



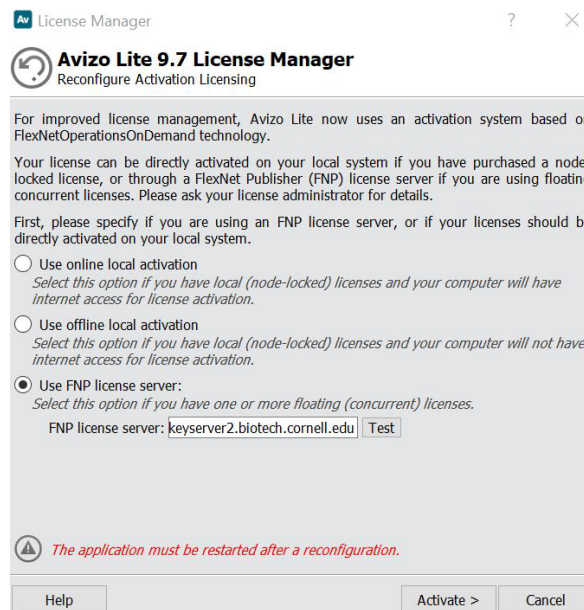
## Avizo Basic User Guide

Avizo is an image analysis software package designed to work smoothly with a variety of 3D imaging formats. Its particular strengths lie in its ability to perform a wide variety of analytical tasks on a particular data set, and in showing those analytical steps using a graphical workflow.

The screenshots included here were taken from Avizo v. 9.7.0 and may be slightly different than what you see if the software has been updated, but should be similar enough to be easy to follow.

The instructions below are designed to provide a general overview that will acquaint most users with the basics that they need to get started, based on BRC-Imaging staff's experience with a lot of different data types and researcher requests. However, Avizo is a huge software package with a lot of depth to it and a somewhat steep learning curve. The methods outlined below are far from the only way to obtain useful data. If you have a method you prefer, feel free to share, and if you have questions, feel free to stop by the lab or contact us at [brc\\_imaging\\_ct@cornell.edu](mailto:brc_imaging_ct@cornell.edu). If you have a particular task not covered here but are new to Avizo, please refer to the application notes after reviewing this document.

**A note for first time users:** The first time you load Avizo on either the B45 or B46 computer, you will need to enter the name of the server that the computer needs to ping to confirm a current license. Click the “Use FNP license server” button and enter our FNP license server address, which is **keyserver2.biotech.cornell.edu**. Click Activate. Avizo should now start up normally with the correct FNP license.



## 1) Download and unzip data folder.

For users on the B46 computer, please put your data onto the H: (RAID) drive in the “All User Images Here” folder, and delete it from the “downloads” folder or desktop as the C: drive has limited space. (Your data will load and process more smoothly if it is local rather than networked or from an external hard drive.) Once you’ve finished your analysis, back up your project, either on the fileshare (Z: drive) or somewhere else of your choosing. The BRC will not archive data on lab computers. We will delete data that has been on lab computers for more than a month, though we will usually contact you if your folder is clearly labeled with your name.

## 2) Load dataset in Avizo.

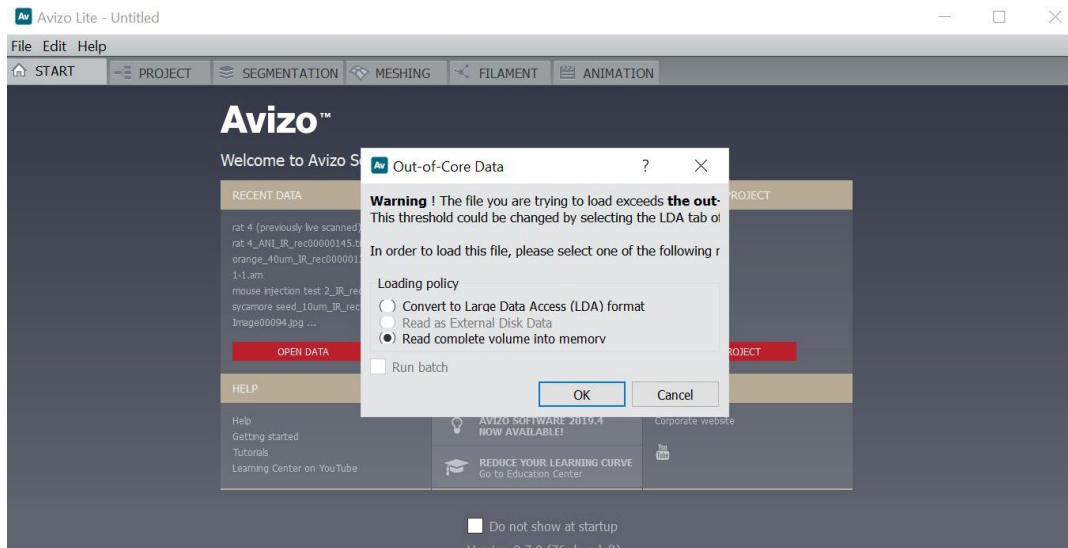
Start up the Avizo software. (If the License Manager window pops up, please refer to the instructions at the top of this manual for first time users.) Click the Open Data button or click File→Open Data, and select all of the files that comprise your data set.

A few notes on opening your data set:

- The folder will need to be unzipped if it isn’t already.
- Your downloaded folder might include non-data files (such as text files that include the scanning/reconstruction recipe, specimen information, or screenshots of the reconstructed data set). Don’t include these—the software is not smart enough to parse these and filter them out.
- The selected files need to be part of a continuous set (i.e., starting from file\_0001.dcm and going to file\_1000.dcm, with no jumps.) The software is not smart enough to parse discontinuities, except to warn you that they exist. If there are missing files, try downloading and unzipping the data again, and/or discuss this with the staff member who performed the scan. Note that the data CAN start or end at any arbitrary number, everything in the middle just needs to be continuous.
- A single .am file (Avizo’s data format) will contain all of the slices in it. If you’re working with a .am file you’ll only have one to open.

Note: Projects will be discussed later, but if you have one you want to open, you’ll just need to open that one file to get the whole project loaded.

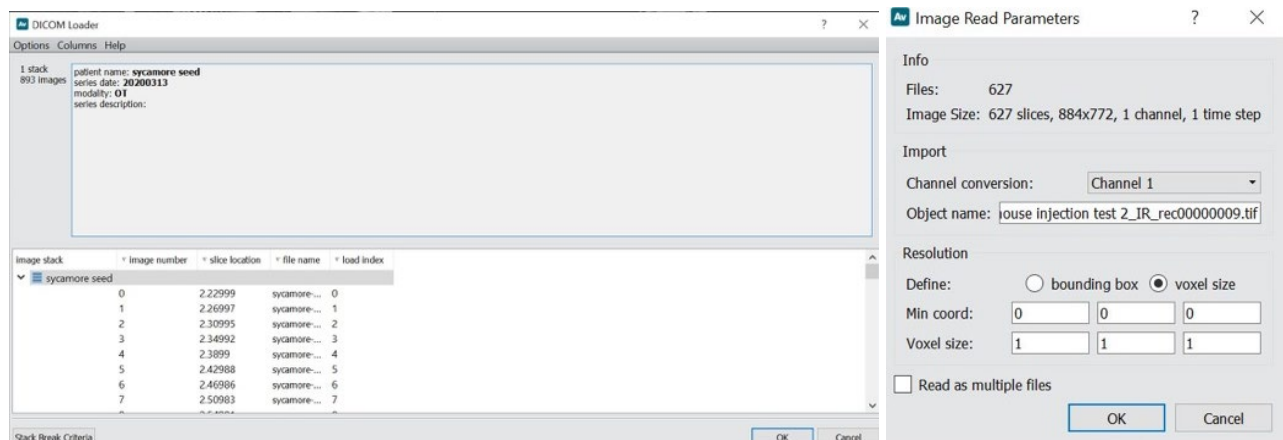
For files larger than about 1GB, you will get an Out-of-Core Data warning:



For files up to about 30GB in size, you can read the complete volume into memory. The B46 computer has plenty of processing power, so you don't need to convert your data.

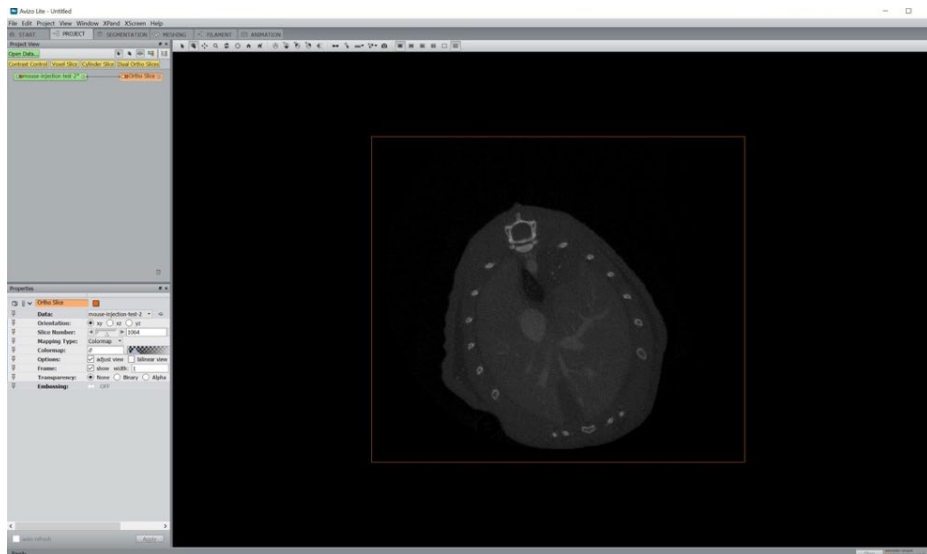
For DICOMs, a window will pop up that shows all of the slices through the data. Click OK to continue. (If the slices are showing up as red instead of black, or if the files don't load correctly, contact BRC staff for assistance.)

For file types that lack metadata about resolution, the Image Read Parameters window will pop up. Enter the resolution in x, y, and z, respectively in the voxel size boxes (units are mm).



### 3) Visualize your data in 2D in the Project Tab

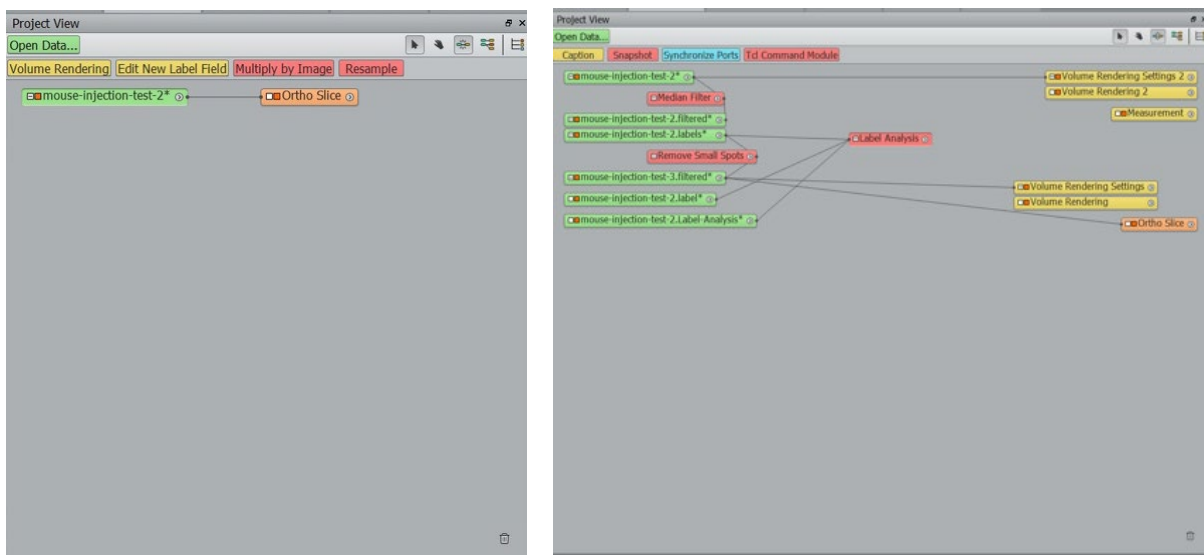
When you load your data, you will move to the Project Tab, which will typically be set up as seen below. If it's the first data set being loaded since the software was opened, you may already see a single 2D slice through the object in the visualization window.



**Visualization Window:** The large window is the visualization window, which shows 2D or 3D data.

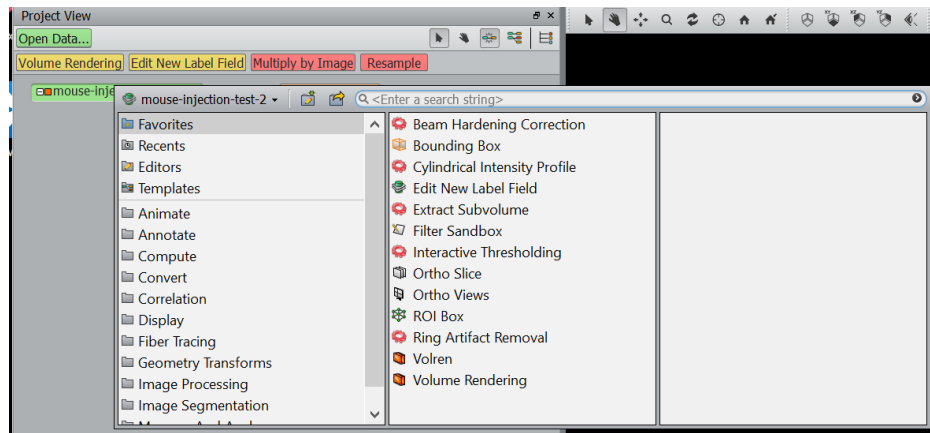
**Project View:** The project view window (upper left window) is a graphical representation of the workflow that had been done so far, and is where you can set up new calculations, visualizations, etc.

Green objects represent data sets, and can be saved in a variety of formats. The images below show two different Project View windows; first, a simple Project View window similar to what you might see after loading data, and a more complex project view window that shows several mathematical functions (in red), savable data files (in green), and multiple 2D and 3D visualizations (in orange and yellow).



To look at the data in 2D (assuming Avizo didn't automatically create the Ortho slice function), Right click on the green data object in the Project View and select "Ortho Slice". You should be

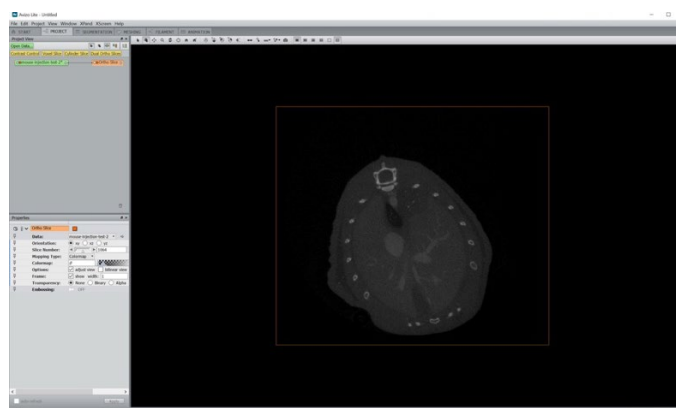
able to find Ortho Slice in the Favorites folder, the Display folder, or you can type it into the search box.



Once selected, it will show up as an orange object in the Project View window, and should appear in the main visualization window. If you don't want to see it in the visualization window, you can either remove the Ortho Slice object entirely by dragging it into the trash can in the Project View window or by clicking the small orange square:



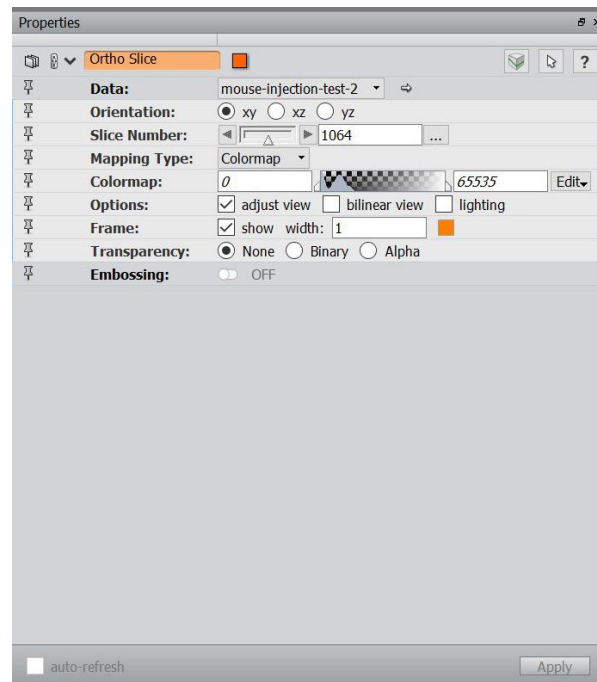
To make further adjustments to the 2D view—for example, to change the location of the 2D slice or brightness/contrast—click Ortho Slice and look at the Properties window in the lower left (if not visible, go to Window->Properties).



**Properties window:** When you click on an object in the Project View Window, the Properties Window will show information about and variables that can be changed on that object.

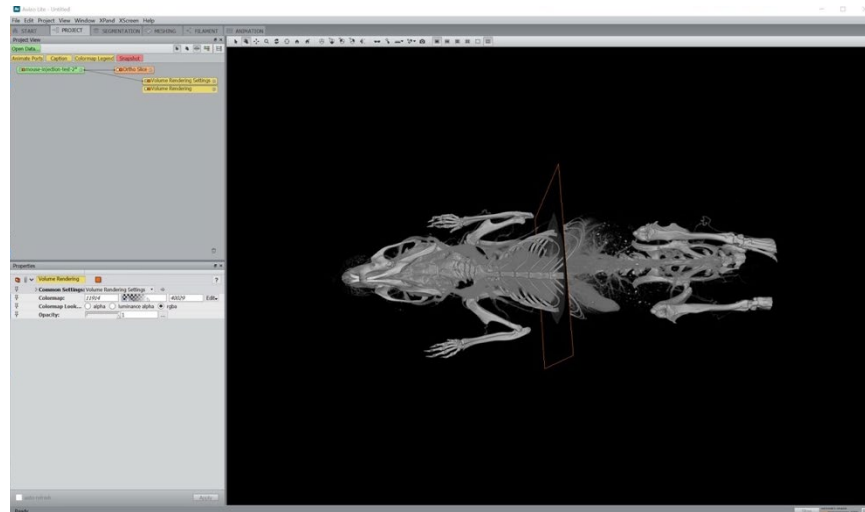
For a green data object, for example, it will show the resolution and other information about the file, and is where you will find options to crop your data set or change its location relative to other images that have been loaded. For a mathematical function which will be applied to the file, it will show the variables that can be applied to the data set before clicking Apply. In the Ortho Slice example we see below, it shows a variety of ways of viewing the data in 2D. These include:

- Changing the plane being viewed (xy, xz, or yz)
- The slice number being shown
- The colormap (brightness, contrast, and color combination), which can be adjusted by dragging the sliders on the histogram or choosing a color look up table from the drop-down menu
- The shading and transparency of the slice
- Help with learning more about these options (the question mark in the corner: the help section can generally be very specific and helpful if your question is “what does this button do,” less so when your question is “how do I make it look like this other image I have?” Feel free to contact BRC staff for assistance with the latter.)



#### 4) View the data in 3D.

Right click on the green data object and select Volume Rendering from Favorites. Two Volume Rendering-related objects will appear in the Project View window, and a 3D rendering will appear in the Visualization window. In the view below, both the Ortho Slice and Volume Rendering are being shown simultaneously. Again, to toggle either view, click the small orange box inside the object.



At the start, the whole scanned region, including air, may be visible. The brightness and contrast can be adjusted by clicking the Yellow Volume Rendering object, and adjusting the handles on the “Colormap” slider in the Properties window. When using any of Avizo’s volumetric colormaps, the transparency will be adjusted automatically as the brightness and contrast are adjusted.

Menu bar keys: To manipulate the volume rendering, you’ll need to use some of the buttons along the top menu bar, which change the function of clicking and dragging the mouse button.



There are some I rarely use, but I will highlight the most useful ones below.



This section adjusts the data set, by rotating, shifting, and zooming the data. The “Set Home” button relocates the centerpoint around which the data rotates to the center of the screen.





This section enables you to do things like create a stereoscopic view of your data for use with 3D glasses, take measurements, and take screenshots

## 5) Segment your data set

There are a variety of reasons you may want to segment your data. Here are a few commonly encountered by BRC users:

- You want to remove or highlight a particular part of your scan for an improved final image.
- You want to select one part of your scan to 3D print.
- You want to analyze some part of your scan based on parameters you'll choose later.

Some objects are easier to segment out than others, and some researcher needs are more stringent than others, but the basic workflow is the same either way.

To start segmenting, right click on the green data set object in the Project View tab and select "Edit New Label Field" from the Favorites folder or search bar. This will send you to the Segmentation tab. (Note: you can go back to the Project tab any time without losing your progress.)

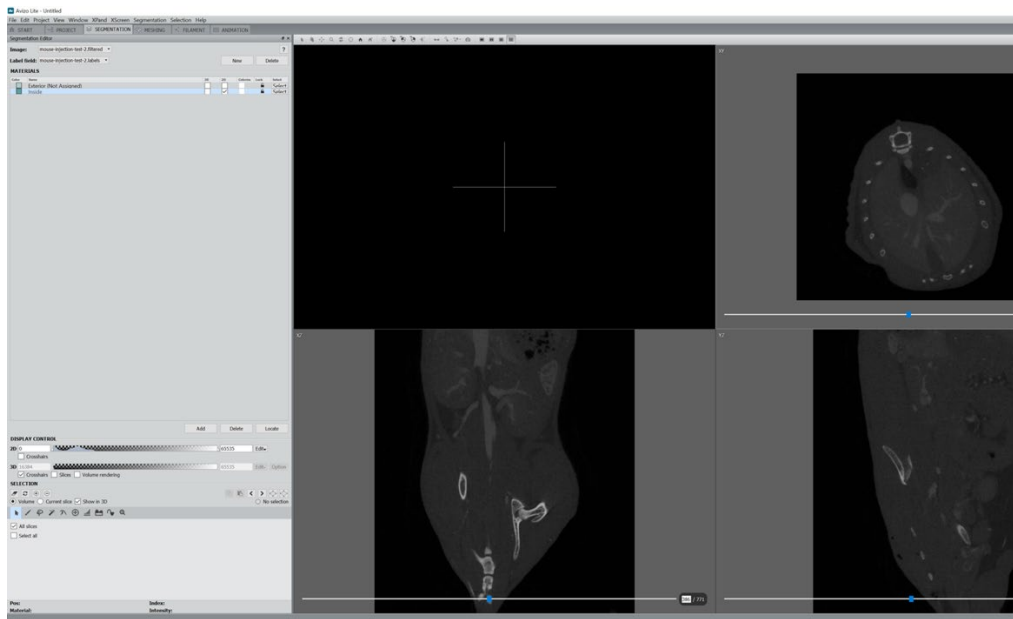
The basic principle of how Avizo does segmentation is as follows: First, it creates a new parallel dataset, of the same dimensions as the original dataset. You can only edit this dataset in the Segmentation tab. (If you go back to the Project tab, you will see a new green object attached to the original data, with the filename extension "filename.labels".) Every voxel in a segmented image is assigned a value. To start with, they are all assigned a value of 0, corresponding to the gray label named "Exterior".

You can select any number of voxels using any combination of the tools in the lower left area. These will show up as a solid red color in both 2D and 3D view, but it's important to note that selecting the data does not change the dataset unless you have told Avizo what to do with the selection.

Once a region is selected, you can choose to add it to a label, subtract it from a label (subtracting it will reassign it to the "Exterior" label and give it a value of 0), or click the eraser button to undo the selections you've made. You can make any number of labels, and voxels in those labels will be assigned a value of 1, 2, 3, etc. Once the selected voxels are assigned to a label, you will see a shaded area overlaid the original grayscale data in the 2D views.

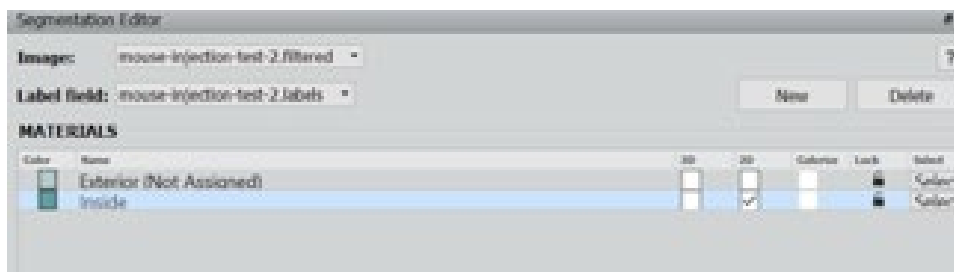


An overview of the Segmentation tab:

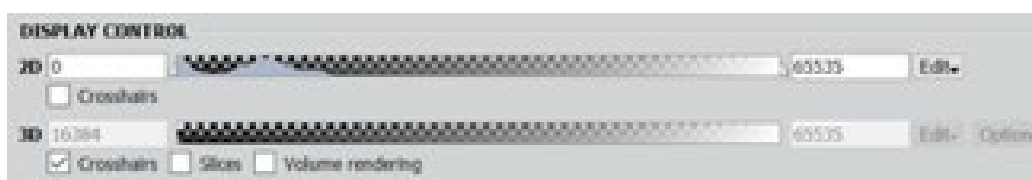


As in the Project view, the large main window displays the data. Here, the upper left corner will show a 3D view, and the other three windows are the xy, yz, and xz slices through the data, respectively. The 3D view can be manipulated using tools along the top bar just as in the Project tab.

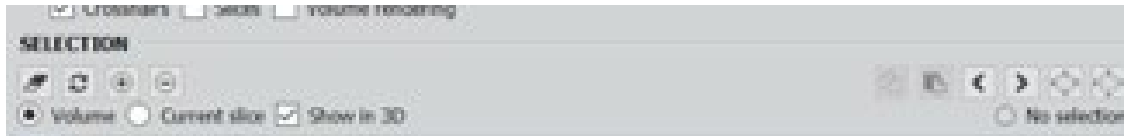
The upper left area shows any labels you have created and their respective color codes. By default, Avizo gives you two, “Exterior” (value of 0) and “Interior” (value of 1). You can add labels, or change label names and colors by right clicking in this area. By default, the labels have a value of 0, 1, 2, 3, etc. You can also choose to display your selections in 2D or 3D using the toggle buttons on the right side of the image below.



The middle-left section adjusts the brightness and contrast of the displayed data.



The middle left section, “Display Control”, allows you to adjust the brightness and contrast in the main window.



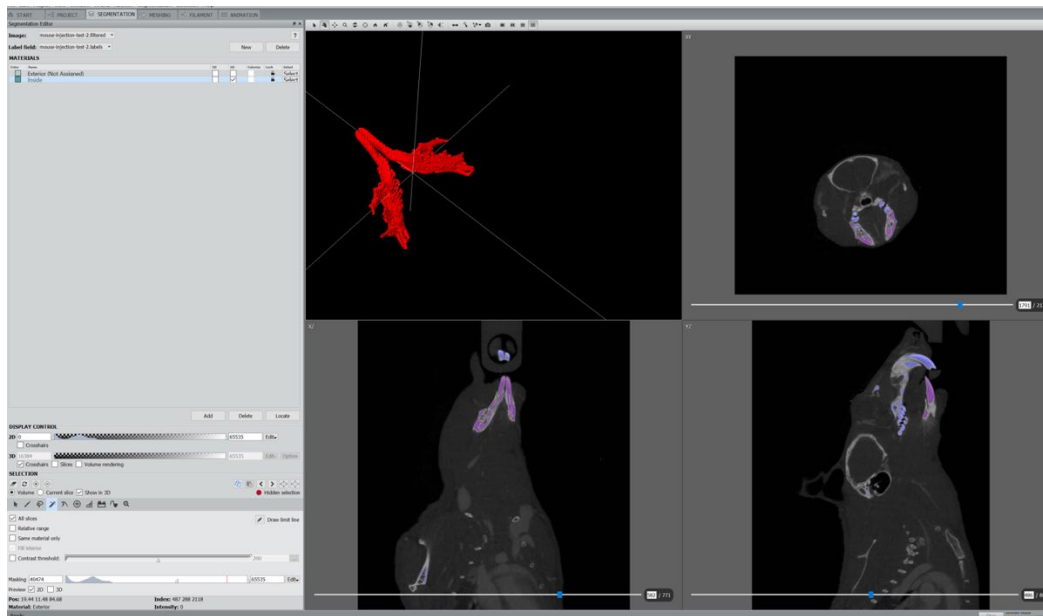
The “Selection” section shows all of the things you can do once you select some portion of the data. Options include:

- Clearing the selection (i.e., not adding or removing it from any existing label field)—farthest left “eraser” button
- Adding or removing it from a label field (to do this, click on the label you want to modify and then + or -)
- Grow or shrink the selection, which takes the entire selection and shrinks or grows it by 1 pixel in every dimension—farthest right buttons, showing squares with small arrows pushing in (shrink) or pushing out (grow)



The bottom toolbar displays all the ways to select data.

- Paintbrush and Lasso: Allow you to manually select an area of interest on a particular slice. To select a larger 3D region, you don’t need to manually select every single slice. Just pick slices with whatever spacing you like and then click Selection→Interpolate. Avizo will interpolate the initially-empty slices based on the information in the filled slices.
- Magic Wand and Threshold: Segment based on brightness. The threshold tool will select every pixel within a particular brightness range. Use the handles on the bottom histogram to select the brightness range you want. The magic wand tool will select every pixel that is BOTH 1) Within the selected brightness range, and 2) directly or indirectly contacting the spot you click within that brightness range, via any bridging material within that brightness range. In the example below, the lower jaw has been selected using the magic wand tool, by clicking in that area and adjusting the brightness handles to select the region of interest. In the 2D view, everything that is gray is not within the chosen brightness range, and everything that is blue is within the brightness range, but is not linked to the pixel that was clicked via a path of similar brightness. Only the lower jaw, which is of a similar brightness and which does touch that selected voxel, is highlighted in red/purple.



Once the region of interest has been selected, you can add it to your desired material using the plus sign under the Selection bar.

## 6) Viewing and manipulating segmented data

Return to the Project tab at the top. You will see a new green object with the name [filename].labels just underneath the original data set. Right click on the new .labels data object and select Volume Rendering, to view the selection and the original grayscale data at the same time. For example, in the image below, you can see the original grayscale data showing a mouse, and the lower jaw has been selected and added to a label field. The section of the dataset that has been labeled can be analyzed by right-clicking and selecting the “Label Analysis” function.

