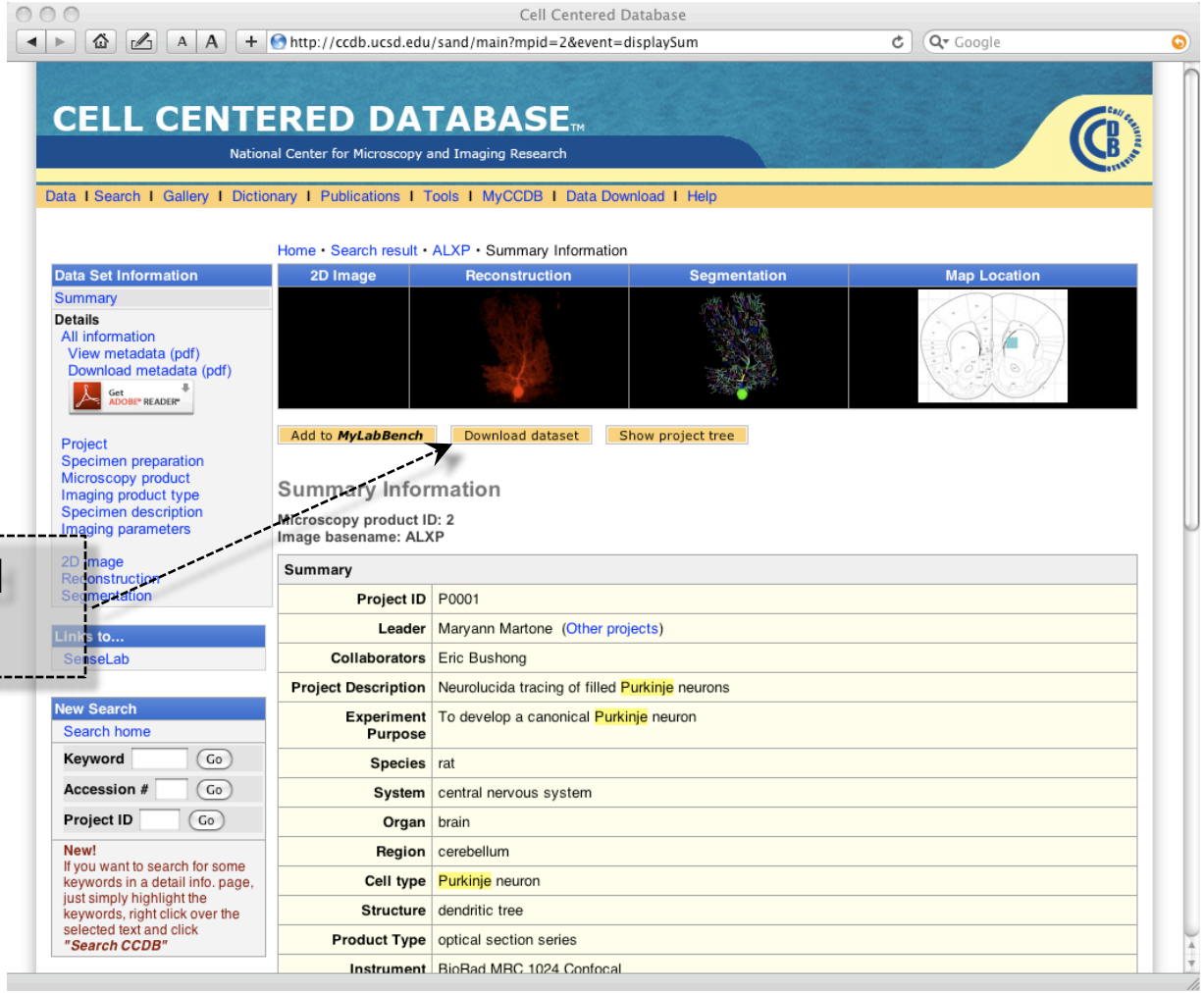
Downloading Sample Data



1) Select Purkinje Cell with ID 2

Downloading Sample Data

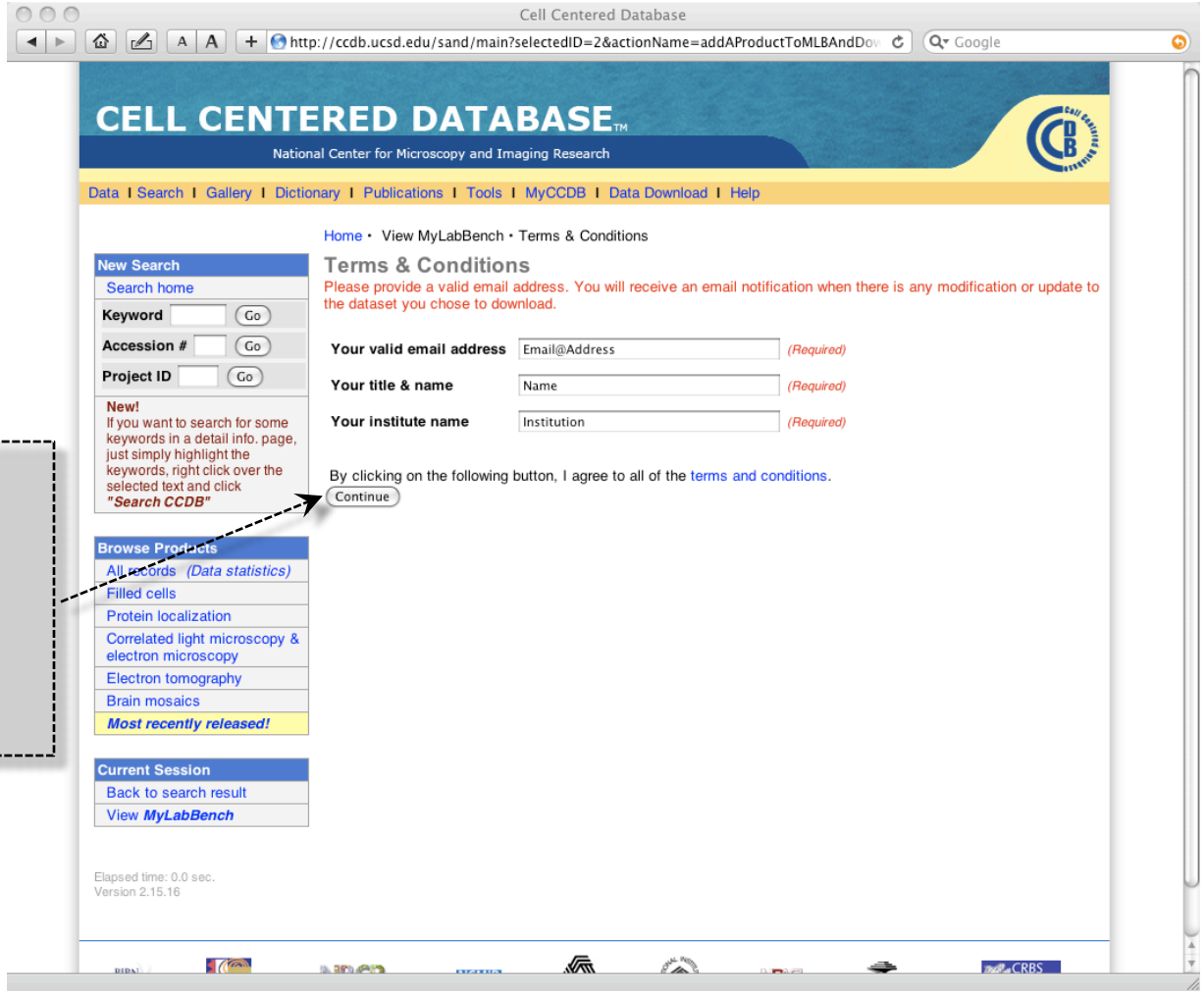
1) Select ‘Download dataset’



Downloading Sample Data

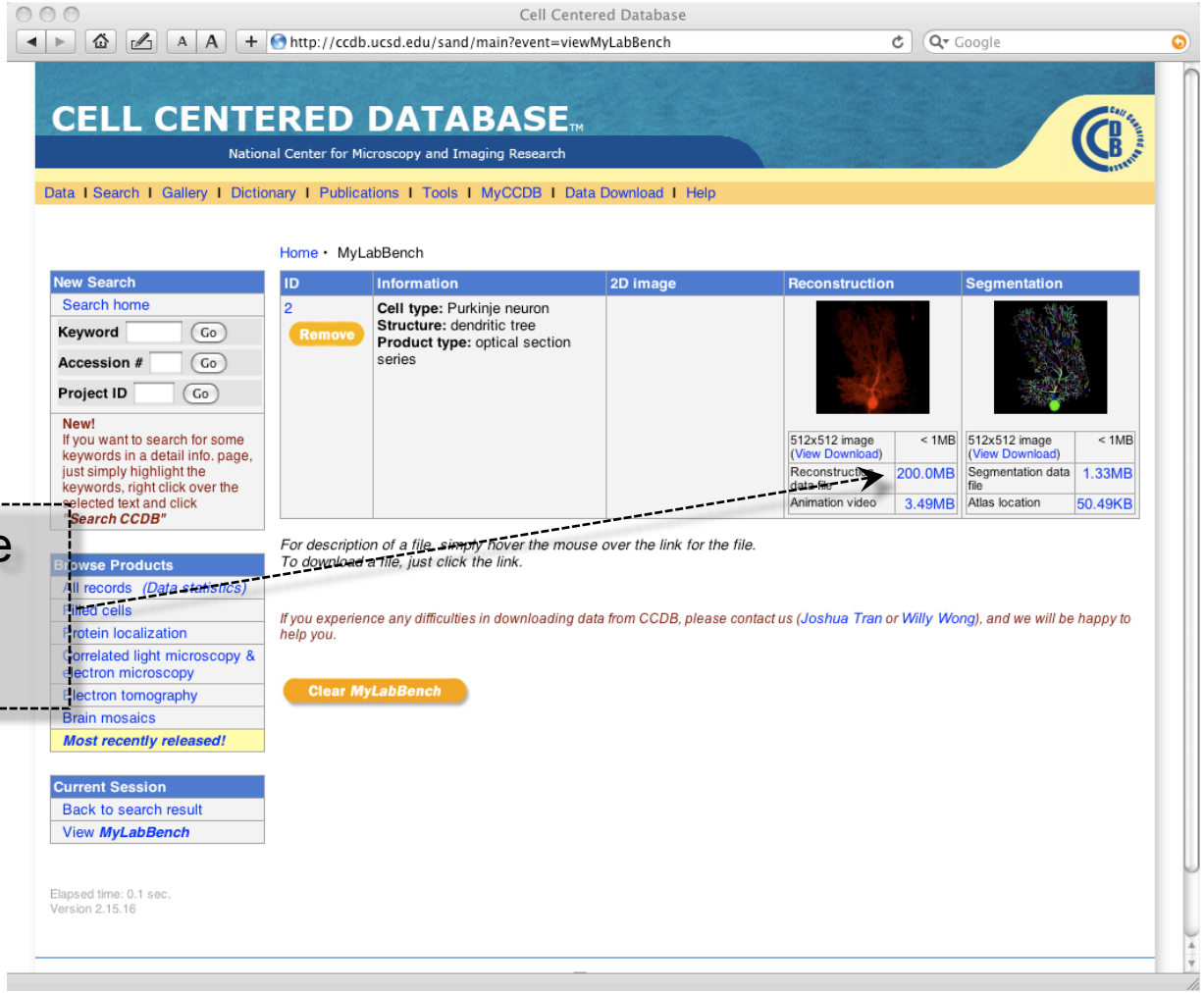
1) Enter required information to receive updates

2) Select 'Continue'

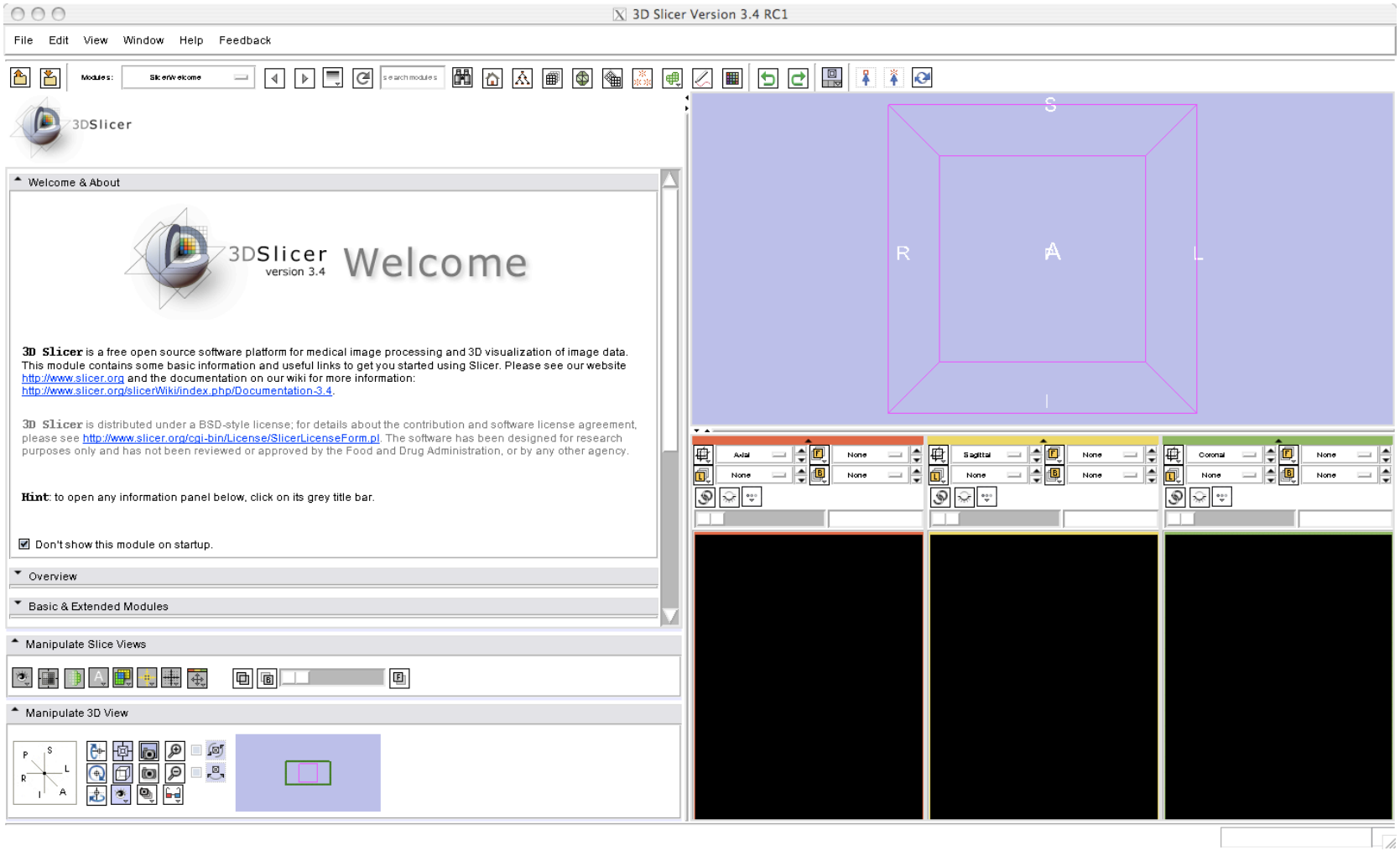


Downloading Sample Data

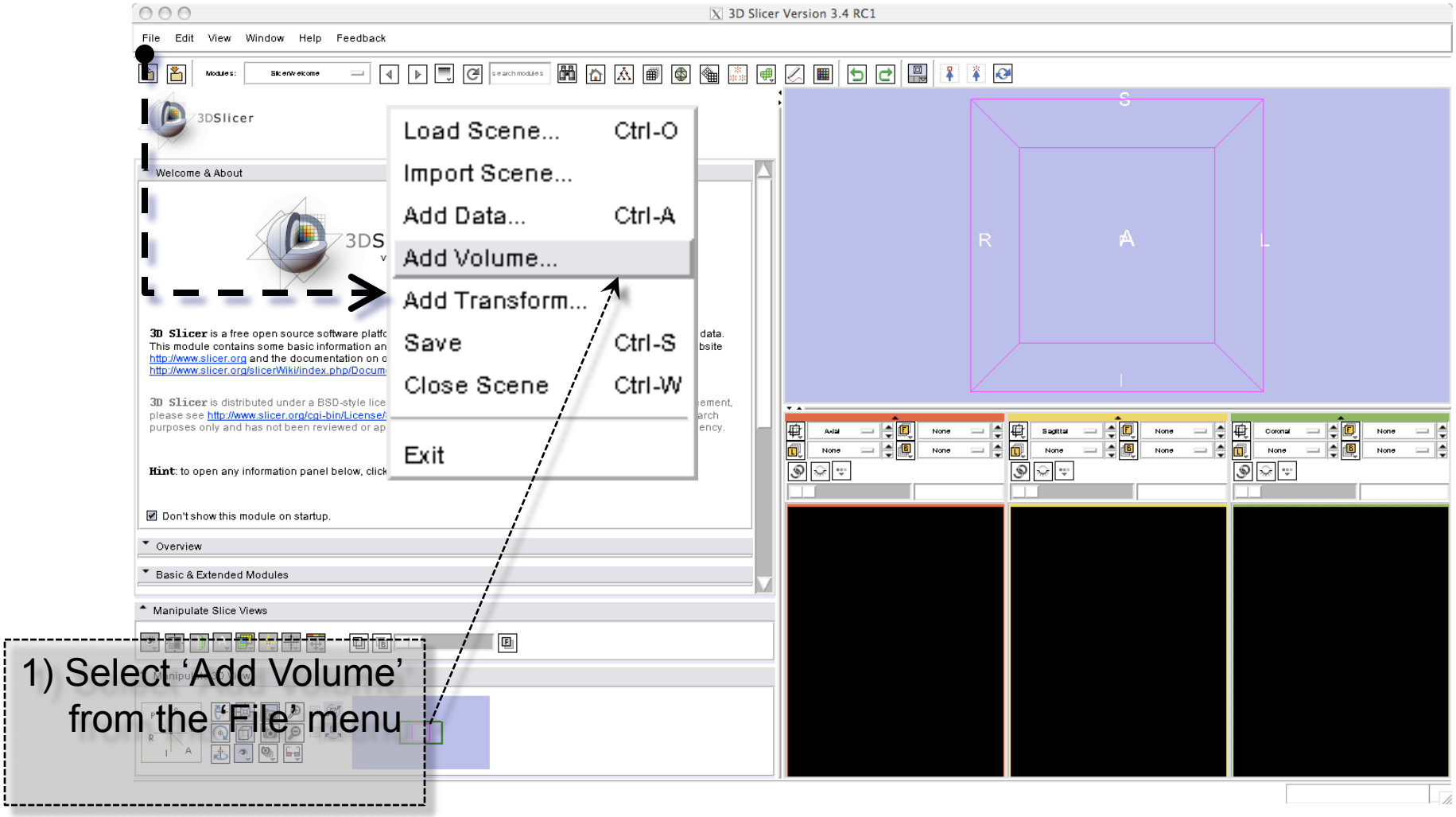
1) Download data file by selecting '200.0MB'



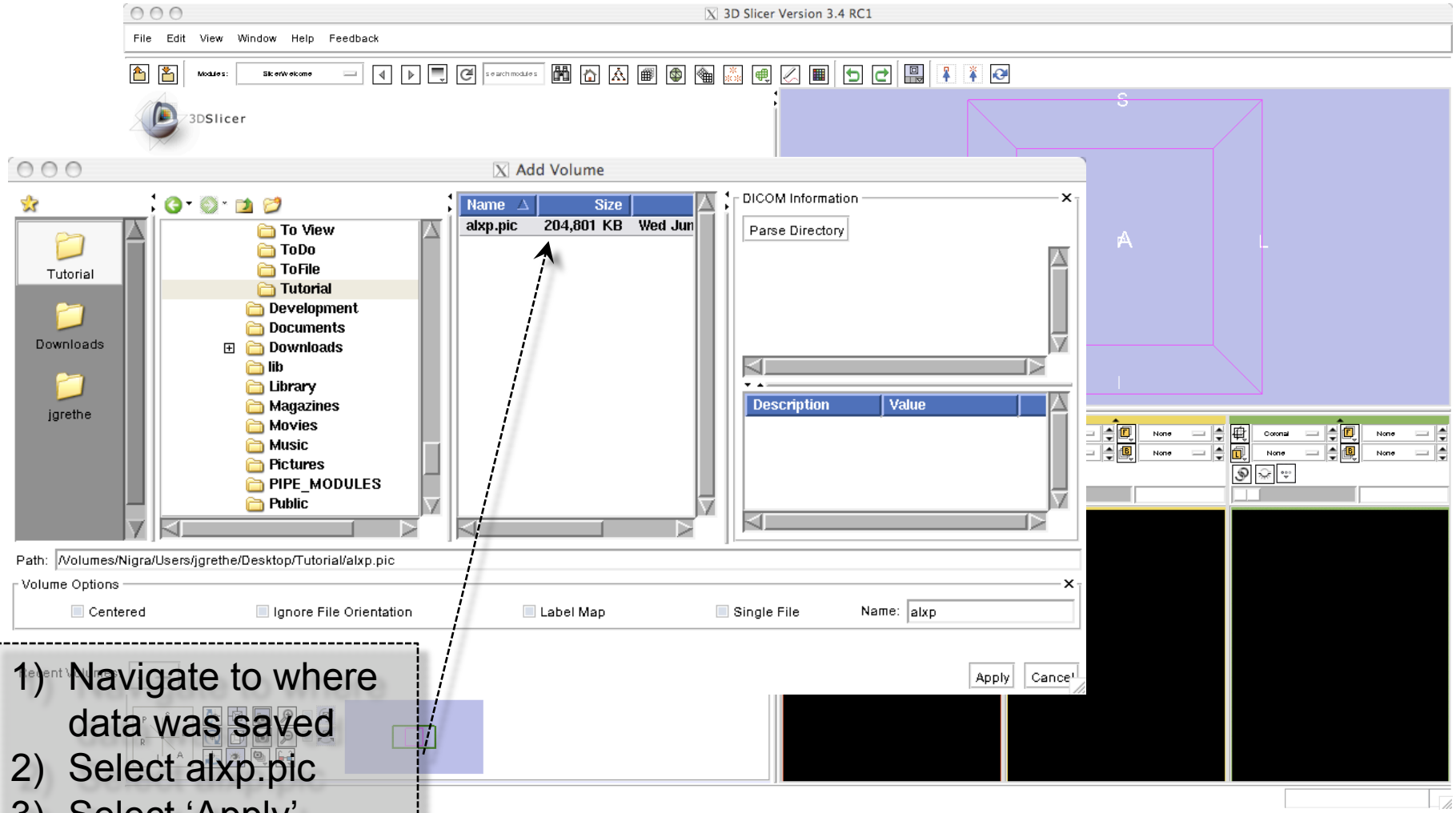
Launch Slicer



Open Purkinje Cell Data

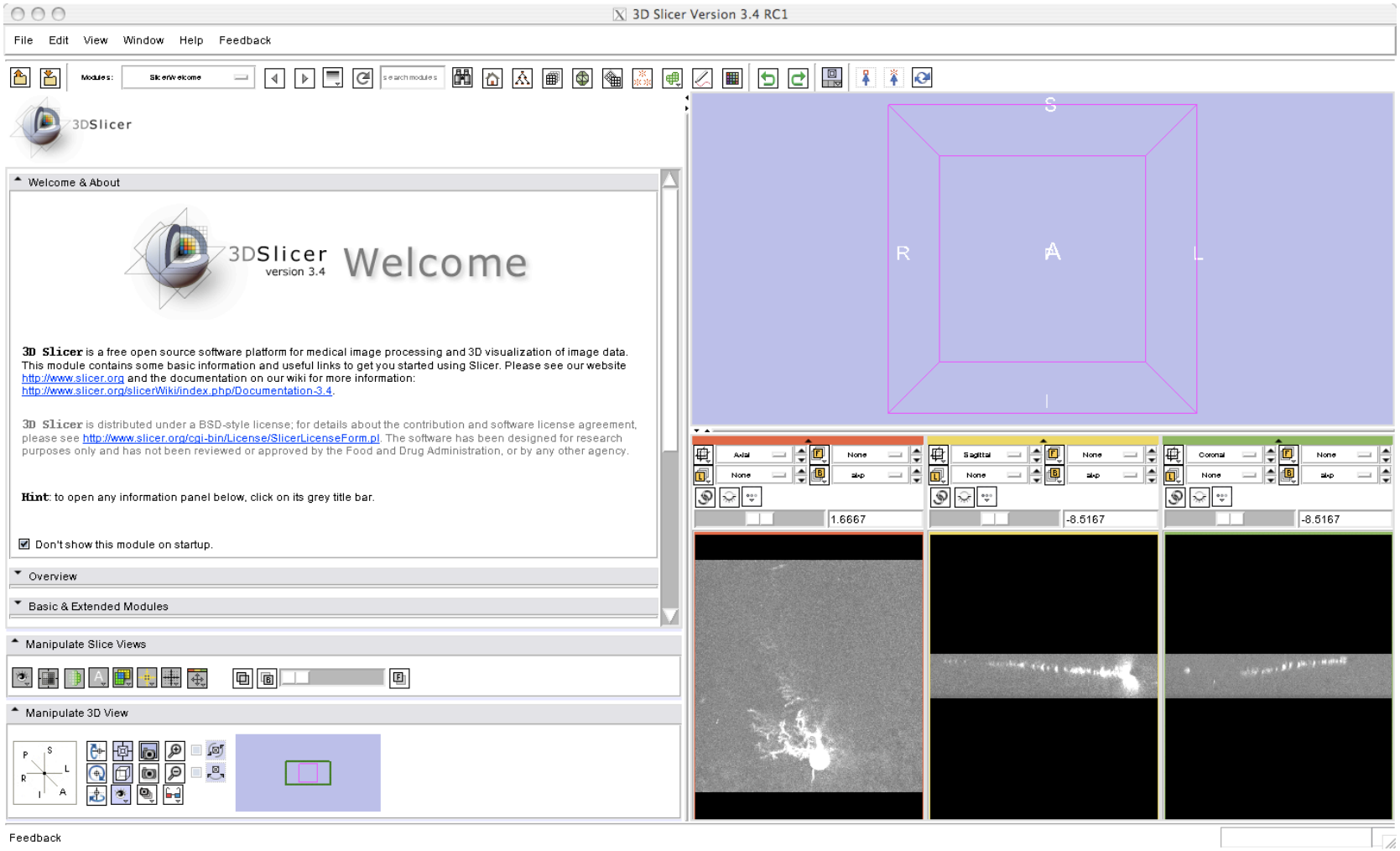


Open Purkinje Cell Data



1) Navigate to where data was saved
2) Select alxp.pic
3) Select 'Apply'

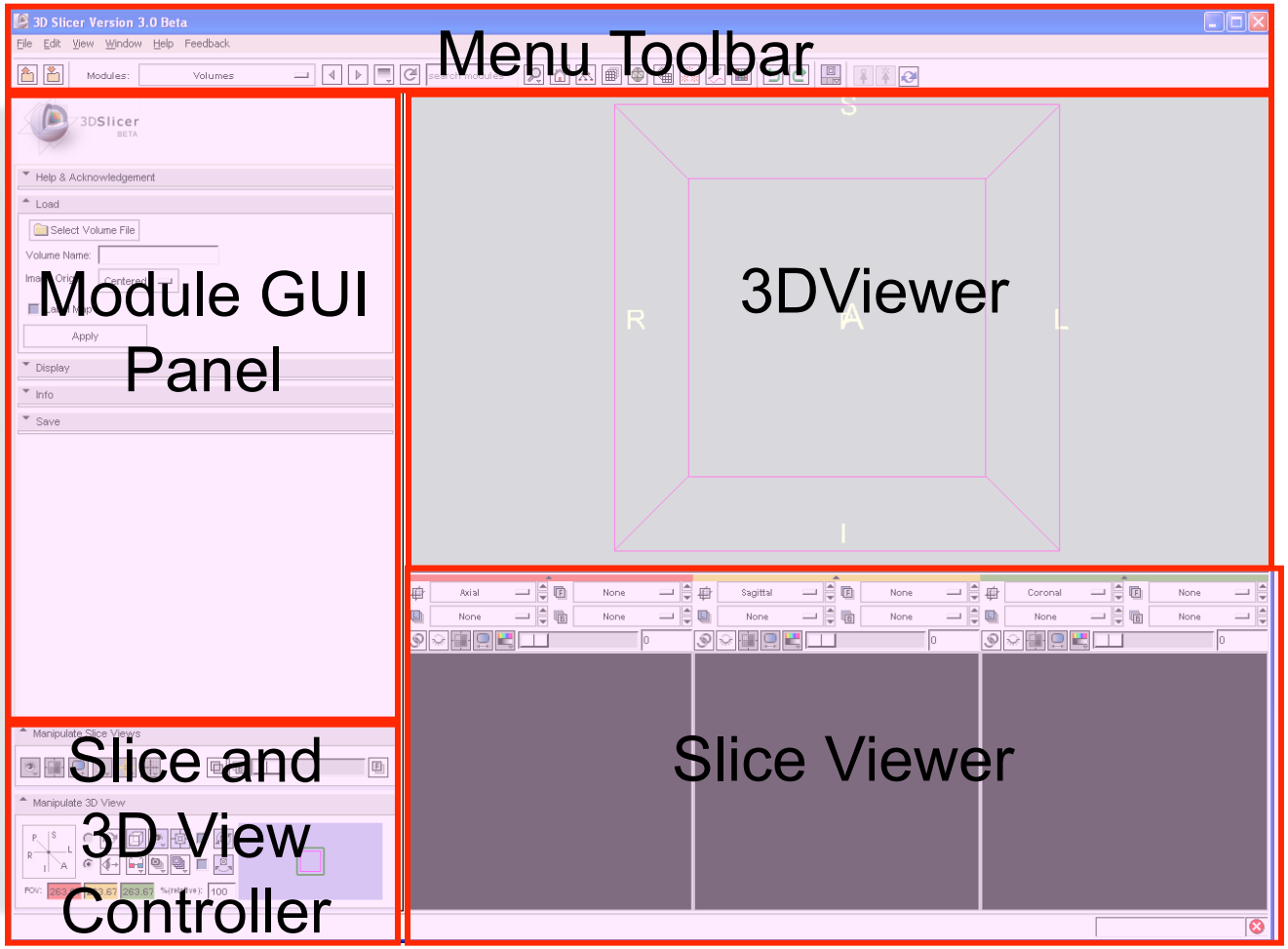
Setup Slicer Layout



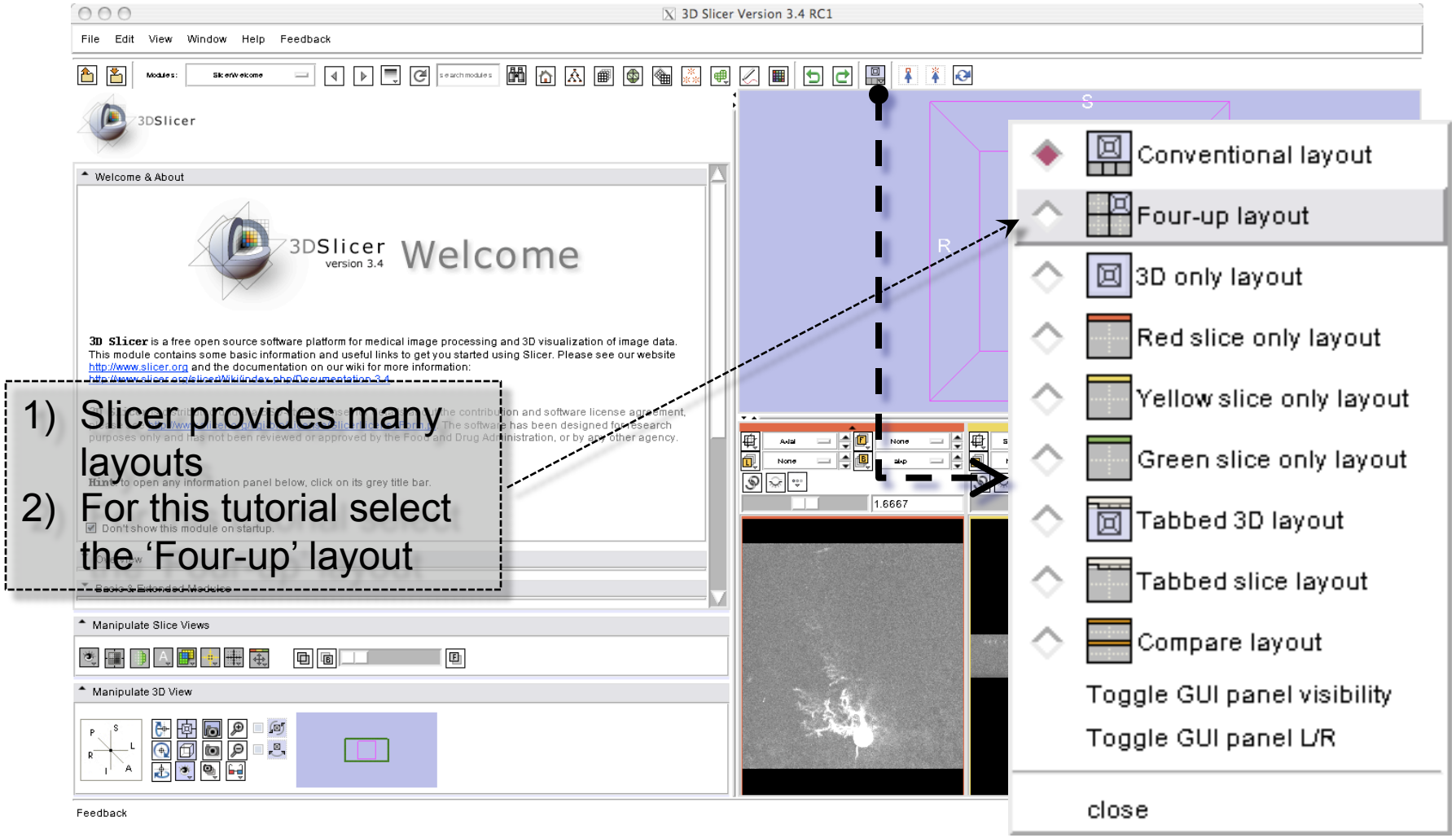
Slicer GUI Layout

The Graphical User Interface (GUI) of Slicer3 integrates five components:

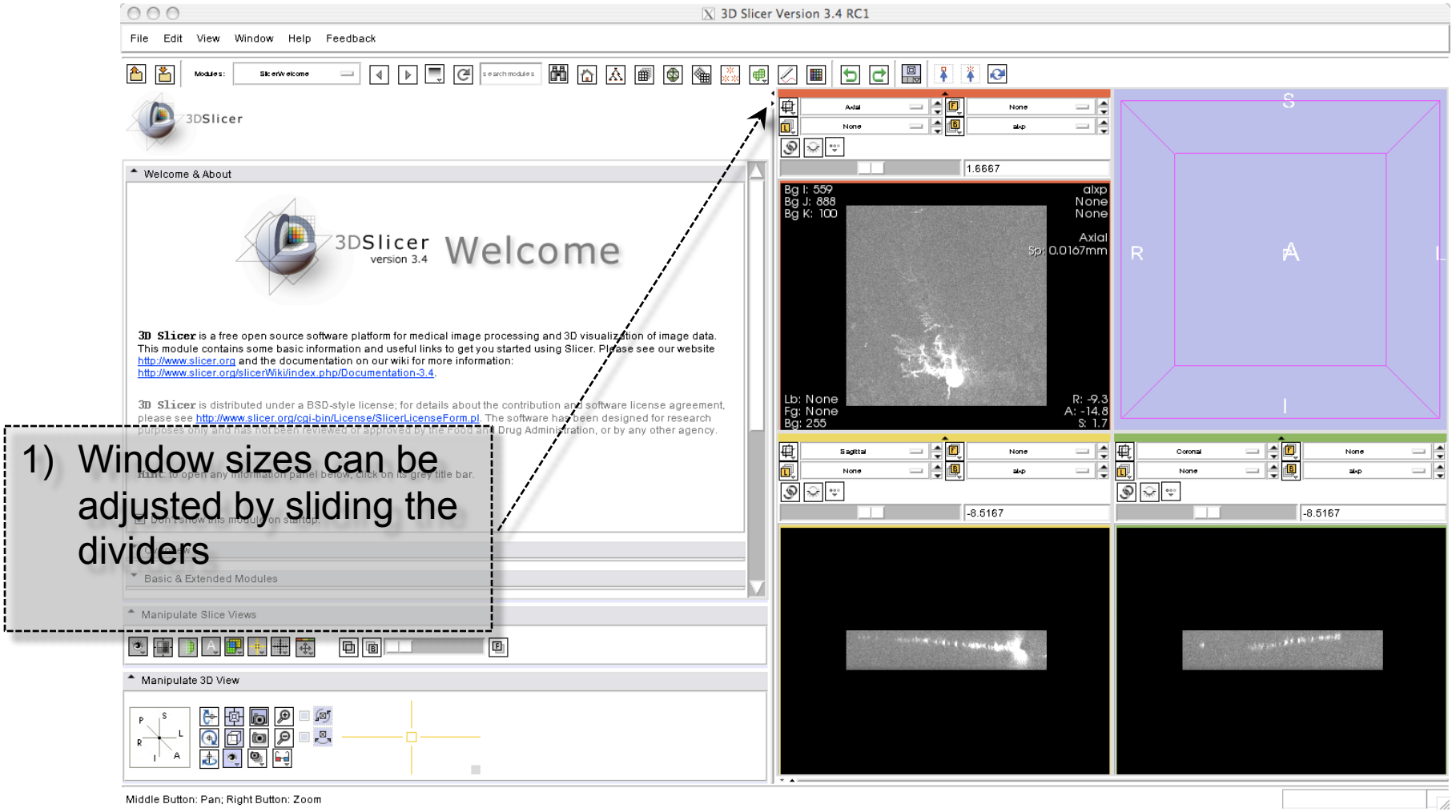
- the Menu Toolbar
- the Module GUI Panel
- the 3D Viewer
- the Slice Viewer
- the Slice and 3D View Controller



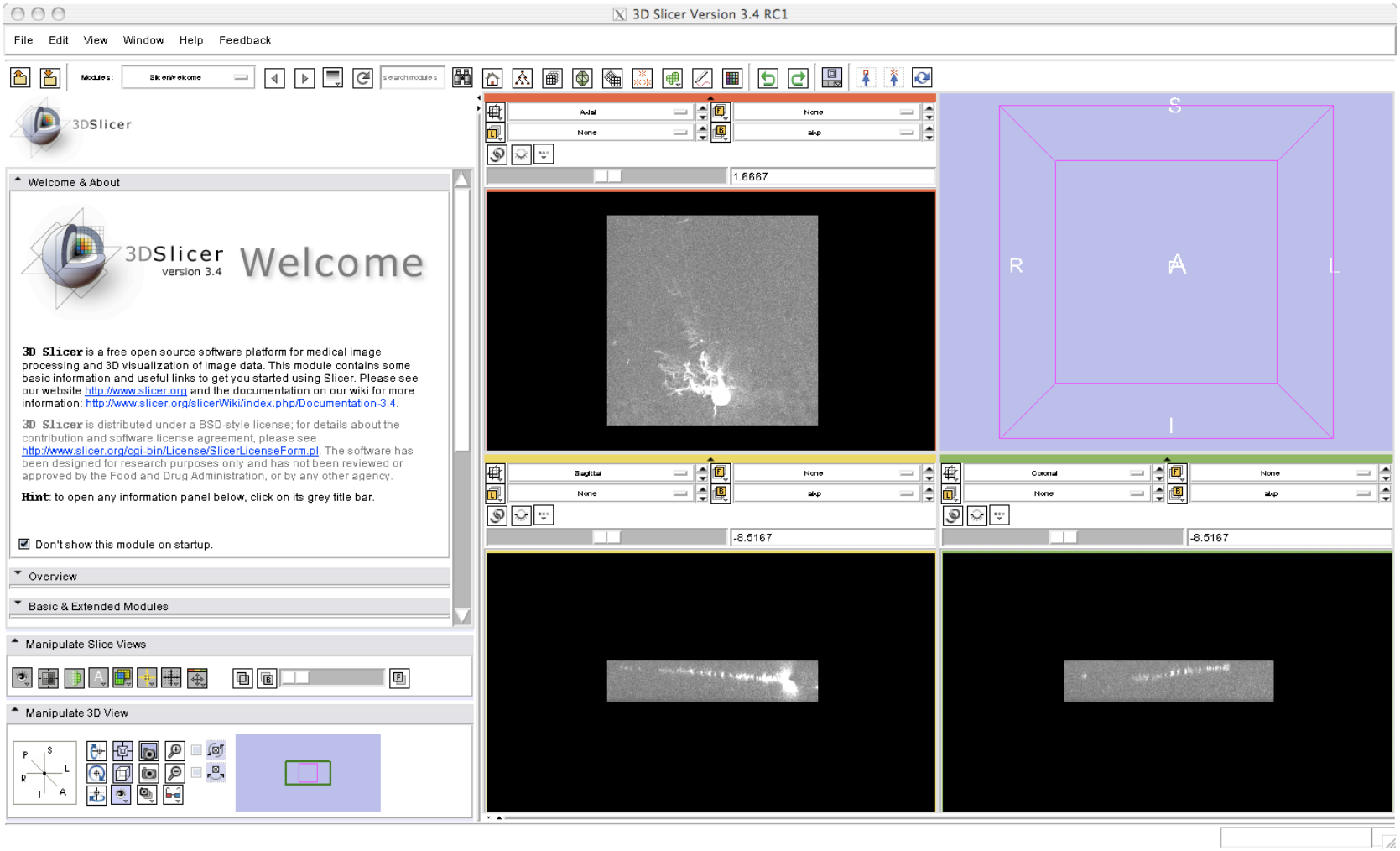
Setup Slicer Layout



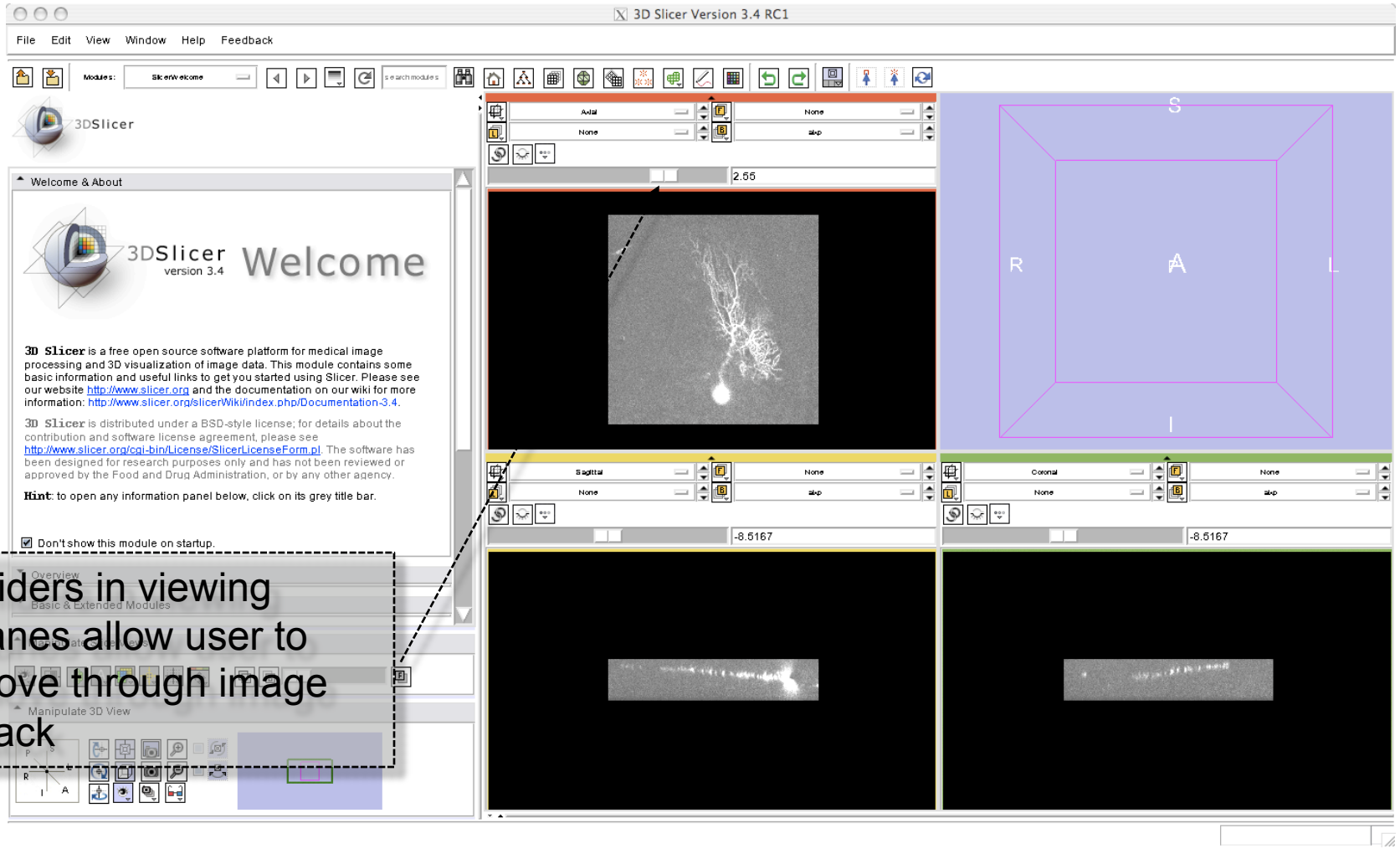
Setup Slicer Layout



Completed Slicer Layout Setup

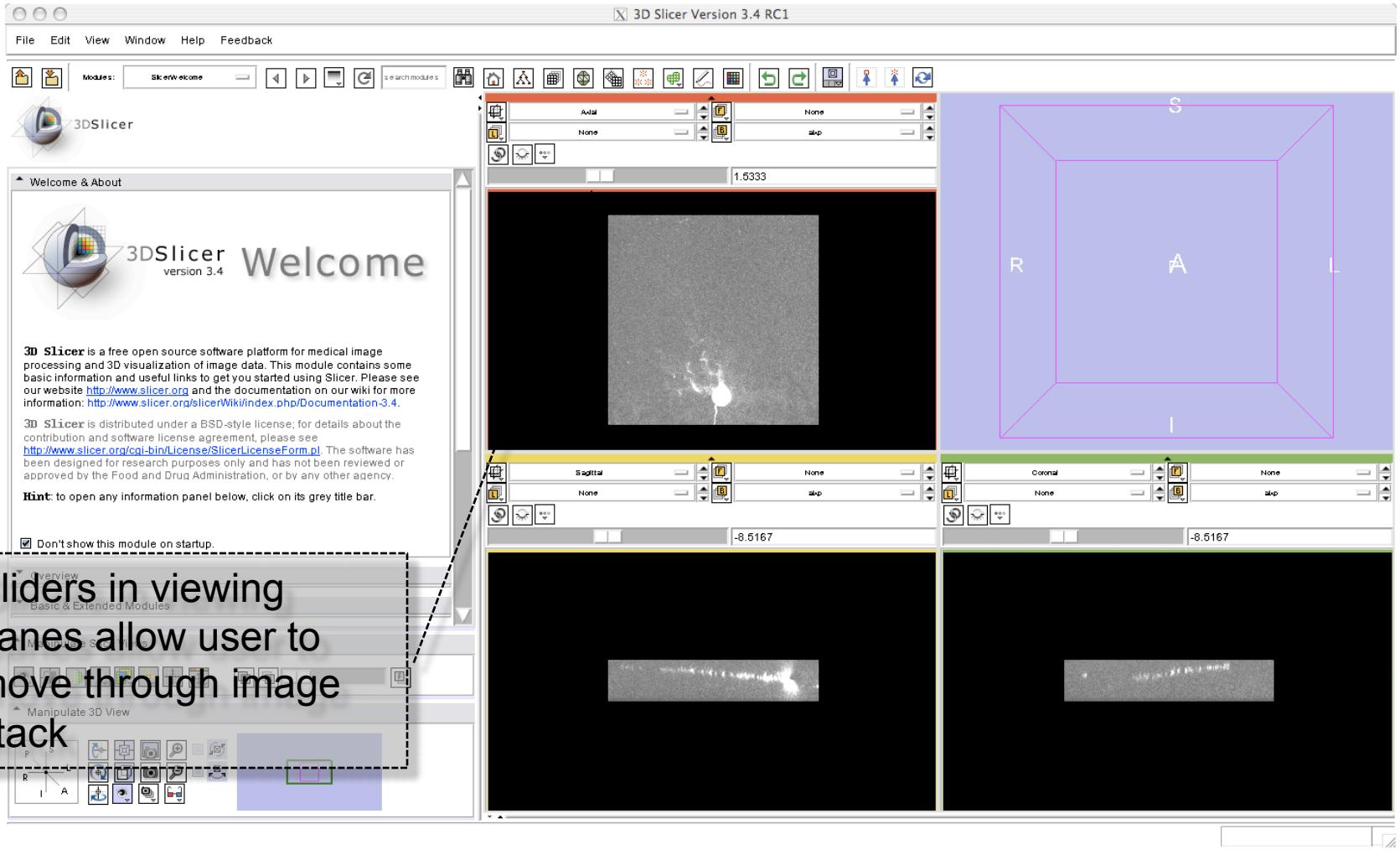


Viewing Confocal Slices



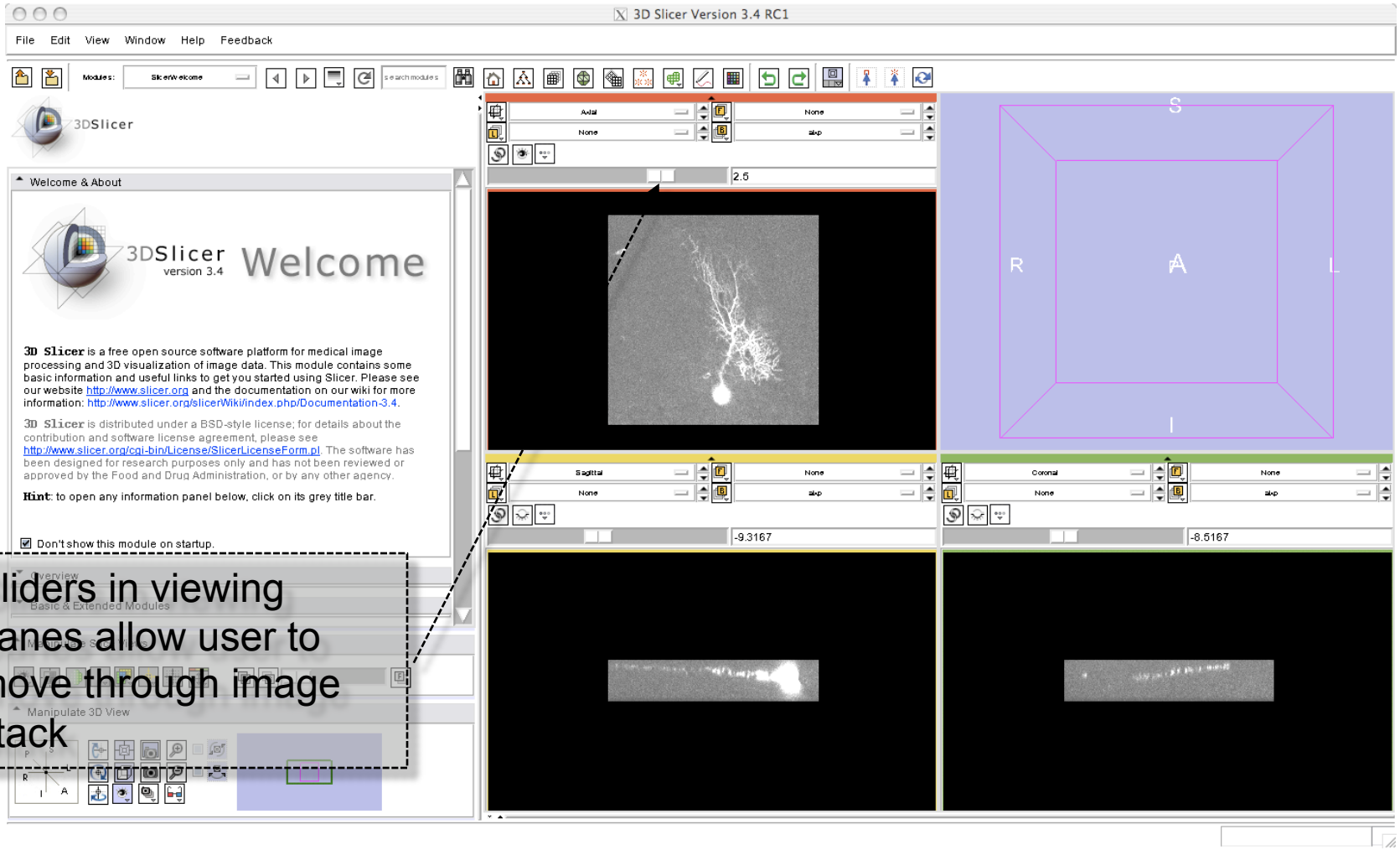
1) Sliders in viewing panes allow user to move through image stack

Viewing Confocal Slices



1) Sliders in viewing panes allow user to move through image stack

Viewing Confocal Slices

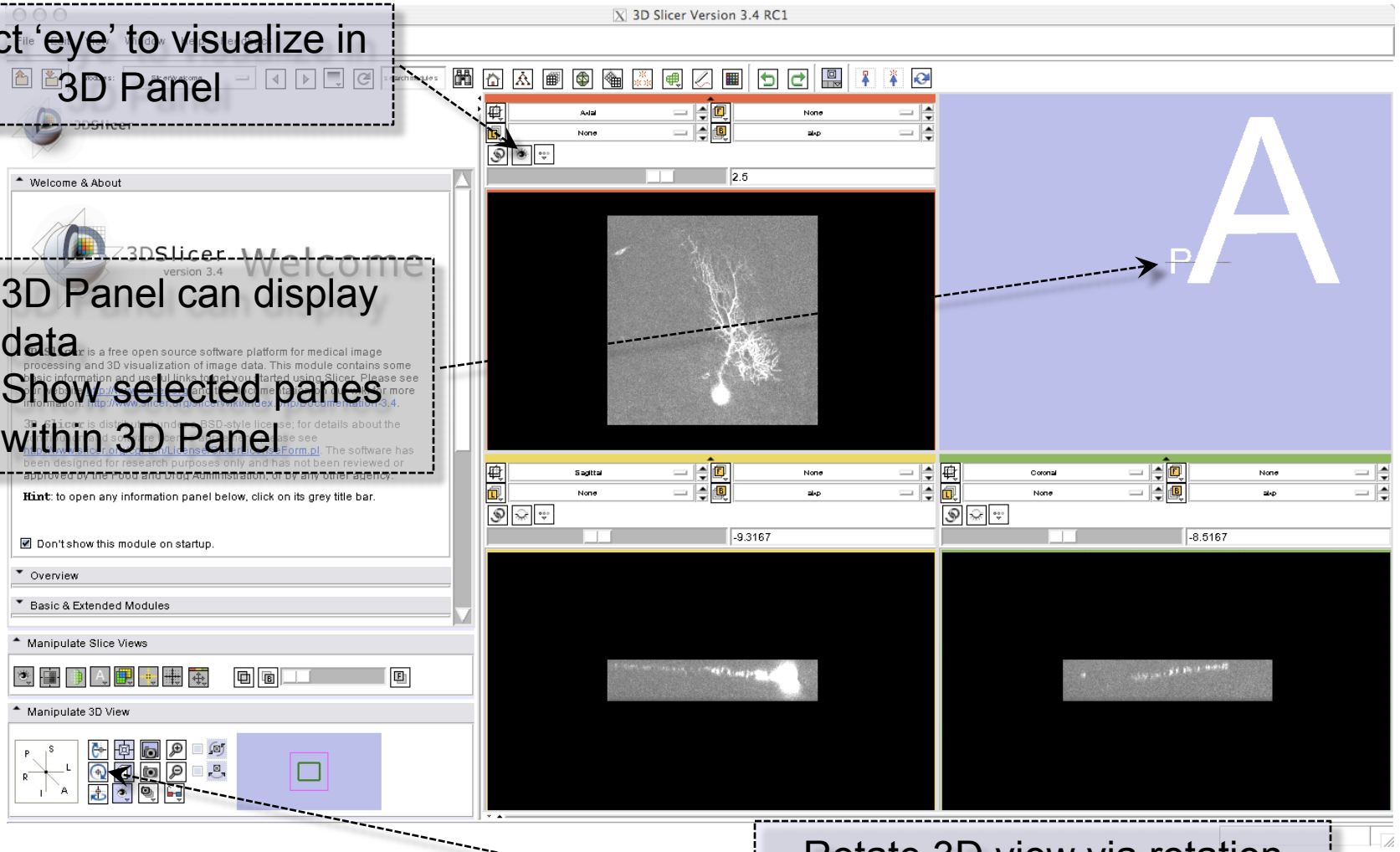


1) Sliders in viewing panes allow user to move through image stack

Basic Viewing in 3D Panel

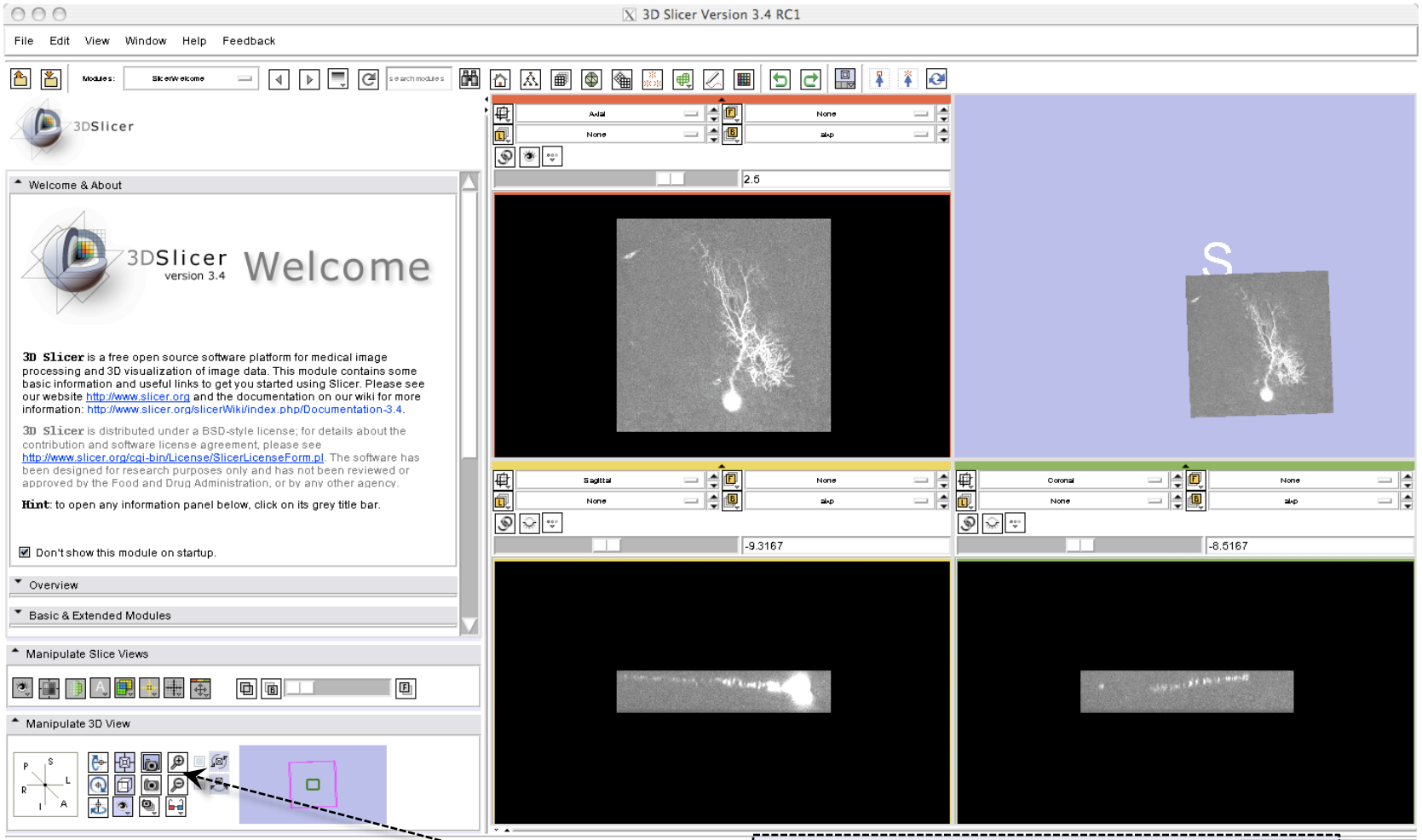
Select 'eye' to visualize in 3D Panel

1) 3D Panel can display data
2) Show selected panes within 3D Panel



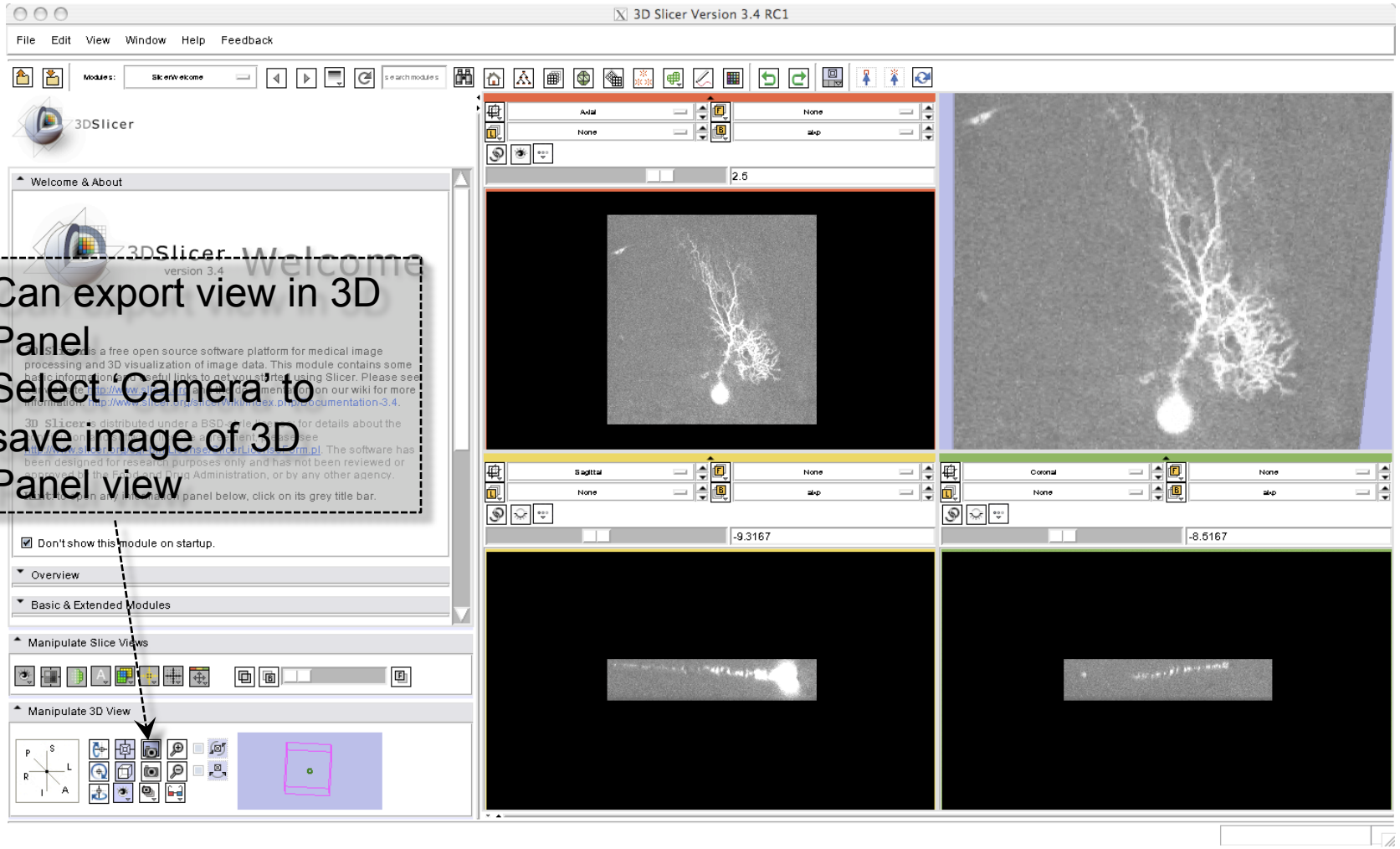
Rotate 3D view via rotation buttons or by clicking and dragging in 3D panel

Basic Viewing in 3D Panel

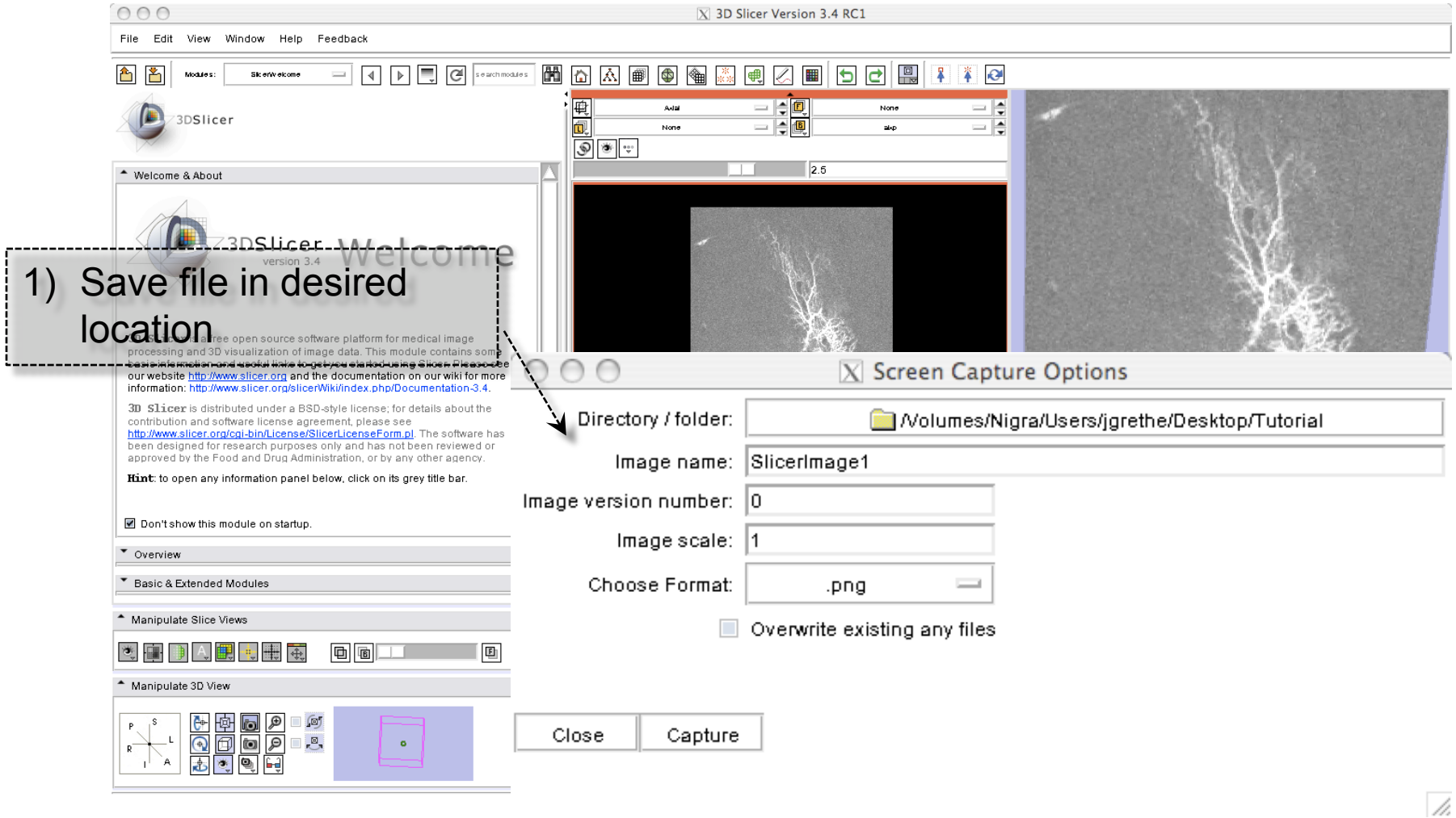


Zoom 3D view via zoom buttons or by clicking in 3D panel

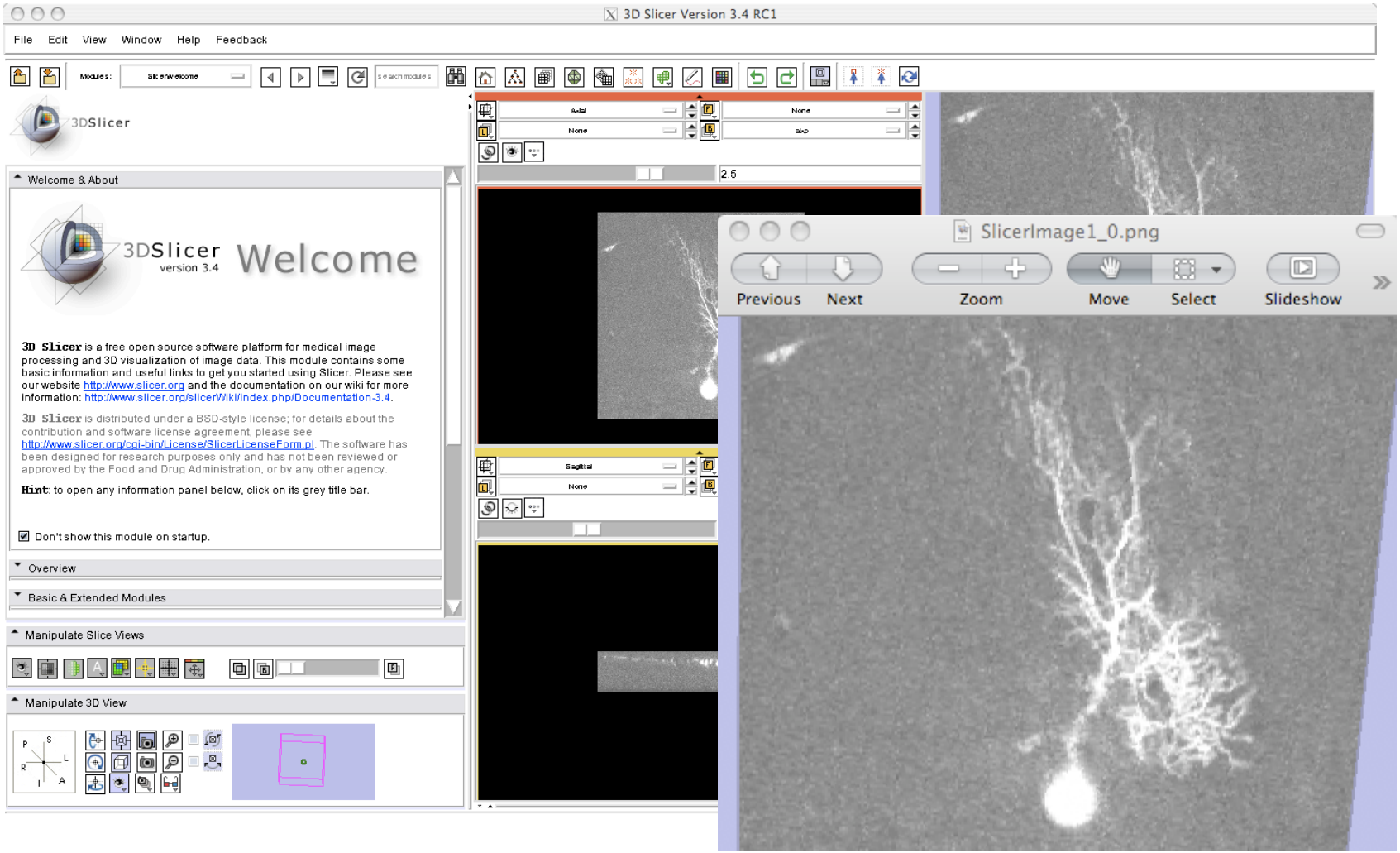
Basic Viewing in 3D Panel



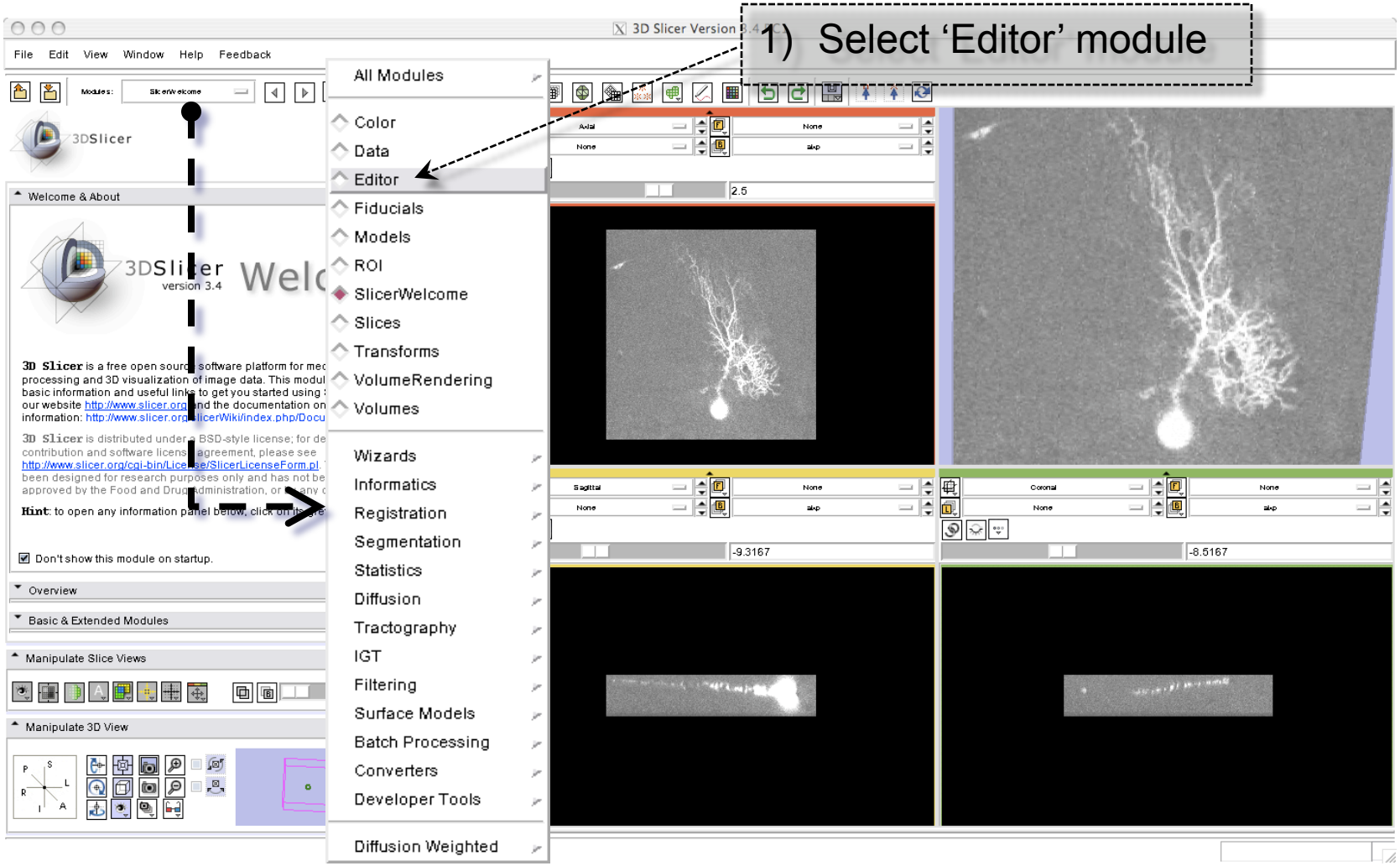
Basic Viewing in 3D Panel



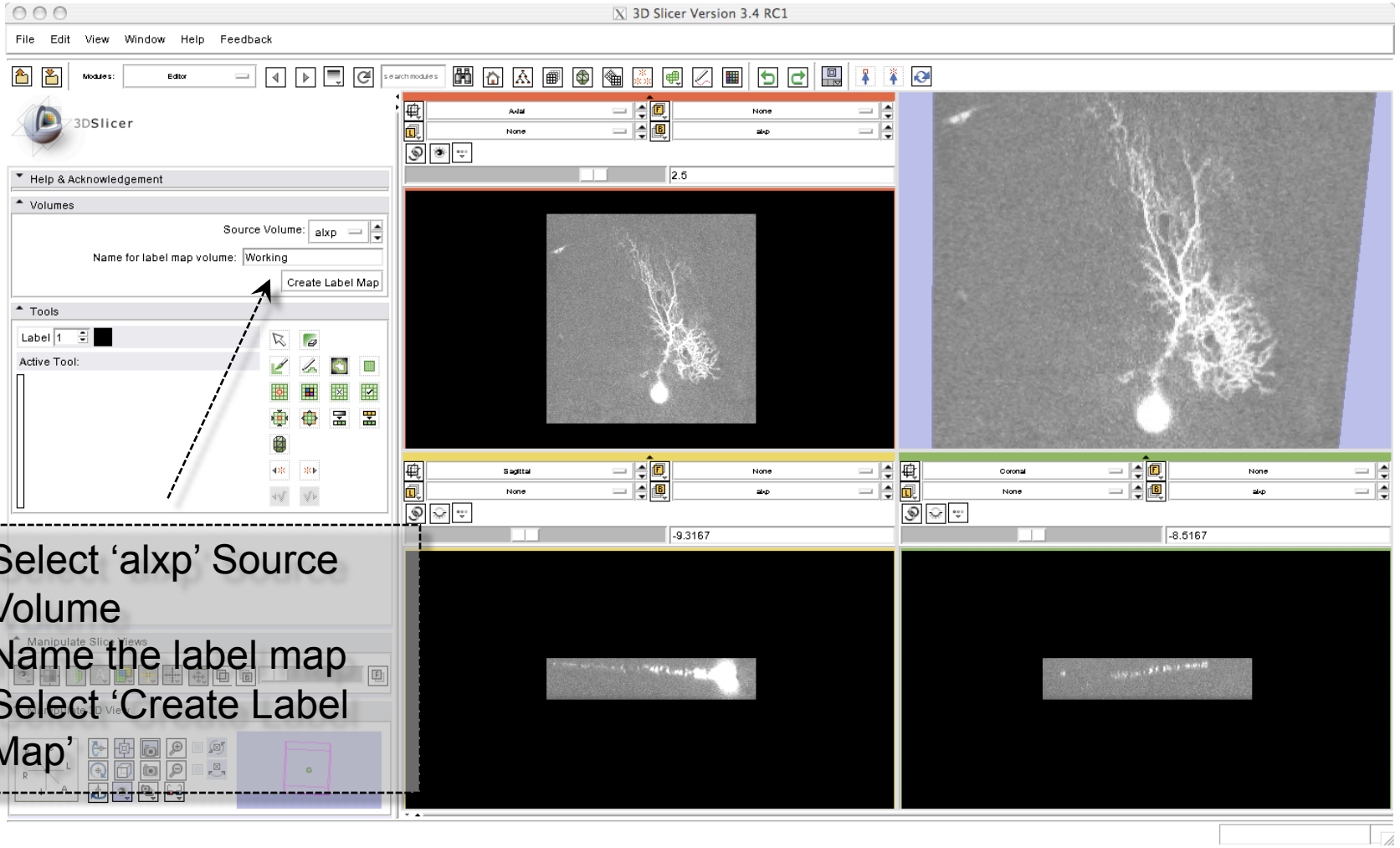
Basic Viewing in 3D Panel



Editing Module

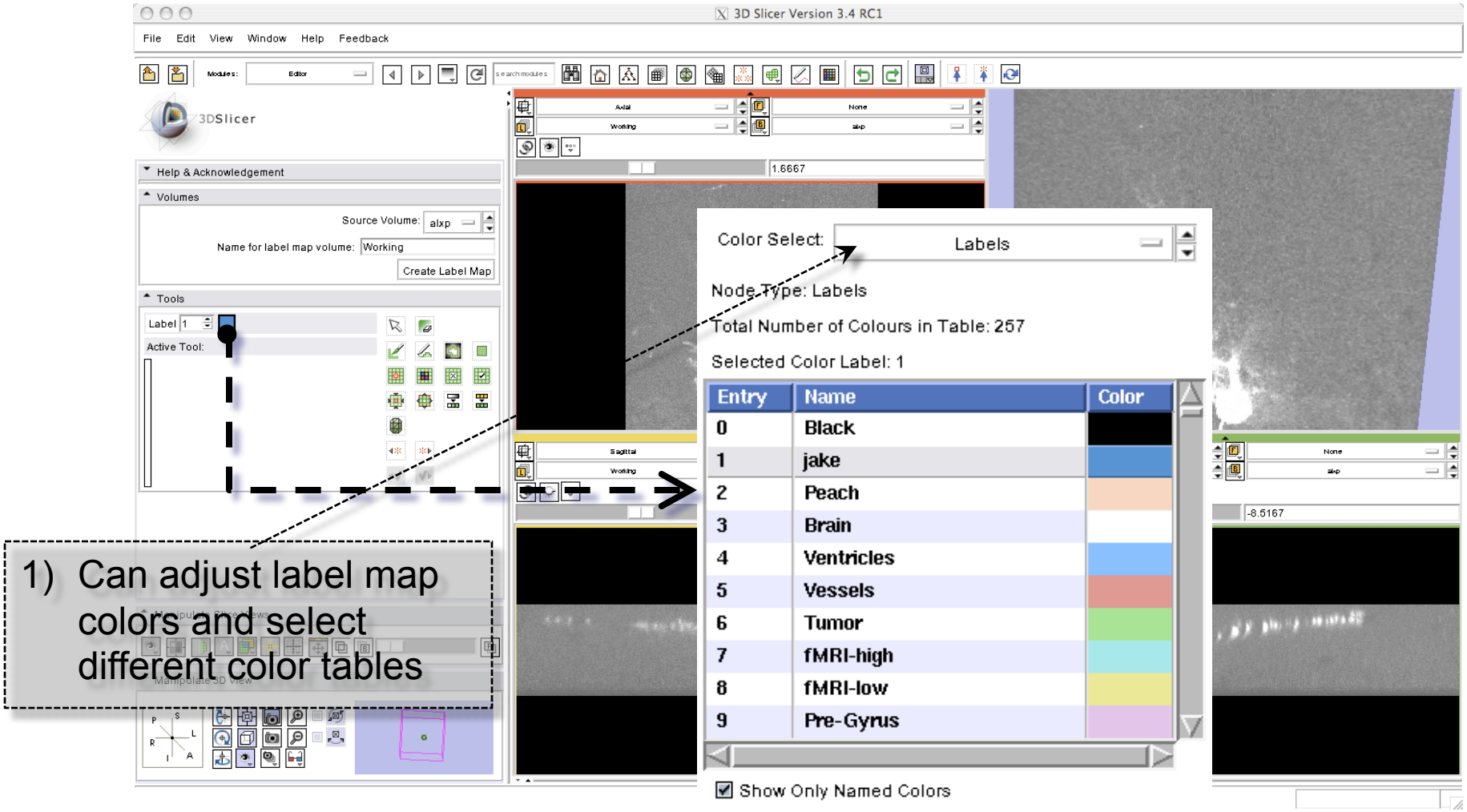


Creating A Label Map

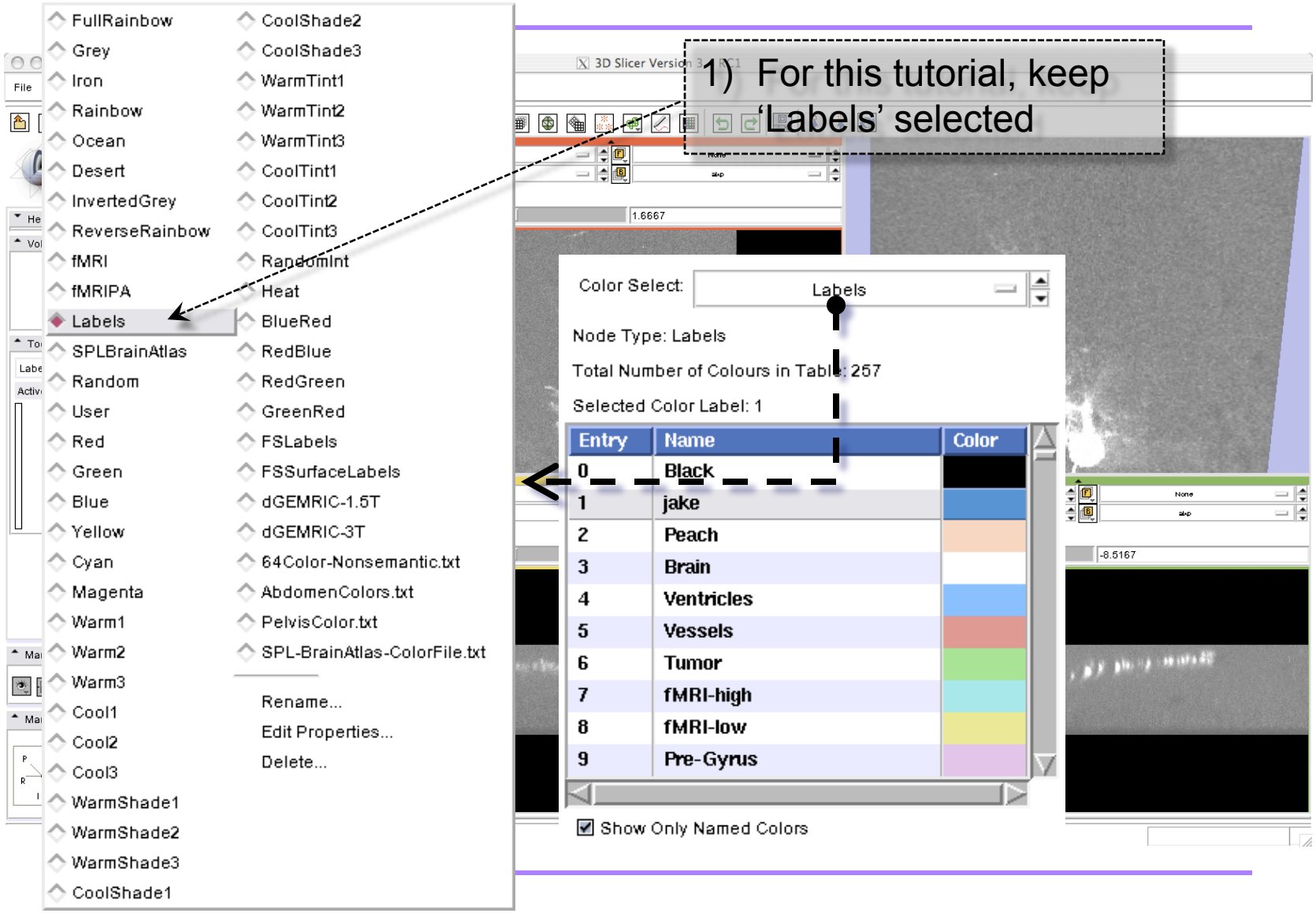


1) Select ‘alxp’ Source Volume
2) Name the label map
3) Select ‘Create Label Map’

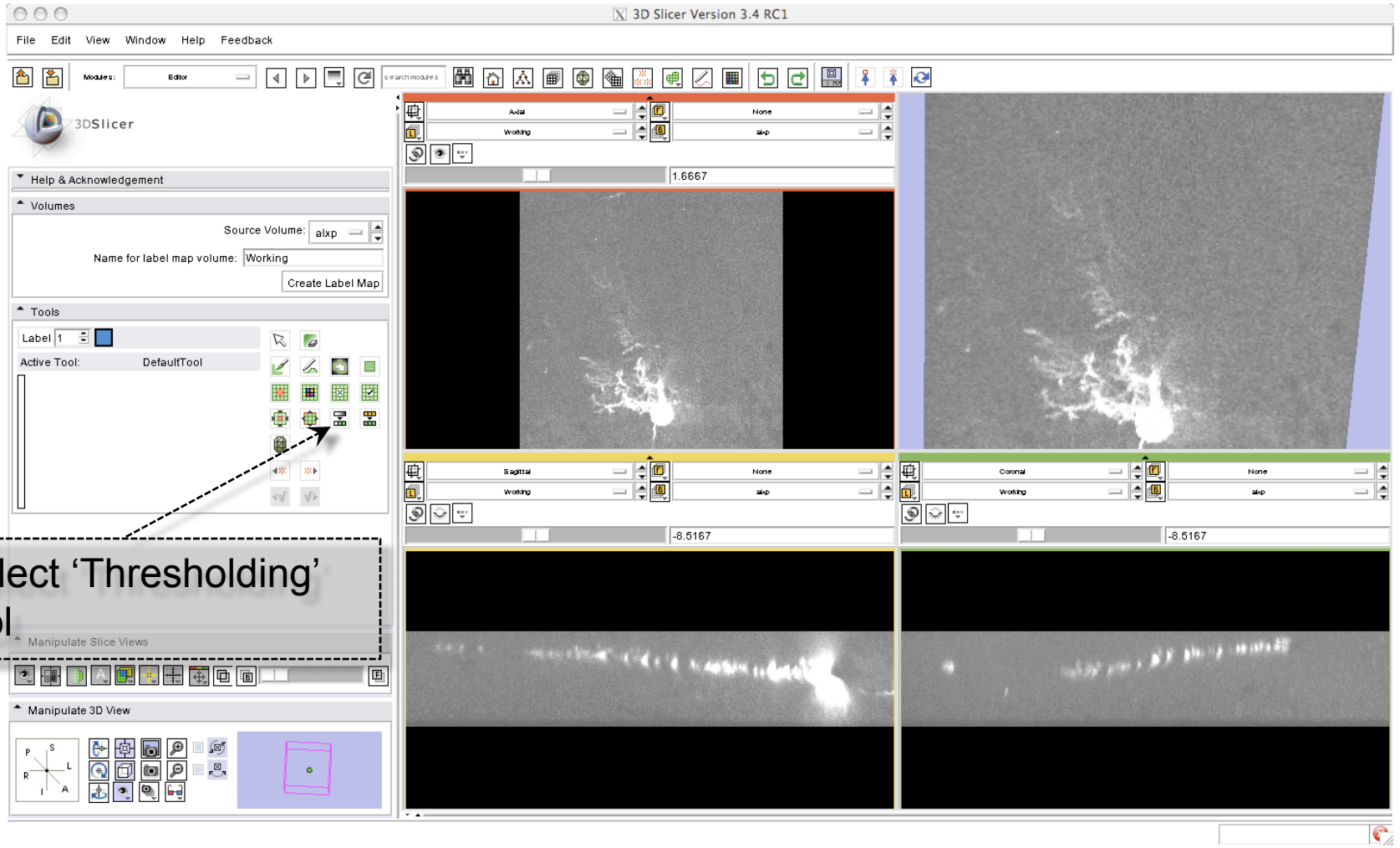
Adjusting Label Map Color



Adjusting Label Map Color

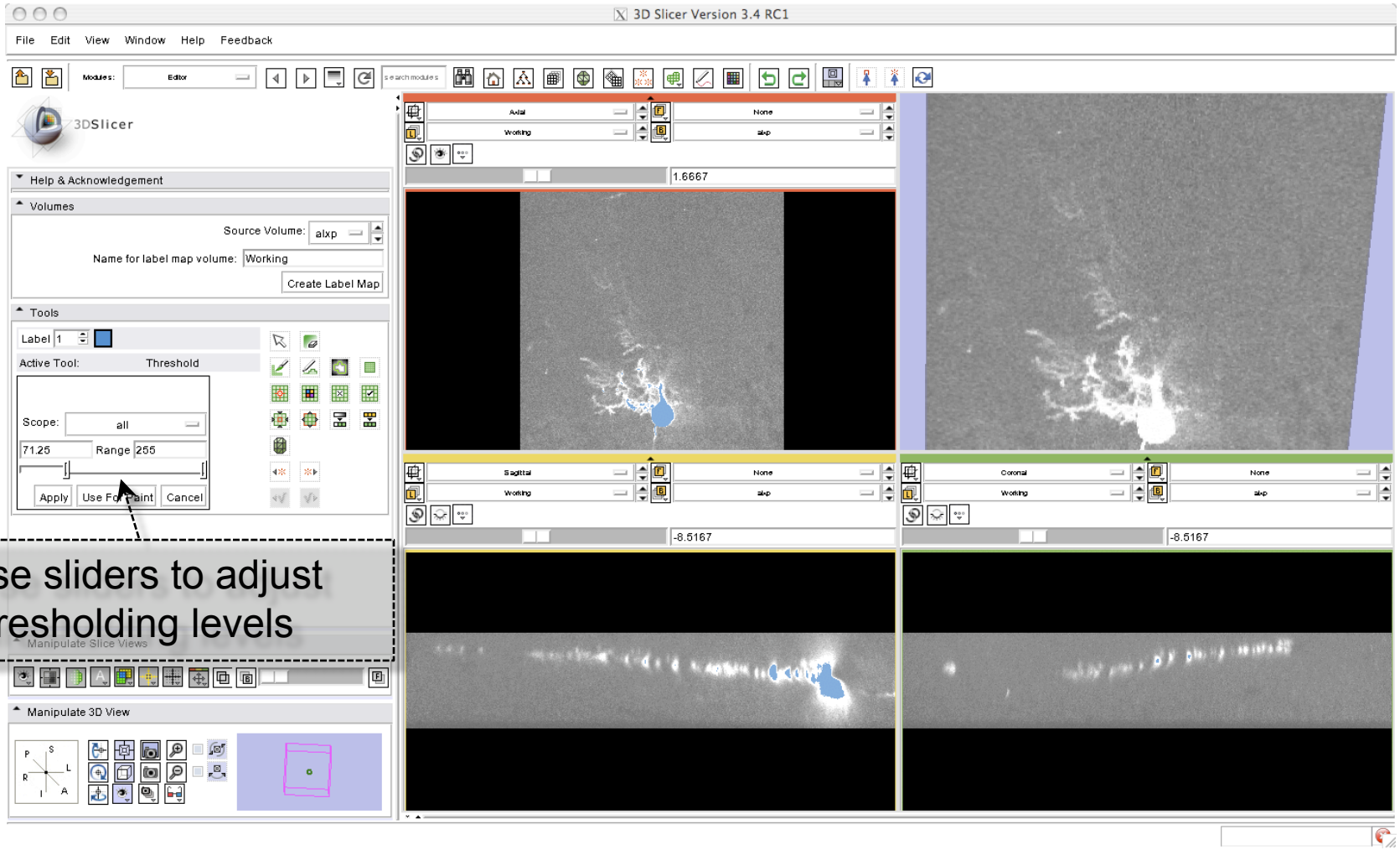


Editing: Basic Thresholding



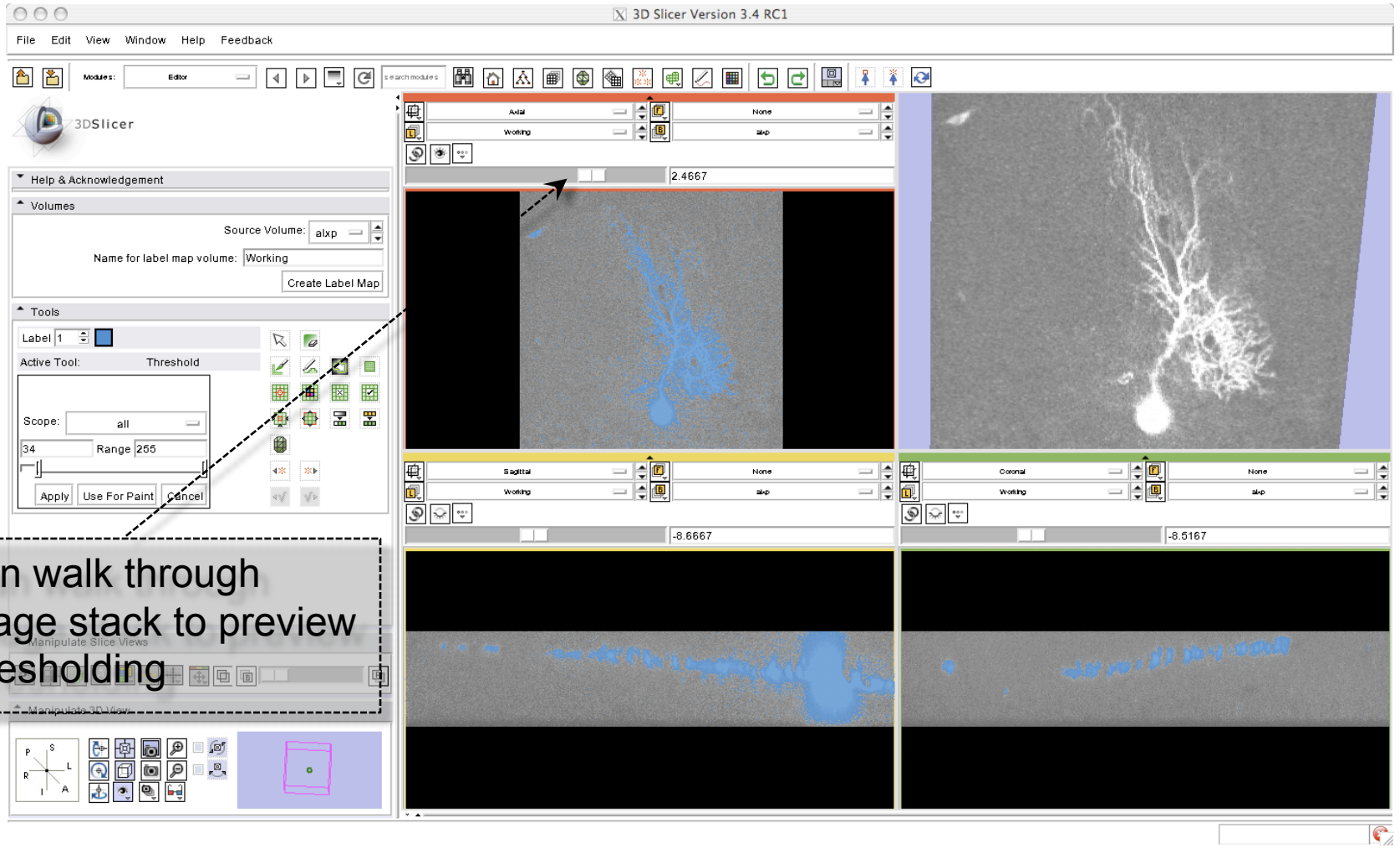
1) Select 'Thresholding' tool

Editing: Basic Thresholding



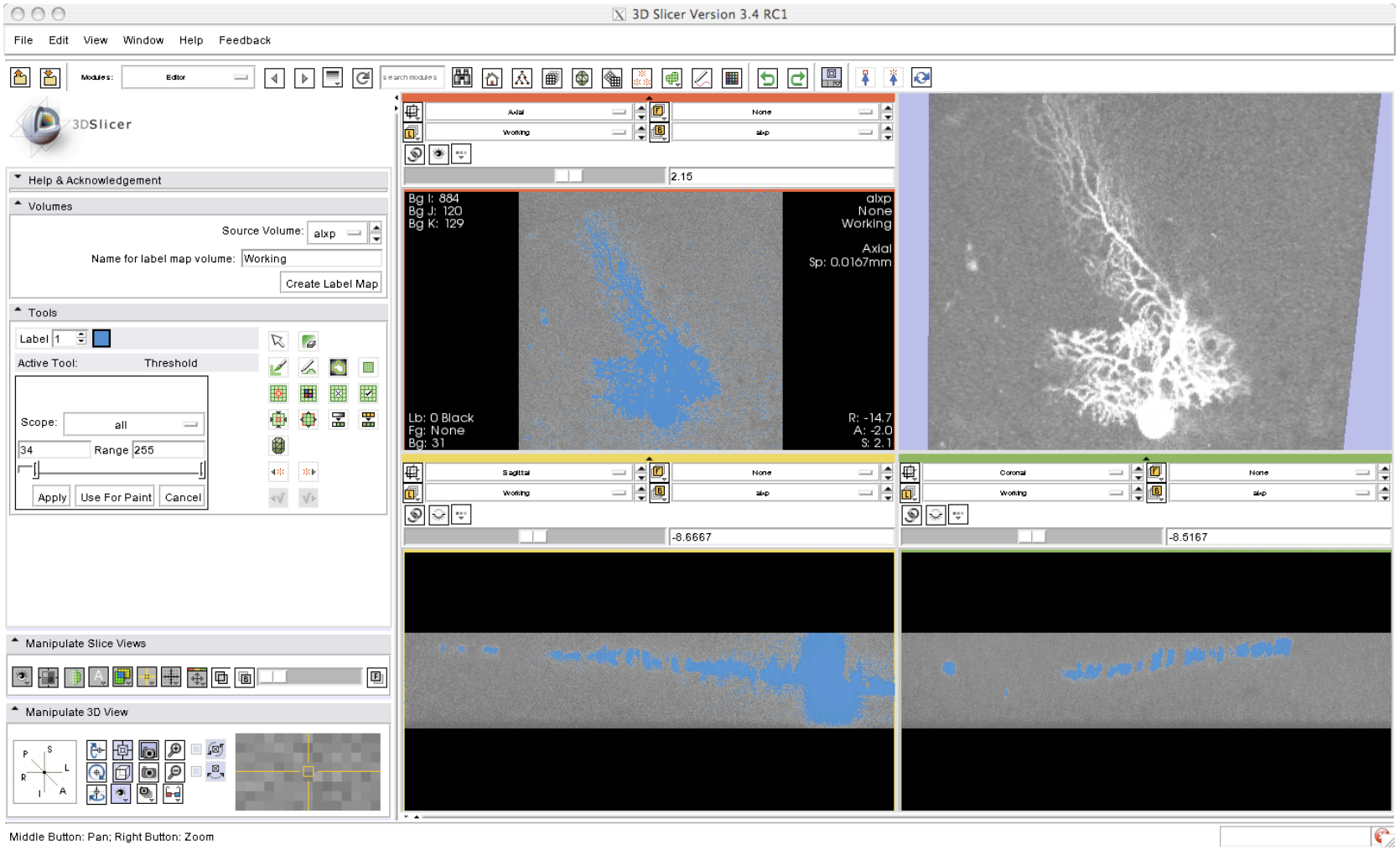
1) Use sliders to adjust thresholding levels

Editing: Basic Thresholding



1) Can walk through image stack to preview thresholding

Editing: Basic Thresholding



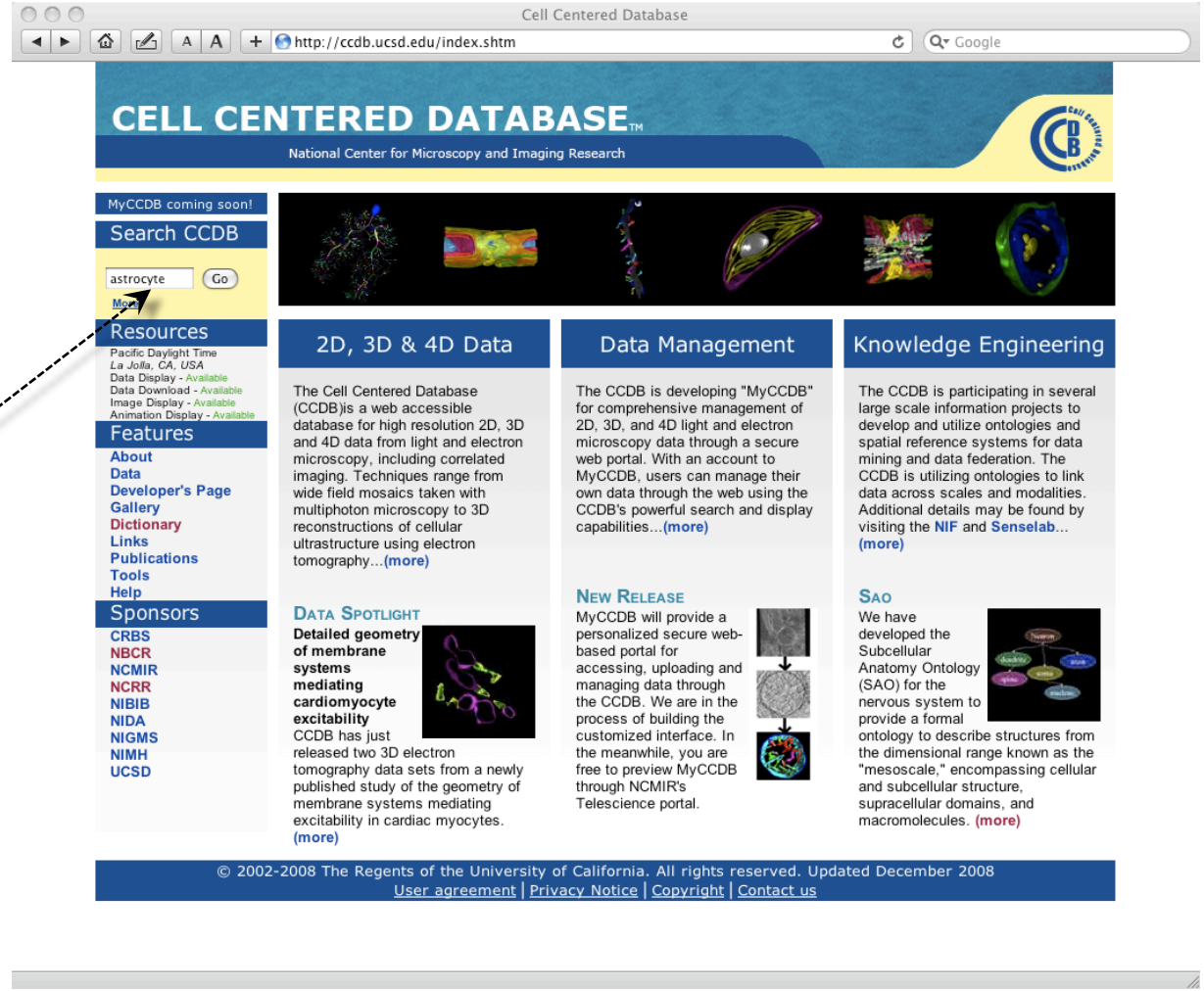
Model Building

Note: this section uses a smaller dataset so that computations can be completed in a reasonable time

Downloading Sample Data

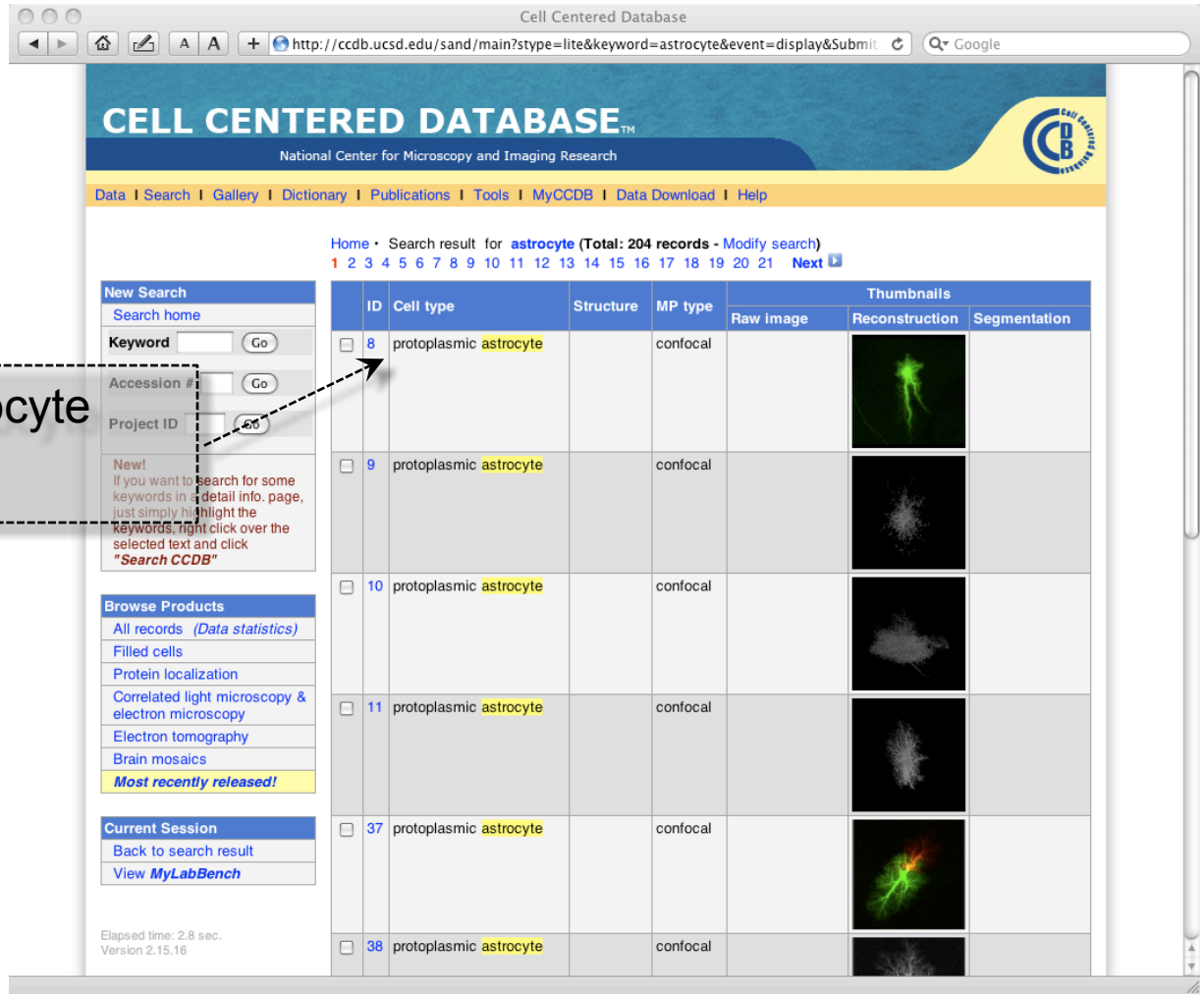
1) Enter Search Term:
   Astrocyte

2) Select 'Go'



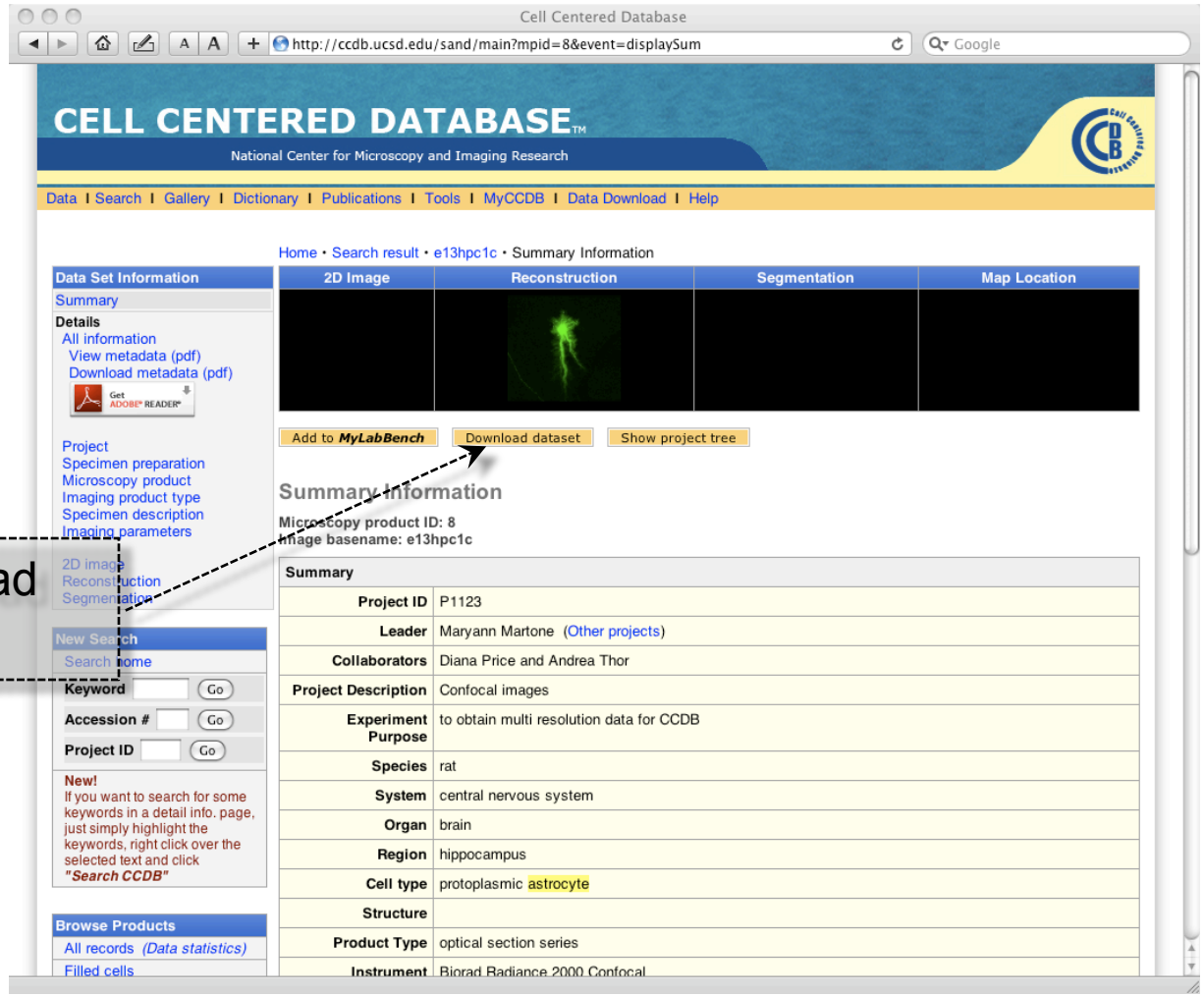
Downloading Sample Data

1) Select Astrocyte with ID 8



Downloading Sample Data

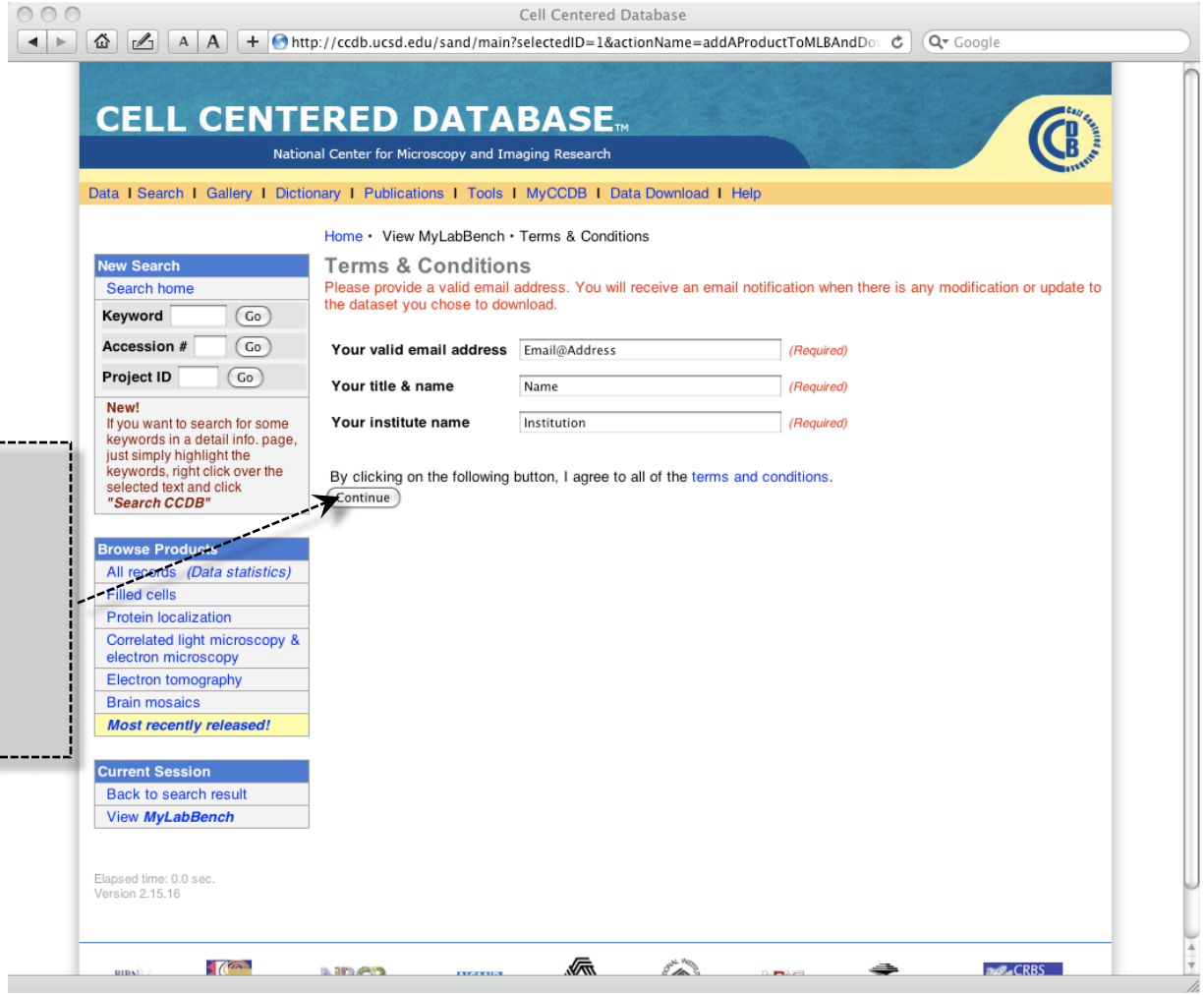
1) Select 'Download dataset'



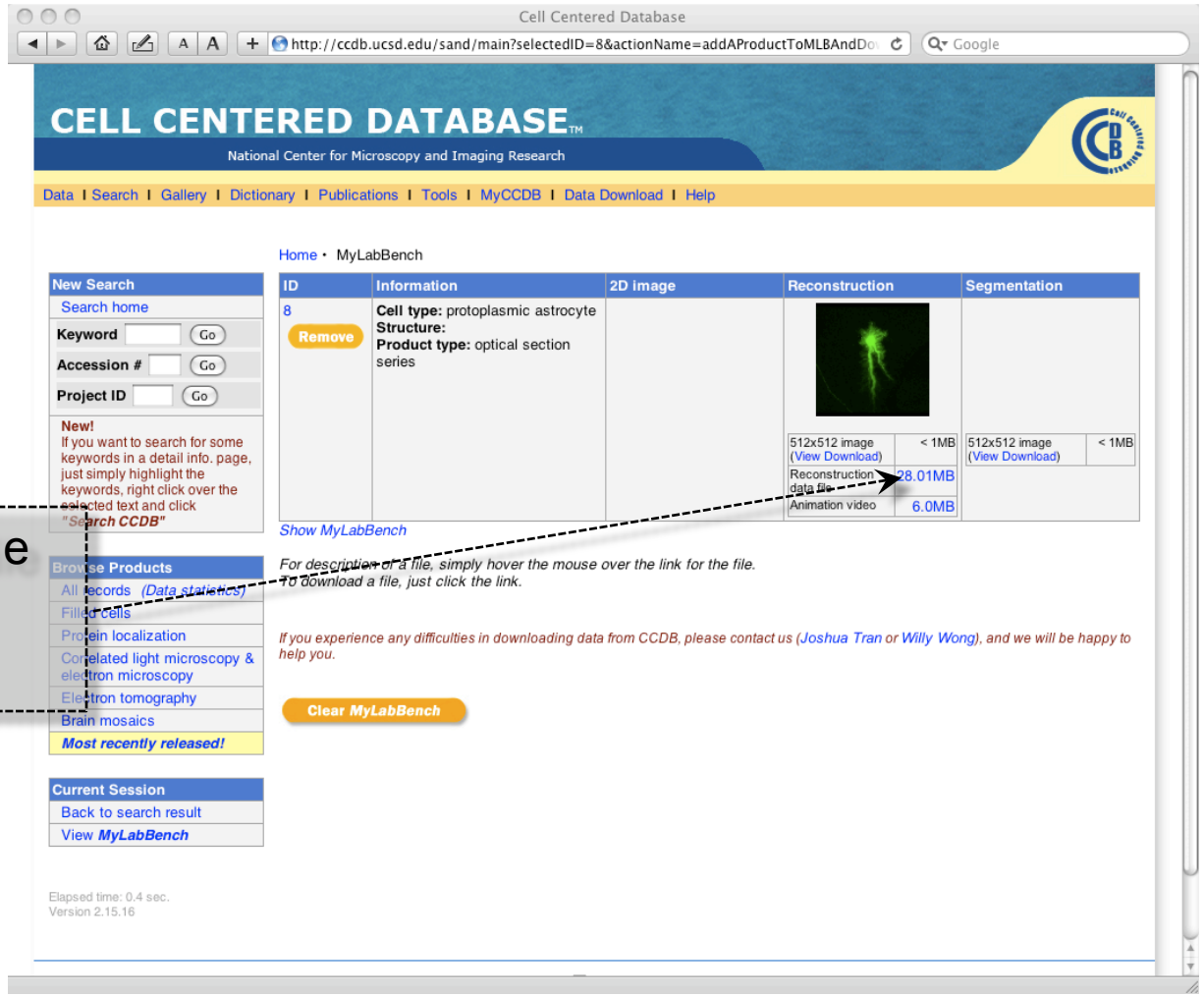
Downloading Sample Data

1) Enter required information to receive updates

2) Select ‘Continue’

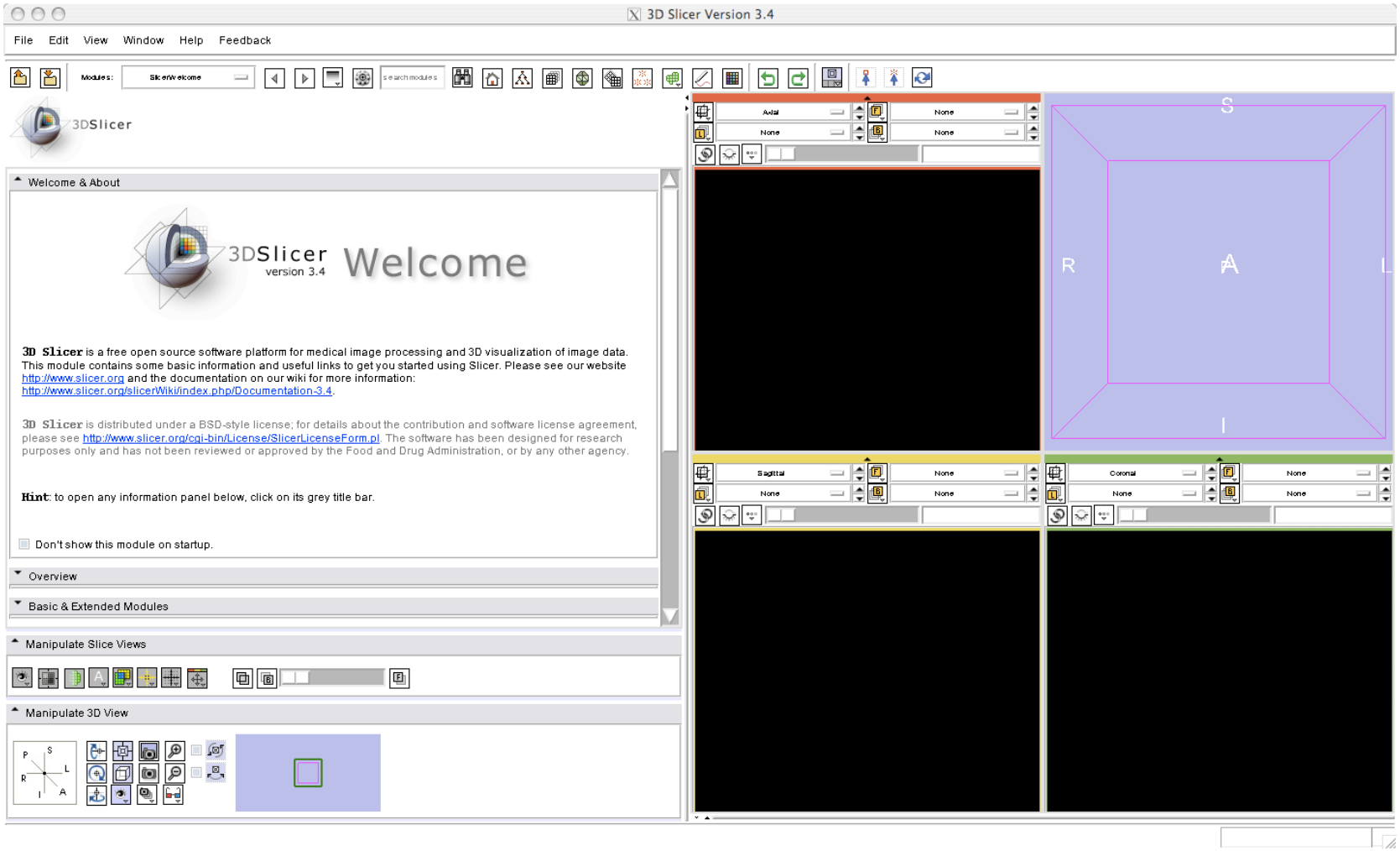


Downloading Sample Data

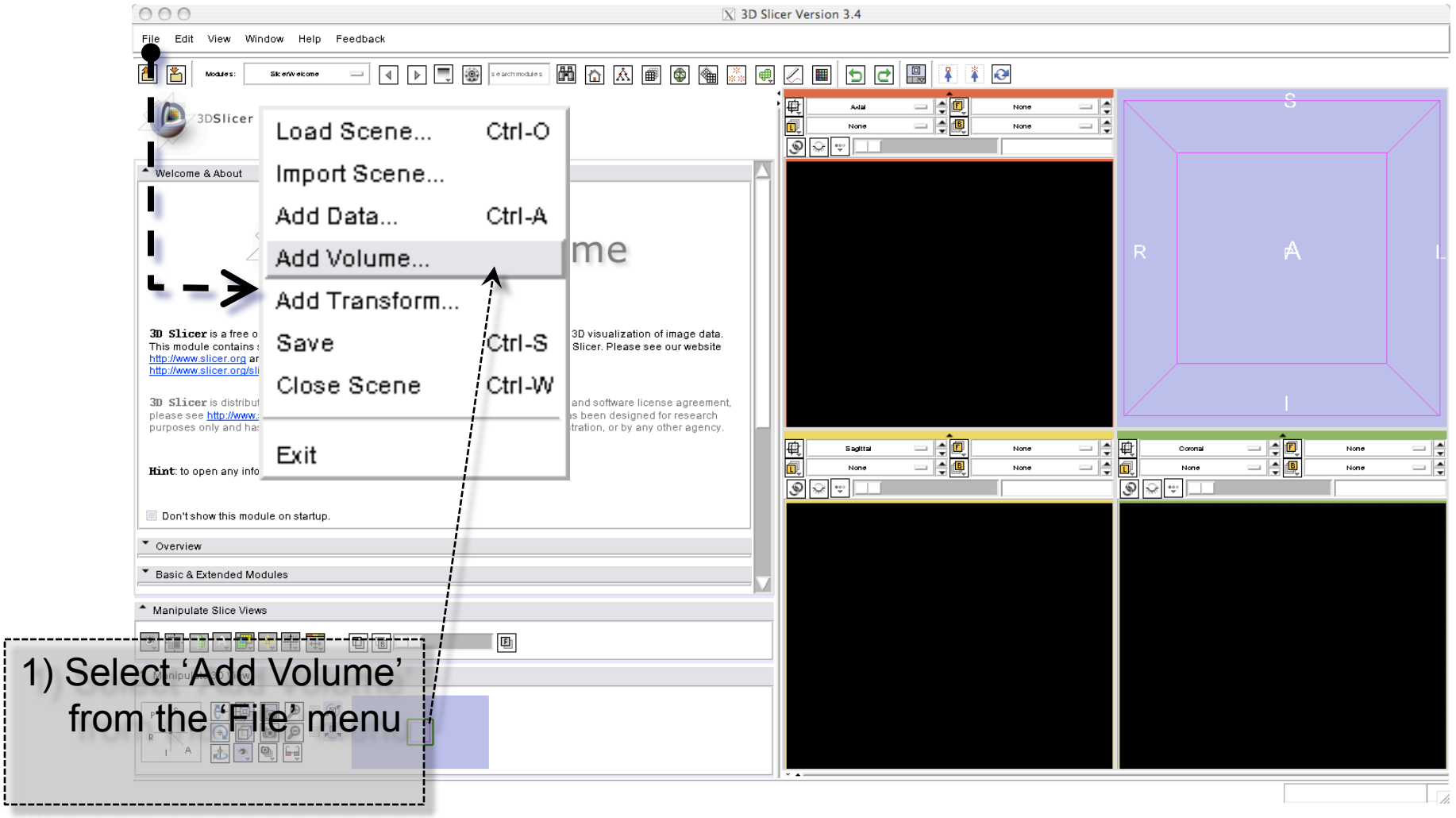


1) Download data file by selecting '28.01MB'

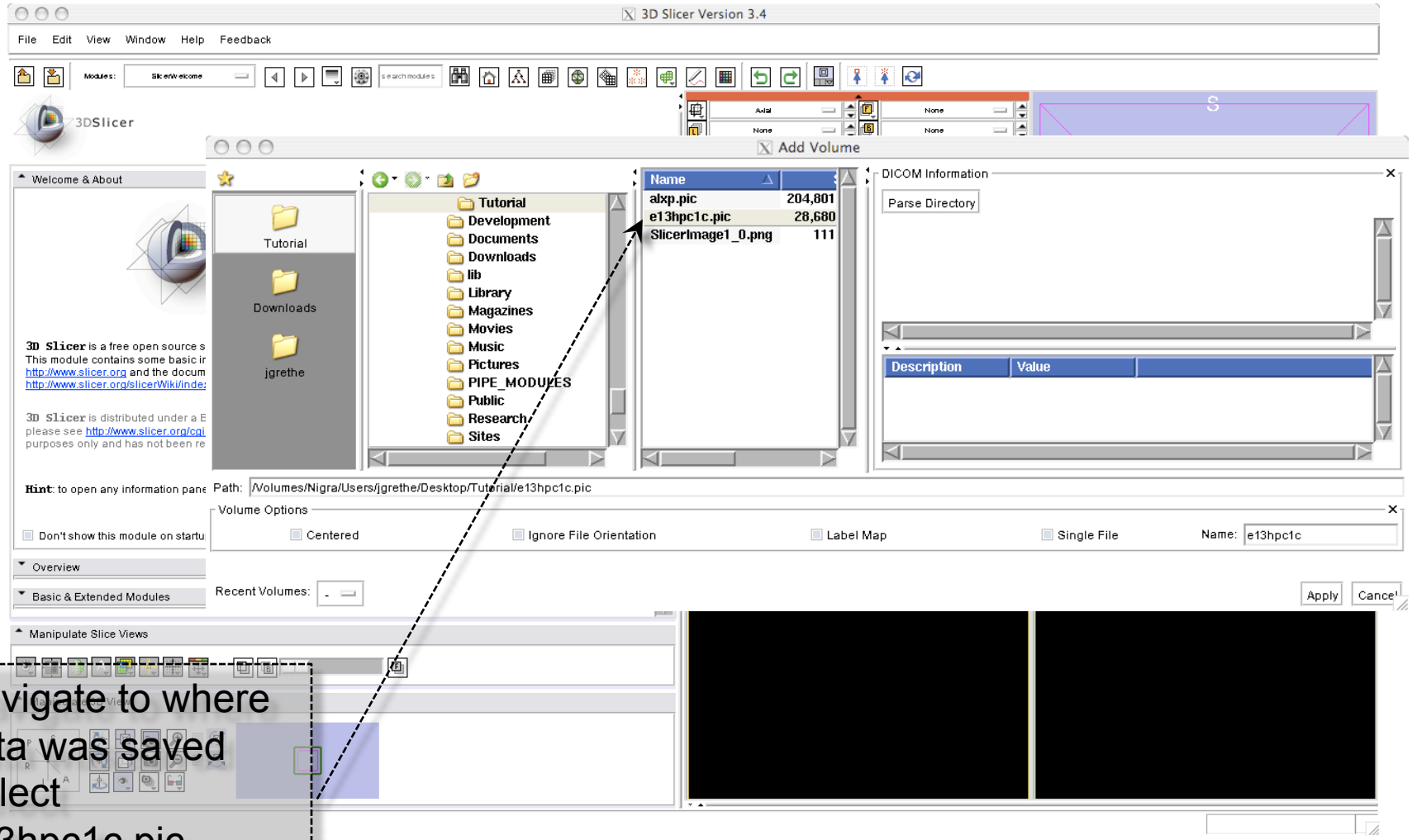
Launch Slicer



Open Astrocyte Data

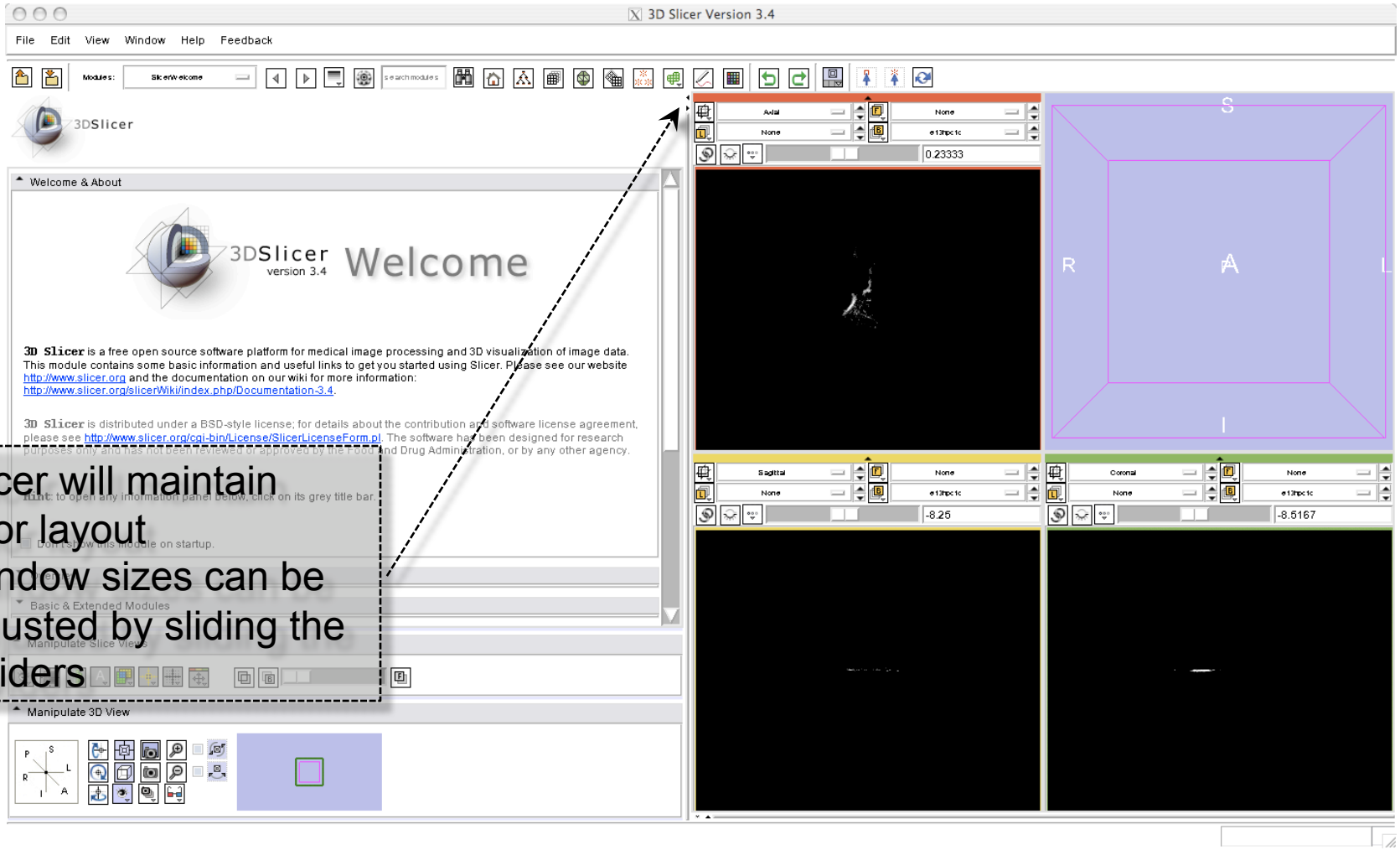


Open Astrocyte Data



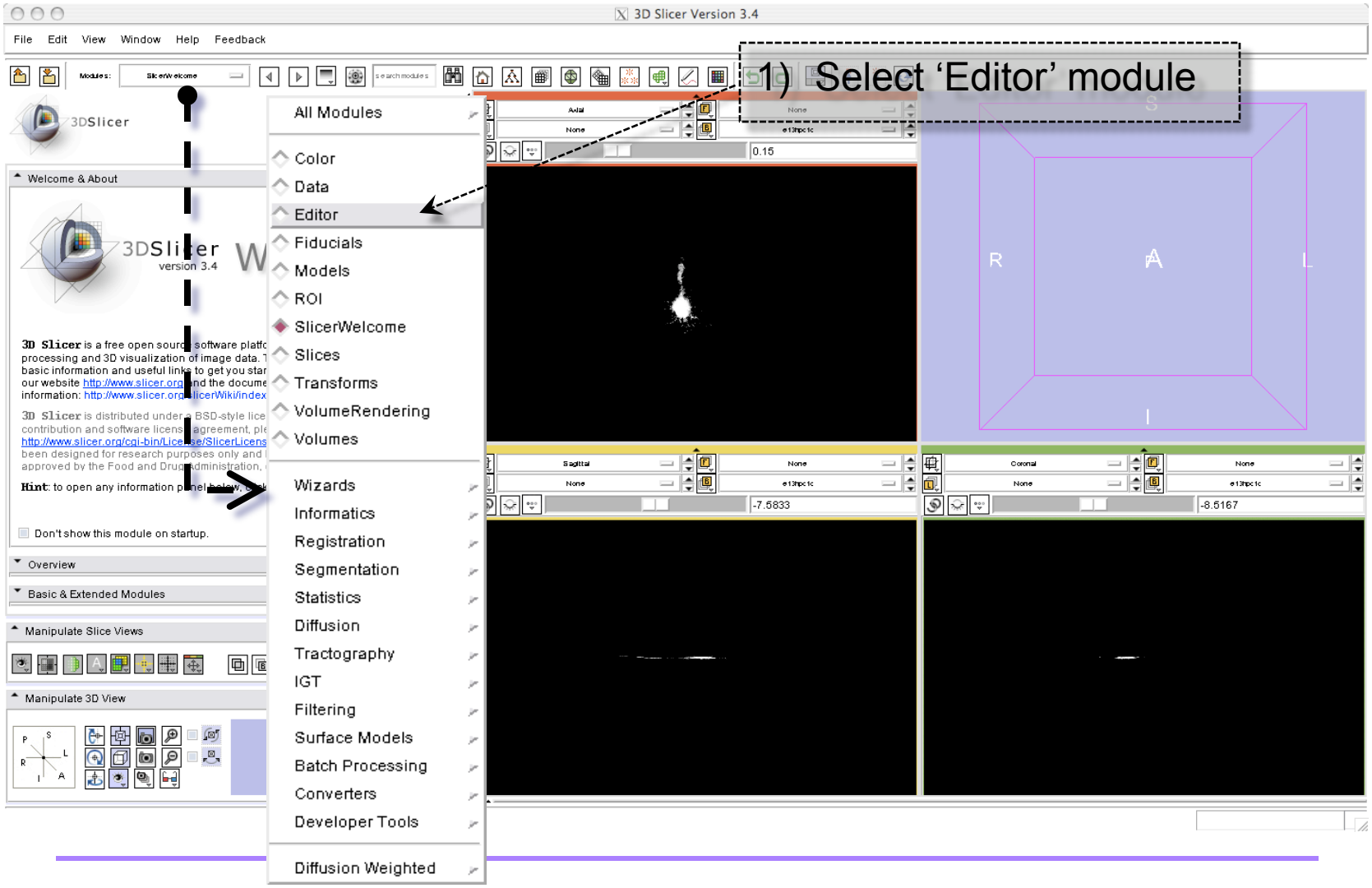
1) Navigate to where data was saved
2) Select e13hpc1c.pic
3) Select 'Apply'

Adjust Slicer Panels

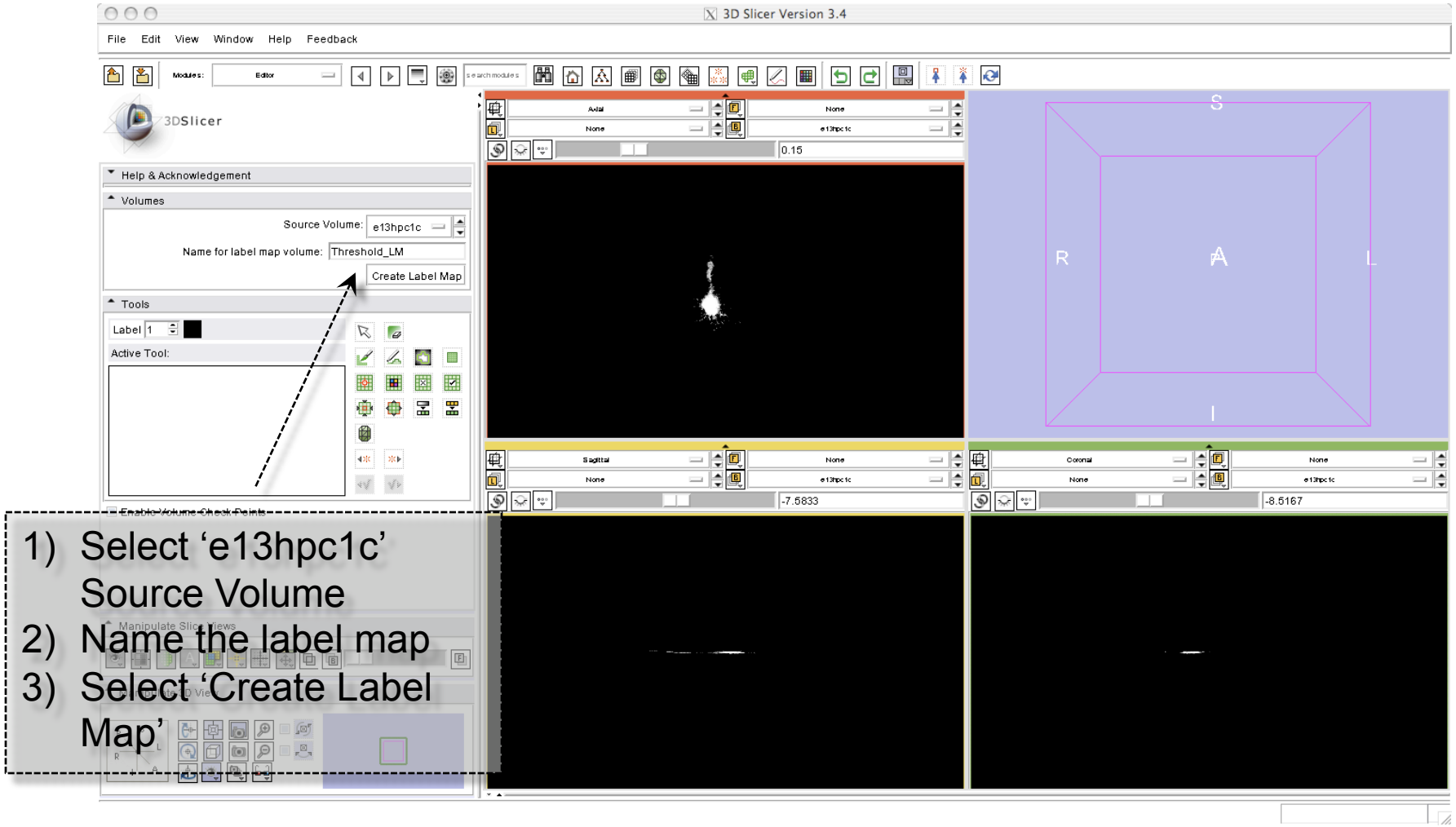


1) Slicer will maintain prior layout
2) Window sizes can be adjusted by sliding the dividers

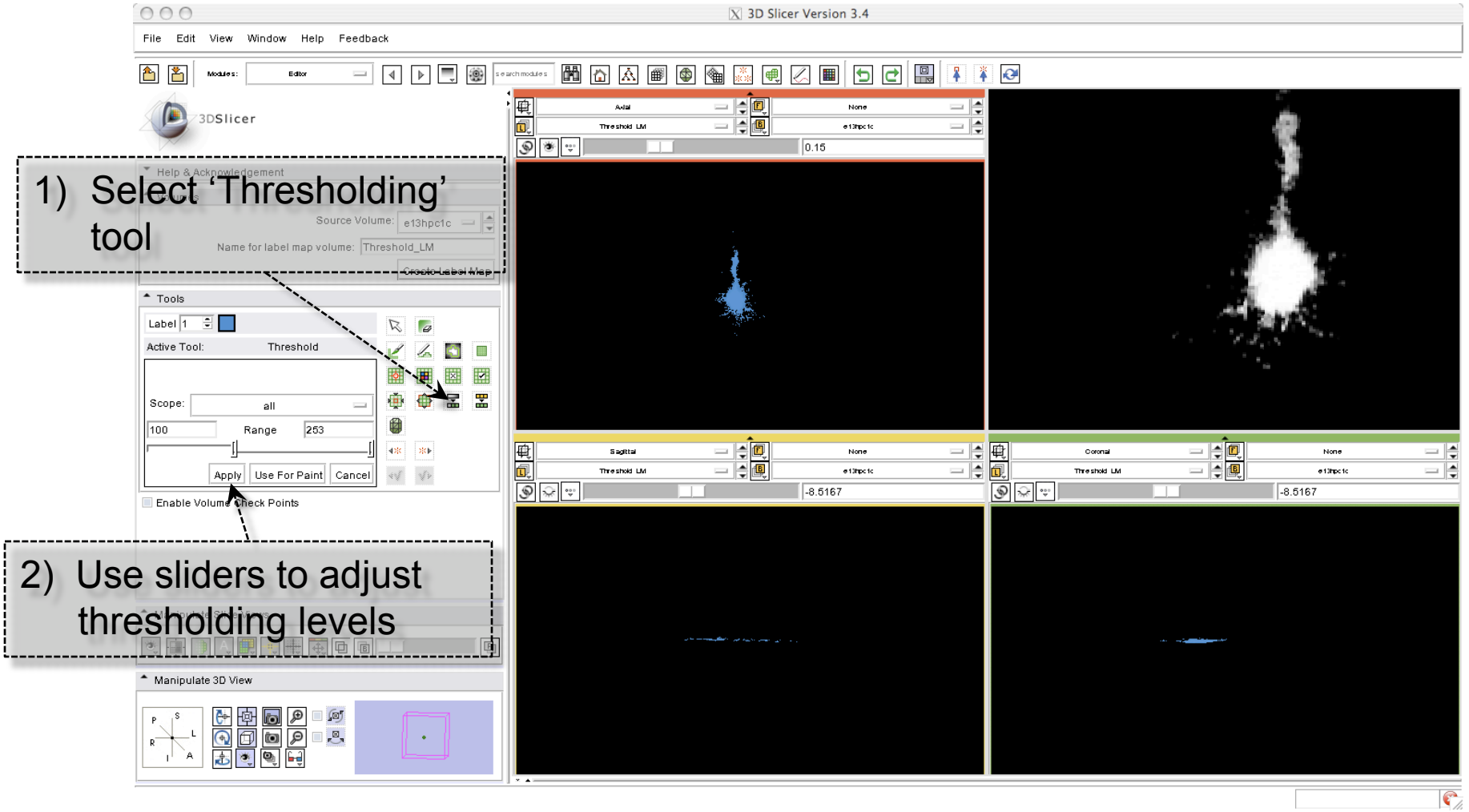
Model Building: Threshold



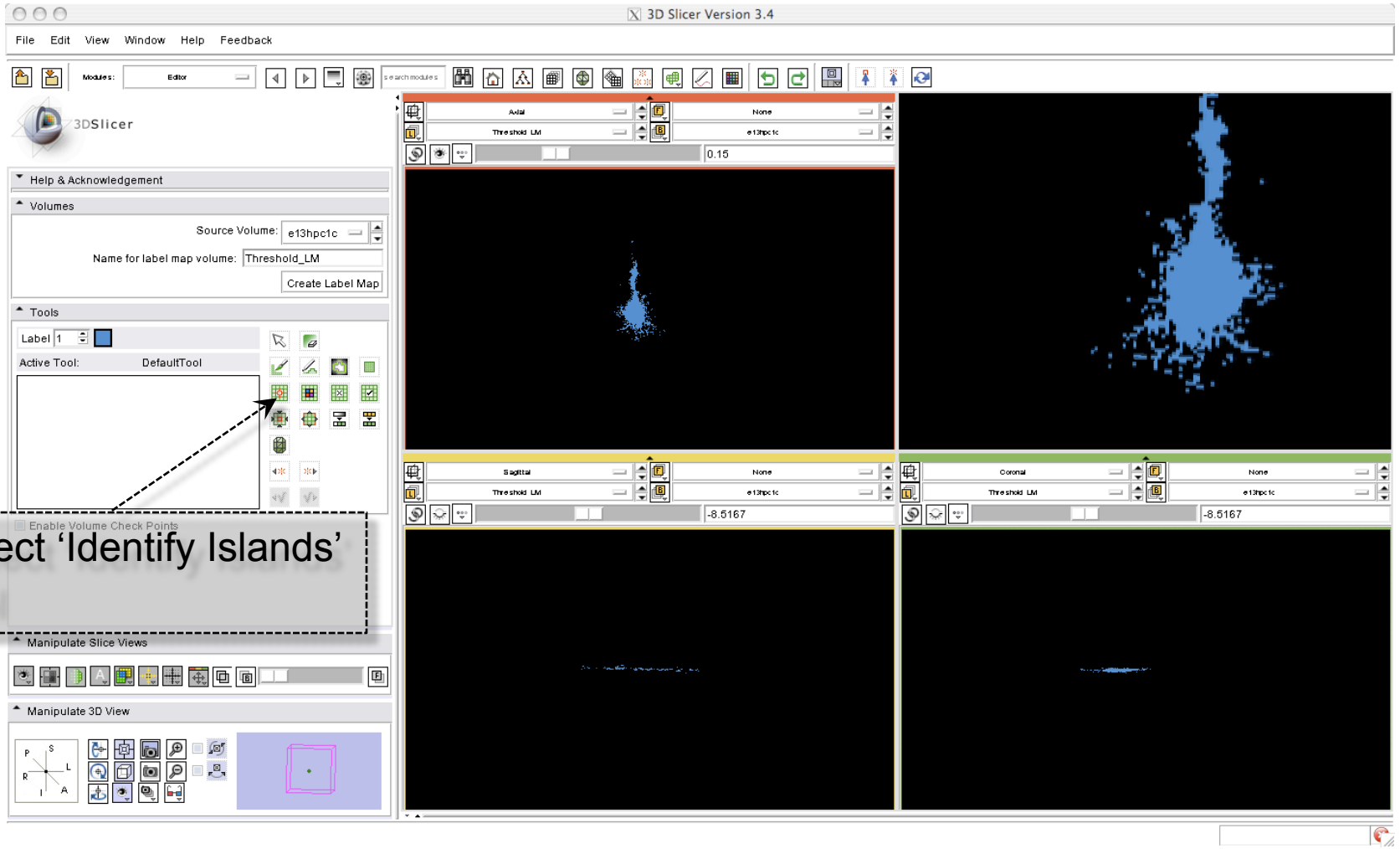
Model Building: Threshold



Model Building: Threshold

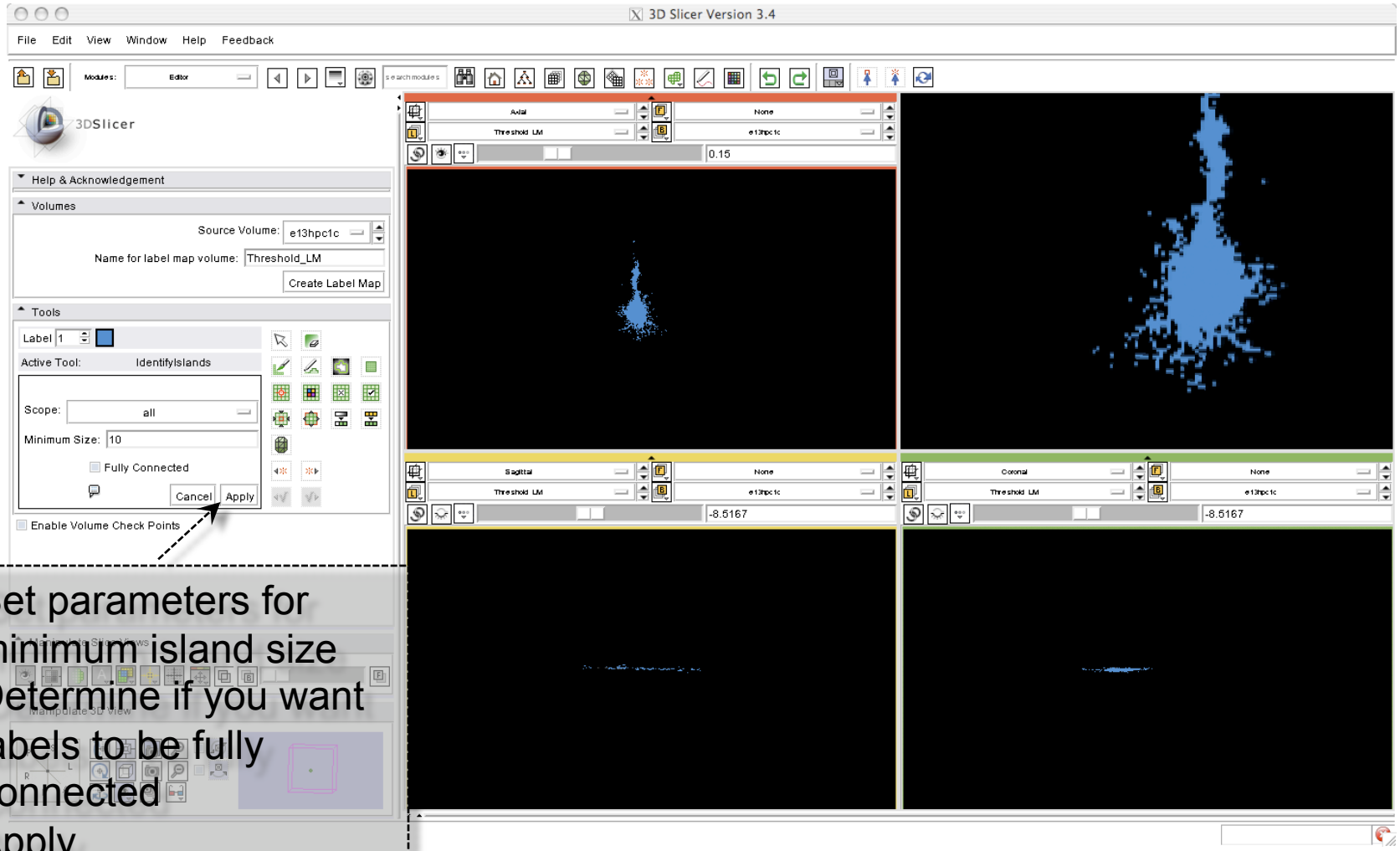


Model Building: Threshold



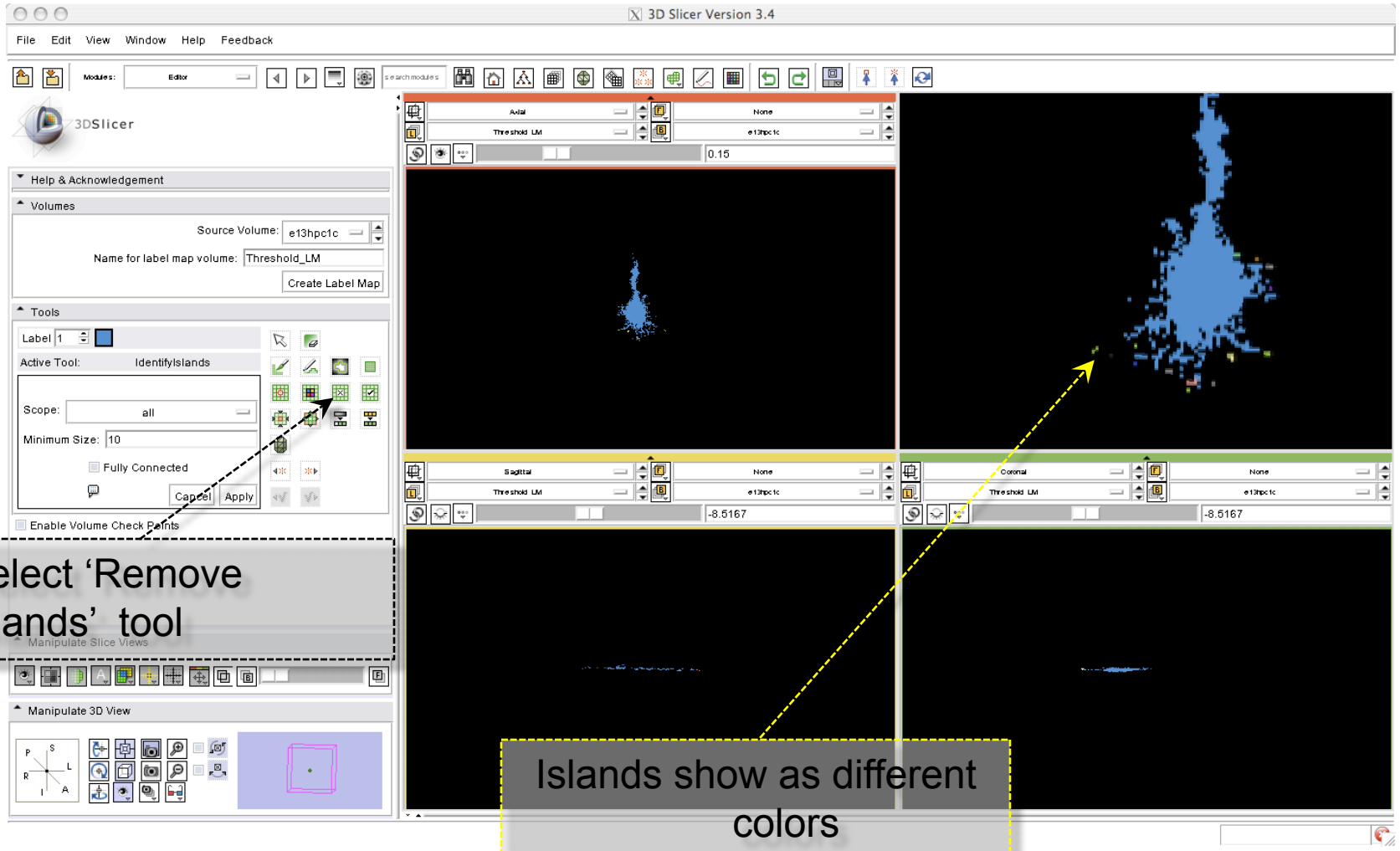
1) Select 'Identify Islands' tool

Model Building: Threshold

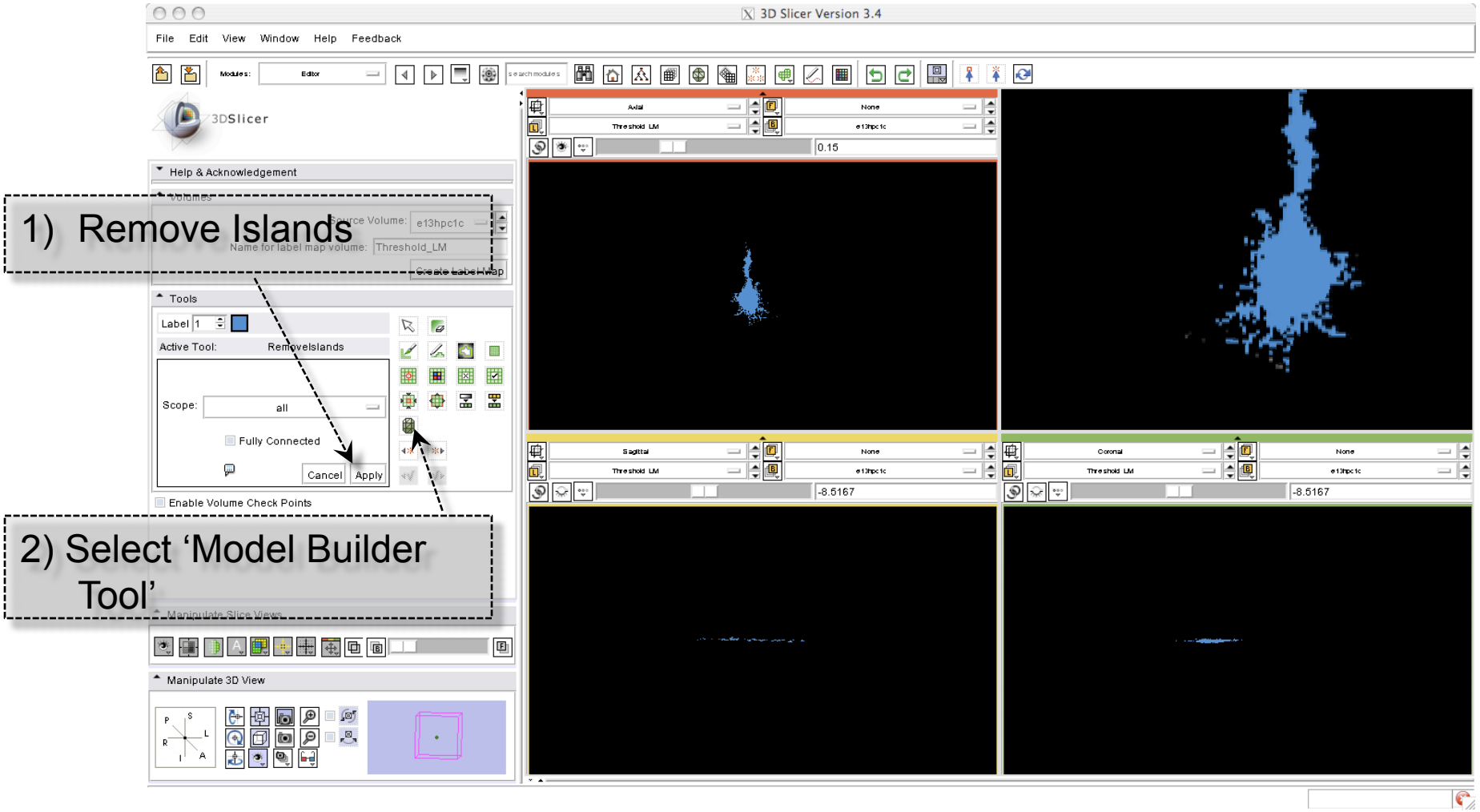


1) Set parameters for minimum island size
2) Determine if you want labels to be fully connected
3) Apply

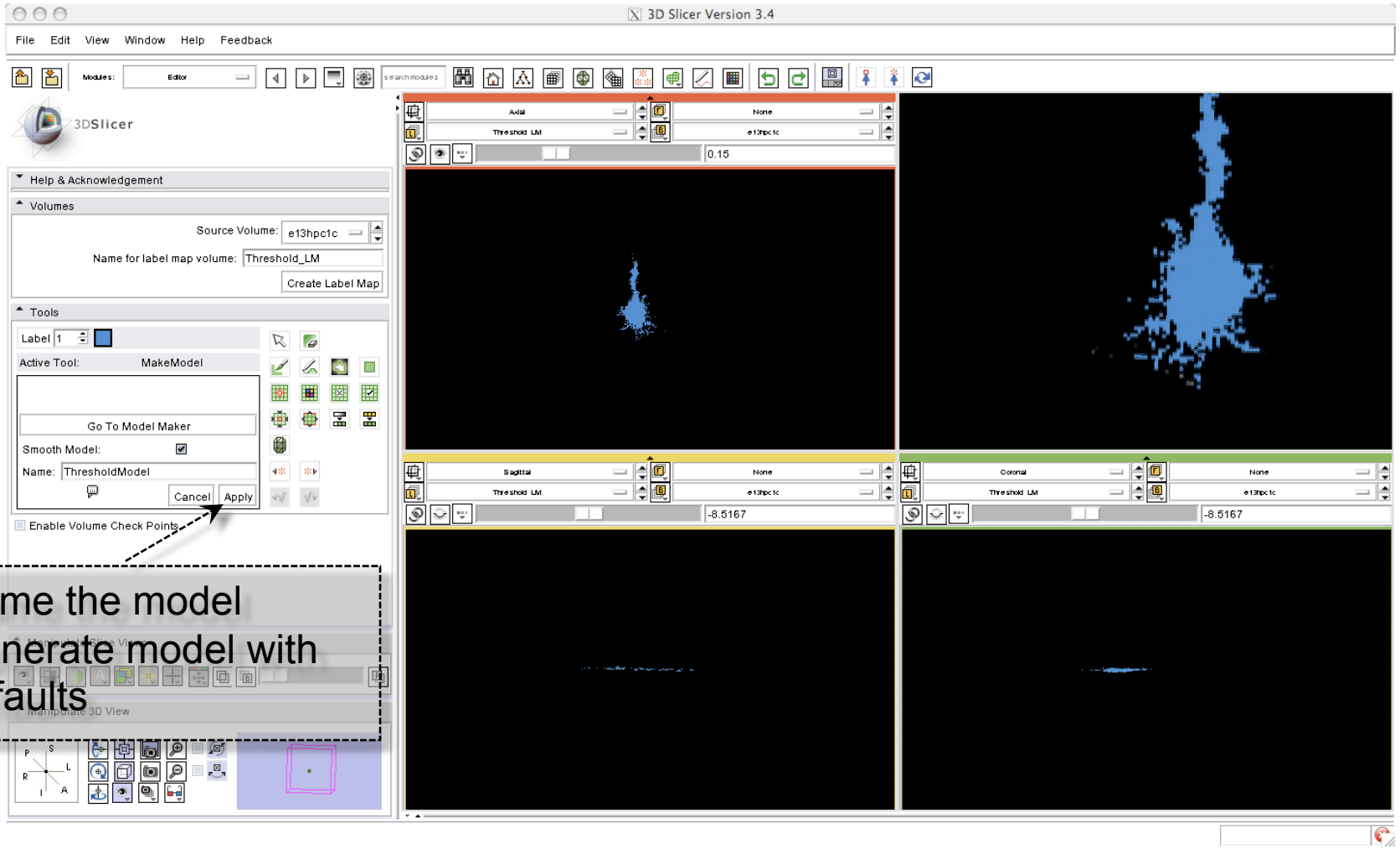
Model Building: Threshold



Model Building: Threshold

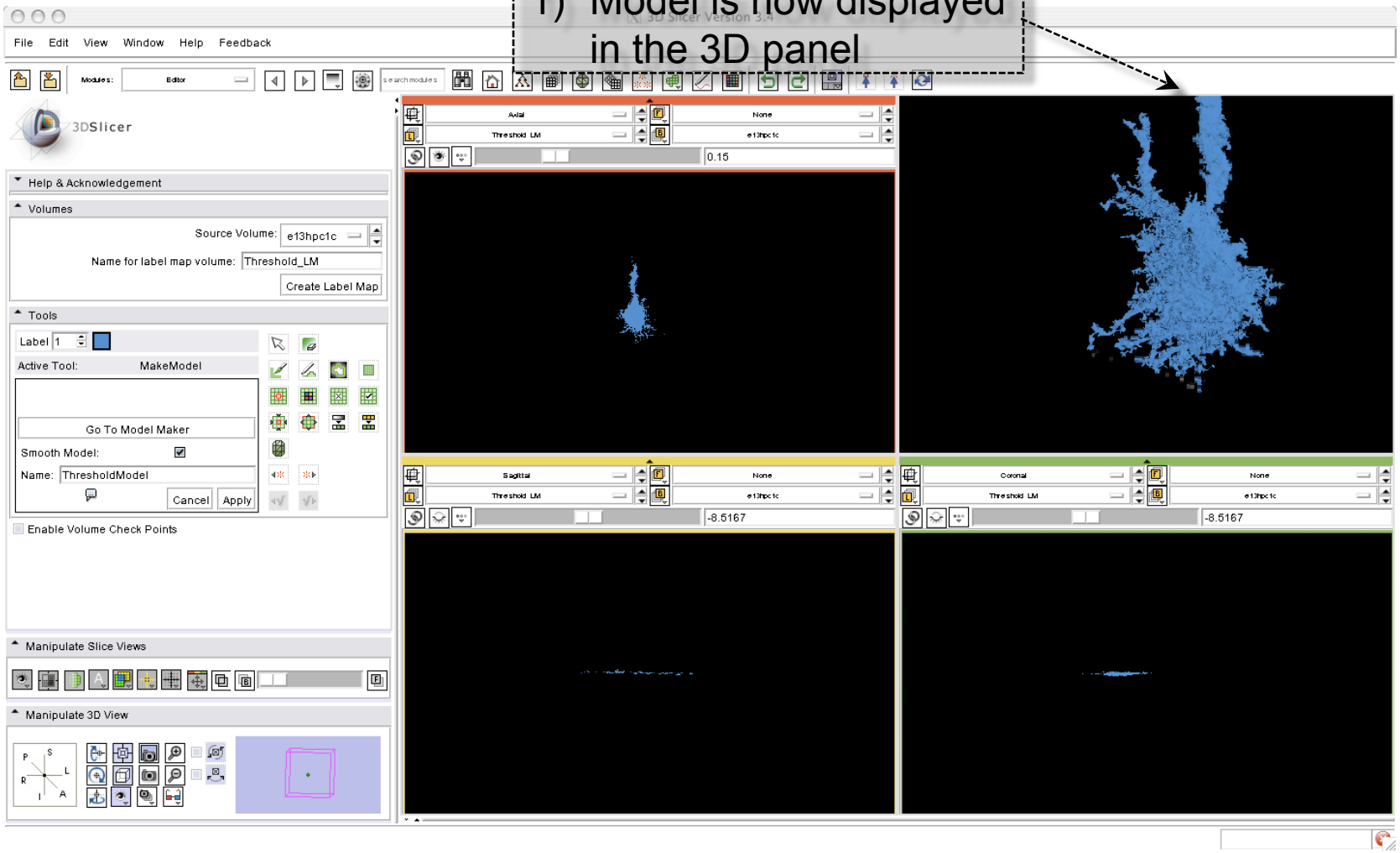


Model Building: Threshold

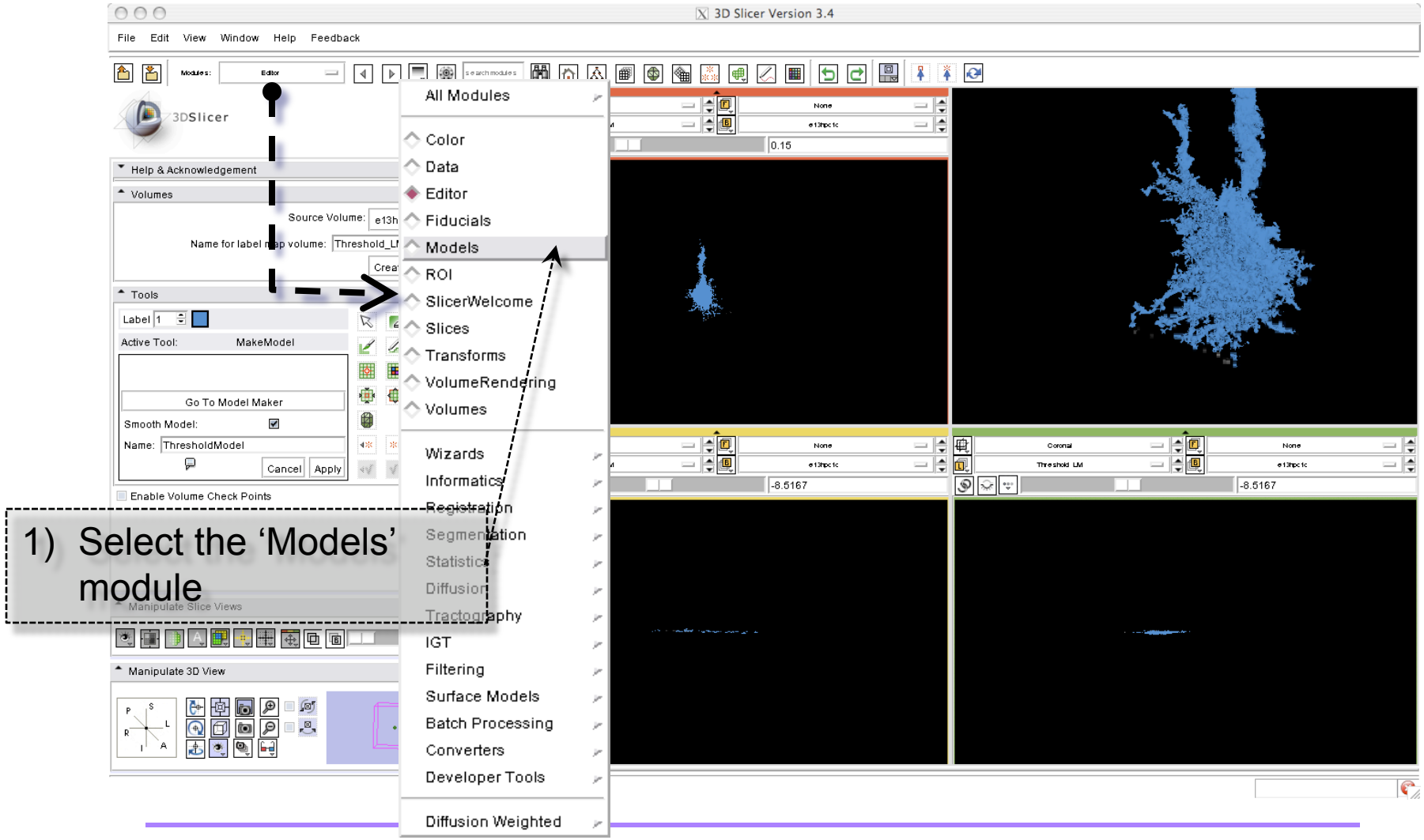


Model Building: Threshold

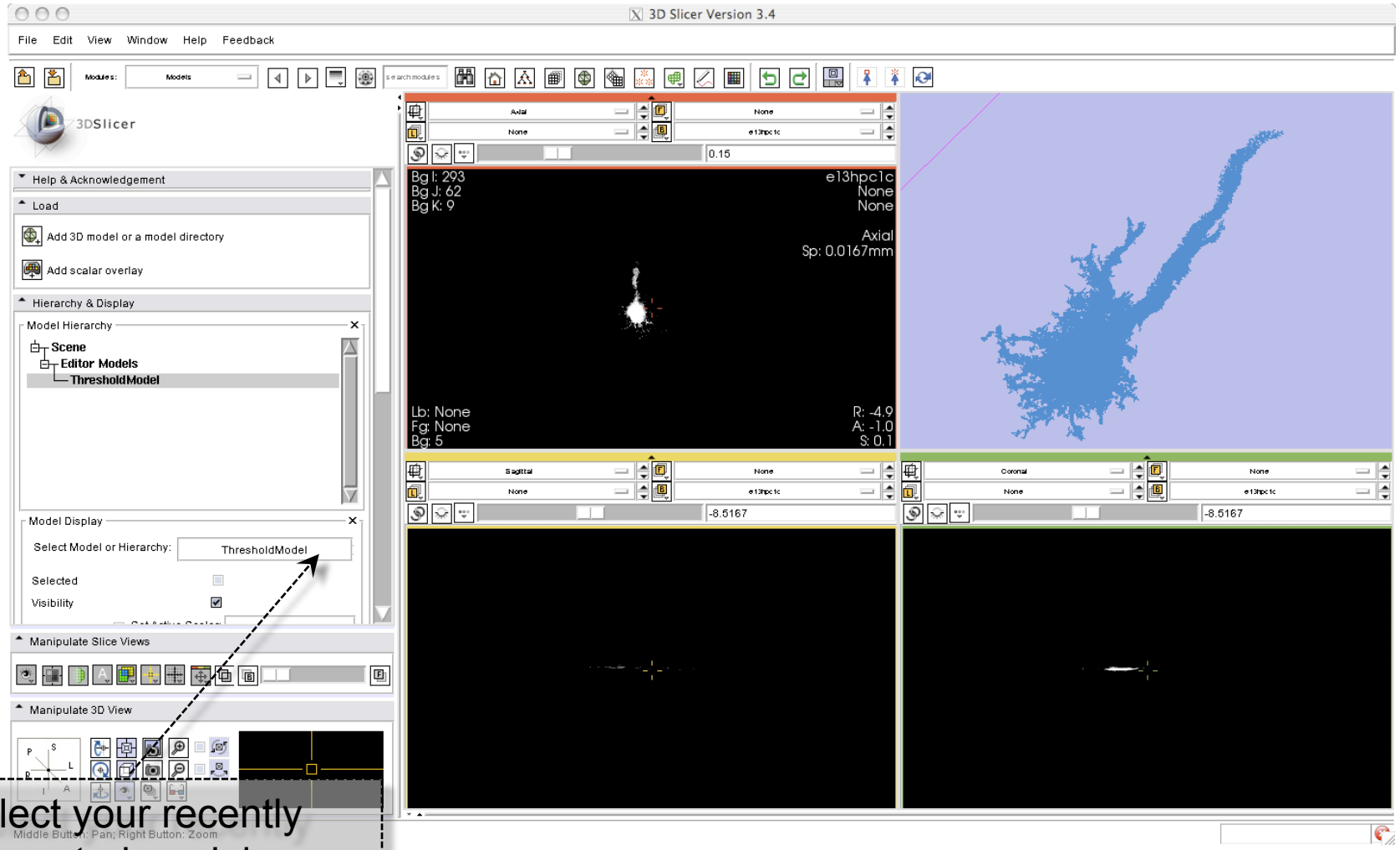
1) Model is now displayed in the 3D panel



Model Building: Threshold

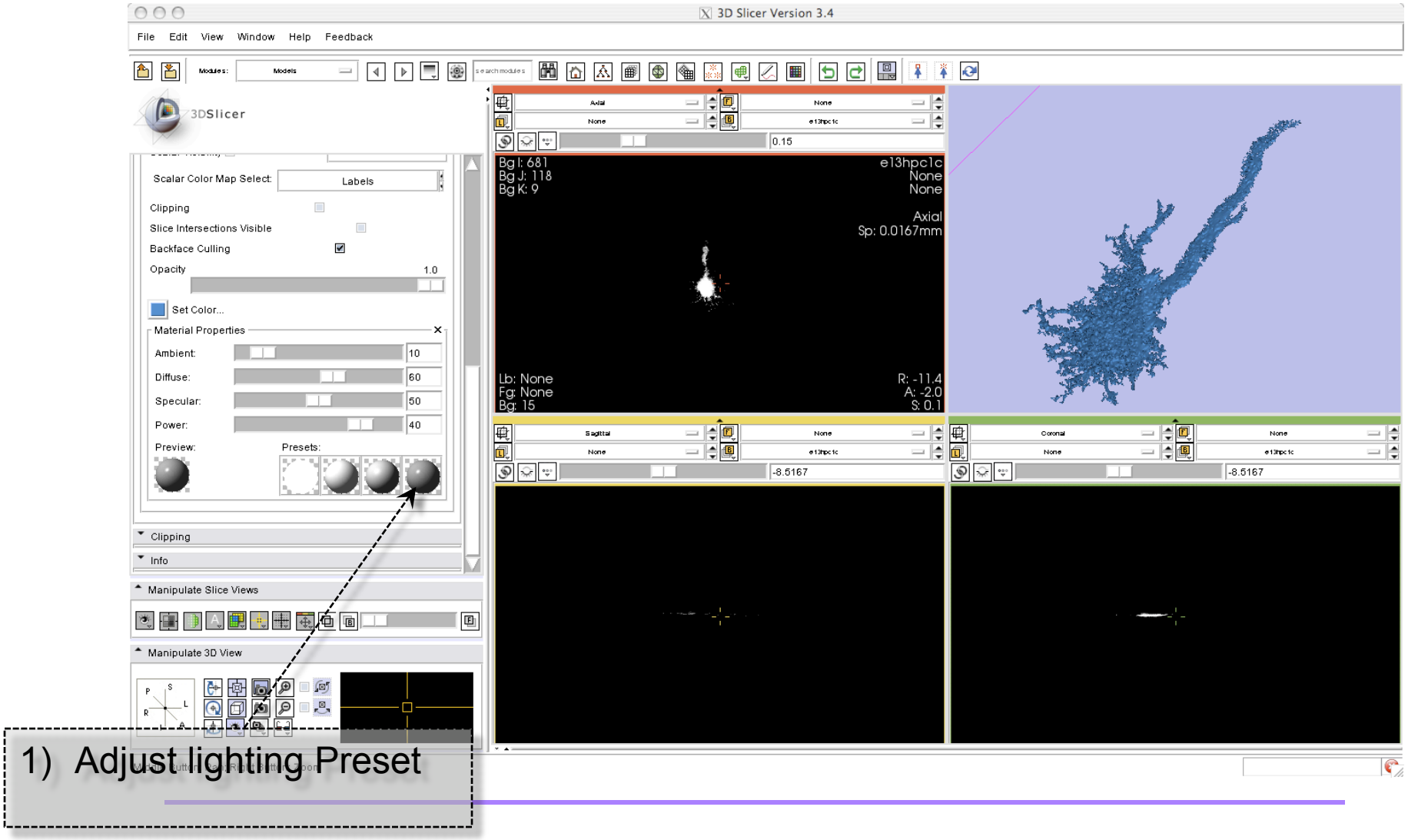


Model Building: Threshold



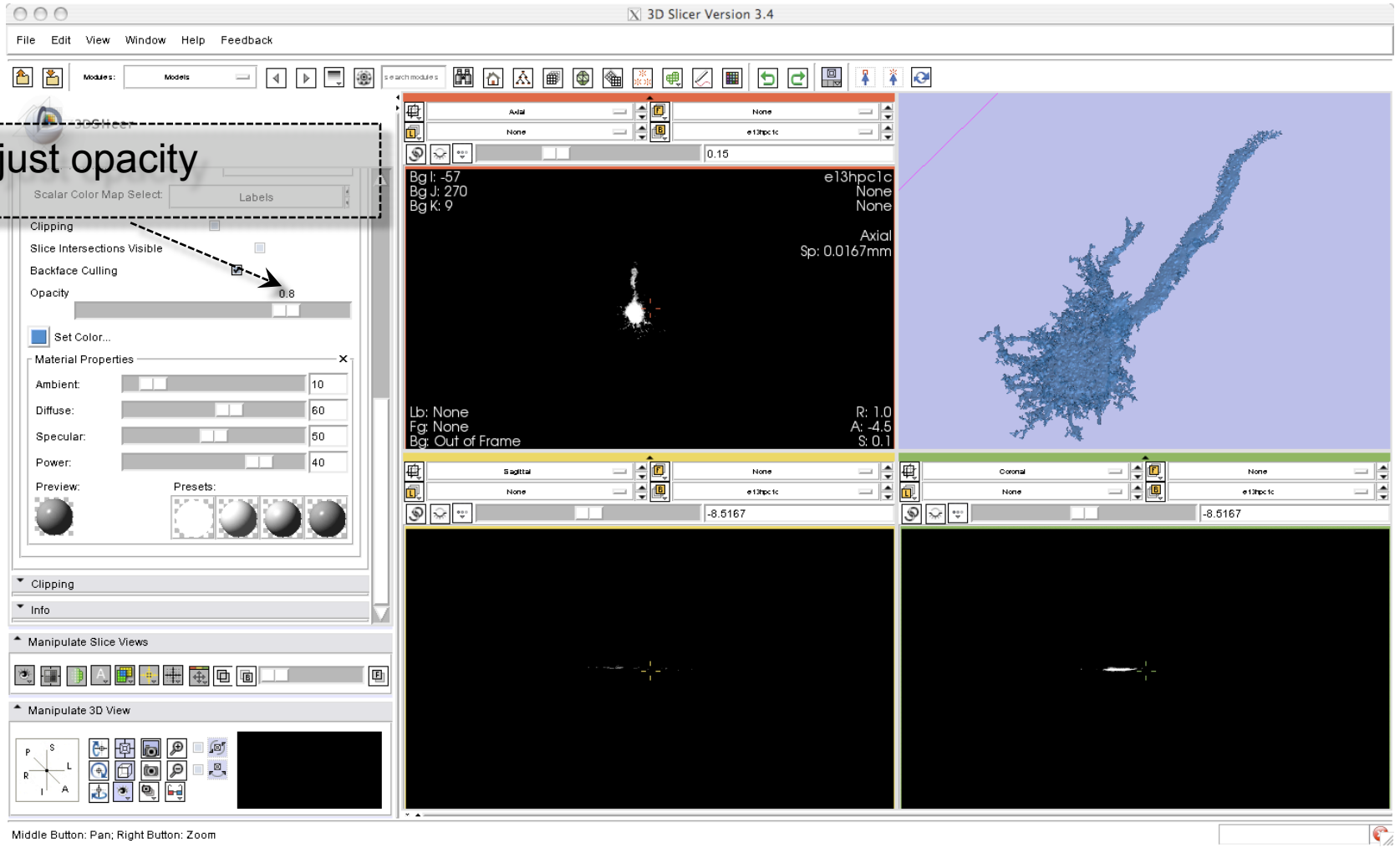
1) Select your recently generated model

Model Building: Threshold



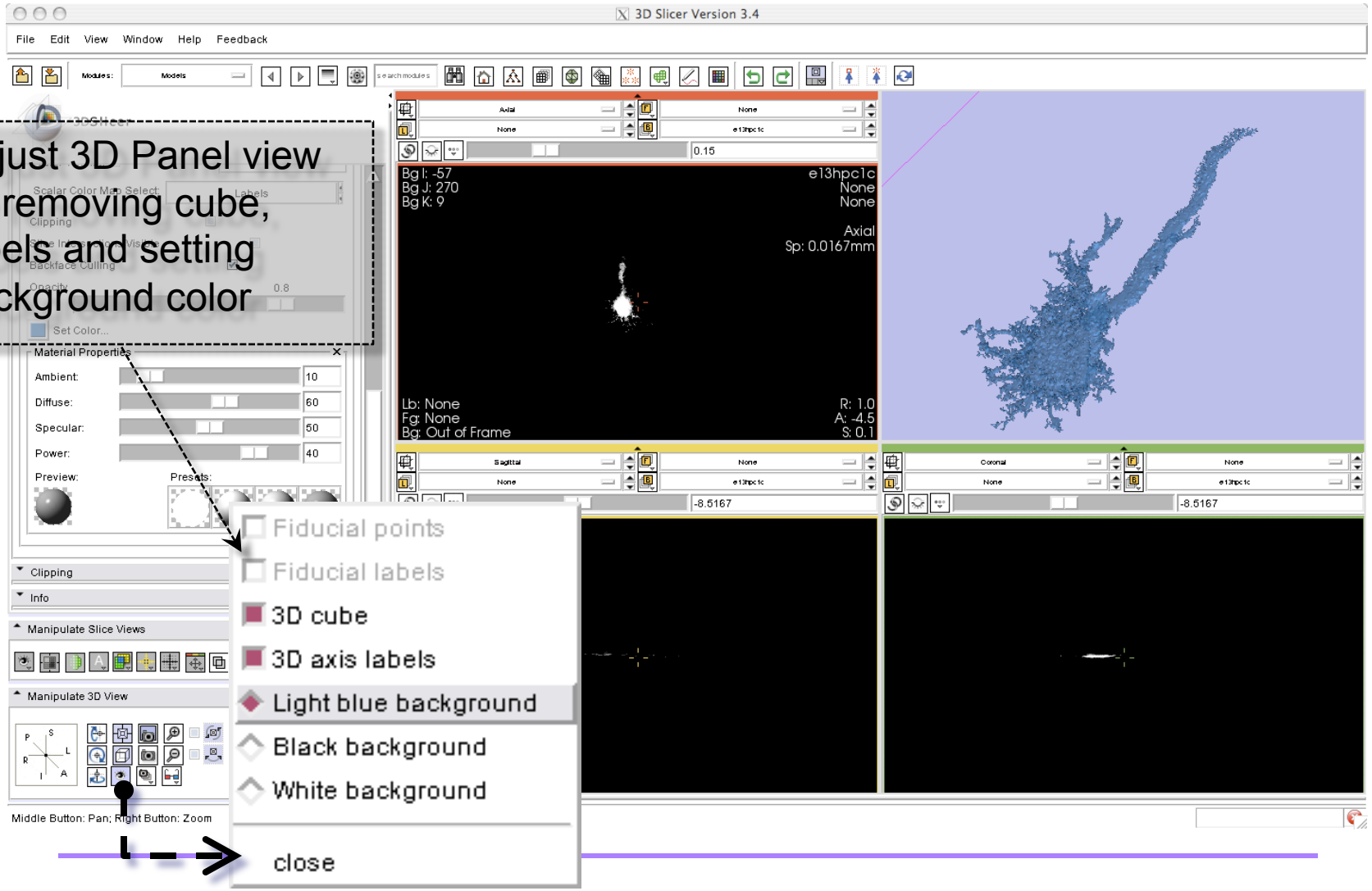
1) Adjust lighting Preset

Model Building: Threshold

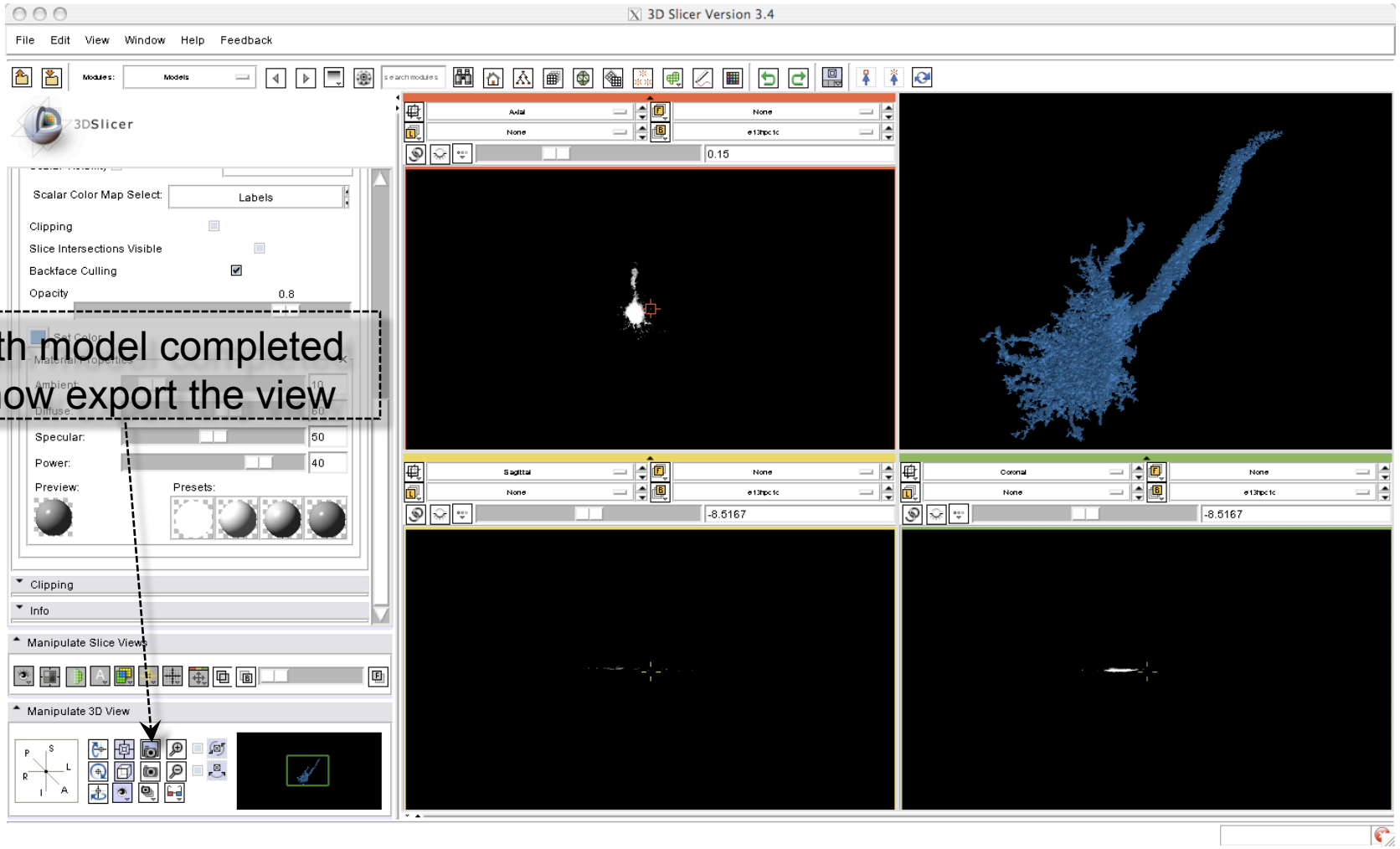


Model Building: Threshold

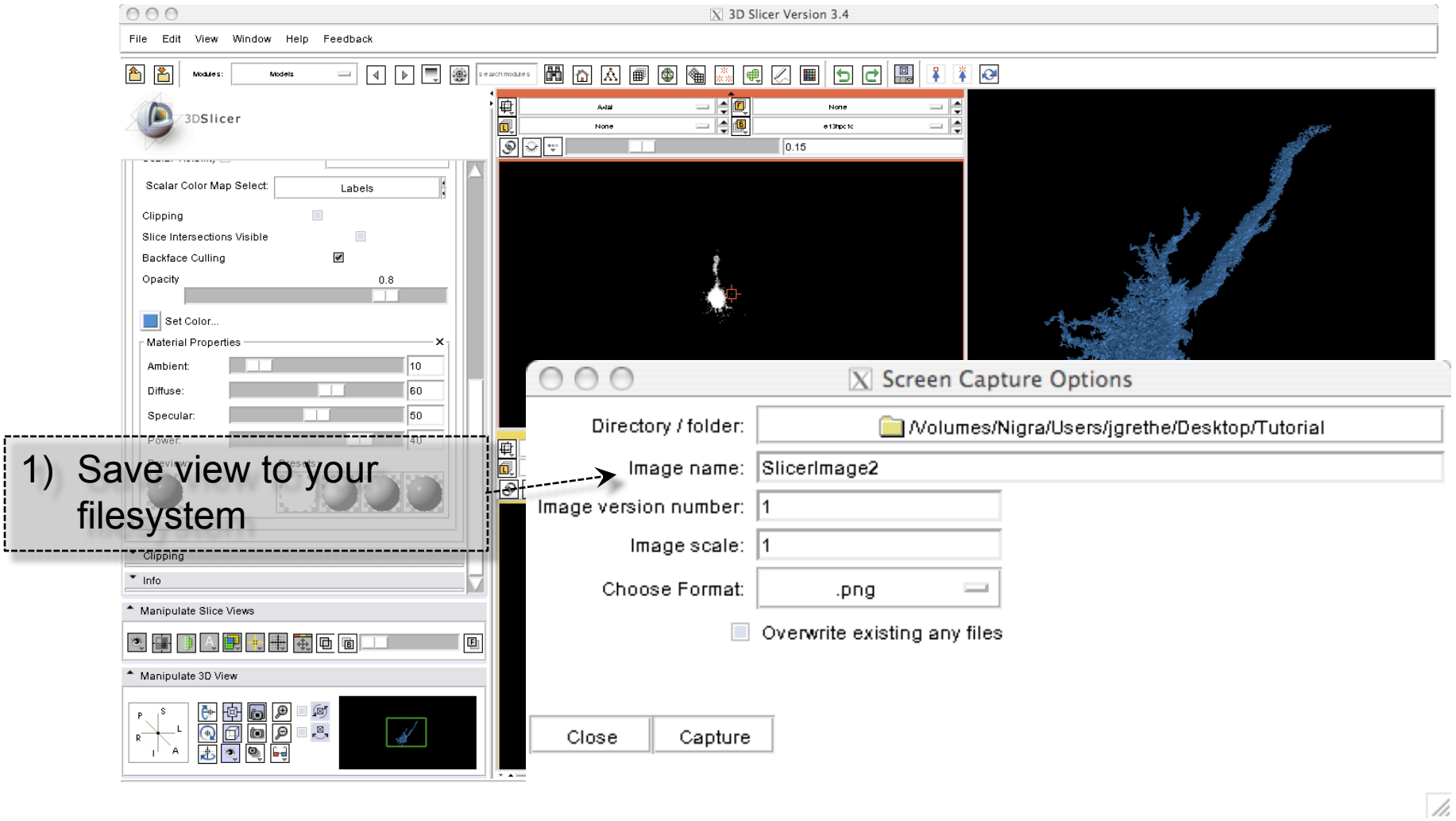
1) Adjust 3D Panel view by removing cube, labels and setting background color



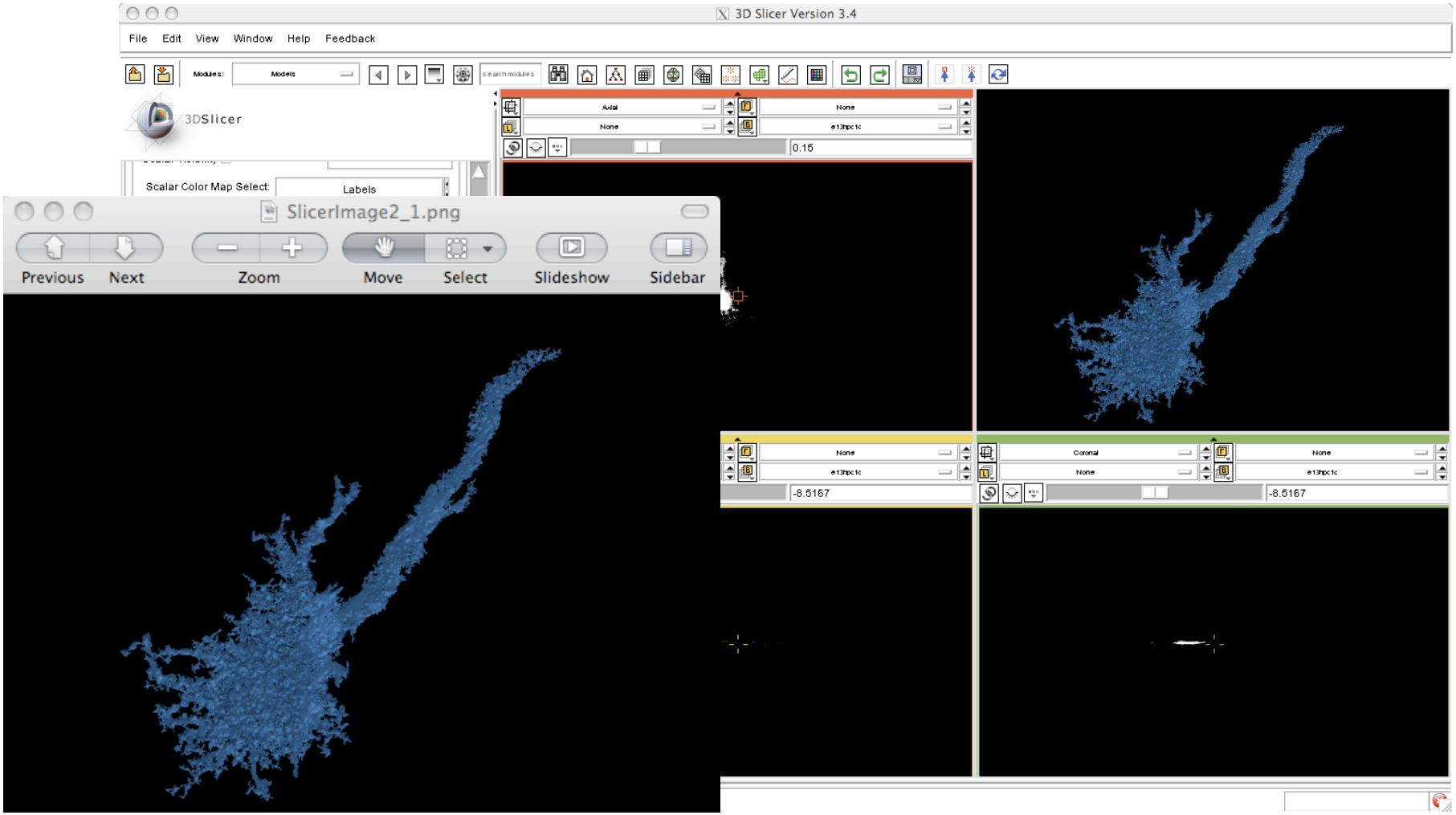
Model Building: Threshold



Model Building: Threshold



Completed Threshold Model Building



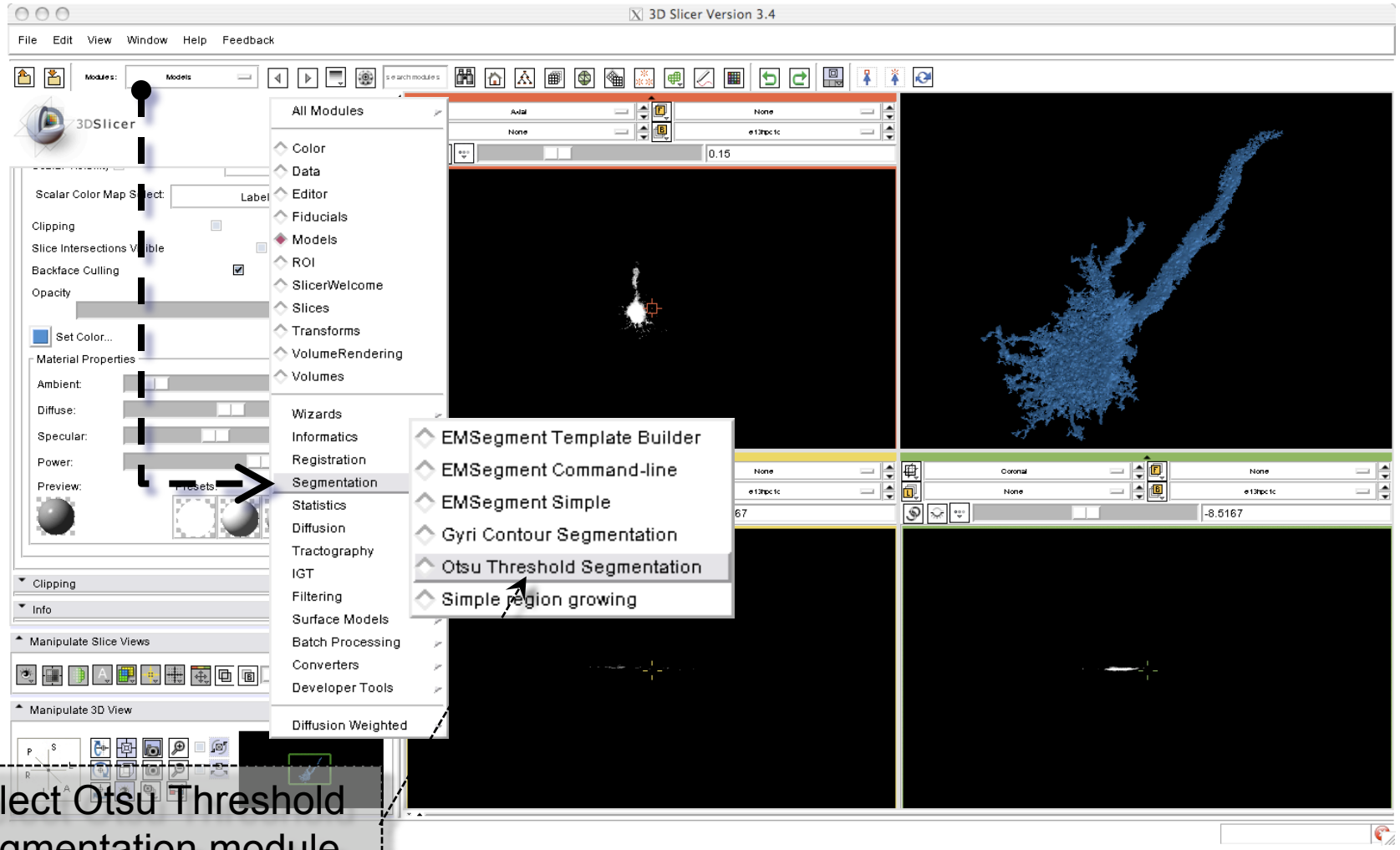
Model Building: Otsu Segmentation

In computer vision and image processing, Otsu's method is used to automatically perform histogram shape-based image thresholding, or the reduction of a graylevel image to a binary image. The algorithm assumes that the image to be thresholded contains two classes of pixels (e.g. foreground and background) then calculates the optimum threshold separating those two classes so that their combined spread (intra-class variance) is minimal.

http://en.wikipedia.org/wiki/Otsu's_method

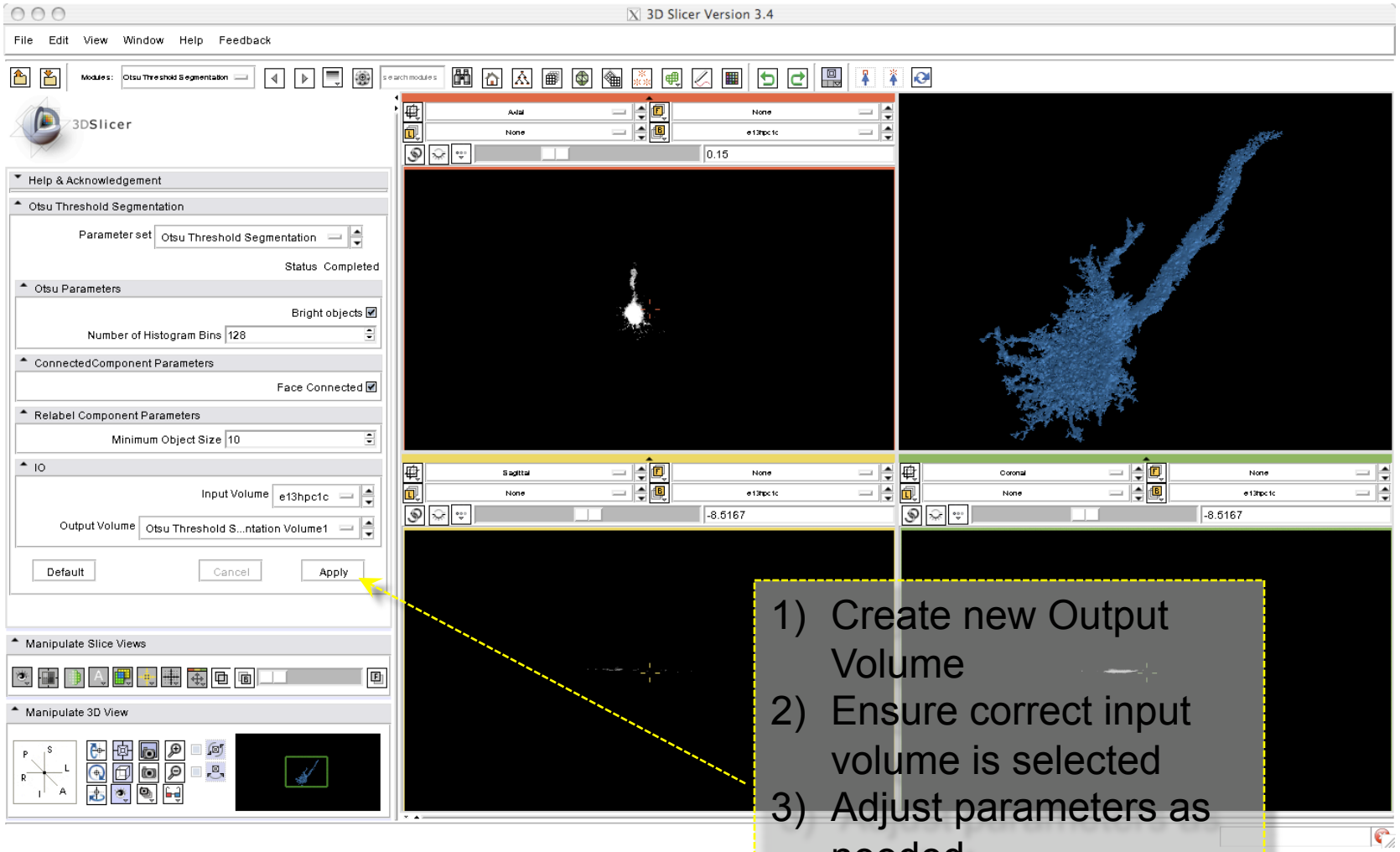
This section of the tutorial will demonstrate the use of Slicer Command Line Modules

Model Building: Otsu Segmentation



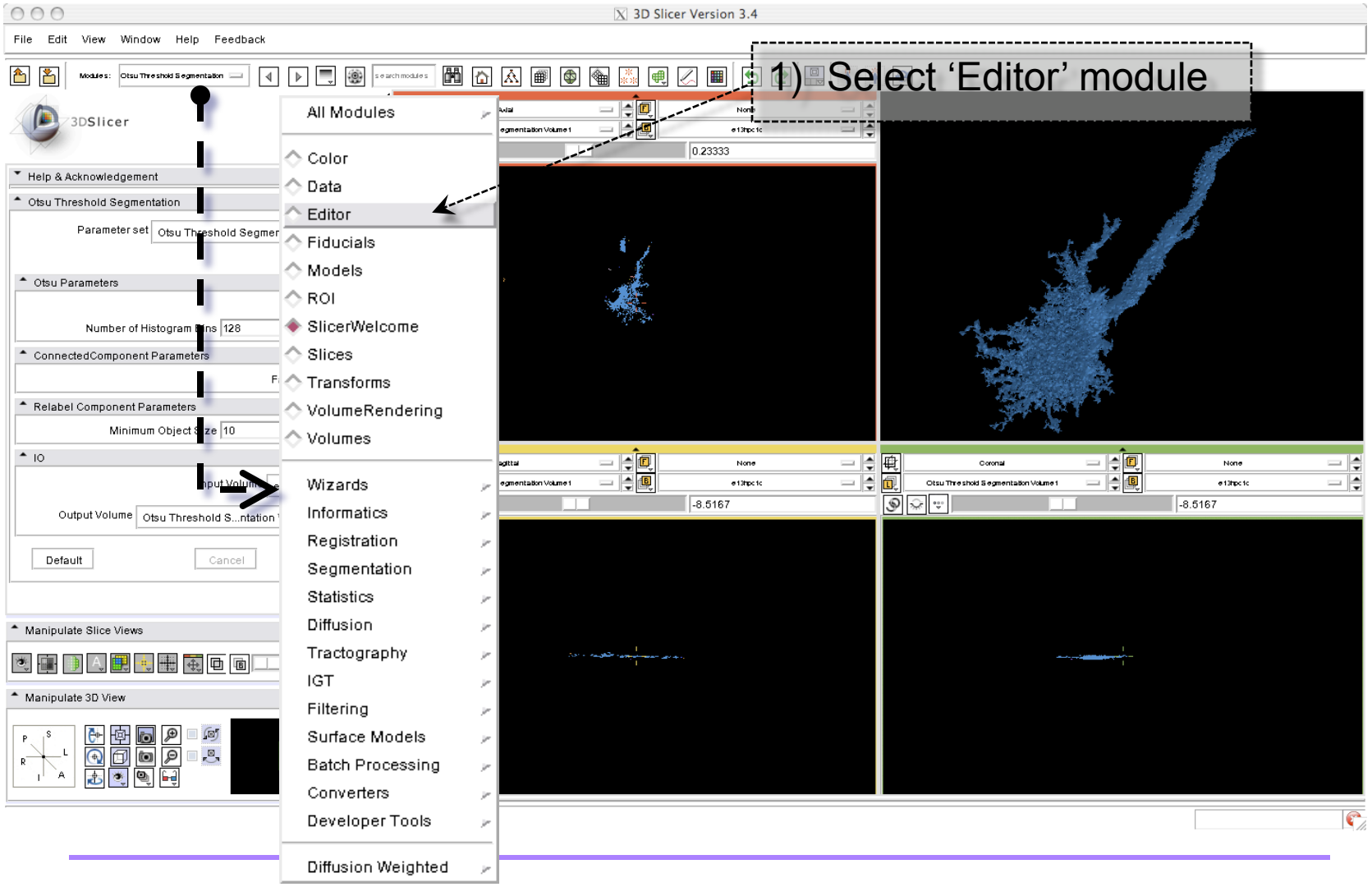
1) Select Otsu Threshold Segmentation module

Model Building: Otsu Segmentation

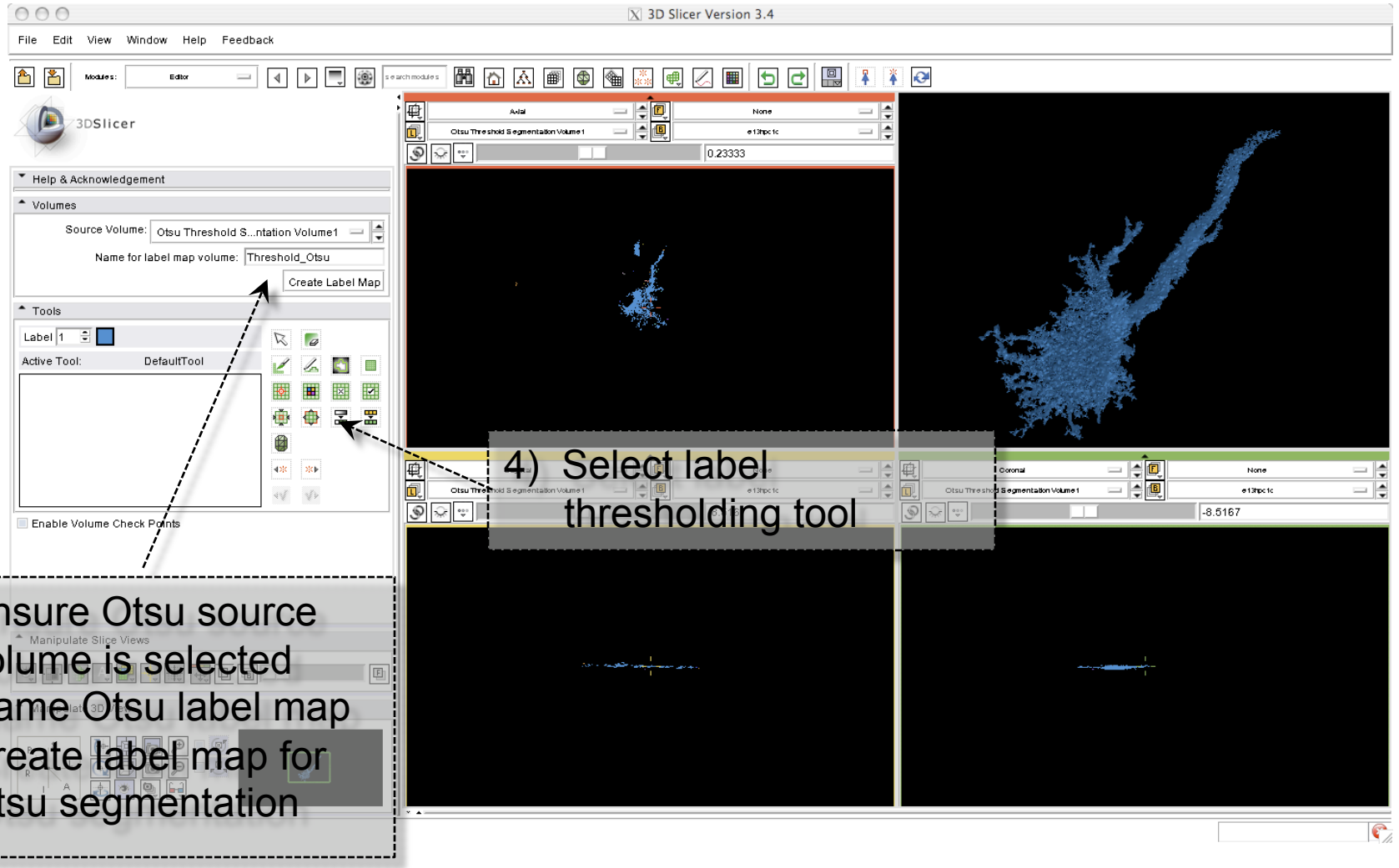


1) Create new Output Volume
2) Ensure correct input volume is selected
3) Adjust parameters as needed
4) Apply Otsu command line module

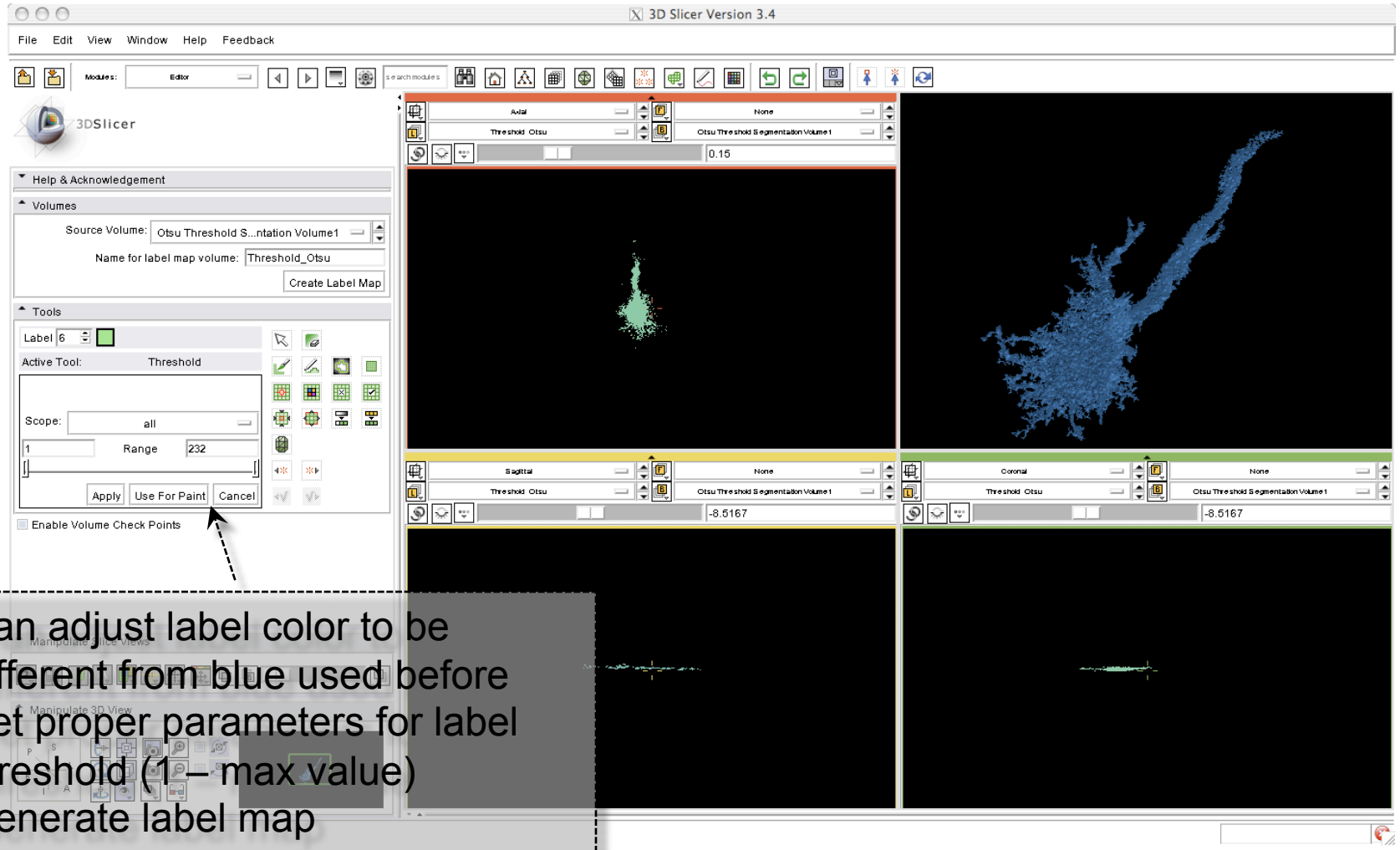
Model Building: Otsu Segmentation



Model Building: Otsu Segmentation

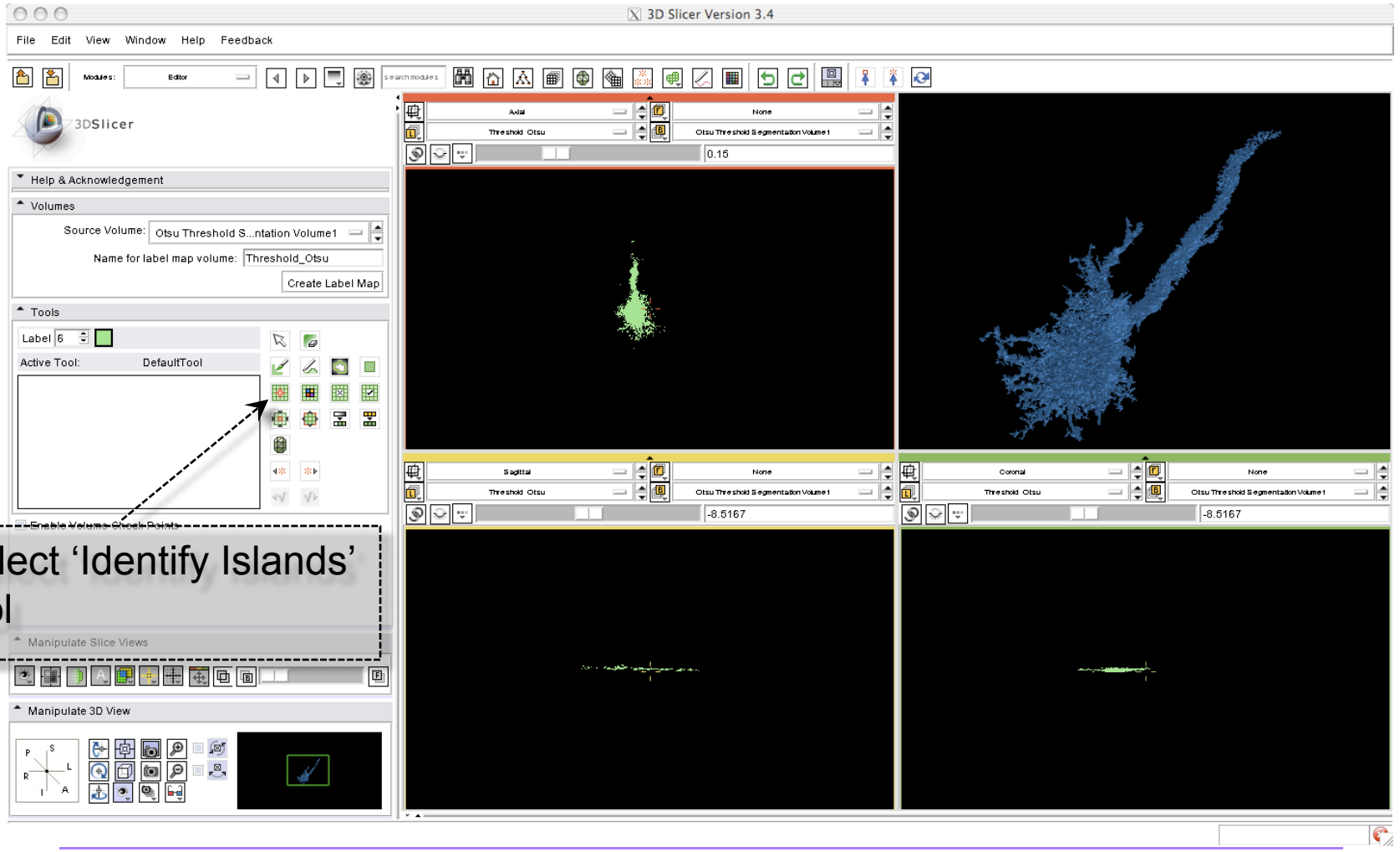


Model Building: Otsu Segmentation



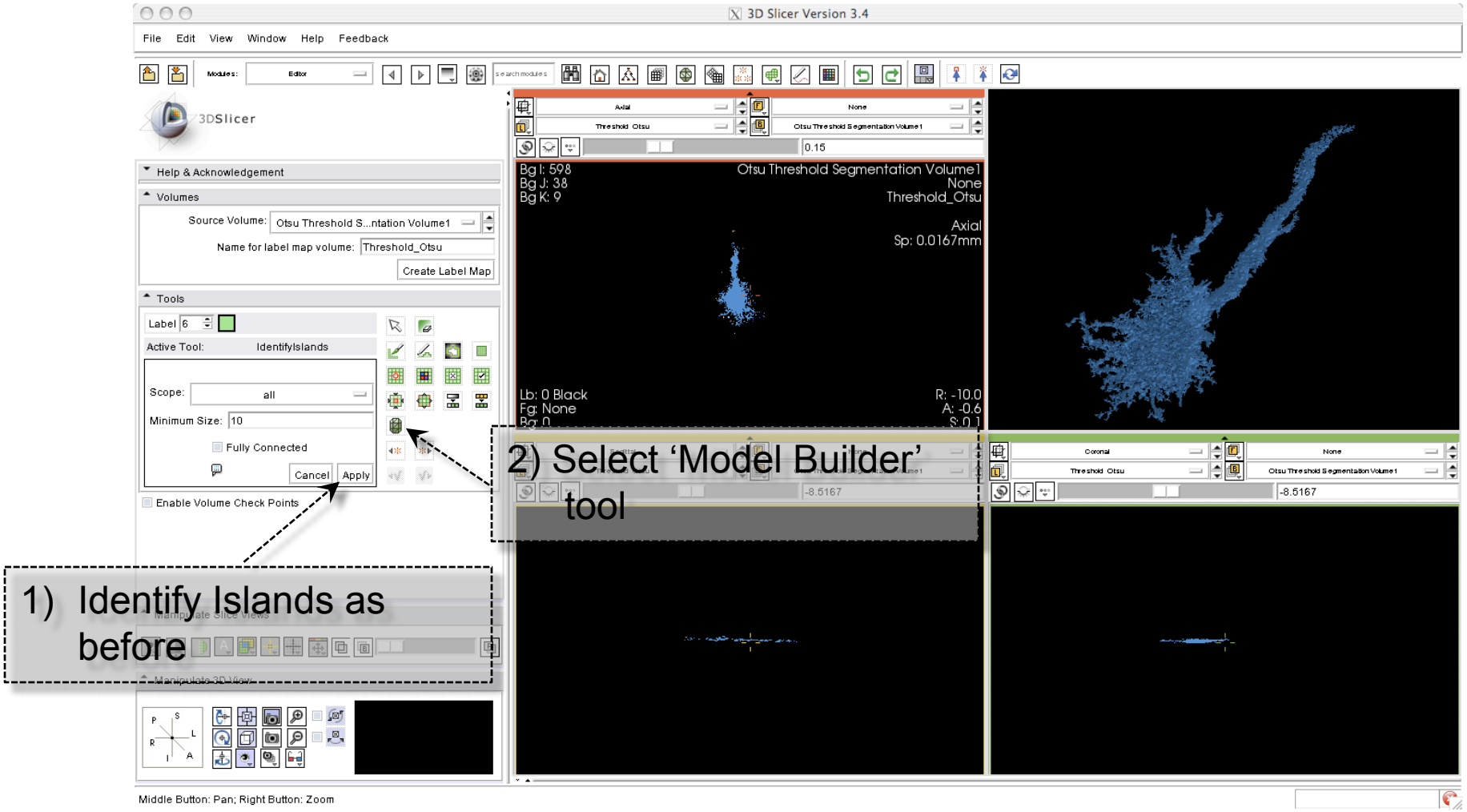
1) Can adjust label color to be different from blue used before
2) Set proper parameters for label threshold (1 – max value)
3) Generate label map

Model Building: Otsu Segmentation

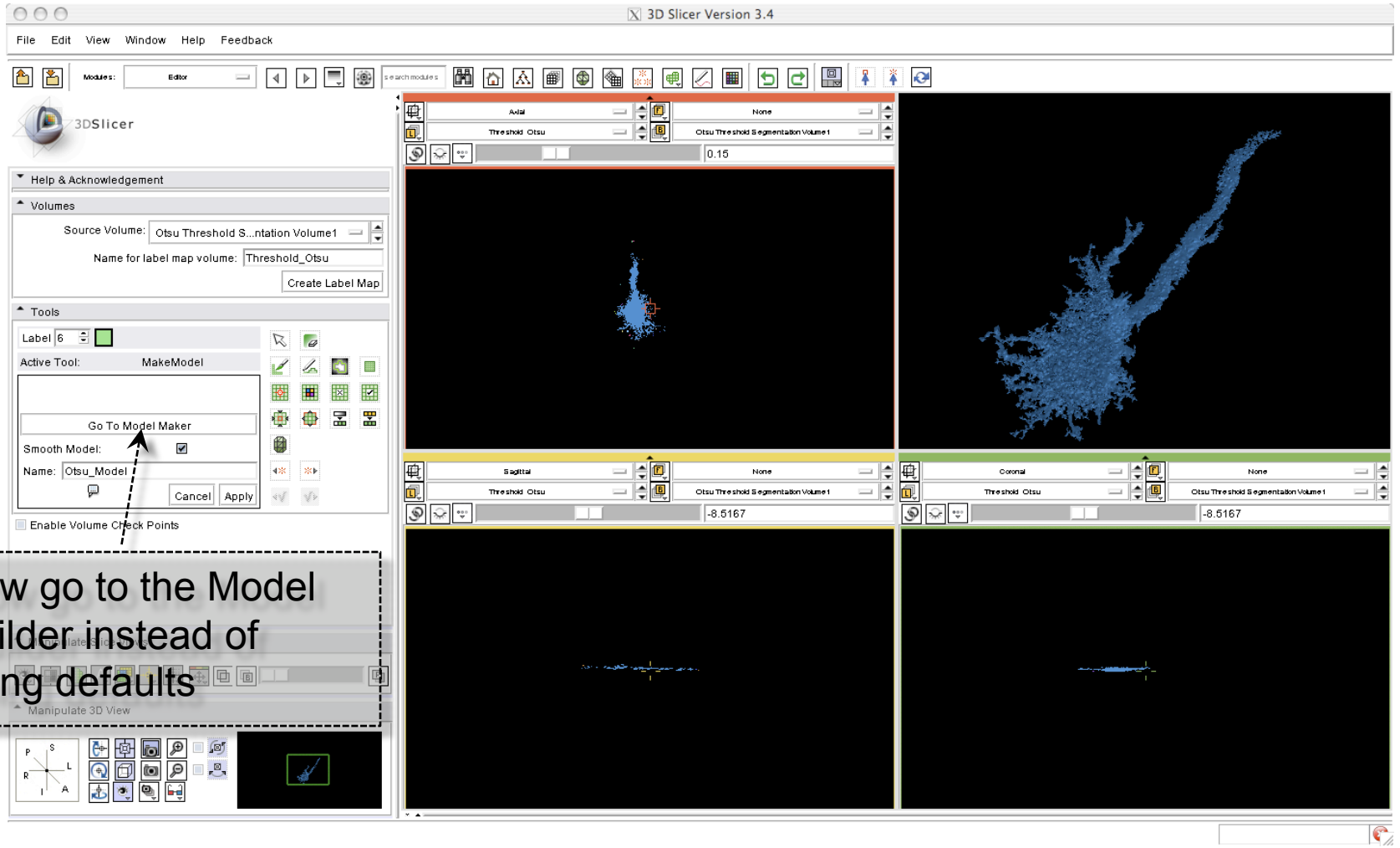


1) Select 'Identify Islands' tool

Model Building: Otsu Segmentation

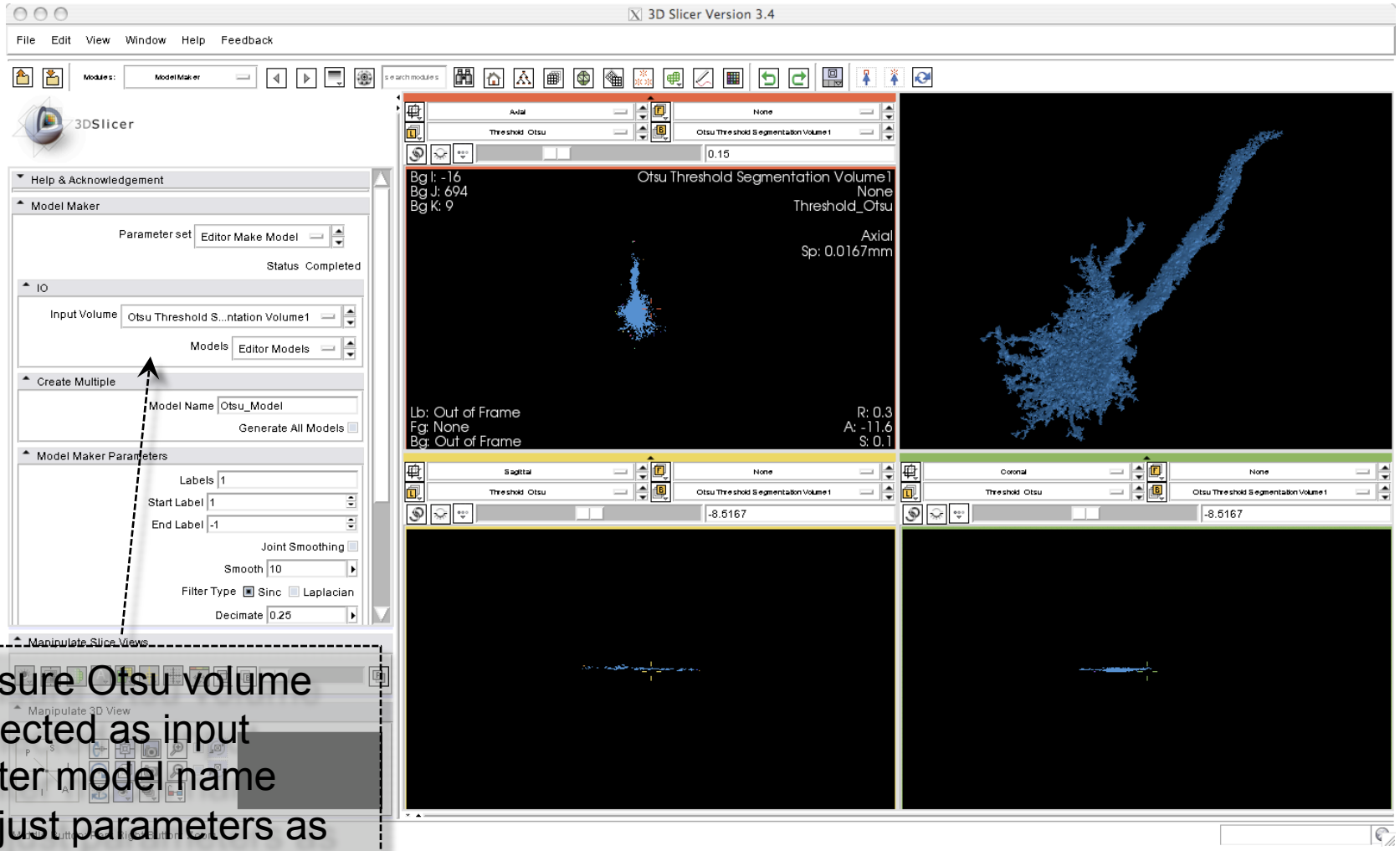


Model Building: Otsu Segmentation



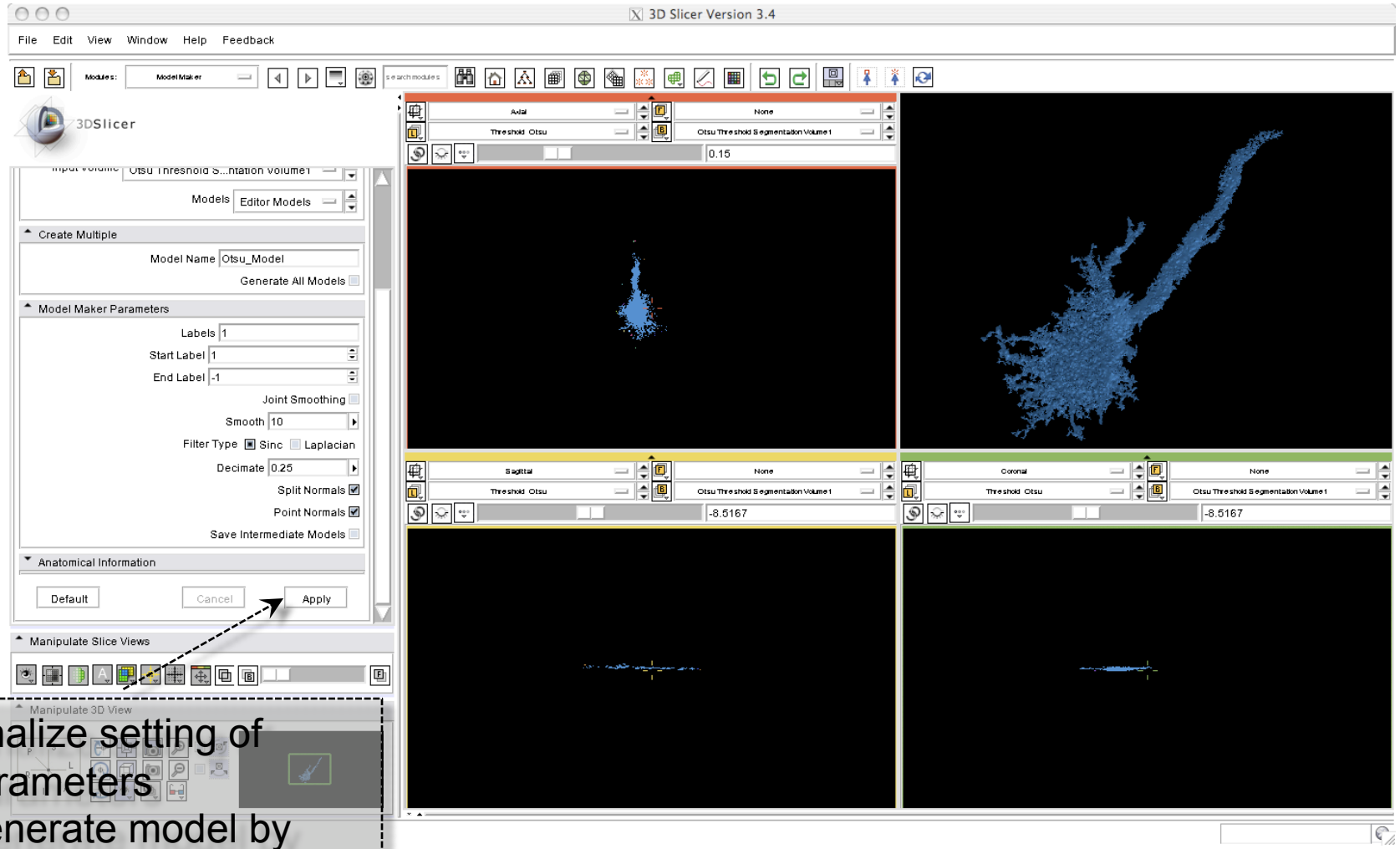
1) Now go to the Model Builder instead of using defaults

Model Building: Otsu Segmentation



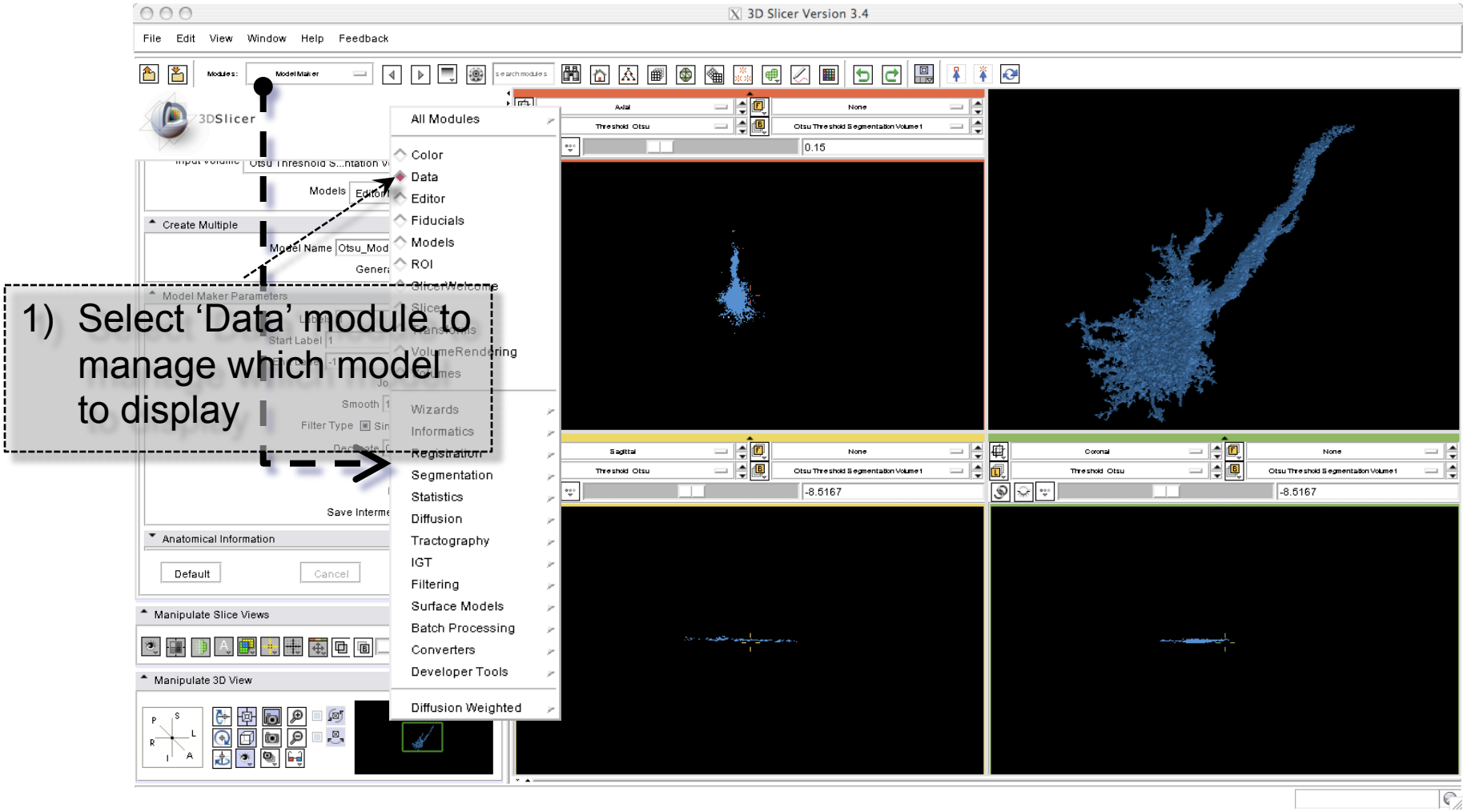
1) Ensure Otsu volume selected as input
2) Enter model name
3) Adjust parameters as needed

Model Building: Otsu Segmentation

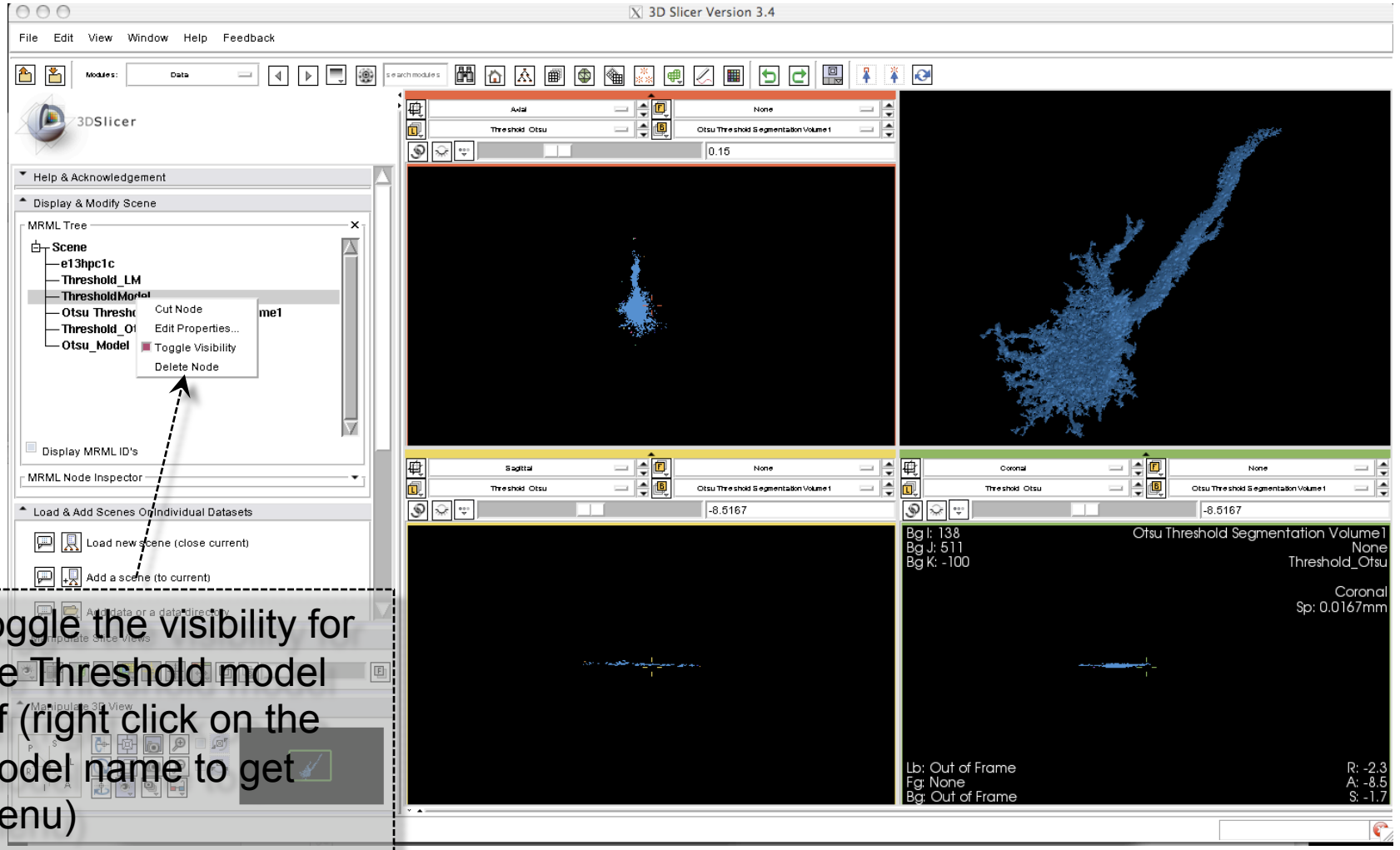


1) Finalize setting of parameters
2) Generate model by selecting 'Apply'

Model Building: Otsu Segmentation

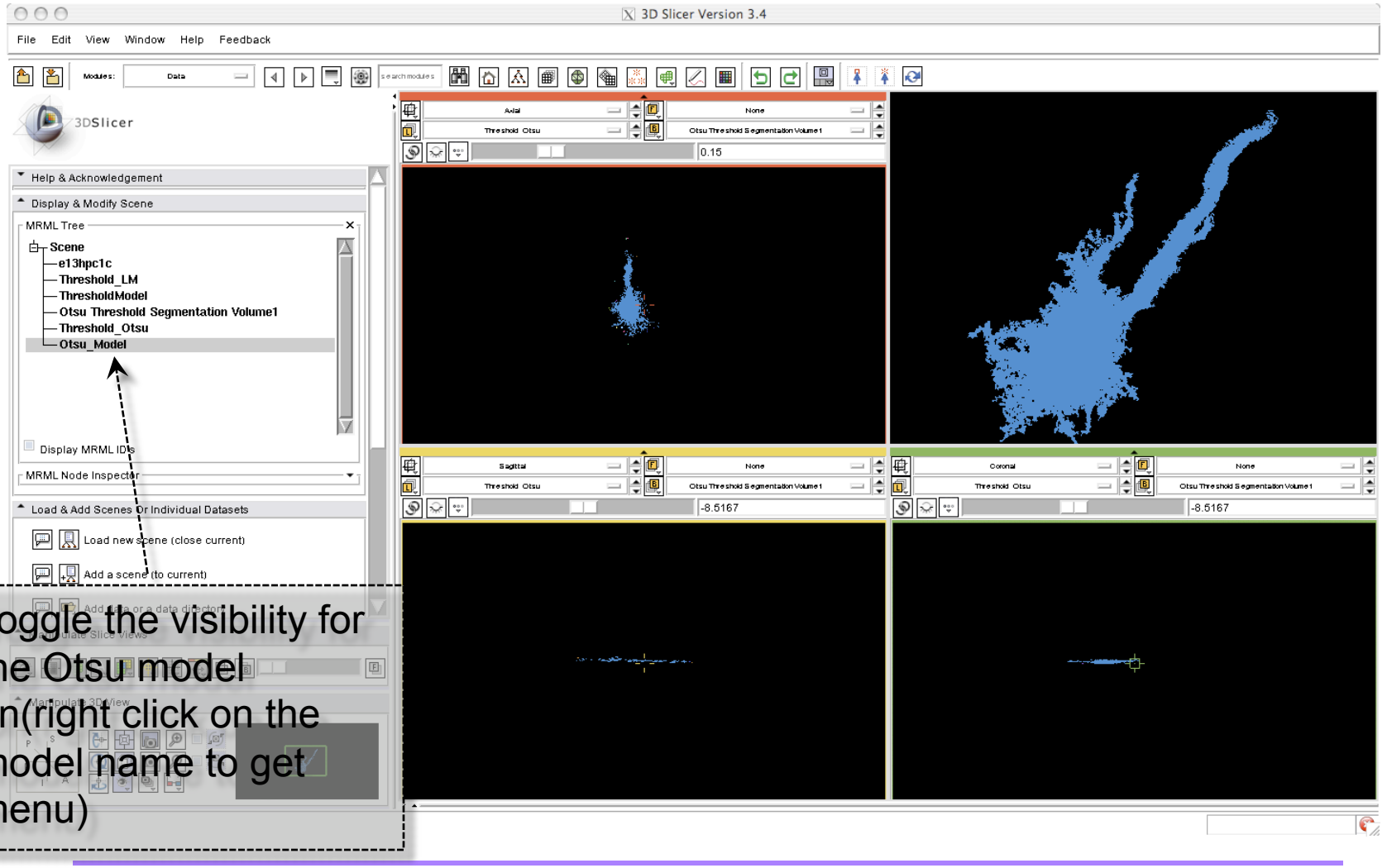


Model Building: Otsu Segmentation



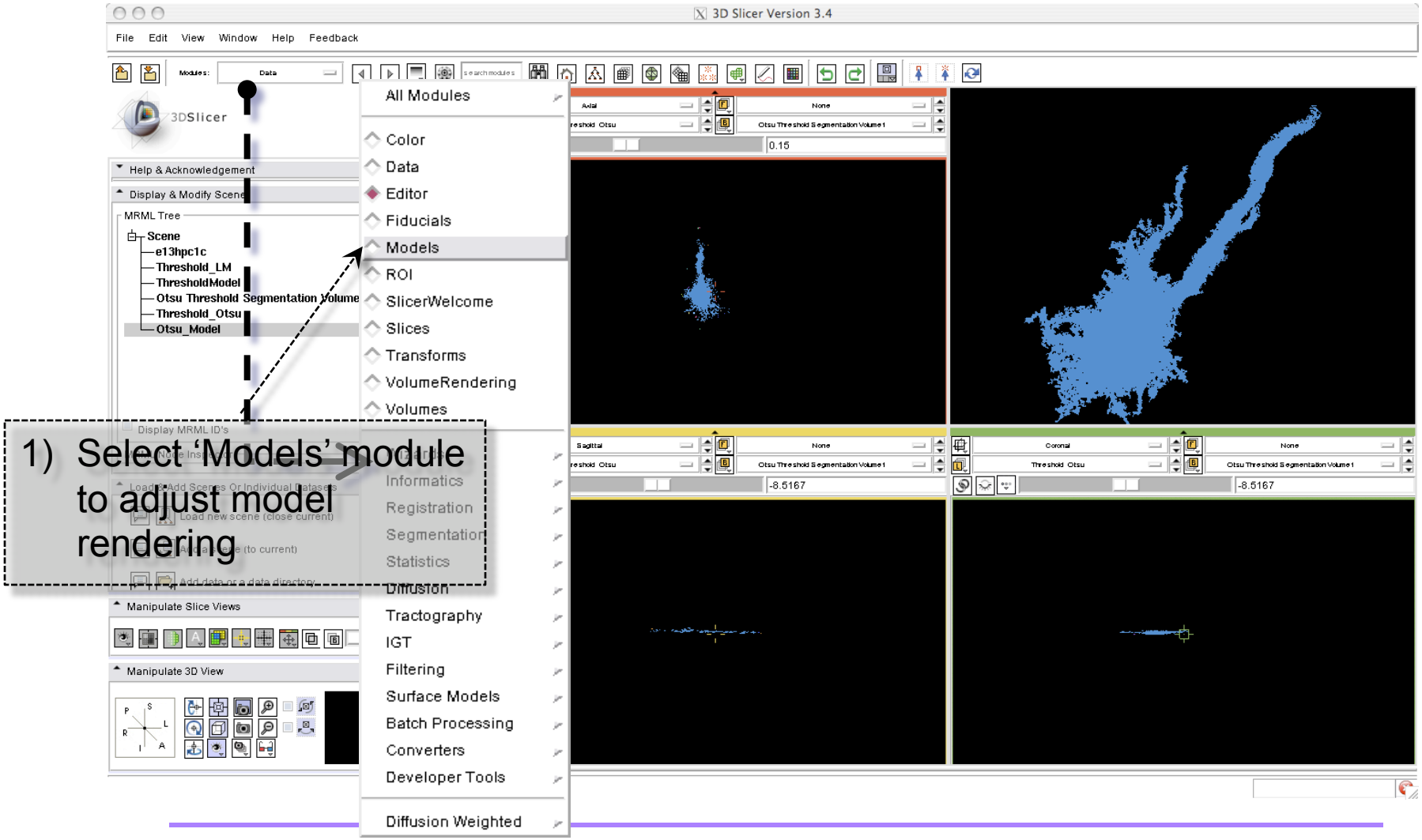
1) Toggle the visibility for the Threshold model off (right click on the model name to get menu)

Model Building: Otsu Segmentation

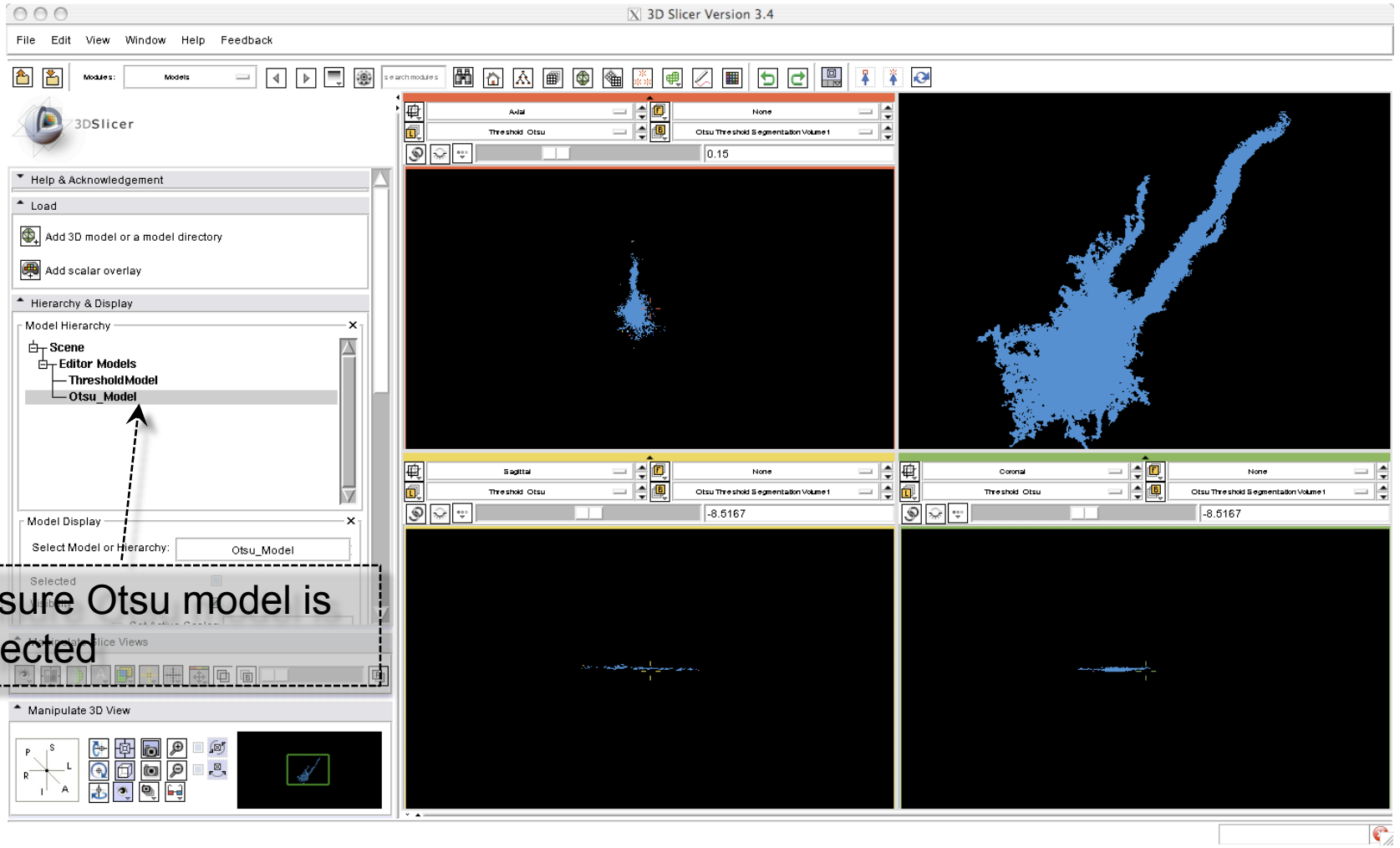


1) Toggle the visibility for the Otsu model on(right click on the model name to get menu)

Model Building: Otsu Segmentation

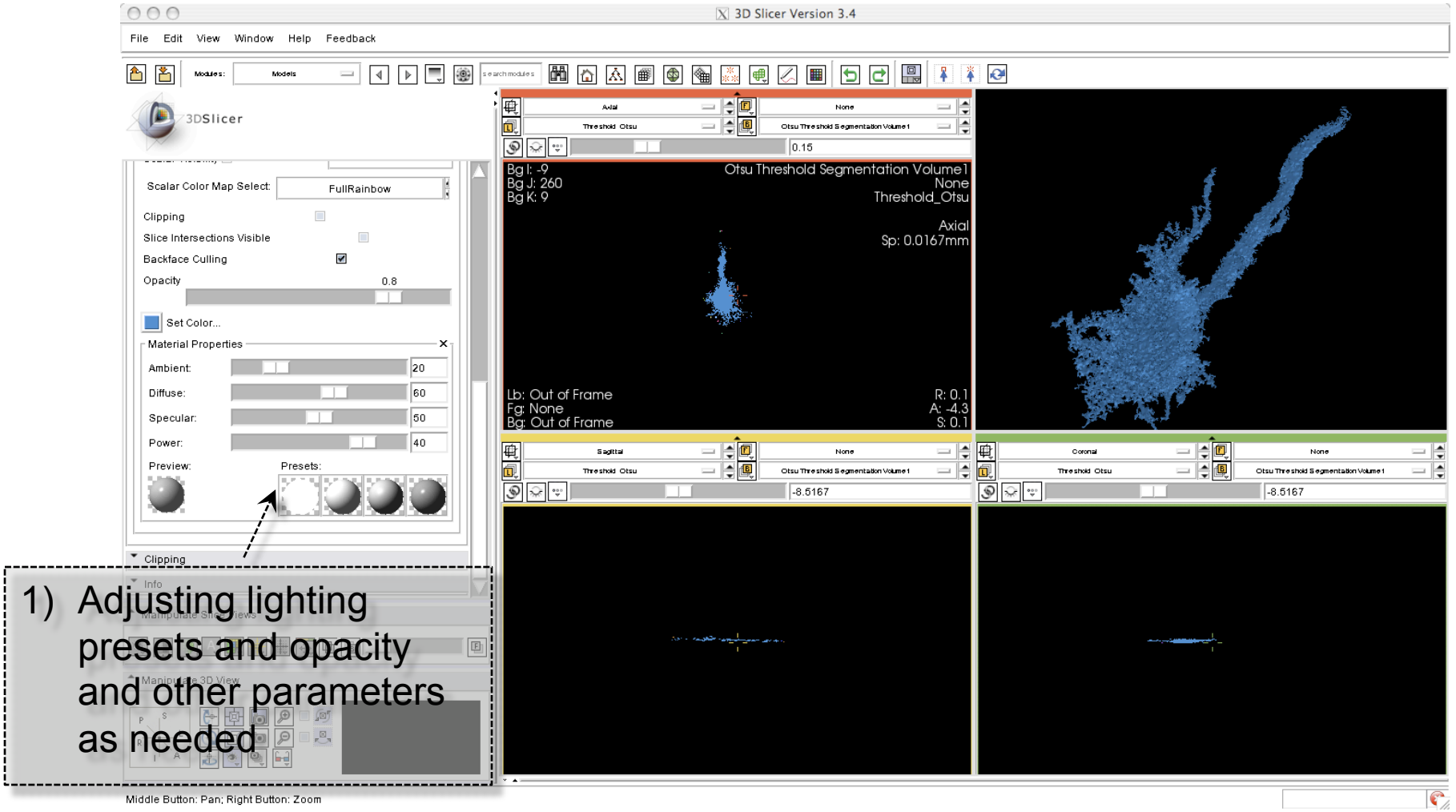


Model Building: Otsu Segmentation



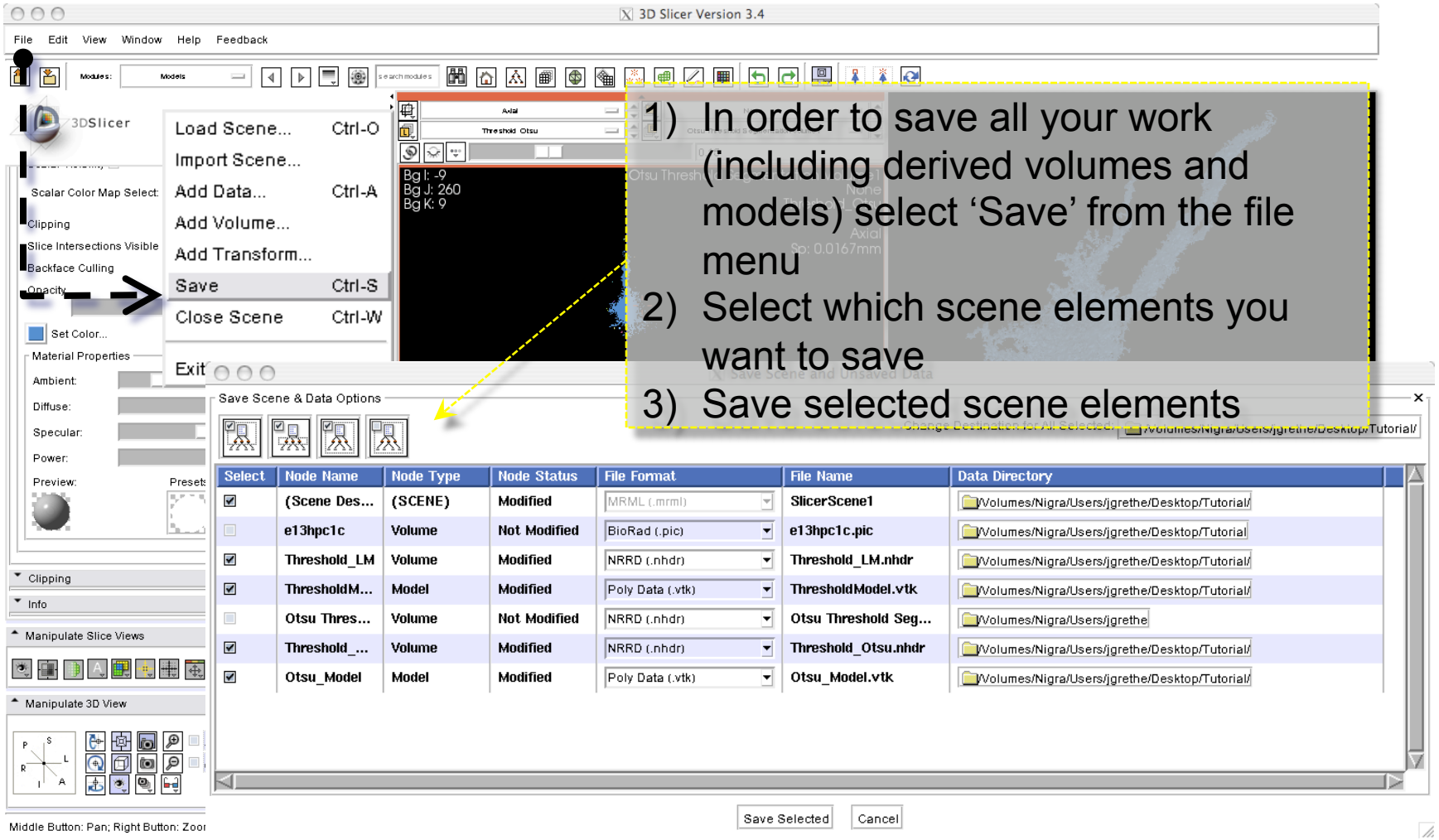
1) Ensure Otsu model is selected

Model Building: Otsu Segmentation

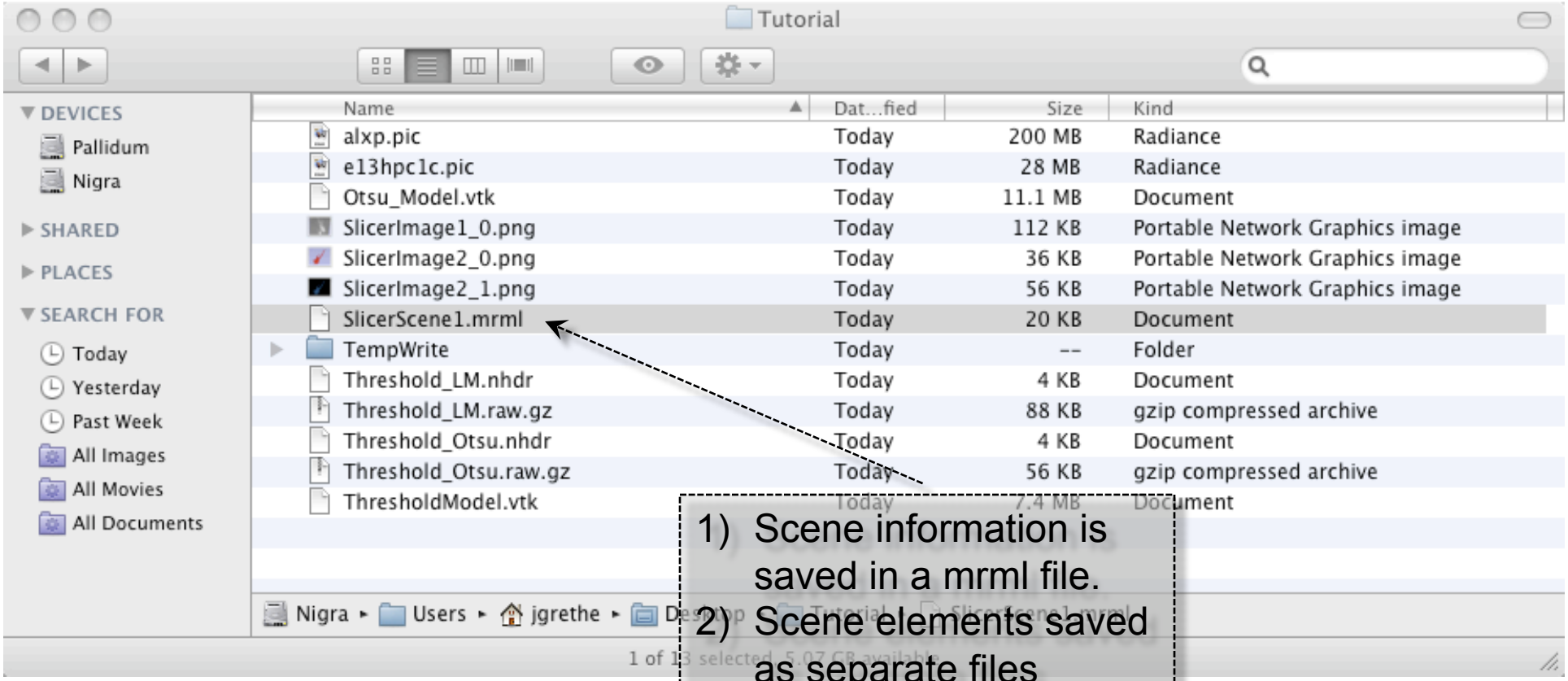


1) Adjusting lighting presets and opacity and other parameters as needed

Saving Your Work



Saving Your Work



Conclusion

- This training module should have provided you with information needed to build models from varying confocal microscopy data

- Slicer provides and intuitive graphical user interface to interact with the data

- Open-source environment and open-access data used in this tutorial

Acknowledgements

National Alliance for Medical Image Computing